Molecular Monitoring of Minimal Residual Disease in Follicular and Mantle Cell Non-Hodgkin's Lymphomas Treated With High-Dose Chemotherapy and Peripheral Blood Progenitor Cell Autografting

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Minimal residual disease (MRD) was evaluated in 30 patients with follicular or mantle cell non-Hodgkin's lymphoma (NHL) undergoing an intensive treatment with high-dose sequential (HDS) chemotherapy and peripheral blood progenitor cell (PBPC) autografting. To minimize the potential tumor cell contamination, PBPC harvests were scheduled at the end of HDS pretransplant phase. All patients had advanced-stage disease and most of them presented with bone marrow (BM) involvement. A tumor marker could be generated in 90% of patients using bcl-2 or Ig heavy-chain genes. MRD was analyzed on PBPC, BM harvests, and after autografting by polymerase chain reaction (PCR). All evaluable follicular and 6 of 9 mantle cell patients achieved clinical complete remission. PCR negativity of PBPC and/or BM harvests was documented in 68% of follicular and 12% of mantle cell lymphomas. Molecular remission of PBPC and/or BM harvests was achieved in 9 of 15 patients with overt marrow involvement and in all patients with only molecular marrow infiltration at onset. Molecular follow-up was conducted on 14 patients: all 7 evaluable patients who received at least one PCR-negative graft maintained the negative status at a median follow-up of 24 months and none of them relapsed so far. Thus, the results show that (1) a molecular marker to monitor MRD can be obtained in most follicular and mantle cell NHL patients, (2) the HDS regimen may provide PCR-negative PBPC and/or BM harvests even from patients with BM disease, and (3) autograft with at least one PCR-negative harvest is associated with a durable clinical and molecular remission.
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HIGH-DOSE CHEMORADIOTHERAPY with autologous bone marrow transplantation (ABMT) is a potentially curative modality for patients with relapsed intermediate/high-grade non-Hodgkin's lymphoma (NHL).[1-7] However, the use of ABMT in forms with less aggressive histology, such as follicular and mantle cell subtypes, has been viewed with concern.[8-10] Patients with advanced stage follicular or mantle cell lymphomas are generally managed with conventional chemotherapy and/or radiotherapy. Despite initial responses achieved with this approach, disease recurrence is the rule and the outcome is ultimately fatal.[10-13] A more intensive approach, using high-dose chemotherapy with ABMT, has been investigated in relapsed patients. However, ABMT toxicity has limited the use of such a procedure to restricted study groups.[8,14-16] The prompt engraftment offered by mobilized peripheral blood progenitor cells (PBPC) has greatly reduced morbidity and mortality linked to autografting programs, widening their applicability.[17-20] Thus, high-dose chemotherapy with PBPC autograft has recently gained growing interest also for the treatment of follicular and mantle cell lymphomas.[21-23]

A major concern in autografting programs is that cell harvests contain occult tumor cells that may contribute to disease relapse. This is particularly relevant in follicular and mantle cell lymphomas in which bone marrow (BM) infiltration is a common feature.[8] For a long time, it has been speculated that PBPC were less contaminated than BM cells.[24] However, several groups have recently shown that PBPC may contain residual tumor cells in hematologic malignancies and some solid tumors.[25-27] Hence, programs of PBPC autografting for low- and intermediate-grade NHL should include an adequate tumor reduction before harvesting, along with a close monitoring of minimal residual disease (MRD). Based on these premises, a high-dose sequential (HDS) chemotherapy program, with PBPC harvest at the end of an intensive pretransplant phase, was designed for patients with advanced-stage follicular or mantle cell lymphoma.[23] A molecular monitoring of MRD was included in the program.

The strategies currently in use to detect MRD are based on polymerase chain reaction (PCR), thus providing high specificity and sensitivity. The rearrangement of the Bcl-2 oncogene is commonly used for PCR detection of residual tumor cells in NHL.[28,29] This rearrangement is found in approximately 75% of follicular and 25% of diffuse lymphomas. The t(14;18) translocation juxtaposes the Bcl-2 oncogene, normally located on chromosome 18, to the Ig heavy chain (IgH) locus on chromosome 14.[30-32] However, in a sizeable fraction of lymphoma patients, the Bcl-2 translocation is not present. For such a reason, we devised an alternative strategy using IgH genes. The rearrangement of variable, diversity, and joining segments (VDJ) of IgH genes generates unique DNA sequences that are specific to each B-cell clone. These unique sequences are called complementarity-determining regions (CDR) and code for the antigen binding site.[33] From the CDR sequences, clone-specific primers and probes were derived and used for the PCR detection of residual lymphoma cells.

