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Stem-cell transplantation in non-Hodgkin's lymphoma: improving outcome

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High-dose therapy with stem-cell transplantation is a potentially curative therapy for younger patients with relapsed aggressive non-Hodgkin's lymphoma (NHL) and is also under investigation in relapsed indolent NHL. There are, however, risks associated with this treatment strategy. Autologous stem-cell transplantation (ASCT) continues to be associated with a high risk of relapse, while graft-versus-host disease is a major limiting factor with allogeneic stem-cell transplantation. The presence of minimal residual disease (MRD) in the harvested, re-infused stem cells, or remaining in the patient following chemotherapy, is associated with relapse after ASCT. As a result, monitoring and eradicating MRD has become a major focus of many studies in NHL. Rearrangement and overexpression of the *bcl-1* and *bcl-2* genes are the hallmarks of mantle-cell and follicular lymphoma, respectively, and evidence suggests that they are promising surrogate markers of MRD. Polymerase chain reaction analysis is a sensitive methodology used to monitor the status of occult lymphoma cells bearing these genetic aberrations, and results from trials of ASCT have shown that clearance of *bcl-1*/JH- and *bcl-2*/JH-positive cells following treatment is associated with a significant improvement in outcome. Rituximab, the anti-CD20 monoclonal antibody, is increasingly used for *in vivo* purging and can effectively eradicate *bcl-1*/JH- and *bcl-2*-positive cells. If the encouraging preliminary results with rituximab are maintained with a longer follow-up, this agent could play a pivotal role in improving outcome after stem-cell transplantation in NHL. [© 2002 Lippincott Williams & Wilkins.]

Key words: rituximab, minimal residual disease, polymerase chain reaction, purging, autologous stem-cell transplantation, graft-versus-host disease.

Introduction

High-dose chemotherapy (HDT) with stem-cell transplantation (SCT) has become an essential treatment modality in non-Hodgkin's lymphoma (NHL). Autologous SCT (ASCT) following myeloablative chemotherapy is standard treatment for patients <55 years old with relapsed aggressive lymphoma.[1] Compared with conventional chemotherapy, ASCT has been shown to produce a survival benefit in patients with relapsed aggressive NHL.[2] ASCT is also under investigation for relapsed indolent lymphoma, as earlier evidence suggested that long remissions may be obtained, particularly in patients who are transplanted during first remission.[3,4] The use of ASCT as first-line treatment is more contentious, although studies are investigating its potential in high-risk patients with aggressive lymphoma who are likely to relapse.

Although ASCT and, to a lesser extent, allogeneic SCT (alloSCT) have in many cases led to significant improvements in both response rates and disease-free survival, both have associated risks: with ASCT the risk of relapse continues to be high, while with alloSCT graft-versus-host disease (GVHD) is a major limiting factor.

The high level of relapse associated with ASCT occurs despite the fact that most patients achieve complete clinical remission. This is due to the presence of residual malignant cells in either the re-infused stem cells, or remaining in the patient following chemotherapy. The ability to monitor the presence of residual malignant cells, known as minimal residual disease (MRD), may be a powerful prognostic tool; it provides important information on the efficacy of treatment and indicates the potential for relapse. In addition, complete clearance of residual malignant cells following ASCT (i.e. molecular remission) is associated with improved disease-free survival.[5]

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An accurate and reliable system for detecting MRD could help reduce the need for extended follow-up and allow rapid and accurate evaluation of treatment efficacy. The challenge remains, however, to identify accurate markers of MRD in NHL. Efforts so far have focused on the genetic anomalies affecting the functions of *bcl-2, bcl-1* and the Ig-B-cell receptor gene. Monitoring the presence of these genetic anomalies by sensitive molecular techniques during treatment and after transplantation provides useful information on the extent of MRD and, thus, treatment efficacy.

