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Minimal residual disease

The role of molecular monitoring in autotransplantation for non-Hodgkin's lymphoma

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Summary:

Seventy-two patients with non-Hodgkin's lymphoma were evaluated for the presence of molecular markers (IgH, bcl-1, bcl-2 rearrangement) on bone marrow, at diagnosis and after PBSCT, and on harvests in order to find a possible predictive role of minimal residual disease on treatment outcome. At diagnosis, 41 (59%) out of 69 available bone marrows showed molecular involvement. Fifty-six percent of leukapheresis were involved, mainly indolent lymphoma ($P = 0.001$) or advanced disease ($P = 0.01$). *Ex vivo* purging cleared only one stem collection out of 31 PCR-positive leukapheresis. Aggressive lymphomas showed both a longer overall survival (OS) ($P = 0.03$) and relapse-free survival RFS ($P = 0.02$) when transplanted with unpurged stem cells, whereas indolent NHL survival was not influenced by *ex vivo* purging. Twenty out of 26 samples taken during follow-up had bone marrow involvement at diagnosis. Of these, 15 cleared their bone marrow; both OS and RFS were significantly longer in the PCR-negative cases ($P = 0.05$ and $P = 0.005$). At 1 year after PBSCT, 75% of patients were PCR negative, with 50% molecular remissions; the relapse rate was 55% for patients still PCR positive *vs* 29% for those who were PCR negative. Thus, after high-dose chemotherapy, close molecular monitoring of MRD using qualitative PCR techniques seems to represent a reliable prognostic indicator.

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High-dose chemotherapy followed by hematopoietic stem cell transplantation has significantly improved the prognosis of non-Hodgkin's lymphomas (NHL).[1] Several clinical parameters, such as the International Prognostic Index (IPI),[2] extra-nodal manifestations[3] and time to relapse,[4] have been proven to be the most statistically significant independent variables predicting outcome. Nevertheless, relapse is frequent, indicating incomplete eradication by conditioning regimens or the presence of residual neoplastic cells in the reinfused graft. Therefore, the identification of parameters other than the previously mentioned clinical ones is needed. In this respect, various molecular markers, ie IgH, Bcl 2, Bcl 1 and TCR rearrangements, are now available for both molecular diagnosis and for the evaluation of minimal residual disease in patients with NHL. Although these molecular assays are sufficiently sensitive and specific, their predictive role in minimal residual disease (MRD) detection is still controversial. Moreover, although many *ex vivo* and *in vivo* purging techniques have been developed to eliminate residual malignant cells from the inoculum, a clear clinical advantage has not been definitely established.

Rates of contamination of harvested progenitors range from 56%[5] to 100%,[6] depending on histology, treatment protocols, purging procedures and molecular assays. At stem cell mobilization after *ex vivo* purging, 62% of patients with aggressive lymphomas achieved molecular remission and a higher continuous remission rate was observed in those patients who received PCR-negative progenitors.[7] Similarly, in indolent lymphomas, several studies have reported an increase in molecular remission rates from 40% up to 93% after *in vivo* purging by anti-CD20 monoclonal antibody.[8] Furthermore, the role of contaminated harvests in terms of future relapse has been demonstrated in patients with both follicular[6] and mantle cell NHL.[9] The effect upon outcome of an uncontaminated graft has also been studied in indolent lymphomas, where a complete remission rate of 38% was found in the group of patients receiving PCR-positive progenitors *vs* 100% remission in patients transplanted with no molecular evidence of malignant cells.[5] Finally, follicular lymphoma cases achieving and maintaining molecular response experienced a longer failure-free survival (FFS) as compared to those who either reverted back to PCR positivity or never achieved a molecular response.[10]

In the present paper, we evaluated 72 patients for the

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prognostic impact on patient outcome of lymphoma-associated molecular markers at diagnosis and at the time of stem cell collection. The role of *in vitro* purging was also evaluated in a subset of patients with NHL.

Patients and methods

Eligibility and therapeutic schedule

Seventy-two histologically confirmed B cell NHL adult patients, classified according to the Revised European American Classification (REAL)[11] and included in various GISL (Gruppo Italiano per lo Studio dei Linfomi) protocols, were retrospectively evaluated. Cases were selected on the basis of availability of mobilized peripheral blood and bone marrow cryopreserved at diagnosis. This GISL study was designed to investigate whether (1) high-dose therapy and autologous transplantation increases molecular remission rate and (2) the quality of molecular remission independently correlates with PFS. Clinical data were analyzed in December 2000. Indolent lymphomas were also included in an ongoing protocol with the aim of evaluating the role of autotransplant even in this clinical setting.

