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Molecular basis for therapeutic decisions in chronic myeloid leukemia patients after allogeneic bone marrow transplantation

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ABSTRACT

Background and Objectives. **Recent progress in the development of diagnostic techniques has greatly facilitated the monitoring of minimal residual disease (MRD) in patients with chronic myeloid leukemia (CML) after allogeneic bone marrow transplantation (BMT), the only curative treatment for this disease. The presence of the P210$^{bcr-abl}$ rearrangement in CML cells has allowed highly sensitive detection of MRD by polymerase chain reaction (PCR). However, complete eradication of the leukemic clone may not be a necessary prerequisite for long-term remission or cure. This observation limits the value of qualitative PCR analysis for prediction of progressive disease and highlights the need to monitor the proliferative activity of the malignant clone in order to permit timely detection of impending relapse and, thus, early therapy. This article discusses the applicability of several molecular methods to the monitoring of treatment efficacy and early assessment of clonal expansion in patients with CML after BMT. It also presents guidelines for clinical use of PCR analyses and the most effective approaches to treat relapsed patients.**

Information Sources. **The authors have been working in this field, both experimentally and at a clinical level, contributing original papers to peer-reviewed journals. The material examined in this review includes articles published in journals covered by MedLine® and reviews from journals with a high impact factor.**

State of the Art and Perspectives. **In view of the very limited value of qualitative PCR in detecting CML patients destined to relapse after BMT, several investigators have developed molecular assays that enable the kinetics of MRD to be monitored over time (e.g. quantitative PCR for P210$^{bcr-abl}$, PCR analysis of whole blood/lineage-specific chimerism and qualitative PCR for P190$^{bcr-abl}$). These molecular strategies closely trace the kinetics of leukemic regrowth. Disease evolution in relapsed patients is consistently characterized by the sequential detection of increasing P210$^{bcr-abl}$ transcript levels, increasing myeloid mixed chimerism and finally, P190$^{bcr-abl}$ positivity preceding cytogenetic relapse. A 10-fold or greater increase in the expression of P210$^{bcr-abl}$ confirmed by a minimun of three independent quantitative PCR analyses and/or a progressive increase in the percentage of host myeloid cells in three consecutive chimerism analyses and/or P190$^{bcr-abl}$ mRNA detection must be regarded as an indication of incipient disease progression and should provide a rationale for initiation of treatment. There are various approaches to the management of the patient who relapses. The first step, if possible, is to reduce or terminate immune suppression. If the patient is not receiving this therapy, he or she can be treated with hydroxyurea or interferon or can be offered a second transplant. However, infusion to the patient of lymphoid cells (DLI) collected from the original donor has the capacity to restore complete remission in 70-80% of cases. Currently, several strategies are being used to minimize the severity of graft-versus-host disease after DLI (optimization of transfused lymphocyte doses, modification of the transfused lymphocyte subsets, administration of lymphocytes in escalating doses or lymphocyte transfection with a suicide gene), to reduce the incidence of marrow aplasia (stem cell support) and to increase the rate of complete responses (cytokines associated with DLI, leukemia-reactive cytotoxic lymphocytes, tyrosine kinase inhibitors or pre-emptive DLI).**

Key words: CML, PCR, BMT, BCR-ABL, relapse, DLI

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High-dose myeloablative chemotherapy followed by allogeneic bone marrow transplantation (BMT) provides the most effective treatment for chronic myeloid leukemia (CML) with 40-60% of patients remaining disease free for more than 5 years post-BMT.[1] The success of this procedure in these patients is related not only to the intensive conditioning therapy but also to the anti-leukemic properties of the donor graft. This critical factor is the so-called graft-versus-leukemia (GVL) effect, and is mediated, at least in part, by mature donor T-cells contained in the marrow graft.[2]

Unfortunately, relapse leukemia remains a major cause of treatment failure after allogeneic BMT and treatment options for relapse are limited. A minority of patients may be cured

after second allogeneic BMT, but the anticipated outcome is disappointing.[3] Donor leukocyte infusions (DLI) can provide a direct GVL reaction and offer an effective approach to relapse that is safer than a second BMT, achieving complete remissions in 60-80% of patients.[4] Among prognostic determinants of response to salvage therapy, disease burden appears to represent a significant factor.[5,6] Thus, minimal residual disease (MRD) evaluation aimed at early detection of relapse has relevant therapeutic implications in this context.

How should patients be monitored after BMT?