Markers of MRD in ASCT

To optimize the outcome in ASCT, residual malignant cells in the patient or the stem-cell harvest must be eradicated, and reliable markers of MRD are required to monitor the efficacy of treatments in achieving this. Two of the most promising markers are the rearrangements of the *bcl-2* and *bcl-1* genes in follicular lymphoma (FL) and mantle-cell lymphoma (MCL), respectively.

bcl-2/JH: a marker of MRD in FL

As an inhibitor of apoptosis, *bcl-2* is upregulated in most FLs and may be an important marker of malignant cells. Upregulation occurs as a result of a t(14;18)(q32;q21) chromosomal translocation, in which the *bcl-2* gene on chromosome 18 comes under the control of the immunoglobulin IgH-J enhancer on chromosome 14, resulting in overexpression of the anti-apoptotic *bcl-2* protein (Figure 1).[6] The highly sensitive polymerase chain reaction (PCR) method is capable of detecting the translocation product of *bcl-2*/JH in one in 10^5–10^6 cells from peripheral blood or bone marrow. Using this method, groups are evaluating the role of *bcl-2*/JH in NHL and its potential as a surrogate marker of MRD to evaluate patient response to treatments such as ASCT and conventional chemotherapy plus monoclonal antibody therapy.

Although the precise clinical significance of the *bcl-2*/JH rearrangement remains unclear, evidence shows that clearance of *bcl-2*/JH is associated with improved clinical outcome.[5,7–9] In the transplant setting, the largest experience was reported by Gribben *et al.*[7] and was recently followed-up by Freedman *et al.*[5] Patients undergoing ASCT for FL were monitored for *bcl-2*/JH status in their peripheral blood using PCR before and after their transplant. Those who converted from *bcl-2*/JH-positive to *bcl-2*/JH-negative following transplantation had significantly longer disease-free survival than those who remained *bcl-2*/JH-positive (Figure 2).[5] This compelling evidence has led many clinical trial investigators to frequently include analysis of *bcl-2*/JH status as a possible surrogate marker of MRD in FL and its clearance as an indicator of treatment efficacy.

bcl-1/JH: a marker of MRD in MCL

MCL is a relatively uncommon lymphoma, comprising about 8% of all NHLs, and is characterized by deregulation and overexpression of the *bcl-1* gene. Overexpression occurs as a result of a t(11;14)(q13;q32) chromosomal translocation, in which the *bcl-1* gene comes under the control of the immunoglobulin IgH-J enhancer on chromosome 14. This leads to deregulation and constant overexpression of cyclin D1, resulting in a higher proliferative capacity and a more aggressive clinical course.[10] MCL has one of the worst prognoses of all NHLs but, in some instances, histopathologic differentiation between MCL and other B-cell lymphomas may be difficult. Therefore, detection of the t(11;14) also indicated as *bcl-1*/JH is of essential diagnostic value for the risk-adjusted management of patients with MCL.[11]

As with *bcl-2*/JH in FL, *bcl-1*/JH rearrangement can be detected by PCR in a subset of patients with MCL,

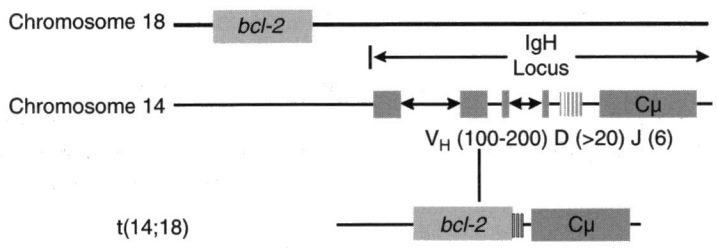

Figure 1. Chromosomal translocation t(14;18) in follicular lymphoma. This translocation creates a *bcl-2*/JH junction and occurs in more than 85% of FLs. Reproduced with permission from Liu *et al.*[6]

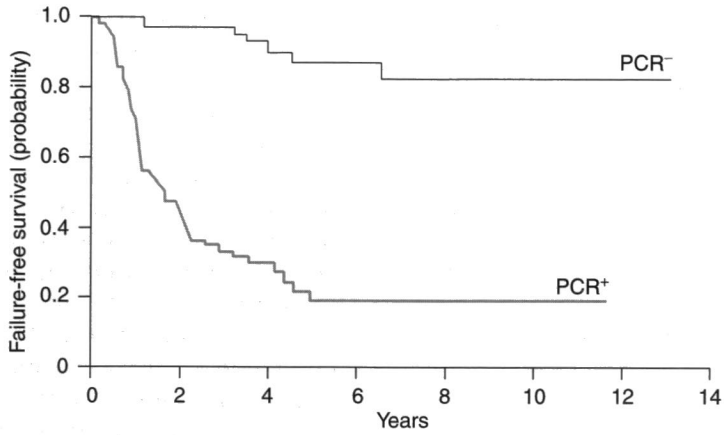

Figure 2. Correlation of *bcl-2*/JH clearance detected using polymerase chain reaction (PCR) with improved clinical outcome in bone marrow transplantation patients. Reproduced with permission from Freedman *et al.*[5]