MRD was evaluated in 30 patients with follicular or mantle cell lymphoma treated with HDS regimen. The aims of the study were (1) to devise a PCR-based strategy able to detect residual disease in most of the cases, (2) to evaluate

Table 1. Patient Characteristics

	Mantle Cell Lymphoma (n = 9)	Follicular Lymphoma (n = 21)
Sex (M/F)	9/0	9/12
Median age in years (range)	46 (32-60)	48 (33-59)
Entry at diagnosis	7	20
Entry at relapse	2	1
Stage III-IV	9	21
Bulky disease	2	6
BM disease	8	16
Median LDH in U/L (range)	350 (246-772)	316 (224-965)
Tumor-related symptoms	7	13

the possibility of having PCR-negative PBPC and/or BM harvests after an intensive pretransplant treatment, (3) to compare tumor contamination of PBPC versus BM, and (4) to evaluate the clinical relevance of PCR analysis on cell harvests and in vivo after autograft.

MATERIALS AND METHODS

Patient characteristics and treatment plan. Since 1990, 30 patients (3 at relapse) with follicular lymphoma, follicular lymphoma with signs of histologic transformation, or mantle cell NHL were enrolled in a pilot HDS chemotherapy program (Table 1). Diagnosis was made by morphologic and immunologic analysis according to the Working Formulation (WF) for Clinical Usage. Diagnosis of mantle cell lymphoma was made according to criteria of Revised European-American Lymphoma classification.[34] All patients had advanced-stage disease and most of them presented with tumor-related symptoms (B symptoms or compression symptoms given by local enlargement of lymph nodes). The HDS program was approved by the hospital ethical committee, and written informed consent was obtained from all patients. The program is organized as follows: 2 cycles of APO (doxorubicin at 75 mg/m² on days +1 and +22; vincristine at 1.4 mg/m² on days +1, +8, and +22; and prednisone at 50 mg/m² on days +1 through day +28) and 1 or 2 courses of DHAP followed by etoposide at 2 g/m², methotrexate at 8 g/m² plus vincristine at 1.4 mg/m², 3 cycles of dexamethasone at 25 mg/m², and, finally, cyclophosphamide at 7 g/m² (Fig 1). Granulocyte colony-stimulating factor (G-CSF; 5 μg/kg) was administered after etoposide and cyclophosphamide.[23] Dexamethasone cycles before cyclophosphamide were used to allow a complete recovery of BM cellularity to obtain a better PBPC mobilization.[35] After cyclophosphamide, a median of three leukophereses were performed to harvest PBPC. Five patients (N44, N45, N66, N103, and N67) had been enrolled in a previous study in which an earlier HDS regimen was used.[17] Two of these latter patients (N44 and N103) had PBPC collection after the first high-dose course, whereas the remaining patients had their cells collected after the final high-dose course. PBPC estimation in leukopheresis product was performed in terms of both CD34⁺ cells as well as myeloid clonogenic cells (colony-forming unit–granulocyte-macrophage [CFU-GM]), as described elsewhere.[35] All patients underwent a back-up BM harvest after HDS therapy. The conditioning regimen consisted of mitoxantrone at 60 mg/m² followed by melphalan at 180 mg/m², except for the 5 patients treated according to the original HDS protocol, who received total body irradiation (13.2 Gy) and melphalan at 120 mg/m². Autologous cryopreserved cells were infused the day after melphalan administra-

tion. Complete remission (CR) was defined as the disappearance of all clinical evidence of active tumor. Partial remission (PR) was defined as a tumor reduction greater than 50%.

Nucleic acid extraction. BM, PB, and lymph node samples were collected during standard diagnostic procedures. Genomic DNA was purified by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation.[36] RNA was isolated using RNAzol B method (Biotecx Laboratories, Houston, TX).