Reverse transcription-polymerase chain reaction (RT-PCR) can detect CML cells through amplification of the unique P210$^{bcr\text{-}abl}$ fusion mRNA transcript which is the molecular correlate of the Philadelphia (Ph') chromosome. However, the prognostic value of detecting the P210$^{bcr\text{-}abl}$ fusion message by conventional RT-PCR amplification after BMT for CML remains a central clinical question because not all patients who are PCR-positive after treatment progress to clinical relapse.[7-24] It is evident that the majority of patients examined during the first few months after BMT are PCR-positive and only become negative in the years following the transplant. Nevertheless, some patients remain constantly or intermittently PCR-positive even after several years and these patients seem to have an increased, but not certain, probability of relapse (Table 1, Figure 1A). Taken together, the data available not only indicate that mere detection of PCR positivity in CML does not permit reliable prediction of the course of disease in individual patients, but also that *cure* of CML by BMT should be understood as a functional process (*functional cure*) rather than the absence of all evidence of disease (*molecular cure*).[25]

In view of the very limited value of qualitative PCR, several groups have developed molecular assays that enable the kinetics of residual disease to be monitored over time.

Quantitative RT-PCR

Several investigators have employed quantitative or semiquantitative PCR assays (Q-PCR) to estimate the amount of MRD in positive specimens, rather than just the simple presence or absence of P210$^{bcr\text{-}abl}$ transcript.[26-32] After BMT, serial Q-PCR analyses of peripheral blood samples can effectively distinguish patients who are destined to remain in remission from those who are destined to relapse. Patients who remain in remission have persistently undetectable, low or falling P210$^{bcr\text{-}abl}$ levels on sequential analyses. Other patients may remain intermittently or persistently PCR-positive for prolonged periods of time without evidence of cytogenetic relapse. The level of detectable P210$^{bcr\text{-}abl}$ transcript in these individuals is usually very low. In contrast, in patients destined to relapse, an increasing or persistently high level of P210$^{bcr\text{-}abl}$ mRNA can be detected on sequential analyses often several months (median 6 months) before the cytogenetic detection of the Ph'-chromosome (Table 2).

Table 1. Qualitative RT-PCR monitoring studies in CML patients after BMT.*

Study	*PCR– (relapse/pts)*	*PCR+ (relapse/pts)*	*Sensitivity*
Martiat *et al.*[7]	0/3	0/2	10^{-5}-10^{-6}
Kohler *et al.*[8]	0/6	1/4	10^{-5}
Hughes *et al.*[9]	0/9	4/9	10^{-5}
Snyder *et al.*[10]	0/1	1/13	NA
Lange *et al.*[11]	0/1	1/8	10^{-5}
Delage *et al.*[12]	0/3	6/19	10^{-6}
Guerrasio *et al.*[13]	0/42	0/6	10^{-5}
Lee *et al.*[14]	0/0	1/4	NA
Roth *et al.*[15]	0/23	10/31	10^{-6}
Miyamura *et al.*[16]	1/11	9/53	10^{-5}
Gaiger *et al.*[17]	0/15	5/15	10^{-6}
Mackinnon *et al.*[18]	0/9	8/17	10^{-5}
Diekmann *et al.*[19]	2/3	5/11	NA
Xu *et al.*[20]	0/18	0/32	10^{-5}
Pichert *et al.*[21]	0/23	24/69	10^{-5}
Radich *et al.*[22]	14/319	33/117	10^{-5}
Santini *et al.*[23]	0/10	0/6	10^{-5}
Roman *et al.*[24]	0/21	7/34	10^{-5}
Total	17/517 (3.3%)	115/450 (25.5%)	

*Abbreviations: NA, not available. *Only patients studied at 2 or more time points have been included.*

Despite these encouraging results, Q-PCR remains labor intensive and costly. Lack of standardization has made it difficult to compare results between different centers.[33] In the near future, however, it is likely that increasing automation (i.e., real time PCR) and the use of appropriate internal controls will enable Q-PCR to become more widespread.