and thus is a sensitive marker of MRD and a potential surrogate marker of treatment efficacy in these patients. In a study of 17 MCL patients who underwent ASCT, the presence of PCR-detectable *bcl-1*/JH post-transplantation was associated with a high probability of relapse, while in the four patients with no *bcl-1*/JH in their re-infused cells, only one had relapsed at 2 years following ASCT.[12] More data from larger studies are now needed to confirm these preliminary results.

Detecting MRD: standard PCR methodologies

The application of PCR to detect MRD is now well established. PCR amplifies specific DNA sequences at the molecular level and is sensitive enough to detect one cell in 10^5–10^6. The technique uses primers that are sequence-specific for the gene translocation or rearrangement of interest. The primers anneal to the DNA on either side of the area of interest, and the encompassed DNA is amplified using a series of temperature-controlled reactions. If the translocation or rearrangement is present, the PCR product can be visualized by polyacrylamide gel electrophoresis.

Drawing comparisons between the results of PCR analysis from different studies is confounded by the non-standardized methods used by different groups, and thus evaluating the importance of study results is difficult. This problem was addressed for acute leukemia by the European BIOMED I Concerted Action 'Investigation of minimal residual disease in acute leukemia: international standardization and clinical evaluation'.[13] Following on from this, lymphomas are being addressed in the European Union BIOMED II Concerted Action (PL96-3936) 'PCR-based clonality studies for the early diagnosis of lymphoproliferative diseases'. A series of PCR protocols for the detection of clonal immunoglobulin and T-cell receptor gene arrangements has been designed and validated by the group of participating laboratories. The group, consisting of 32 laboratories from eight countries, has developed a consensus approach for the detection of the *bcl-1* and *bcl-2* translocations t(11;14) and t(14;18), using a series of standardized PCR primers together with consistent amplification and reaction conditions (van Dongen *et al.*, unpublished data).

The use of standardized PCR methods to detect MRD will allow cross-group comparison of data. This will rapidly increase the possibility of identifying reliable surrogate markers of MRD that can be used to measure treatment efficacy and predict outcome.

Eradicating MRD by purging

The detection of residual disease has been used in a number of settings to predict relapse. It is also possible to detect MRD in autologous stem cells prior to transplantation. To reduce the risk of relapse, treatments have focused on eradicating MRD by manipulating the stem-cell harvest or treating the patient post-ASCT with maintenance therapy designed to inhibit regrowth of residual tumor cells.

Initial attempts to remove lymphoma cells from the transplant involved *in vitro* purging with

monoclonal antibodies, drugs and cytotoxic agents.[7,14,15] ***In vitro*** purging is designed to remove contaminating tumor cells after collection of the transplant and before re-infusion. This method, however, has met with only partial success. While generally effective, ***in vitro*** purging can significantly reduce the stem-cell yield, delay hematopoietic recovery after transplantation, and is both time-consuming and expensive.[16]

In vivo purging with rituximab

More successful results have been achieved by ***in vivo*** purging with the anti-CD20 monoclonal antibody rituximab. This involves depleting the peripheral blood of tumor cells before and/or during stem-cell mobilization, thus preventing contamination of the harvest in the first instance. To maximize the chance of eradicating MRD and further reduce the probability of relapse, purging can be combined with post-transplant rituximab immunotherapy.