PCR amplification of Bcl-2/IgH translocation. Amplification of major (MBR) and minor (mcr) breakpoints was performed using nested PCR. The oligonucleotide primers were the same originally designed by Gribben et al.[28] Briefly, 1 μg of genomic DNA was amplified in 200 μmol/L dNTPs, 1× Taq buffer (50 mmol/L KCl, 10 mmol/L TRIS-HCl, pH 8, 2 mmol/L MgCl₂, 0.1% [wt/vol] gelatin) final concentration, adding 2.5 U of Taq DNA polymerase (Promega, Madison, WI) in a final volume of 50 μL. The amplification was performed as follows: 94°C for 1 minute, 55°C (MBR) or 58°C (mcr) for 1 minute, and 72°C for 1 minute for 25 cycles, followed by 7 minutes of final extension at 72°C. The reamplification for 30 cycles of a 5-μL aliquot of the first reaction was performed using internal primers. Amplified DNAs were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide and were visualized by UV light. Each amplification contained a weak-positive control consisting of 10⁻⁵ dilution of the appropriate cell line. Polyclonal DNAs were used as negative controls.

PCR amplification and sequencing of IgH VDJ. The tumor VDJ was amplified starting from genomic DNA as previously described.[27] Briefly, 1 μg of DNA was amplified in 200 μmol/L dNTPs, 1× Taq buffer (50 mmol/L KCl, 10 mmol/L TRIS-HCl, pH 8, 1.5 mmol/L MgCl₂, 1% [wt/vol] gelatin) final concentration, adding 2.5 U of Taq DNA polymerase (Promega), 10 pmol of VH.L or VH.D primers, and 10 pmol of JH.D primer (without restriction sites) in a final volume of 50 μL. The amplification was performed as follows: 94°C for 1 minute, 62°C for 30 seconds, and 72°C for 30 seconds for 35 cycles, followed by 7 minutes of final extension at 72°C. The prevention of PCR contamination was performed as previously described.[37] Amplified DNA was directly sequenced using VH.L, VH.D, and JH.D primers, as previously described.[27] When the sequence quality did not allow a complete reading of CDR regions, DNA was reamplified with primers containing *Eco*RI and *Hin*dIII restriction sites and cloned in Bluescript SK vector (Stratagene, San Diego, CA). Restriction enzyme analysis was performed on plasmid DNAs prepared by the alkaline lysis method, and miniprep plasmid DNAs were sequenced as previously described.[38] The analysis of sequencing data was performed using the PC-GENE software (IntelliGenetics, Inc, Mountain View, CA).

Detection of residual lymphoma cells. PBPC, BM cell harvests, and BM and PB samples after autografting were evaluated for the presence of residual lymphoma cells. One microgram of DNA was amplified using the Bcl-2 or IgH assay. When IgH were used as genetic marker, 20% of the PCR product was analyzed by agarose gel electrophoresis, blotted overnight, and hybridized to CDR3 probes end-labeled with [γ-³²P] ATP, as described previously.[38] To avoid false-negatives, all the DNA samples failing to produce a PCR product were reamplified, and the DNA quality was tested by amplifying the sequence of p53 exon 5 or N-ras exon 2.

Oligonucleotide synthesis. Oligonucleotides were synthesized with a 391 PCR-MATE EP, DNA synthesizer (Applied Biosystems, Foster City, CA) on a 0.2 μmol scale, according to the users' manual.

