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Molecular analysis of patients with relapsed or refractory intermediate-high grade non-Hodgkin's lymphoma with bone marrow infiltration undergoing peripheral blood progenitor cell transplantation

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Background and Objectives. IgH gene rearrangement studies with a polymerase chain reaction (PCR) technique can detect the persistence of clonal cells at molecular level during the remission phase. This persistence of clonal cells can be used to establish the relationship between minimal residual disease (MRD) and clinical outcome. We have developed a three-step single strand conformational polymorphism PCR strategy which is able to detect clonal B lymphoid cells at a frequency as low as 1 clonal cell in 10^6 normal cells.

Design and Methods. Twenty patients with intermediate or high-grade B non-Hodgkin's lymphoma (NHL) were evaluated. Patients were pre-treated with a median of two (range 1-4) conventional chemotherapy lines before high-dose cyclophosphamide (HDCY). All patients had their bone marrow (BM) involved by disease (median 10%; range 5-50%). Nineteen patients were offered high-dose therapy followed by peripheral blood progenitor cells (PBPC) autografting.

Results. MRD analysis was performed for each patient at the end of conventional chemotherapy and every three months after high dose therapy. All these patients achieved complete response (CR) after high dose therapy (HDT). Six patients relapsed after a median time of 24.5 months. All the studied apheresis samples were positive at the molecular analysis. All 6 patients still positive at the molecular analysis after PBPC autografting relapsed. The remaining 13 patients who were negative maintained CR.

Interpretation and Conclusions. Whereas the detection of clonal cells in the apheresis samples did not predict an unfavorable outcome, the disappearance of the clonal rearranged band from the BM sample after HDT proved to be a favorable prognostic factor and was associated with long-lasting disease-free status.

Key words: PCR, MRD, high-risk NHL, PBPC transplantation.

Combination chemotherapy for diffuse, intermediate and high-grade non-Hodgkin's lymphoma (NHL) results in long-term cure for about 40 % of patients and the majority need further salvage treatment.[1-3] The management of patients with refractory or relapsing NHL is usually disappointing and the long-term survival rate is less than 10%.[4-6] In these patients high dose therapy (HDT) followed by autologous bone marrow transplantation (ABMT) can be useful in increasing survival and disease-free survival (DFS).[7,8] However, ABMT is not feasible in patients who have hypoplastic marrow following conventional treatment, or whose bone marrow (BM) is still involved by disease. Peripheral blood progenitor cell (PBPC) autografting may be feasible for these patients and more purified progenitor cells can be mobilized after chemotherapy-induced neutropenia.[9] High-dose cyclophosphamide (HDCY) followed by growth factor administration is the most widely used regimen for the collection of PBPC.[10-12]

Polymerase chain reaction (PCR)[13] based studies of clonality can be useful for monitoring minimal residual disease (MRD) through the subsequent therapeutic phases and might help in defining

prognosis. Rearrangements of immunoglobulin heavy chain (IgH) genes occur at an early stage of B-lymphocyte development and can be exploited as a marker for clonality in the vast majority of B lymphoid malignancies.[14,15] Since disease relapse following complete remission is thought to stem from neoplastic cells that escape chemotherapy,[16,17] IgH gene rearrangements studied with a PCR technique can demonstrate the persistence of clonal cells at molecular level during the remission phase. This persistence of clonal cells can be used to establish the relationship between MRD and clinical outcome. We have developed a three-step single strand conformational polymorphism polymerase chain reaction (SSCP-PCR) (Figure 1)[18-20] strategy which is able to detect clonal B lymphoid cells at a frequency as low as 1 in 10^6 cells (Figure 2). SSCP is based on the fact that variations as small as one base exchange in the sequence alter the secondary structure of single strand DNA, e.g. by different intramolecular base pairing. The change in the sequence causes modifications of electrophoretic mobility, which are observed as band shifts. Differential transient interactions of the bent and curved molecules with the gel fibers during electrophoresis cause the various sequence isomers to migrate differently. Since this method proved to be simple, rapid and reliable, it might be considered an alternative to clono-specific primer-based PCR[21-28] in monitoring patients with B-lymphoproliferative disorders in complete hematologic remission (CR). The fundamental biological and clinical question concerning whether all neoplastic cells have to be killed to *cure* the patient is, in fact, still unresolved and might be better elucidated using the most sensitive approaches now available.[29-31]

In this paper we present the clinical and molecular analysis of twenty patients with refractory and relapsed aggressive B-NHL who underwent high-dose therapy. The main aim of this study was to evaluate the value of MRD in predicting the outcome for this category of poor prognosis patients.