At the beginning of therapy, all patients underwent staging procedures, including history, physical examination, hematology and serum chemistry profile, total body computerized tomography, bone marrow aspiration and biopsy. All patients had measurable or evaluable disease, an ECOG performance status <2, white blood cell (WBC) count $>2 \times 10^9$/l, platelets $>100 \times 10^9$/l and adequate heart, liver and kidney function. Clinical characteristics are listed in Table 1. Histology was defined according to the REAL classification in 62 patients. Among the 36 indolent lymphomas, 21 were classified as follicular and five as mantle cell. Of the remaining 26 cases with aggressive disease, 24 were diffuse large cell lymphomas (DLCL). At diagnosis, the majority of the patients (71%) were at an advanced disease stage and 85% had a low or low–intermediate International Prognostic Index (IPI).

Table 1 Characteristics of the non-Hodgkin's lymphoma patients studied

	No (%)
Total No. of patients	72
Evaluable for outcome	60
Evaluable for clinical features	62
Sex	27 M/45 F
Median age/range	42/18–61
Histology	
Indolent	36 (58)
Aggessive	26 (42)
Stage	
I–II	18 (29)
III–IV	44 (71)
IPI	
Low/low–intermediate	53 (85)
High–intermediate/high	9 (15)
Median follow-up (months)/range	57/6–114
Harvests (done)	66 (92)
PBSCT (done)	50 (81)
Median follow up from PBSCT (months)	46
Disease status	
CR/PR	38/8 (63/13)
Progression–relapse	7 (12)
Deaths	7 (12)

All patients received CHOP or CHOP-like treatment as first-line therapy before starting the high-dose protocol. According to the protocol of Gianni *et al*,[12] patients received CTX 7 g/m^2 i.v. either at the beginning or at the end of the following sequence of drugs (VP-16 2 g/m^2 i.v., MTX 8 g/m^2 i.v. and VCR 2 mg i.v.), based on the absence or presence of histologically-proven bone marrow infiltration, respectively. Patients received G-CSF 5 μg/m^2 s.c. and CD34$^+$ cells were monitored daily by flow cytometry as the WBC started to increase after aplasia, with a target yield of $\geqslant 5.0 \times 10^6$/kg CD34$^+$ selected cells. One or two harvests from each patient was evaluated for minimal residual disease by molecular assay.

Conditioning included mitoxantrone 60 mg/m^2 i.v. on day −5 and melphalan 180 mg/m^2 on day −4. In 31 cases (15 aggressive and 16 indolent), *ex vivo* purging was performed on leukapheresis products using anti-CD20 antibody (negative selection) incubation followed by anti-CD34 immune adsorption (positive selection).

Complete remission (CR) was defined on the basis of the resolution of all symptoms and signs of lymphoma and bone marrow clearing; partial remission (PR) required $>50\%$ decrease of lesions; patients who did not achieve a CR or PR were considered resistant.

Statistical methods

All calculations were performed using the SPSS for windows, release 9.0, 1999. Time to relapse or progression was analyzed by the Kaplan–Meier method. Difference in PFS between prognostic groups was evaluated on univariate analysis by the log-rank test. Comparisons of clinical response data by individual prognostic variables were performed using the Fisher's exact test.

Molecular assays

PCR analyses were performed on bone marrow and leukapheretic samples. Mononuclear cells were isolated by fractionation on Ficoll/Hypaque (Axis-Schield, Oslo, Norway) gradient; high molecular weight DNA was extracted and suitable aliquots were utilized for PCR tests after spectrophotometric quantitative evaluation.

Two consensus primers were designed for the VH-DH-JH region of the immunoglobulin heavy chain gene (IgH), as previously described;[13–14] the downstream primer was 5′ labeled with 6-FAM fluorochrome.

The sequences were as follows: VH region primer (third framework region): 5′-ACA CGG CYS TGT ATT ACT GT-3′; VH region primer (second framework region): 5′-TGG [A/G]T CCG [C/A]C AG[G/C] C[T/C][T/C] CNG G-3′; JH consensus external primer: 5′-ACC TGA GGA GAC GGT GAC CA-3′; JH consensus inner primer: 5′-ACC AGG GTC CCT TGG CCC CA-3′.