RESULTS

Treatment feasibility and clinical outcome. PBPC collection was satisfactory in 24 patients with more than 20 × 10⁴ CFU-GM/kg harvested with a median of 3 leukophereses
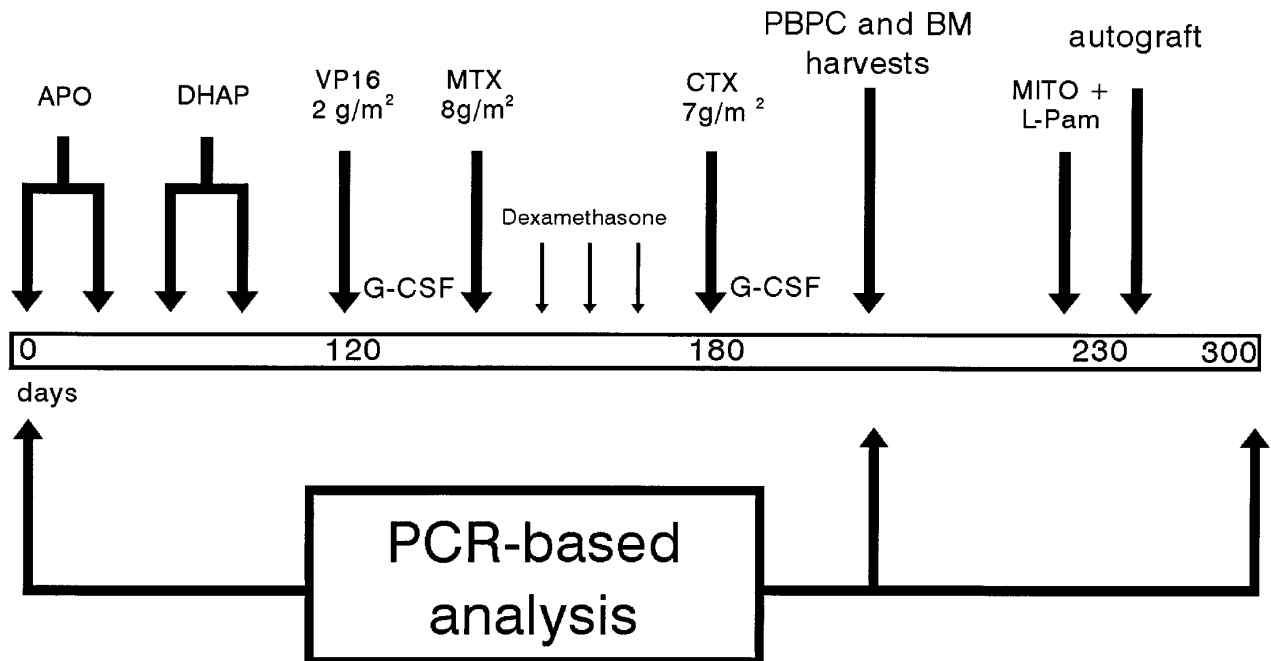
Fig 1. Schematic representation of HDS program. APO, see the Materials and Methods; VP16, etoposide; MTX, methotrexate; CTX, cyclophosphamide; MITO, mitoxantrone.

(range, 1 to 4). Four patients (N15, N45, N66, and N67) had low yields ($<20 \times 10^4$ CFU-GM/kg); in two more patients (N156 and N165), there was virtually no collection due to heavy marrow infiltration. Five patients were not able to undergo the final autografting phase because of (1) the occurrence of esophageal carcinoma immediately before autograft (N60), (2) transplantation refusal (N154), or (3) persistence of overt BM lymphoma cell infiltration after HDS chemotherapy (N165, N193, and N156).

The treatment was well tolerated. There was one treatment-related death from intracranial bleeding secondary to severe thrombocytopenia during the postgraft period (day +21). Of note, this was in the patient (N45) who received a reinfusion of a suboptimal dose of PBPC ($<10 \times 10^4$ CFU-GM/kg) and BM cells.

The regimen proved to be effective in terms of tumor reduction, although follow-up is short. Clinical outcome before autografting was evaluated in 27 of 30 patients. In follicular lymphomas, 10 CR (55%) and 8 PR (44%; 3 PR had only a residual marrow infiltration) were obtained. In patients with mantle cell lymphoma, 4 CR (44%) and 5 PR (55%; 4 PR had disease persistence at marrow level) were achieved. Sixteen of 21 patients with follicular lymphoma have been autografted so far and are evaluable for clinical results (5 patients are waiting for the final autografting phase). All evaluable patients achieved CR. Fourteen patients (87%) are presently alive in continuous CR (CCR) at a median follow up of 19 months (range, 3 to 66 months). All patients with mantle cell lymphoma are evaluable: 6 achieved CR and 3 (33%) are presently in CCR at a median follow up of 18 months (range, 3 to 40 months); 3 patients achieved PR only.

Overall, 28 patients (20 follicular and 8 mantle cell) are alive at a median follow-up of 24 months (range, 3 to 66 months).