Design and Methods

Patients and treatments

Twenty patients with intermediate or high-grade B-NHL[32] were evaluated. All the patients, with relapsed or refractory disease and bone marrow involvement, were enrolled in a pilot study held between July 1991 and July 1995 at the First Division of Hematology of San Martino Hospital.[33,34] All patients had received extensive conventional treatment prior to HDCY, including CVP, CHOP, ProMACE-MOPP, MACOP-B, and VACOP-B.[35] Patients had been pre-treated with a median of two and a range of 1 to 4 conventional chemotherapy lines before HDCY (6 patients, 1 line; 11 patients, 2 lines; 2 patients, 3 lines; 1 patient, 4 lines). Twelve patients had achieved a partial response (PR) before HDCY, and were considered to have chemosensitive disease. Six patients had relapsed after treatment, four patients were defined as having sensitive relapse and two as resistant relapse. Two patients were non-responders (NR) to conventional treatment. The BM of all patients was involved by disease (median 10%; range 5-50%).

The patients underwent autografting after having received HDCY+G-CSF in order to collect PBPC. Cyclophosphamide (CTX) was administered at the dosage of 7 g/m^2 and was followed by granulocyte colony-stimulating factor (G-CSF) (5 µg/kg in continuous i.v. infusion) until the achievement of

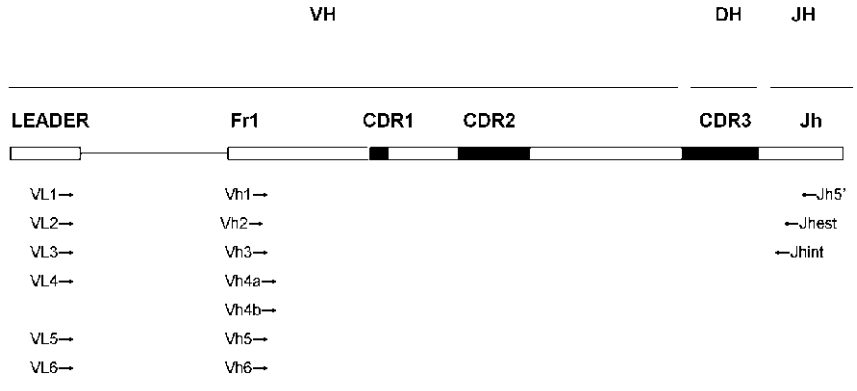

Figure 1. Schematic organization of the rearranged IgH gene. The position of the primers is indicated.

Table 1. Patients' characteristics. The histology is indicated according to the Working Formulation definitions.

UPN	Age	Sex	Histology (WF)	% BM infiltration	Apheresis (studied)	DNA source (BM)	Specific primer
1	54	M	H	20	9 (9)	fresh sample (refractory, no "rescue")	Vh3
2	27	M	F	10	5 (1)	fresh sample	Vh3,4a
3	36	M	H	10	4 (0)	archival slide	Vh4a,6
4	33	M	F	40	5 (5)	fresh sample	Vh3
5	37	M	F	10	9 (2)	archival slide	Vh3
6	45	F	F	10	6 (0)	archival slide (sensitive/relapse)	Vh1,3
7	41	F	F	10	2 (0)	bone biopsy	Vh4a
8	29	M	G	30	4 (1)	fresh sample (refractory)	Vh1,3
9	50	M	F	10	3 (2)	fresh sample	Vh1,4a
10	49	M	H	10	5 (1)	archival slide	Vh2,6
11	40	F	G	10	5 (0)	fresh sample	Vh3
12	54	M	F	10	5 (0)	fresh sample	Vh2,4a
13	43	M	F	5	6 (6)	archival slide	Vh6
14	43	F	F	20	7 (7)	fresh sample (resistant/relapse)	Vh3,5,6
15	40	M	H	5	4 (0)	fresh sample (sensitive/relapse)	Vh4b
16	19	M	J	5	4 (0)	fresh sample	Vh3
17	37	M	F	10	4 (2)	bone biopsy (sensitive/relapse)	Vh3
18	52	F	F	50	8 (8)	archival slide (resistant/relapse	Vh4a,4b
19	33	F	F	5	4 (0)	fresh sample	Vh4a,5
20	44	F	H	5	4 (0)	fresh sample (sensitive/relapse)	Vh2,4b

Table 2. Comparison of molecular results with clinical state.