PCR fragment length ranged from 65 to 130 bp for the CDR3 and from 240 to 280 bp for the FR2. PCRs were performed in a Gene Amp PCR System 2400 (Applied

Biosystem, Warrington, UK). Total reaction volume was 25 μl, containing 1.5 units Taq DNA Polymerase (Eurobio, Les Ulis, France), 67 mmol/l Tris-HCl (pH 8.8), 16 mmol/l $(NH4)_2SO_4$, 0.01% Tween 20, $MgCl_2$ 1.5 mM, 200 μmol/l dNTPs, 0.4 μmol/l of each primer and 1 μg of DNA. Every PCR procedure included distilled water instead of DNA as a negative control and DNA carrying monoclonal IgH rearrangement as a positive control.

Each individual cycle consisted of denaturation at 95°C 30 s, annealing at 55°C 30 s for first round PCR or 58°C 30 s for second-round PCR and a final extension at 72°C of 30 s. These cycles were repeated 30 times, preceded by 2 min of denaturation at 95°C and followed by 5 min of chain elongation at 72°C.

In order to amplify BCL2-IgH rearrangement, nested PCR reactions were performed in a final volume of 25 μl using 1 μg of DNA, 0.4 μmol/l of oligonucleotide primers, 200 μmol/l each of dNTPs, $MgCl_2$ 2 mM and 1.5 U Taq polymerase (Eurobio) in the 1 × PCR buffer (67 mmol/l Tris-HCl, pH 8.8, 16 mmol/l $(NH4)_2SO_4$, 0.01% Tween 20).

The first round of PCR was performed for 25 cycles and the second for 30 cycles; primers for MBR and MCR detection and reaction conditions were previously described by Gribben *et al*,[15] employing an inner JH primer 6-FAM labeled.

In order to amplify BCL1-JH rearrangement, PCR reactions were performed in a final volume of 25 μl using 1 μg of DNA, 0.4 μmol/l of oligonucleotide primers, 200 μmol/l each of dNTPs, 2 mM $MgCl_2$ and 1.5 U Taq polymerase (Eurobio) in the 1 × PCR buffer (67 mmol/l Tris-HCl, pH 8.8, 16 mmol/l $(NH4)_2SO_4$, 0.01% Tween 20). Each amplification included a consensus IgH consensus primer, as reported above, and a forward primer, as described by Molot *et al*.[16]

PCR-amplified products were resolved by 2.5% agarose gel and then by capillary electrophoresis. Samples were prepared for analysis by mixing 0.8 μl of PCR products with 15 μl of deionized formamide (Sigma, St Louis, MO, USA) and 0.5 μl Gene Scan 500 Tamra-labeled internal standard (Applied Biosystem). This mixture was denatured at 95°C for 2 min and then quickly cooled on ice. Capillary electrophoresis and fluorescence detection with a virtual filter C was performed on ABI Prism 310 Genetic Analyzer (Applied Biosystem).

Runs were executed with the module GS STR POP 4 (1 ml) C with 10-s and 15 kV injection and run voltage, 60°C constant temperature, 24 min run time, using polymer POP 4 and the running buffer Genetic analyzer 1 × (Applied Biosystem).

Genescan 2.1 software was then used to analyze the PCR products, with accurate sizing and quantification of the peak areas. This allows accurate prediction of the equivalence point of PCR products from competitor and target molecules. The sensitivity of molecular assays resulted in $10^{-4}/10^{-5}$.

Results

Clinical outcome

Seventy-two patients were enrolled in this study and 60 were evaluable for outcome. Overall response was seen in 77%, with 63% complete remissions. Clinical responses were not significantly correlated with age ($<$ or $\geqslant$50 years), sex, histology (indolent or aggressive), IPI (low/low–intermediate or high/high-intermediate), stage ($<$ or $\geqslant$ III), molecular status at diagnosis, harvest contamination or molecular status after transplant (data not shown).

Fifty-three patients (88%) remain alive at a median follow-up of 57 months. No treatment-related deaths occurred; seven patients died, six relapsed and one because of an infectious complication after more than 100 days from the graft. Overall survival (OS) of the entire patient population was 87% (95% CI, 80–107) with a significant advantage in favor of the indolent group (96% *vs* 74%, $P = 0.06$); overall, patients showed a relapse-free survival (RFS) of 72% (95% CI, 55–85). Although not significant, RFS was longer in patients with aggressive disease (73% *vs* 62%).