Hematologic chimerism analysis

As a further approach to monitoring post-BMT outcome, several investigators have employed chimerism analysis using highly polymorphic loci detection.[34,35] These techniques allow the relative proportions of host and donor cells in the post-BMT period (mixed chimerism MC) to be identified and quantified. Characterization of this phenomenon might be of special importance in patients transplanted for leukemia, because the presence of recipient cells might reveal reappearance of the malignant clone. Although chimerism analysis cannot assess whether or not a re-emerging endogenous

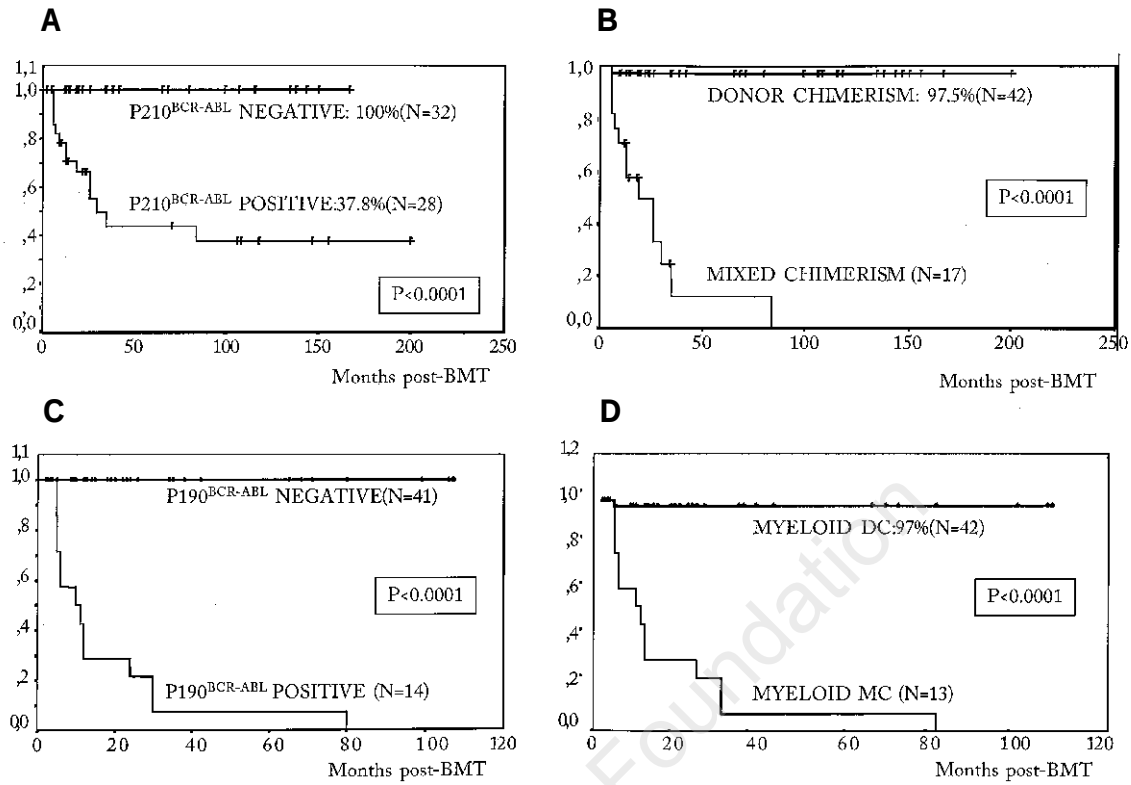

Figure 1: Cordoba BMT group experience in monitoring CML patients after allogeneic BMT. Influence of P210$^{bcr-abl}$ status (A), whole blood chimerism (B), P190$^{bcr-abl}$ status (C) and myeloid chimerism (D) on the Kaplan-Meier probabilities of disease-free survival.

population contains leukemic cells, samples taken at intervals can provide evidence that the expansion rate of a re-emerging clone is consistent with malignant growth.

In recent studies, we and others[18,24,34,36-43] have shown that full donor chimerism (DC), as detected by PCR assay, is associated with prolonged disease-free survival and identifies patients with a low risk of leukemic relapse after BMT for CML, whereas MC is significantly associated with cytogenetic or hematologic relapse in both unmanipulated and T-cell depleted grafts. Moreover, host cells can be detected between 3-6 months before cytogenetic relapse and between 5-21 months before hematologic relapse (Table 3, Figure 1B). However, chimerism studies after BMT have been hampered by the use of whole blood instead of lineage-specific hematopoiesis. This latter issue is particularly relevant in the setting of CML patients. In fact, the disease is predominantly expressed in the myeloid compartment and T-lymphocytes rarely belong to the leukemic clone. Moreover, T-cells frequently survive the conditioning regimen and thus, they may affect interpretation of the chimerism findings concerning prognostic impact.