UPN	Pre CTX		Post-CTX		Pre PBPC reinfusion		Post PBPC reinfusion		Last evaluation		Follow-up (months from ABMT)		DFS (months from ABMT)
	CS	MS	CS	MS	CS	MS	CS	MS	CS	MS	CS	MS	
1	res/r	+	NR	+	no rescue	+		died/sepsis		-			
2	PR	+	CR	+	CR	+	CR	+	alive/rel	+	68	12	16
3	PR	+	PR	+	PR	+	CR	+	died/NHL	+	55	27	29
4	PR	+	PR	+	PR	+	CR	+	alive/IICR	+	76	48	46
5	PR	+	PR	+	PR	+	CR	+	alive/IICR	+	76	5	16
6	sens/r	+	CR	+	CR	NA	CR	+	alive/IICR	+	61	18	23
7	PR	+	CR	NA	CR	NA	CR	+	died/NHL	+	35	12	25
8	res/r	+	PR	+	PR	+	CR	-	alive/CR	-	72	0	72
9	PR	+	CR	-	CR	+	CR	-	alive/CR	-	70	39	73+
10	PR	+	CR	+	CR	+	CR	-	alive/CR	-	59	25	59+
11	PR	+	CR	NA	CR	NA	CR	-	alive/CR	-	54	24	55+
12	sens/r	+	CR	+	CR	NA	CR	-	alive/CR	-	58	0	65+
13	PR	+	CR	-	CR	+	CR	-	alive/CR	-	67	36	68+
14	res/r	+	CR	-	CR	-	CR	-	died in CR	1	1	3	
15	sens/r	+	CR	+	CR	+	CR	-	alive/CR	-	59	0	60+
16	PR	+	CR	+	CR	+	CR	-	alive/CR	-	64	0	74+
17	sens/r	+	CR	NA	CR	NA	CR	-	alive/CR	-	69	24	71+
18	res/r	+	PR	+	PR	NA	CR	-	died/aplasia	-	5	3	5
19	PR	+	CR	NA	CR	NA	CR	-	alive/CR	-	79	0	79+
20	sens/r	+	CR	+	CR	NA	CR	-	died/AML	-	55	0	41

Abbreviations: CTX: cyclophosphamide, PBPC: peripheral blood progenitor cells; ABMT: autologous bone marrow transplantation; DFS: disease-free survival; CS: clinical status; MS: molecular status. PR: partial remission; CR: complete remission; NR:no response; NA: not available.

>1×10⁹/L WBC for 3 consecutive days. Hematopoietic precursors (CD34⁺ cells and CFU-GM) were collected and evaluated as previously reported.[36] One patient died of *Pseudomonas aeruginosa* sepsis during the aplastic phase following HDCY. Nineteen patients were offered high-dose therapy followed by PBPC autografting at a median of 2 months (range 2-4 months) after CTX. Eleven patients received melphalan (120 mg/kg) + total body irradiation (8 Gy in a single dose), and 8 patients received BCNU, etoposide, cytarabine and melphalan (BEAM regimen). Further clinical details are reported in Tables 1 and 2.

DNA sources

DNA was extracted from bone marrow samples before therapy (Table 1), at the end of conventional chemotherapy, after HDCY and every three months after high-dose therapy. When fresh bone marrow samples were not available, DNA was extracted either from unpreserved, unstained cover slips prepared during bone marrow aspirates, or from 10-µ slices of preserved paraffin-embedded bone biopsy.[37] Genomic DNA was prepared as previously described.[38] The integrity of extracted DNA was tested by PCR amplification of X-linked HUMARA gene.[39]

Synthesis of oligonucleotides and PCR reaction conditions

Oligonucleotides were synthesized and purchased from TIB MOLBIOL s.r.l. CBA, Genoa, Italy. All the rounds of PCR were hot started. Special precautions were taken to prevent cross contamination, as recommended by Kwok.[40] Furthermore, a control sample containing all reagents except DNA template was included in each amplification battery to detect contamination in any of the reagents or cross-contamination among tubes. Samples were amplified at least twice to ensure consistency and to prevent sporadic false-positive results.