Molecular results

Bone marrow samples were available for molecular analysis from 69 patients at diagnosis; in 62 cases the histology was defined according to the REAL classification. Twenty-eight patients were PCR-negative and 41 (59%) showed a clonal IgH rearrangement; 16 out of 21 follicular and 10 out of 24 DLCL cases showed a bcl2/JH rearrangement; bcl1/JH rearrangement was found in 3/5 mantle cell lymphomas. Out of the remaining 12 cases, eight were classified as lymphoplasmocytic lymphomas, two as marginal zone NHL, one as Burkitt, one as CLL. PCR positivity was more frequent in the indolent than in the aggressive group (68% *vs* 40%) ($P = 0.02$) and was significantly associated with an advanced stage of neoplasia ($P = 0.04$), while no significant correlation was found with age, sex or IPI. Molecular bone marrow involvement at diagnosis failed to show any significant impact on either OS and RFS.

Out of 66 evaluable harvest products, 37 (56%) were contaminated on PCR assay, 25 in the indolent and 12 in the aggressive group. Again, PCR positivity was significantly associated with indolent lymphoma ($P = 0.001$) and with advanced disease stage ($P = 0.01$). Of note, lymphoma-free harvests did not give any clinical advantage in terms of relapse rate, OS or RFS.

Harvests from 34 cases with PCR-positive bone marrow were evaluated (Table 2). Twenty-eight harvests were contaminated, while five indolent and one aggressive lymphoma (18%) were PCR-negative. Of 26 bone marrows PCR negative at diagnosis, eight (31%) contained contaminated stem cell precursors on mobilization ($P < 0.01$).

The power of *ex vivo* purging procedures was assessed in 31 PCR-positive leukapheresis products (16 indolent and 15 aggressive lymphomas). Twenty-two were at an advanced disease stage. Only one stem cell collection was cleared by purging, and *ex vivo* purging failed to remove the malignant cells from contaminated apheresis products in 3/8 cases with a negative bone marrow at diagnosis.

Table 2 Clinical outcome and PCR monitoring of the 26 patients also molecularly tested after PBSCT

Patient No.	*Histology (according to REAL)*	*Molecular status at diagnosis*	*Molecular status of harvests*	*Molecular status 3 months after PBSCT*	*Molecular status 6 months after PBSCT*	*Molecular status >12 months after PBSCT*	*Clinical outcome*
1	indolent	PCR positive	PCR positive	PCR positive			relapse
2	indolent	PCR positive	PCR positive	PCR negative			remission
3	indolent	PCR positive	PCR positive	PCR negative	PCR negative	PCR negative	remission
4	indolent	PCR positive	PCR positive	PCR negative	PCR negative	PCR negative	remission
5	indolent	PCR positive	PCR positive	PCR negative	PCR negative	PCR negative	remission
6	indolent	PCR positive	PCR positive	PCR positive			relapse
7	indolent	PCR positive	PCR positive	PCR positive	PCR negative	PCR positive	relapse
8	indolent	PCR positive	PCR positive	PCR negative	PCR positive	PCR positive	remission
9	indolent	PCR positive	PCR positive	PCR negative			remission
10	indolent	PCR positive	PCR positive	PCR negative			relapse
11	indolent	PCR positive	PCR positive	PCR negative			remission
12	indolent	PCR positive	PCR positive	PCR negative			remission
13	aggressive	PCR positive	PCR positive	PCR positive		PCR negative	remission
14	aggressive	PCR positive	PCR positive	PCR positive			remission
15	aggressive	PCR positive	PCR positive	PCR negative		PCR negative	relapse
16	aggressive	PCR negative	PCR negative	PCR negative		PCR negative	remission
17	aggressive	PCR negative	PCR negative	PCR negative			relapse
18	aggressive	PCR positive	PCR positive	PCR positive			relapse
19	aggressive	PCR positive	PCR positive	PCR positive	PCR positive	PCR negative	remission
20	indolent	PCR positive	PCR positive	PCR positive	PCR positive	PCR negative	remission
21	indolent	PCR positive	PCR positive	PCR negative		PCR negative	remission
22	indolent	PCR positive	PCR positive	PCR negative	PCR negative	PCR negative	remission
23	aggressive	PCR negative	PCR negative	PCR negative			remission
24	aggressive	PCR negative	PCR negative	PCR negative			remission
25	indolent	PCR negative	PCR negative	PCR negative			remission
26	aggressive	PCR negative	PCR negative	PCR positive			relapse