Identification of patients with a molecular marker. A molecular marker was obtained from the diagnostic tissue in 27 of 30 patients (90%). A PCR-amplifiable Bcl-2/IgH translocation was detected in 16 of the 21 patients (76%) with follicular lymphoma: 15 involving the MBR and 1 the mcr breakpoint (Tables 2 and 3). To detect residual lymphoma cells, a nested PCR was used. In 5 patients, the Bcl-2 breakpoint amplified with nested PCR was also hybridized to a specific probe (data not shown). As previously reported, no differences in assay sensitivity were found; thus, subsequent cases were analyzed by nested PCR only.[29] The assay sensitivity was tested diluting appropriate cell lines (RL and DHL-16; kindly provided from Dr J.G. Gribben, Division of Hematological Malignancies, Dana-Farber Cancer Institute, Boston, MA) with normal BM cells. Nested PCR for Bcl-2 could detect 1 lymphoma cell in 10^5 normal cells.

Lymphoma VDJ sequence was obtained in 11 of 14 Bcl-2−negative patients (79%). The VDJ was amplified using sense consensus primers derived from the leader (VH.L) or first framework (VH.D) region of IgH genes and an antisense primer from JH region.[27] The lack of amplification in 3 patients may be explained either by the presence of somatic mutations or by the use of unknown variable regions that prevent correct primer/template annealing. Amplified VDJ regions were then sequenced. Direct sequencing analysis provided a good quality sequence in 8 cases. For the remaining patients, amplified VDJs were cloned in a plasmid vector. For each patient, 10 clones were sequenced, and,

Table 2. PCR Analysis of Residual Tumor Cells in Follicular Lymphomas

UPN	Histology (W.F.)	Molecular Marker	Status/Stage	BM Before Treatment Histology	PCR	BM Harvests	PBPC Harvests 1	2	3
N44	C	Bcl-2/MBR	D/IV	+	+	+	+	+	NA
N234	C	Bcl-2/mcr	D/IV	+	+	+	+	+	+
N54	C	Bcl-2/MBR	D/IV	+	+	+	+	+	+
N14	G (transf)	Bcl-2/MBR	D/IV	+	+	+	−	−	NA
N45	B	Bcl-2/MBR	D/IV	+	+	−	−	−	NA
N15	F (transf)	Bcl-2/MBR	D/IV	+	+	−	−	−	−
N60	F (transf)	Bcl-2/MBR	D/IV	+	+	−	−	−	−
N245	C	Bcl-2/MBR	D/IV	+	+	−	−	−	−
N281	B	Bcl-2/MBR	D/IV	+	+	−	+	+	+
N41	C	IgH	D/IV	+	+	+	+	+	+
N101	D (transf)	IgH	R/III	−	+	−	−	−	+
N404	G (transf)	Bcl-2/MBR	D/III	−	+	−	−	−	−
N347	D (transf)	Bcl-2/MBR	D/IV	+	+	−	+	+	+
N66	G (transf)	NA	D/III	−	NA	NA	NA	NA	NA
N228	C	NA	D/IV	+	NA	NA	NA	NA	NA
N357	B	Bcl-2/MBR	D/IV	+	+	−	+	+	+
N103	G (transf)	IgH	D/III	−	+	−	+	+	+
N360	C	Bcl-2/MBR	D/IV	+	+	+	+	+	+
N427	D	Bcl-2/MBR	D/IV	+	+	−	+	+	+
N373	C	Bcl-2/MBR	D/IV	+	+	+	+	+	+
N379	C	Bcl-2/MBR	D/III	−	+	−	−	−	−

Abbreviations: D, diagnosis; R, relapse; transf, transformed histology; NA, not available.

together with a few heterogeneous clones, a predominant clone was present in all cases. Concordant results were obtained by both direct sequencing of amplified DNA and after cloning in a previous study.[27] For all patients, a CDR2 sense primer and a CDR3 probe were synthesized and used with a JH region primer to detect residual lymphoma cells. Several polyclonal lymph nodes were used as negative controls, and no false-positive results were detected. Our IgH PCR-based assay could detect 1 tumor cell in 10^5 normal BM cells. Such a sensitivity was achieved performing a serial dilution of a Burkitt cell line (BJAB) with normal cells (data not shown).