Assessment of clonality

Clonal DNA amplification of the pre-CTX sample (i.e. tumor cell rich samples) was tested in different reactions containing each of the seven Vh specific primers.[41] Amplification was performed in a total volume of 20 µL containing 0.1-1 µg of genomic DNA, 200 mMol/L each of deoxynucleotidetriphosphate (dNTPs), 1.5 mMol/L $MgCl_2$, 150 µg/mL of bovine serum albumin (BSA), 0.5 U Taq DNA polymerase (DyNAzyme™ II, FINNZYMES OY, FIN), 1 mM of Vh and Jint amplimers[41,42] (Figure 1). Following an initial denaturation step at 95°C for 5 min, 25 cycles were performed (cycle conditions: denaturation step at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec). In the last cycle the extension step was increased to 5 min. Five microliters of each reaction were analyzed by gel electrophoresis in 2% high resolution agarose stained with ethidium bromide.

Clonal Jh rearrangement resulted in a distinct band within the expected size range of ~300 bp. This clonal band corresponds to the PCR product that we should sequence in order to construct clono-specific primers or probes.[25,26] These bands have their own electrophoretic mobility and represent the disease-specific marker. Vh specific primers which are able to amplify the clonal band were used for all follow-up samples from the same patient.

MRD analysis

MRD analysis was performed for each patient at the end of conventional chemotherapy, after HDCY and every three months after high-dose therapy. In our series only bone marrow samples were analyzed. We applied our three step SSCP-PCR (Figure 1), which is made up of the following steps:

First PCR round: VL specific oligonucleotides (sense) and JH 5' specific amplimer (antisense) chosen from 6 different V leader and JH consensus regions were used at a concentration of 1 mM (Figure 1).[43,44] The reaction buffer also contained: 200 mMol/L each of dNTPs, 1.5 mMol/L $MgCl_2$, 150 µg/mL bovine serum albumin (BSA), 0.5 U Taq DNA polymerase, 1 µg template DNA and 1× reaction buffer as supplied by the manufacturer in a final volume of 20 µL. After initial denaturation at 95°C for 5 min, 25 cycles of amplification were performed using an automated thermocycler (Omnigene, Hybaid, Teddington, Middlesex, UK). Each cycle consisted of denaturation at 95°C for 20 sec, annealing of amplimers at 50°C for 20 sec and extension at 72°C for 30 sec. In the last cycle the extension step was increased to 5 min.

Second PCR round: nested PCR was performed in a final volume of 20 µL containing 1 µL of first step product , 200 mMol/L of dNTPs, 1.5 mMol/L $MgCl_2$, 150 µg/mL of BSA, 1 mM of the corresponding Vh family specific and Jh est. consensus primers (Figure 1) , 0.5 U of Taq DNA polymerase. Following an initial denaturation step at 95°C for 5 min, 25 cycles were performed (cycle conditions: denaturation step at 95°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec). In the last cycle the extension step was increased to 5 min.

Third PCR round: an aliquot of 1 µL of the amplified product from the second step was reamplified in a PCR reaction solution containing 40 pmoles of

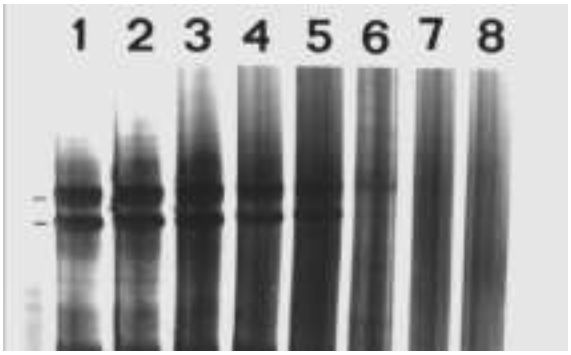

Figure 2. Third step amplification of dilution of clonal plasma cells into polyclonal cells. Lanes 1-8: serial dilutions from 1×10^{-1} down to 1×10^{-8}.

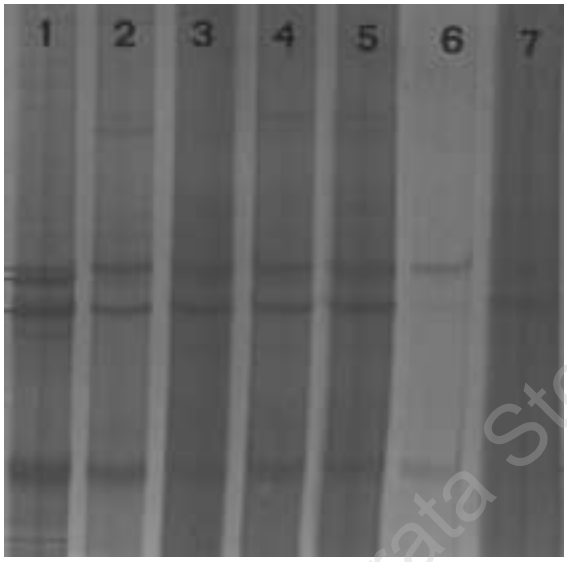

Figure 3. Third step amplification of the samples of patient #5: lane 1: preCTX phase; lane 2,3: apheresis; lane 4: post-CTX phase; lane 5: prePBPC reinfusion; lane 6: postPBPC reinfusion; lane 7: last evaluation 5 months after BMT. The clonal bands (arrows) are present in all the samples.