The role of rituximab in eradicating MRD from the peripheral blood and bone marrow has been established by several studies in NHL (Table 1).[9,17–20] Rituximab has been shown to be capable of clearing *bcl-2*/JH-positive cells from the peripheral blood and also the bone marrow of patients. In the pivotal study of rituximab single-agent therapy in relapsed and refractory indolent lymphoma, rituximab cleared *bcl-2*/JH-positive cells from the peripheral blood in 62% of patients who were *bcl-2*/JH-positive at baseline.[21] In a study of 50 patients with FL and a low tumor burden, *bcl-2*/JH was successfully cleared from 57% of positive patients by four infusions of rituximab; *bcl-2*/JH clearance was associated with a significantly improved progression-free survival (Figure 3). Combining rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) has yielded response rates of 95%, with a long duration, in indolent lymphoma. This immunochemotherapy regimen also resulted in high rates of *bcl-2*/JH clearance from both blood and bone marrow.[20]

These results have led the way for ***in vivo*** purging with rituximab during ASCT for FL. In FL, Buckstein

Table 1. Clearance of *bcl-2*/JH by rituximab

Reference	No. of patients	CR rate (%)	*bcl-2*/JH clearance	
			n/total	%
Piro *et al.*[17]	37	14	9/18	50
Ghielmini *et al.*[18]	76	3	15/29	52
Foran *et al.*[19]	70	3	13/21	62
Czuczman *et al.*[20]	70	7	36/70	51
Colombat *et al.*[9]	50	26	17/30	57

CR, complete response.

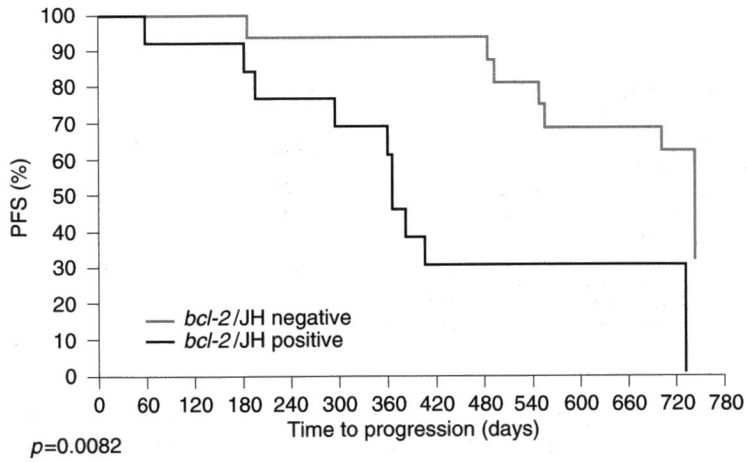

Figure 3. Clearance of *bcl-2*/JH by rituximab correlates with improved progression-free survival (PFS).

et al.[22] at the Toronto-Sunnybrook Regional Cancer Centre (TSRCC) evaluated the safety and efficacy of *in vivo* purging and post-transplant consolidated immunotherapy with rituximab and HDT followed by ASCT. Patients were treated with a single infusion of rituximab during stem cell mobilization, followed by consolidative immunotherapy with two 4-week cycles of rituximab at 2 and 6 months post-transplant (Figure 4).[23] In addition to maintaining a $CD34^+$ stem-cell yield comparable to the unpurged controls, *in vivo* purging with rituximab did not adversely affect stem cell function. At 9 months follow-up, 12 out of 13 patients were in molecular remission (PCR negative) in the blood and/or bone marrow and one patient had died at 7.2 months of septic causes. At a median follow up of 23.8 months, one patient became PCR positive for *bcl-2*/JH at 18 months and had clinical relapse at 21 months post-transplant (Figure 5).

Given the poor clinical outcome of first-line chemotherapy, ASCT is increasingly considered as a therapeutic option for patients with MCL. Also at the TSRCC, a clinical trial of ASCT following *in vivo* purging with rituximab and post-transplant immunotherapy (Figure 4), is evaluating the potential of *bcl-1*/JH status as a surrogate marker of MRD in MCL. The *bcl-1*/JH translocation was identified in 4 of 10 patients before *in vivo* purging. Initial results show that all of the 10 patients who have undergone a transplant are in complete clinical remission or unconfirmed remission. For the six patients who did not have PCR detectable *bcl-1*/JH, PCR was used to detect clonal immunoglobin gene rearrangements in the diagnostic sample. It was possible to develop patient-specific-VDJ PCR primers from the DNA sequences of the PCR products in four of these patients[12] and consensus primers for VH framework regions were used on the remaining two patients ('Clonal IgH'). Patient-specific PCR was then used to assess MRD. At 9 months follow-up, five of seven patients were PCR negative following treatment (Figure 6). Although a longer follow-up is necessary to establish the role of *bcl-1*/JH as a surrogate marker of MRD in MCL, these results are encouraging, and suggest that *bcl-1*/JH clearance may be associated with improved clinical response.