We have overcome this drawback by analyzing lineage-specific chimerism in highly purified cell fractions.[24,42] Two clearly defined groups of patients with MC can be observed after BMT for CML:

a) a group defined by MC and P210$^{bcr-abl}$ negativity. None of these patients relapses. Lineage specific analysis of chimerism in these patients indicates that this MC reflects the transient persistence of recipient T-cells that escape control

Table 2. Quantitative RT-PCR monitoring studies in CML patients after BMT*.

STUDY	N	PCR– (relapse/pts)	PCR+ (relapse/pts)
Cross *et al.* [28]	28	0/17	11/11
Lion *et al.* [29]	28	0/23	5/5
Lin *et al.* [31]	98	1/69	21/29
Total	154	1/109 (0.9%)	37/45 (82%)

**PCR+ indicates increasing or persistently high BCR-ABL transcript levels; PCR- indicates undetectable, decreasing, or low BCR-ABL transcript levels.*

Table 3. Chimerism PCR monitoring studies in CML patients after BMT.

Study	N/BMT Type	MC (%)	Relapse	Relapse/MC
Lawler *et al.* [18]	4/TCD	3 (66%)	1/4	1/3
	2/UM	1 (50%)	0/2	-
Roux *et al.* [36]	16/TCD	8 (87%)	7/16	7/8
Mackinnon *et al.* [24]	30/TCD	22 (37%)	10/30	9/22
	2/UM	0	0	-
Elmaagacli *et al.* [37]	28/UM	18 (64%)	4/28	4/18
Gardiner *et al.* [38]	4/TCD	4 (100%)	3/4	3/4
	14/UM	3 (21%)	1/14	1/3
Roman *et al.* [39]	15/UM	2 (13%)	2/15	2/2
Cordoba BMT Group [40-42]	11/TCD	7 (63%)	7/11	7/7
	48/UM	10 (20%)	8/48	7/10
TOTAL	65/TCD	44 (68%)	28/65 (43%)	27/44 (54%)
	109/UM	34 (31%)	15/109 (14%)	14/34 (41%)

Abbreviations: TCD, T-cell depleted graft; UM, unmanipulated graft; MC, mixed chimerism.

by allogeneic immune effector cells. These patients show decreasing or stable low levels of autologous signals over time;

b) a group of patients with MC and P210$^{bcr-abl}$ positivity all of whom relapse. Regardless of the origin of T-cells, all of these patients show MC in the myeloid population as demonstrated by cell-lineage specific analysis. Moreover, they show increasing amounts of autologous cells in contrast to patients who developed MC in the recovery phase (Table 4, Figure 1D).

RT-PCR for P190$^{bcr-abl}$ mRNA

Recently, the P190$^{bcr-abl}$ transcript, which is classically associated with Ph'-positive acute lymphoblastic leukemia, has been detected at diagnosis in virtually all patients with CML, in whom it occurs as a consequence of alternative or mis-splicing events in the BCR gene[44-46]. Because the amount of P190$^{bcr-abl}$ has been correlated with that of P210$^{bcr-abl}$ corresponding to 0.02-30% of the total bcr-abl transcripts, one could speculate that a rising tumor burden is needed for P190$^{bcr-abl}$ to become detectable by RT-PCR during hematologic remission.

Recently, we tested the hypothesis that P190$^{bcr-abl}$ mRNA detection could be used, in addition to other markers, as an indicator of disease evolution in the post-BMT outcome of CML patients.[42] Out of 55 CML transplant recipients, fourteen relapsed. P190$^{bcr-abl}$ was detected 1-6 months prior to cytogenetic relapse in 11 patients, and concomitantly with cytogenetic relapse in 3 patients. In the remission group, all patients tested invariably negative for P190$^{bcr-abl}$. In contrast to P210$^{bcr-abl}$, P190$^{bcr-abl}$ mRNA emerges from our study as a novel marker of CML evolution after BMT. In fact, P190$^{bcr-abl}$ positivity by non-quantitative RT-PCR was associated with impending cytogenetic relapse in the majority of patients. Moreover, P190$^{bcr-abl}$ mRNA was not detected in any patient as a reversible finding nor was it ever found in long-term survivors (Figure 1C). Therefore, P190$^{bcr-abl}$ detection (if confirmed by other studies) could be a simple way to detect relapse early after BMT without the need for expensive quantitative techniques.

As we can see, combinations of all the above-specified molecular strategies closely trace the kinetics of leukemic regrowth after allogeneic BMT. In fact, disease evolution in relapsed patients is consistently characterized by the sequential detection of P210$^{bcr-abl}$ transcripts, increasing amounts of myeloid chimerism, and finally P190$^{bcr-abl}$ positivity preceding impending cytogenetic and hematologic recurrence. During this period of time, increasing levels of P210$^{bcr-abl}$ mRNA are observed (Figure 2).