Bone marrow samples, available at 3 (26 cases), 6 (8 cases) and 12 months (12 cases) after transplant, underwent molecular analysis during follow-up after PBSCT. Twenty out of all 26 cases had bone marrow involvement at diagnosis. Molecular bone marrow remission was achieved in 12 cases (60%) after 3 months from transplant, including eight cases that had received contaminated precursors. Two other cases achieved molecular remission at 6 months; thereafter, one of them reverted to PCR positivity and then relapsed. One year from transplant, 15 (75%) patients with molecular involvement of the bone marrow were PCR negative. Fifty percent of patients transplanted with contaminated stem cells achieved molecular bone marrow remission, independent of bone marrow PCR status at diagnosis.

Molecular remission status after transplantation did not significantly correlate with histology, age, sex, IPI, or disease stage. The relapse rate of patients still PCR positive after transplantation was higher as compared to that seen with PCR-negative patients (55% *vs* 29%), but the difference was not statistically significant ($P = 0.23$).

Figure 1 shows the influence of bone marrow PCR positivity after transplantation on OS. In fact, median OS was 85 months in the PCR-positive cases and it was not achieved in the PCR-negative ones ($P = 0.05$). Similarly, RFS (Figure 2) was significantly longer for PCR-negative cases (median RFS not reached *vs* 21 months in the PCR-positive group, $P = 0.005$).

Regardless of the efficacy of the procedure, 26% of patients who received a purged apheresis product relapsed *vs* 39% transplanted with unpurged precursors ($P = 0.12$).

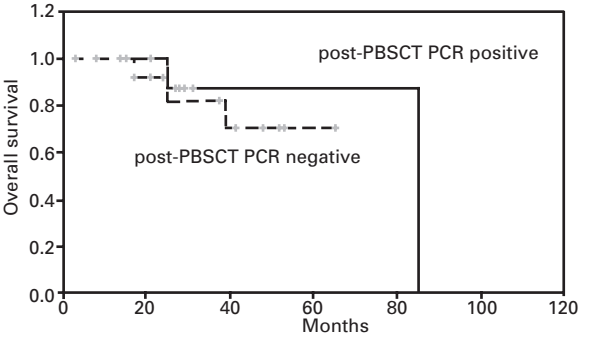

Figure 1 Overall survival of non-Hodgkin's lymphoma patients receiving a blood stem cell harvest according to molecular status achieved after PBSCT: the median was 85 months for patients still PCR positive *vs* a median not achieved for PCR-negative patients ($P = 0.05$).

For the entire series, a trend for longer OS ($P = 0.07$) was observed in patients transplanted with unpurged apheresis products. In particular, 98% of unpurged cases were still alive at 50 months as compared to 70% of cases transplanted with purged precursors (Figure 3). As far as RFS is concerned, no statistical difference was observed between the two groups (80% *vs* 58%, Figure 4). We analyzed the role of purging on OS and RFS in the aggressive and indolent cases separately. Aggressive lymphomas showed both a longer OS ($P = 0.03$, Figure 5) and RFS ($P = 0.02$, Figure 6) when transplanted with unpurged stem cells, whereas indolent NHL survival was not influenced by *ex vivo* purging.

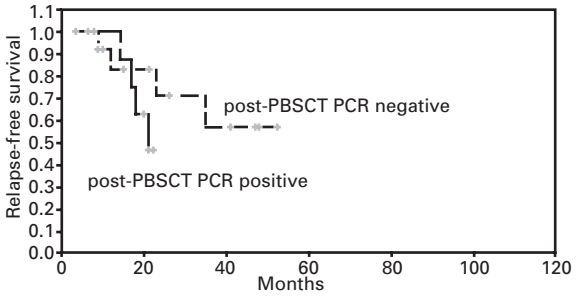

Figure 2 Relapse-free survival of non-Hodgkin's lymphoma patients receiving a blood stem cell harvest according to molecular status achieved after PBSCT: the median was 21 months for patients still PCR positive *vs* a median not achieved for PCR-negative patients ($P = 0.005$).