Detection of residual lymphoma cells in PBPC and BM harvests. PCR-based analysis of MRD was performed on PBPC, and BM harvests were performed in 27 patients. All patients had a PCR-positive BM before treatment, and in 22 overt lymphoma, infiltration was present at morphologic examination of trephine BM biopsy. A total of 73 PBPC and

27 BM harvests were analyzed. Twenty-three of 73 PBPC and 13 of 27 BM harvests were PCR negative (Tables 2 and 3). In 7 patients (26%), lymphoma cells were not detected in both PBPC and BM harvests. In 7 patients (26%), either PBPC or BM harvests were PCR negative. In 13 patients (48%), both PBPC and BM harvests contained residual lymphoma cells. Nine of 14 patients (64%) with PCR-negative harvests had an overt BM infiltration at diagnosis. No statistical correlation could be found between the degree of BM infiltration at diagnosis and PCR negativity of cell harvests.

The analysis of MRD showed a marked difference between follicular and mantle cell lymphoma: 13 of 19 (68%) patients with follicular lymphoma had at least one PCR-negative harvest, whereas only 1 of 8 (12%) patients with mantle cell histology had PCR-negative harvests.

Detection of residual lymphoma cells after transplanta-

Table 3. PCR Analysis of Residual Tumor Cells in Mantle Cell Lymphomas

UPN	Molecular Marker	Status/Stage	BM Before Treatment Histology	PCR	BM Harvests	PBPC Harvests 1	2	3
N67	IgH	D/IV	+	+	+	+	+	+
N154	IgH	D/IV	+	+	+	+	+	+
N140	IgH	D/IV	+	+	+	+	+	+
N165	IgH	R/IV	+	+	+	+	NA	NA
N70	IgH	D/IV	−	+	−	−	−	NA
N193	IgH	R/IV	+	+	+	+	+	+
N156	IgH	D/IV	+	+	+	+	NA	NA
N362	IgH	D/IV	+	+	+	+	+	+
N68	IgH	D/IV	+	NA	NA	NA	NA	NA

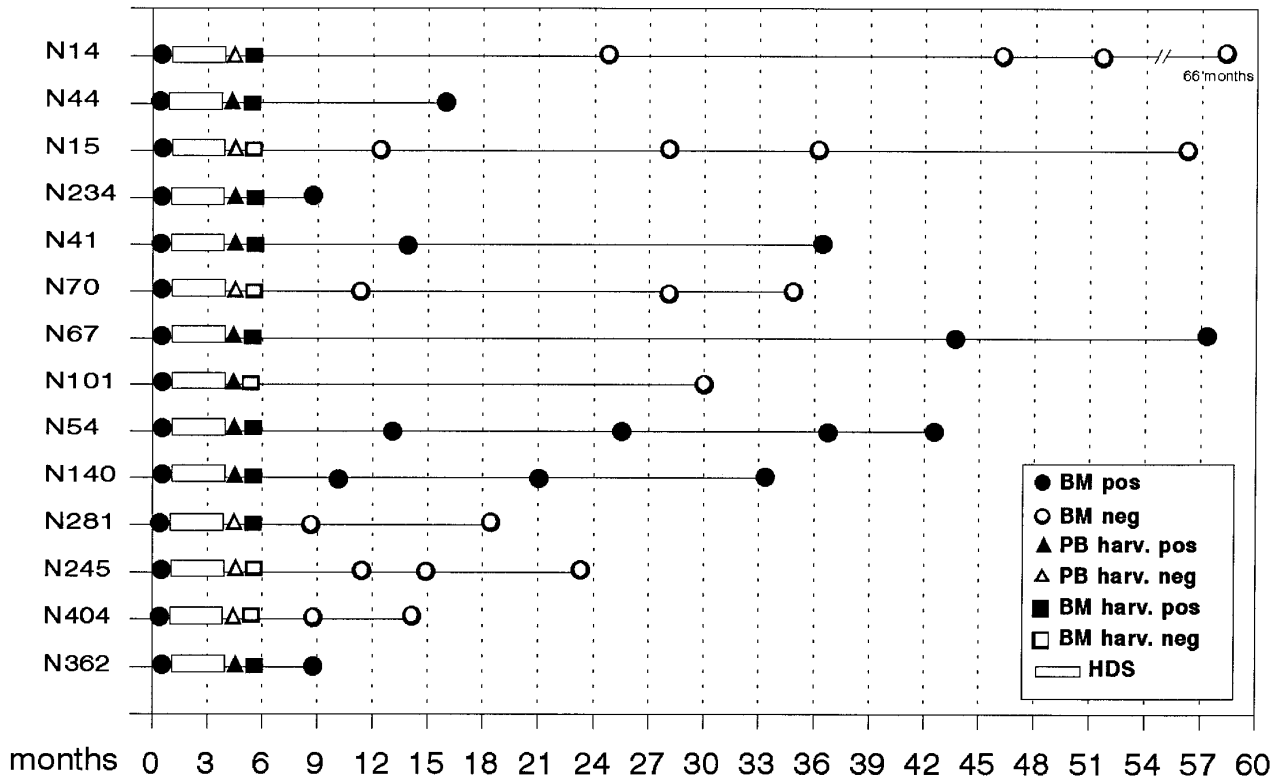Abbreviations: D, diagnosis; R, relapse; NA, not available.