Table 4. Myeloid specific chimerism monitoring studies in CML patients after BMT.

Study	Relapse/N	Myeloid MC/Relapse
Roux *et al.*[36]	5/5	5/5
Verdonck *et al.*[43]	2/11	2/2
Cordoba BMT Group[40-42]	14/60	14/14
TOTAL	21/76	21/21(100%)

Abbreviation: MC, mixed chimerism.

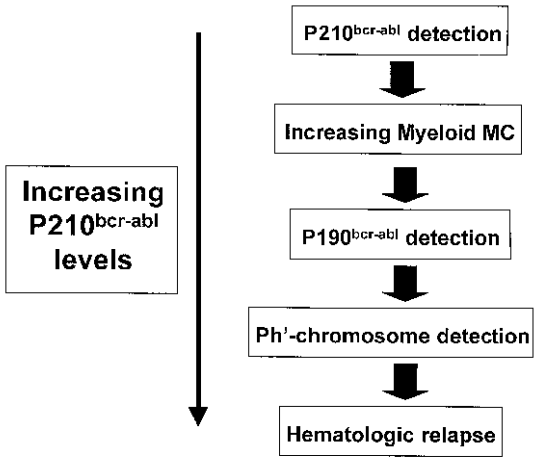

Figure 2. Molecular kinetics of leukemic regrowth in CML patients after allogeneic BMT.

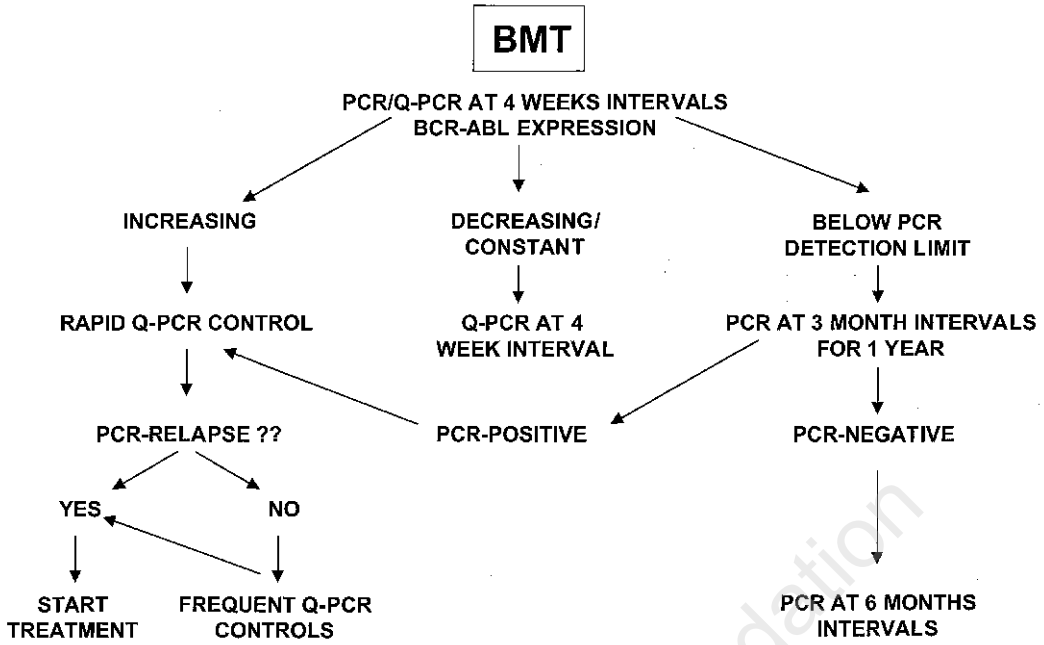

Figure 3: Guidelines of the EICML group for clinical use of quantitative PCR analysis for P210$^{bcr-abl}$ in CML patients after BMT.

The excellent correlation between Q-PCR findings and clinical course of the disease provided a basis for general recommendations by the group of *European Investigators on CML* (EICML group)[30] on the use of Q-PCR for monitoring of MRD after BMT (Figure 3): patients are followed-up by qualitative PCR at 3-monthly intervals in the first year after BMT, when two-step PCR results are negative, and at 6-monthly intervals during further years of PCR negativity. In patients with persistent post-BMT or reappearing P210$^{bcr-abl}$ positivity, Q-PCR must be performed at 1-month or shorter intervals.