Rituximab was also assessed in conjunction with sequential HDT (sHDT). Successful *in vivo* purging and complete clinical and molecular remissions were documented in all seven previously untreated patients.[24] A follow-up study of 28 patients continued to show successful *in vivo* purging and impressive efficacy with this regimen.[25] Complete remission was achieved by 97% of patients, and the projected event-free and overall survival at 3 years was 85%. This shows a marked improvement over that previously achieved in MCL, indicating that sHDT with rituximab *in vivo* purging offers the possibility of improving the current poor prognosis in MCL.

Long-term follow-up will determine whether the encouraging early results of rituximab *in vivo* purging and maintenance will improve the outcome of patients treated with ASCT.

AlloSCT: preventing GVHD

AlloSCT can be used in patients who are incapable of mobilizing sufficient numbers of stem cells as well as in other circumstances. It removes the risk of malignant cell contamination of the graft, but is associated with a high transplant-related mortality through graft failure and GVHD. Traditional strategies to combat GVHD have largely concentrated on the elimination or inactivation of T-cells, which are

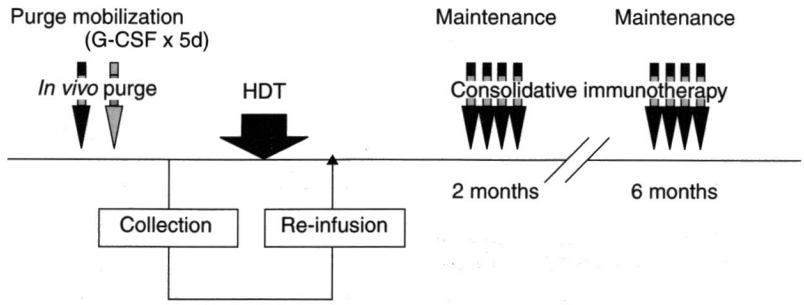

Figure 4. Treatment schedule of rituximab purging *in vivo* and consolidative immunotherapy in patients (18–65 years) with recurrent FL or newly diagnosed mantle-cell lymphoma. *In vivo* purging consisted of a single infusion of rituximab (Rituxan) (375 mg/m^2) and for consolidative immunotherapy an infusion of rituximab (375 mg/m^2) was given once-weekly for 4 weeks. HDT, high-dose chemotherapy, G-CSF, granulocyte colony stimulating factor.