Fig 2. PCR-based monitoring of MRD after autografting. harv,harvests. (□) PCR-negativity; (■) PCR-positivity.

tion. Seventeen of 27 patients having a molecular marker have been autografted so far. Molecular monitoring of BM samples by PCR was possible in 14 patients (Fig 2). Patient N45 died from intracranial bleeding at day +21 after autografting, patient N103 refused restaging examinations, and patient N379 has been just autografted. Median follow-up from autografting was 25.5 months (range, 3 to 61 months). It is noteworthy that the subset of 7 patients who received at least one PCR-negative harvest maintained the negative status during molecular follow-up (longer follow-up of 61 months). Patients receiving only PCR-positive harvests remained positive (longer follow-up of 52 months; Fig 2). None of the PCR-negative patients relapsed so far; 3 relapses (N67, N54, and N140) occurred in the PCR-positive group. Clearly, the follow-up is short and, at the present time, the patient groups are too small for anything other than a descriptive statistical analysis.

DISCUSSION

Follicular and mantle cell lymphomas share some clinical features: (1) an indolent course, with a median survival ranging from 3 to 9 years; (2) incurability with conventional chemotherapy regimens; and (3) frequent marrow involvement at onset.[8-13] Because long-term life expectancy is relatively poor, we designed an innovative HDS chemotherapy regimen for young patients with advanced-stage follicular or mantle cell NHL. High tumor reduction and prolonged disease-free survival after intensive chemoradiotherapy and

autologous transplantation have been recently reported in high-risk follicular lymphomas.[14-16,21,22] At present, these promising results do not allow definite conclusions on disease curability. In fact, clinical studies require a long follow-up as a consequence of the indolent course characterizing follicular lymphomas.

A major concern in autografting programs is the reinfusion of occult tumor cells contained in the graft. PCR-based techniques are essential to evaluate the contamination of residual tumor cell in harvests for autografting purposes.[39] In addition, MRD analysis would provide an early end-point to assess the curative potentiality of novel intensive chemotherapy approaches in indolent lymphomas. The predictive value of PCR-based analysis emerged from a recent study in follicular lymphomas in which in vitro-purged BM cells were used for autograft. Patients autografted with PCR-negative harvests seldom relapsed, whereas the opposite occurred in positive cases.[29,40] These observations prompted us to develop a strategy aimed to molecular monitoring of NHL patients undergoing PBPC autograft. A two-step experimental strategy was developed: first, patients were screened for the presence of bcl-2 gene rearrangements; second, lymphoma-specific markers were derived from IgH gene rearrangement in bcl-2–negative cases. The PCR assay using IgH genes offered a sensitivity and specificity similar to a translocation marker such as Bcl-2.[27] The combined use of bcl-2 and IgH gene rearrangements was successful in providing a molecular marker for 90% of the patients.

The particular feature of our program was PBPC collection after repeated high-dose chemotherapy courses. Previous extensive administration of myelotoxic drugs could impair the subsequent progenitor mobilization.[35] However, with the introduction of a chemotherapy-free interval before the final mobilizing course, adequate PBPC collections could be restored in 80% of patients. The result is of particular interest considering that most patients presented with marrow involvement, a feature often associated with poor PBPC mobilization.[41] Moreover, the extensive pretransplant treatment provided PCR-negative PBPC and/or BM harvests in 14 of 27 evaluable patients. Therefore, the designed schedule allowed satisfactory PBPC collections in terms of both quantity and quality.