Based on our own observations,[42] we propose alternative and/or complementary guidelines for clinical use of PCR analyses in CML after BMT (Figure 4): patients should be monitored during the entire post-transplant course of disease by qualitative RT-PCR for detection of P210$^{bcr-abl}$ fusion message. Negative RT-PCR results allow gradual extension of the time intervals between PCR analyses. PCR follow-up during the first year after achievement of a PCR negative status should entail analyses every 3 months. Subsequently, the intervals could be increased to a maximum of 6 months. As long as a patient tests P210$^{bcr-abl}$ positive, chimerism analyses should be performed at least once every four weeks. If MC is observed, blood samples should be collected and analyzed as rapidly as possible to assess increasing myeloid MC and P190$^{bcr-abl}$ status.

One important issue is that the frequency of PCR analyses required for efficient monitoring of MRD greatly reduces the possibility of using bone marrow as the preferential source of cell material. However, Lin *et al.*[47] and Huss *et al.*[48] found a high degree of concordance between bone marrow and peripheral blood in terms of sensitivity in the assessment of MRD and chimerism by PCR analyses. The reliance on analyses of peripheral blood cells does not, therefore, seem to adversely affect the sensitivity of PCR testing in CML patients, facilitating the follow-up of such patients.

When should patients be treated after BMT?

DLI therapy has resulted in a remission rate in excess of 70% in patients with relapse of CML following BMT.[49] Data confirm that patients entering remission with DLI for chronic-phase relapse of CML are less likely to recur than patients treated for advanced-phase relapse, supporting recommendations to treat patients early in the course of relapse. Furthermore, response rates appear to be higher in patients with only cytogenetic relapse.[50] Because disease burden appears to represent a determinant fac-

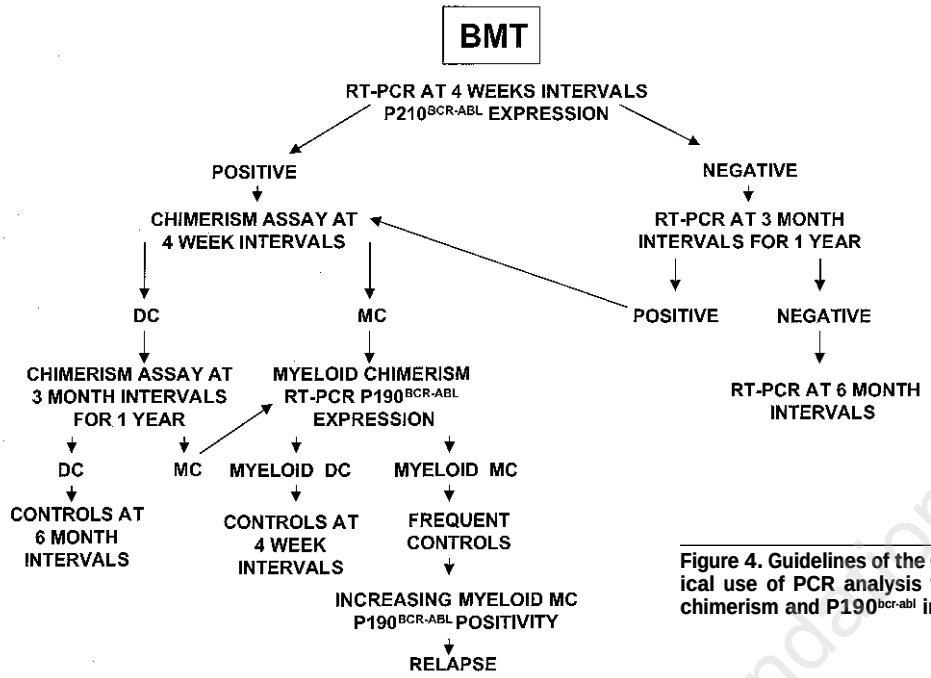

Figure 4. Guidelines of the Cordoba BMT group for clinical use of PCR analysis for P210$^{bcr-abl}$, hematologic chimerism and P190$^{bcr-abl}$ in CML patients after BMT.

tor in response to salvage therapy, DLI should be considered in patients with CML at the time of *molecular relapse* in order to achieve two important beneficial effects: a) early treatment may result in a better success rate in accomplishing cure of the disease as well as requiring lower doses of DLI, thus possibly eradicating all host-type tumor cells with a smaller chance of GVHD[51], and b) a state of MC is frequently observed at this early relapse-phase and is prognostically important since persistence of PCR detectable donor cells prior to DLI is associated with molecular remission without risk of severe aplasia, whereas absence of MC correlates with the occurrence of severe myelosuppression.[6]