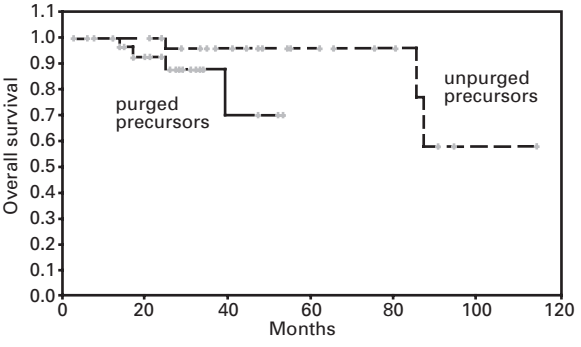

Figure 3 Overall survival of non-Hodgkin's lymphoma patients receiving a blood stem cell harvest according to *ex vivo* purging procedure: 50-month OS was 98% for patients reinfused with unpurged precursors *vs* 70% for patients receiving purged stem cells ($P = 0.07$).

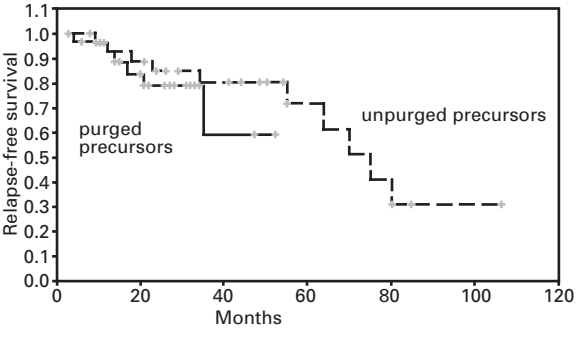

Figure 4 Relapse-free survival of non-Hodgkin's lymphoma patients receiving a blood stem cell harvest according to *ex vivo* purging procedure: 50-month RFS was 80% for patients reinfused with unpurged precursors *vs* 58% for patients receiving purged stem cells ($P = 0.32$).

Discussion

Molecular evaluation of MRD in patients with NHL at diagnosis and during several treatment phases has repeatedly been suggested to be a possible predictor of clinical outcome. Bone marrow samples at diagnosis, harvest products and bone marrows during follow-up after high-dose chemotherapy have been assessed for MRD in a series of patients with aggressive and indolent NHL, with the aim of evaluating the efficacy of *ex vivo* purging and the clinical relevance of molecular MRD assessment at diagnosis and after PBSCT.

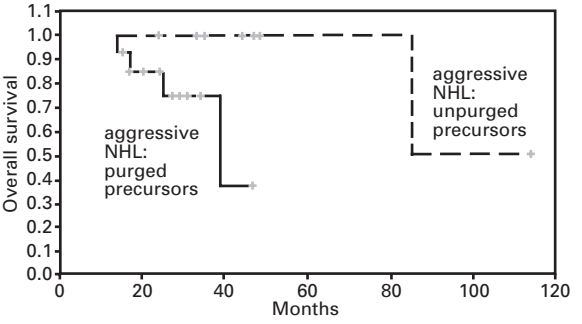

Figure 5 Overall survival of patients with aggressive NHL according to the *ex vivo* purging procedure: the median was 40 months for patients reinfused with purged precursors *vs* 84 months for those engrafted with unpurged cells ($P = 0.03$).

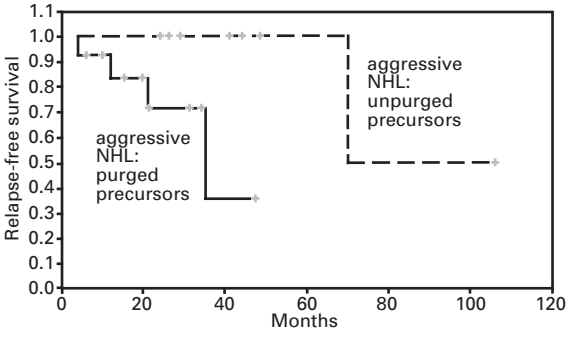

Figure 6 Relapse-free survival of patients with aggressive NHL according to the *ex vivo* purging procedure: median was 33 months for patients reinfused with purged precursors *vs* 72 months for those engrafted with unpurged cells ($P = 0.02$).