The collection of PCR-negative PBPC in follicular lymphomas treated with high-dose chemotherapy has been recently reported by two groups.[42,43] In the study reported by Haas et al,[42] the proportion of patients with PCR-negative PBPC was lower compared with our results. The intensive pretransplant phase of our program was probably responsible for the higher rate of PCR negativity. In addition, our molecular analysis was performed also on BM harvests, whereas in the report by Haas et al,[42] there is no mention about the possibility of having PCR-negative BM harvests. Hardingham et al[43] reported a percentage of PCR-negative PBPC harvests slightly higher than ours. However, some PCR-negative PBPC were collected from patients in PR, and none of them had long-term survival in CR. This unexpected observation could be partially related to a lower sensitivity in MRD detection. In fact, the sensitivity of Hardingham et al's IgH assay was less efficient than ours, because it was not based on the use of lymphoma-specific primers and probes.[25,42]

The efficacy of our HDS program was proved by the high rate of CRs documented in all evaluable follicular and in 6 of 9 mantle cell patients. However, the achievement of molecular remission was influenced by marrow histology at diagnosis: 9 of 15 patients with overt marrow involvement had BM and/or PBPC harvests negative at PCR analysis, whereas all patients presenting with only molecular infiltration without histologic evidence of marrow involvement had PCR-negative harvests. This is one more proof that BM lymphoma cells are somehow less susceptible to cytotoxic agents than cells from other sites.[44] The achievement of PCR negativity did also positively correlate to follicular histology. PCR negativity of PBPC and/or BM harvests was documented in 68% of follicular patients; on the contrary, only 1 patient with mantle cell lymphoma achieved PCR negativity. The differences between follicular and mantle cell lymphoma cannot be merely explained by a different degree of marrow involvement. They probably reflect an intrinsic low sensitivity of mantle cell subtype to chemotherapy, even when delivered at high doses.[45]

PCR negativity was more frequent in BM than in PBPC harvests. In particular, 5 patients had PCR-negative BM harvests despite positive PBPC. This observation seems to contradict the never-proved hypothesis that PBPC are less contaminated by lymphoma cells than BM.[24,46] A possible explanation might be that, during growth factor mobilization,

all the cells present in the marrow, including lymphoma cells, are driven into circulation; by contrast, during BM harvest, only a restricted area of the bone is tested and the amount of lymphoma cells collected might be below the threshold of PCR detection.

Clearly, the follow-up is short, and a longer period of observation is required to conclusively evaluate the clinical relevance of molecular analyses. However, preliminary data from the molecular follow-up on 14 patients suggest that the reinfusion of some harvests devoid of PCR-detectable lymphoma cells might be critical to mantain a PCR-negative status after transplantation. The collection of PCR-positive and -negative harvests in the same patient is a sign of pronounced tumor reduction, and it may be speculated that the molecular status is close to the threshold of detection of MRD assay. All 7 evaluable patients who received at least one PCR-negative graft maintained the negative status with a follow-up of 24 months. All PCR-negative patients are at present in CCR. The durable PCR-negativity achieved by patients who received also some positive grafts may have different explanations: reinfused cells could have been irreversibly damaged by chemotherapy, losing their clonogenic potentiality; alternatively, the relatively small number of re-infused lymphoma cells could have been controlled in vivo by the immune system. Whatever the explanation, our results are in line with those from Gribben et al[29,40] showing that reinfusion of a PCR-negative BM graft, after in vitro purging, correlates with a durable PCR-negativity. Thus, HDS chemotherapy can provide an in vivo purging similar to the one obtained using in vitro immunologic purging. These data suggest that the achievement of PCR-negativity might predict a prolonged disease-free survival, if not even cure, and support the use of in vitro purging when both BM and PBPC are positive for PCR-detectable lymphoma cells.[47,48]

In conclusion, our results show that (1) it is possible to obtain a molecular marker to monitor residual disease by PCR in most of follicular and mantle cell NHL patients, (2) the HDS regimen may provide PCR-negative PBPC and/or BM harvests even from patients with BM disease, and (3) autograft with at least one PCR-negative harvest is associated with a durable clinical and molecular remission. Based on these findings, we started a pilot study using the HDS regimen and in vitro immunologic purging for follicular and mantle cell patients having only PCR-positive harvests.

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