However, an important issue is how should molecular relapse be defined, taking account of the fact that low-level P210$^{bcr-abl}$ positivity after allografting may not herald clinically relevant disease recurrence. Introduction of the term *PCR relapse* has been proposed by the EICML group based on the dynamics of P210$^{bcr-abl}$ expression. PCR relapse has been defined as a 10-fold or greater increase in the relative expression of the marker gene detected and confirmed by a minimum of 3 independent, consecutive Q-PCR analyses. The proposed definition has been designed to account for the possibility of transient changes, such as fluctuating P210$^{bcr-abl}$ expression, and the inaccuracy inherent in the technique.

Our group has also established criteria for *molecular relapse*. In our experience, a progressive increase in the percentage of host myeloid cells in three consecutive chimerism analyses and/or P190$^{bcr-abl}$ mRNA detection are points of no return and infer impending *cytogenetic relapse*.[42]

The presence of an increasing number of leukemic cells according to both definitions of molecular relapse can be regarded as an indication of incipient disease progression and should provide a rationale for initiation of treatment.

How should patients in relapse be treated?

An important number of patients in relapse after BMT can still obtain a cytogenetic/molecular response with different treatment modalities.

Cyclosporin A (CyA) withdrawal

Patients who relapse under immunosuppressive therapy with CyA can be treated by its immediate discontinuation. In the majority of patients the response to CyA withdrawal develops in cytogenetic and hematologic relapse. In contrast, patients with more advanced disease at time of relapse respond poorly and in a transient way. The interval to complete remission is about two months after CyA discontinuation.[52,53]

Interferon alpha (IFNα) therapy

IFNα therapy can achieve a complete cytogenetic remission in 30% of patients in chronic phase hematologic relapse after allogeneic BMT.[54,55] This remission-inducing effect of IFNα is clearly less in relapse after T-cell depleted

BMT, with response rates of 10%, perhaps due to an insufficient cell effector mechanism.[56] IFNα may be more effective when the tumor burden is smaller and there is enough recipient bulk of donor T cells as occurs in cytogenetic relapse in the non-T-cell depleted BMT. In this sense, Higano *et al.*[57] reported a high complete cytogenetic response (80%) in a group of 14 patients with these characteristics (the median time to cytogenetic response was 7.5 months). However, most patients do not obtain a molecular remission and will require long-term therapy with IFNα to maintain the level of response.

Donor lymphocyte infusions

In 1990, Kolb *et al*.[58] reported that use of DLI was effective in three relapsed CML patients transplanted with marrow from HLA-identical siblings. All of them achieved a complete cytogenetic response. Other initial studies confirmed these results.[59-64] Complications described associated with DLI are acute or chronic GVHD and severe myelosuppression.

In 1994, Van Rhee *et al*.[50] reported complete responses in seven patients in molecular/cytogenetic relapse. All these patients entered complete remission without developing associated myelosuppression.

Results from data of European centers analyzed by the EBMT[4] and from a multicenter survey in North America[65] showed a 80% of response in early relapse or chronic phase relapse. Patients in more advanced disease at relapse obtained a lower percentage of response. As favorable prognostic factors for a response, the following have been pointed out: the type of relapse, transformed versus chronic phase, occurrence of post-BMT acute and/or chronic GVHD, time interval between BMT to DLI of less than 2 years, acute and chronic GVHD post-DLI and high percentage of donor T-cell chimerism.[4,65,66] The donor type, unrelated versus sibling DLI, had no significant effect on the response rate or the incidence of GVHD.[67,68]

The median time to cytogenetic response is 3 months, although molecular responses occur as late as 8 months.[4,65,69]

Different strategies have been used to reduce the high rate of GVHD reactions and toxicities after DLI:

a) *modification of the transfused lymphocyte subsets.* The $CD8^+$ T-cells have been implicated as the principle mediator of GVHD in humans. Two studies have tried to assess whether CD8-depleted DLI could induce remission in patients with relapsed CML after BMT while minimizing the incidence of GVHD.[69,70] Both of them showed a reduced percentage of acute GVHD, while the DLI antitumor effect was maintained;