the Vh family specific primer and the most internal Jh primer (Figure 1), in 100 µL final volume including 1×PCR buffer supplied by manufacturer and 2 U of Taq DNA polymerase with proofreading activity (Vent$_R$ DNA Polymerase, New England BioLabs, USA). Samples were *hot start* amplified with initial denaturation at 95°C for 5 minutes followed by 25 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, extension at 72°C for 10 sec with a final single extension step at 72°C for 5 minutes.

Five microliters of the PCR product were mixed with 5 µL of the sequencing gel-loading buffer (98% deionized formamide, 10 mM EDTA [pH 8.0] 0.025% xylene cyanol FF and 0.025% bromophenol blue) and denatured at 95° C for 5 min. This mixture was loaded on non-denaturing 5-20% gradient acrylamide/bisacrylamide gel (49:1). Electrophoresis was performed on a horizontal system ($260\times220\times1$ mm) at 12 mA with constant cooling at 12° C for 15-16 hours and then stained by silver salts.

Each follow-up sample amplification was always electrophoresed in the presence of the corresponding diagnosis clonal product.

Sensitivity assessment

In order to estimate the sensitivity of the method serial dilutions of clonal and polyclonal cells were prepared. The plasma cells present in the pleural effusion of a patient affected by multiple myeloma were used as the clonal sample. One million clonal plasma cells were serially diluted in 10 million polyclonal BM mononuclear cells of healthy donors in proportions ranging from $1/10^1$ to $1/10^8$. The cell mixture was washed, pelletted and resuspended in 5 mL of phosphate-buffered saline. Genomic DNA extraction was performed according to the standard method. DNA was diluted in 250 µL of water; then 25 µL of each dilution sample were amplified using VL and Vh neoplasia-specific primers in a final volume of 50 µL. Two units of Taq polymerase were employed while the other reagents were used at the previously described concentrations. Five microliters of third step product were used for SSCP analysis. Dilutions down to $1/10^6$ were detectable after silver staining (Figure 2). Anyway, in MRD detection, we use 1 µg of template DNA,which represents 2×10^5 genomes so our sensitivity level is realistically 10^{-5}.

Results

Clinical results

All patients were evaluated for response after HDCY. One patient died of sepsis during neutropenia following HDCY. A complete re-staging, including CT scan and two posterior iliac crest biopsies, was performed for all patients before PBPC transplant. This showed that 14 out of 19 patients achieved CR before HDT. Patient #3 was considered a partial responder because bone marrow infiltration disappeared but lymphoadenomegaly persisted. Both physical examination and bone biopsy showed residual disease in the other 4 patients. Nineteen patients underwent HDT and PBPC autografting. All these patients achieved CR. Three patients died in CR post-HDT of lung fibrosis (at 3

months), sepsis (at 5 months) and secondary AML (at 41 months). Six patients relapsed after a median time of 24.5 months (range 16-46). All the other patients are alive and disease free, with a median follow-up of 70 months (range 55-79).

Molecular results

In all the cases a marrow tumor-rich sample was available (12 fresh marrow samples, 6 marrow smear slides, 2 paraffin-embedded bone biopsies) (Table 1).

We were able to identify the neoplastic monoclonal rearrangement in all cases.

In 8 patients we found only one monoclonal rearrangement, in 11 two different clones. One patient had three different clones (the characteristics of the diseases are shown in Table 1). Vh3 was the most represented Vh family specific primer (10 out of 20 cases); Vh4a was present in 7 cases; Vh6 in 4; Vh1 in 3; Vh2 in 3; Vh4b in 3; Vh5 in 2. The MRD study was performed separately for each clone in the various phases of therapy.

After SSCP migration each monoclonal band usually resolves into two distinct bands, due to the denaturation of the two strands. Amplification of the Jh gene from a polyclonal lymphoid population does not show a preferential PCR product.

Nineteen out of twenty patients completed the whole therapeutic program. One patient experienced disease progression after PBPC collection.

All the analyzed apheresis samples were positive at the molecular analysis.