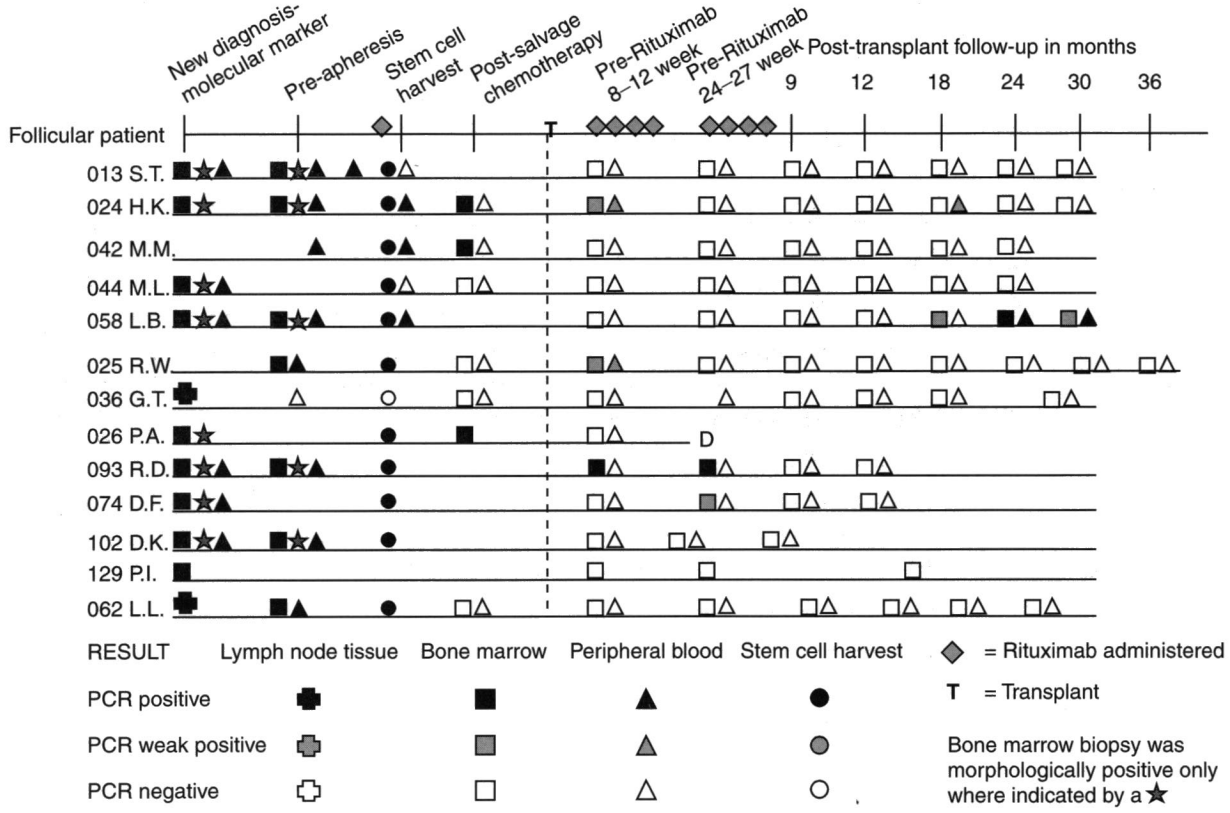

Figure 5. Preliminary molecular diagnosis results for follicular lymphoma using polymerase chain reaction (PCR) monitoring of autologous SCTwith rituximab *in vivo* purging and post-transplant maintenance.

acknowledged to be involved in its development, but this often results in other complications.[26,27] For example, anti-CD52 therapy can effectively prevent GVHD, but the associated immunosuppression and high incidence of viral infection may offset the benefits.[27]

More recently, the role of the B-lymphocyte in the pathogenesis of GVHD has been examined. B-lymphocytes may act as antigen-presenting cells capable of triggering CD4 T-cell activation, ultimately resulting in interleukin-2-mediated proliferative responses. B-cell-targeted therapy, therefore, could be important in managing GVHD, and a retrospective analysis has shown that patients treated with rituximab for remission induction experienced significantly fewer episodes of GVHD than patients not treated with rituximab (Table 2).[18–20,28]

This has led researchers to reconsider their approach to the management and treatment of GVHD. A phase II trial has been proposed to further evaluate the clinical benefits of rituximab in the prevention of GVHD in the setting of allogeneic peripheral blood SCT. The results of this trial could have a major impact on improving the outcome to alloSCT.

Discussion

bcl-1/JH and *bcl-2*/JH are emerging as important surrogate markers of MRD in MCL and FL, respectively. Monitoring the status of these markers in the peripheral blood and bone marrow appears to offer a valuable tool for measuring treatment efficacy and predicting patient response.

As there are increasing numbers of studies evaluating the role of *bcl-1*/JH and *bcl-2*/JH as surrogate markers for MRD, the need to implement a standardized approach to PCR becomes increasingly urgent. The PCR methods developed by the BIOMED II Concerted Action Group provide an opportunity for investigators to standardize their methods across study centers. This will make comparisons between studies feasible, and greatly enhance our ability to rapidly assess the efficacy of treatments such as rituximab for eradicating MRD.