In the entire series, OS and RFS were 87% and 72%, respectively. In particular, RFS appeared advantageous for aggressive histologies, in accordance with the situation described in the literature.[17,18]

As expected, indolent histology and advanced disease stage were significantly associated with molecular bone marrow involvement at diagnosis and contaminated apheresis products. Patients with bone marrow involvement were more likely to have contaminated stem cell harvests, whereas lack of malignant bone marrow cells at diagnosis was significantly associated with lymphoma-free harvests. Nevertheless, 18% of PCR-positive cases harvested PCR-negative progenitors and 31% of patients with PCR-negative marrow harvested PCR-positive peripheral blood stem cells, indicating that the molecular status of the bone marrow could not be unequivocally used as a convenient predictor of tumor-free leukapheresis products. These data are in accordance with those observed by Magni *et al*[8] who found that 44% of their patients with indolent NHL, who were peripheral blood PCR, negative just before mobilization, harvested contaminated precursors.

The appearance of contaminated precursors can be explained either by their having been mobilized from residual peripheral masses or for technical reasons. In our series, lack of correlation between quality of remission and molecular status of the harvest may be explained by the high sensitivity of the molecular detection method

employed in this study, and one could argue that the higher concentration of residual neoplastic cells or the reduction in lymphocytic polyclonal background observed in the harvests might justify the finding of a high rate of PCR positivity.

Whether *ex vivo* purging procedures are of clinical relevance in either indolent or aggressive NHL is still a matter of debate. In this regard, the European Blood and Marrow Transplant Registry (EBMTR) reported a slightly longer 5-year OS for patients who received purged marrow as compared to that of patients receiving unmanipulated stem cells (54% *vs* 48%), without differences in progression-free survival.[19] In a series of 116 relapsed follicular lymphoma patients with contaminated aphereses, the Dana Farber Cancer Institute reported 42% of molecular remissions after *ex vivo* purging. The relapse risk for patients transplanted with contaminated precursors was 11.7 times higher than that of patients receiving PCR-negative grafts. The 8-year FFS in purged patients was 19% for the patients whose bone marrow remained PCR positive *vs* 83% for patients receiving PCR-negative bone marrow.[20] Other studies demonstrated a molecular remission rate ranging from 14% to 83%.[18,21,22] Therefore, *in vitro* purging in autologous transplantation for NHL should be further investigated. In our series, we observed 18% of molecular remissions after *ex vivo* purging procedures. Relapse rates were 26% and 39% for patients receiving purged and unpurged aphereses, respectively.

In our series, in addition to the B cell-negative selection, CD34-positive purging was performed. The expected benefits of this procedure are related to the removal of tumor cells lacking CD34 expression. However, the delay in T lymphocyte recovery after $CD34^+$ transplantation may adversely contribute to clinical outcome. In fact, although grafts were depleted of several logs by purging, a trend for longer survival for patients receiving unpurged harvests was found. This result seems to be more significant in patients with aggressive lymphoma. However, these data should be confirmed in a larger cohort of patients. Overall, the feeling is that MRD status of the harvest should not be considered the only clinico–biological variable to influence both rate and duration of response. New, recently published *in vivo* monoclonal antibody purging approaches indicate that disease eradication may be more easily achieved than by *ex vivo* purging[8] methods.

The finding that molecular remission after PBSCT was achieved in 75% of patients with molecular bone marrow involvement at diagnosis and in 50% of cases transplanted with contaminated progenitors suggests that transplantation *per se*, through reducing residual disease offers a key advantage in improving the clinical outcome of NHL patients. This is in line with a recent report dealing with follicular lymphomas, in which the risk of disease progression was 19.2 times higher for non-transplanted as compared to transplanted patients.[22]

The role of MRD after PBSCT has also been studied. Voso *et al*[3] reported a relapse rate 4.5 times higher in follicular lymphomas PCR positive after transplantation than in negative cases. Similarly, the Dana Farber Cancer Institute[20] reported that 36/39 cases PCR positive after BMT relapsed. These data are in line with our results, demonstrating a significantly longer RFS and OS for patients achieving molecular remission after PBSCT. Thus, after high-dose chemotherapy, close molecular monitoring of MRD using qualitative PCR technique seems to represent a reliable prognostic indicator.

In the near future, quantitative, rather than qualitative assays for the evaluation of MRD may provide further prognostic information in patients with NHL.

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