b) *administration of lymphocytes in escalating doses at long intervals.* Mackinnon *et al*.[5] reported a group of patients who received initially low doses of DLI (1×10^5/kg) followed by progressive dose escalation if no toxicity or response was documented. Doses of 1 to 5×10^7/kg were particularly effective for patients in molecular/cytogenetic relapse, with a 100% response, and none of the patients developed acute GVHD. Higher doses were associated with a diminished incidence of acute GVHD in comparison with previous reports. In the study reported by Dazzi *et al.*[71], the infusion of escalating doses of DLI at 3-monthly intervals showed a better efficacy with a lower incidence of acute and chronic GVHD in comparison to a single bulk DLI;

c) *stem cell support*. Another major complication of DLI is pancytopenia and its consequences. Pancytopenia derives from suppression of host normal and leukemic hematopoiesis induced by the transfused T-lymphocytes.[72] A possible alternative to avoid the development of myelosuppression in patients in whom donor hematopoiesis is not detected is to infuse G-CSF mobilized donor mononuclear cells (PBSC). However, different studies[73,74] and our personal experience have shown that PBSC cannot prevent pancytopenia in all cases;

d) *other approaches.* Lymphocyte transfection with a suicide gene[75] or the transfusion of leukemia-reactive cytotoxic lymphocytes[76] have also been reported as approaches to reduce the risk of GVHD.

Other strategies have been used to increase the rate of complete response or to control the residual clonogenic tumor cells:

a) *treatment with different cytokines, concomitant with or after DLI.* In the two large DLI retrospective studies,[4,65] IFNα had no apparent influence on response. However, some patients who failed to respond to DLI obtained a remission when IFN was added to the treatment.[5] Interleukin-2 (IL-2) has shown a GVL effect associated with allogeneic BMT. Its administration *in vivo* in combination with IL2-activated DLI has been shown to be effective in several patients who did not respond previously to DLI;[51]

b) *Pre-emptive DLI.* In patients who are at high risk of relapse, post-BMT DLI may provide an additional GVL effect against this impending relapse. Patients undergoing T-cell-depleted BMT have been eligible for this treatment because of the increased risk of relapse.[77,78] At present, several trials are investigating the use of pre-emptive DLI. The preliminary results suggest an advantageous effect but with a significant incidence of GVHD.[79]

The GVL effect of DLI is sustained in the majority of patients who obtain a complete

remission. However, a significant proportion of patients will still relapse. The relapse rate in patients in complete response after DLI is approximately 25% at 3 years.[80] Many of these patients will enter remission with a second course of DLI.

Second myeloablative therapy

Second BMT in hematologic malignancies is associated with a high therapy-related mortality and survival rates of about 30% at 4 years.[3,81,82] Nevertheless, the results of second BMT are better in CML patients with an overall survival of 46% at 2 years, and in patients who relapse more than 6 months after the first transplant.[83]

Conclusions

The burden of Ph^+ clonogenic cells at relapse in patients with CML who have undergone BMT is the cornerstone in achieving a durable complete remission with the different treatments. Because of this, it is critical to establish a precise definition for *molecular relapse* after BMT.

$P210^{bcr\text{-}abl}$ positivity does not permit reliable prediction of cytogenetic/hematologic relapse. Other molecular criteria which appraise the kinetics of residual disease have been proposed. The EICML group define relapse as at least a 10-fold increase in the relative expression of the marker gene detected, confirmed by a minimum of three independent, consecutive Q-PCR analyses. However, Q-PCR is a labor-intensive assay limited to research laboratories. Other alternative approaches can be followed. Detection of a mixed chimerism will herald a cytogenetic relapse in the majority of patients. The sensitivity of this assay can be improved by analyzing lineage-specific chimerism in highly purified myeloid cell fractions. $P190^{bcr\text{-}abl}$ is a disease burden marker and its detection after BMT implies imminent cytogenetic relapse. We consider that the association of these two phenomena is a signal to treat the patient immediately.

The therapeutic strategy will be determined by the time between BMT and relapse. Patients who relapse under immunosuppressive treatment may obtain a remission with CyA withdrawal. The remaining patients should be treated with DLI with progressively escalating doses at relatively long intervals. The administration of IFNα associated with DLI might have a positive effect on the remission rate. In patients with high risk of relapse because of a T-cell-depleted allograft, the pre-emptive transfusion of donor lymphocytes might prevent relapse. However, this approach may result in toxicity in a significant group of patients cured by BMT. Close molecular follow-up with early treatment of any sign of imminent relapse might be a better option.

Other therapies currently under study, such as tyrosine kinase inhibitors, deserve evaluation in this clinical setting.

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JR and MAA contributed equally to this review. AT is the head of the Department and participated in writing the paper.

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