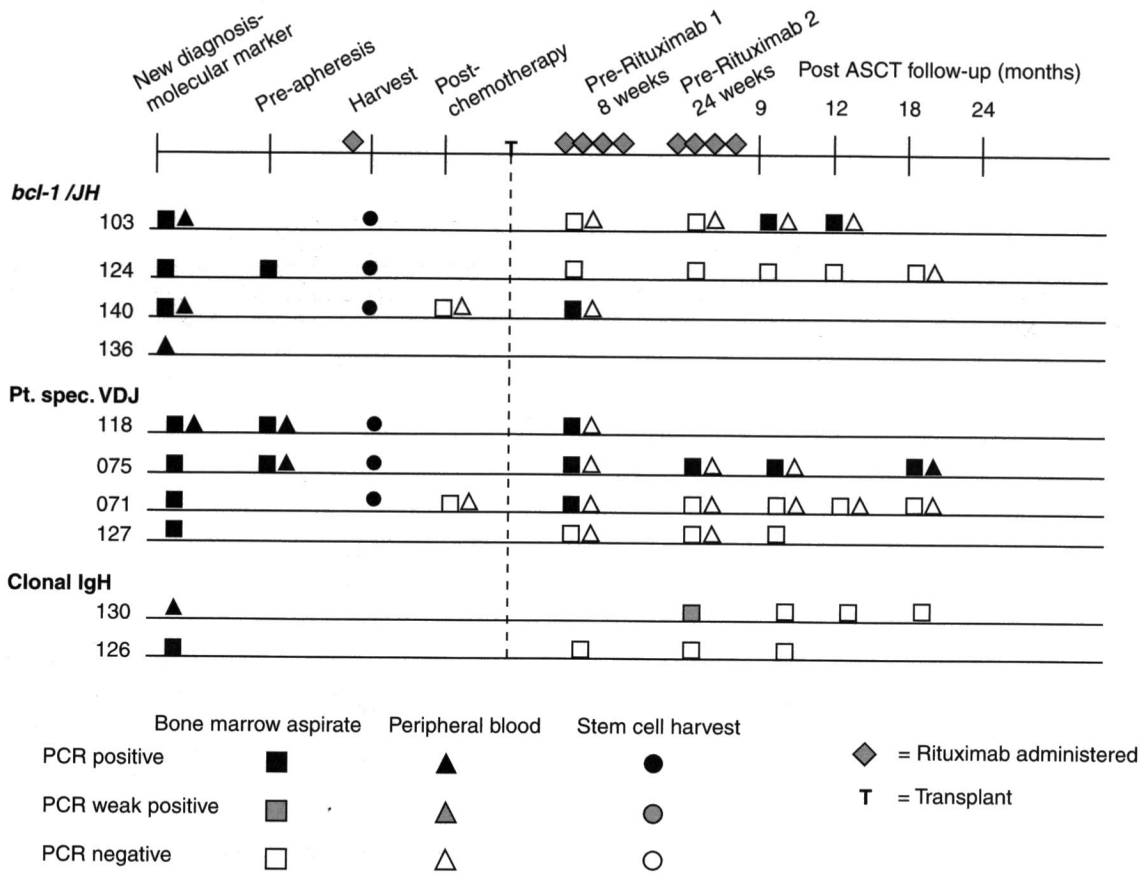

Figure 6. Molecular diagnosis results for mantle-cell lymphoma using polymerase chain reaction (PCR) monitoring of autologous SCT (ASCT) with rituximab *in vivo* purging and post-transplant maintenance. *bcl-1*/JH, primers for t(11;14); pt. spec. VDJ, patient specific VDJ primers/JH; clonal IgH, primers for VH framework regions/JH.

Table 2. Rituximab in allogeneic SCT: effect on clinical outcomes. Reproduced with permission from Ratanatharathorn *et al.*[28]

Clinical endpoints	Rituximab (%) ($n = 14$)	No rituximab (%) ($n = 23$)	p value
Acute GVHD (II–IV)	18	51	0.05
Disease progression[18]	25	32	0.90
Progression-free survival[19]	56	37	0.72
Overall survival[20]	71	36	0.33

GVHD, graft-versus-host disease.

Evidence suggests that improving the outcome of ASCT in the treatment of NHL is achievable, and eradicating MRD is crucial for this. Emerging data show promising results with rituximab *in vivo* purging and consolidative immunotherapy post-transplant in combination with HDT – suggesting rituximab could have a pivotal role in improving the outcome of SCT. Encouraging clinical and molecular results for both indolent and mantle-cell NHL have been reported. The challenge now is to collect more data, especially from randomized, controlled trials, to determine whether these regimens should become standard when treating NHL with transplantation.

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