The clinical and molecular responses after CTX therapy (16 patients studied) are shown in Table 2. In 3 out of 16 patients studied at the molecular level (patients #9, 13, 14) monoclonal Jh rearrangement was not detected. Only patient #14 mantained PCR negativity at the analysis performed before PBPC reinfusion. Patients #9 and #13 became PCR positive at the analysis before transplantatrion. This molecular behavior is perhaps related to the impossibility of our method to detect clonal cells because of low DNA concentration in the studied samples. In patient #14 the PCR result truly represents a reduction of neoplastic burden under the sensitivity threshold of our technique. Nevertheless patients #9, 13, 14 had a sustained clinical and molecular complete response after PBPC reinfusion (patient #14 died of pulmonary fibrosis). In six out of 19 patients reaching CR after BMT the monoclonal band was still detectable (Figure 3). In the others it disappeared. As shown in Table 2, the patients still positive at the molecular analysis eventually relapsed. When relapse occurred, the clonal bands identified at diagnosis and in the apheresis samples were always detected. In our study we did not observe any subclonal evolution which would have indicated a clonal variation. In three cases, samples were studied with both the SSCP method and the ASO-PCR method.[25] Comparable results were obtained.

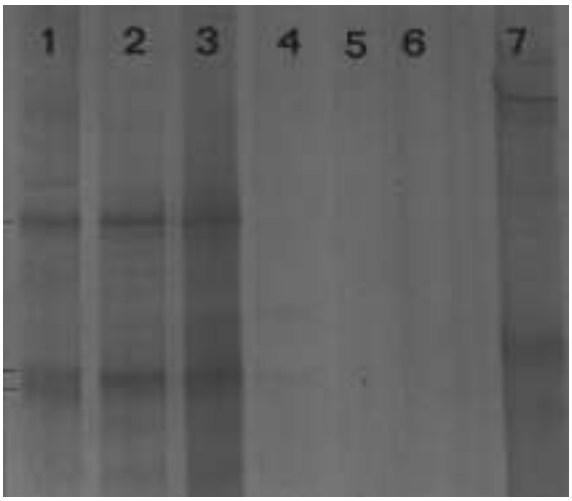

Figure 4. Third step amplification of the samples of the samples of patient #17: lane 1: preCTX phase; lane 2,3: apheresis; lane 4: post-PBPC reinfusion; lane 5,6,7: follow-up phase.

Molecular-negative patients after BMT maintained CR. The type of conditioning regimen (melphalan plus TBI or BEAM) did not affect clinical or molecular results.

Biological samples for long-term molecular follow-up (median 24 months/ range 1-39 months) were available in only 7 cases. No modifications of the molecular status reached after PBPC reinfusion were recorded (Figure 4).

Discussion

The present study concerns MRD analysis in patients with aggressive B-NHL and bone marrow involvement undergoing PBPC transplantation. The main purpose of the study was to correlate the molecular data with the clinical follow-up. In this poor-risk subset of patients, high-dose therapy may result in a high percentage of CR,[33,34] but some patients relapse a variable time after the therapy. It is not clear whether relapse is caused by chemorefractory neoplastic cells persisting in the patient after HDT or by the reinfusion of clonal cells with the apheresis product.[20-22]

Our study showed that malignant B-lymphoid cells were detectable in all the PBPC collections

studied. Nevertheless this did not prevent HDT inducing durable complete clinical and molecular responses.

Our data disagree with previous reports as far as concerns the poor prognostic value of the persistence of clonal cells in the apheresis collections.[9,16,17,27-29,45] Gribben[16,17,27-29] and Corradini[45] performed MRD analysis of low-grade NHLs using either bcl2 gene rearrangement or VDJ clonal rearrangement. Sharp *et al.*[46] employed low sensitivity methods to study the prognostic value of minimal tumor in the marrow harvest or in the apheresis product of patients with high-grade NHL receiving HDT. He found that patients receiving tumor negative harvests had a significantly better outcome than those who did not. It must be outlined that in Sharp's report the positivity of molecular analysis reflected a heavy neoplastic contamination of harvest product and perhaps a large amount of residual neoplastic cells in the patient. We studied advanced stage/refractory intermediate-high grade NHLs with a high sensitivity method detecting JH clonal rearrangements. Whereas the detection of clonal cells in the apheresis did not predict an unfavorable outcome, the disappearance of the clonal rearranged band in the BM sample after HDT proved to be a favorable prognostic factor and was associated with long-lasting disease-free status. The dilution of clonal cells contaminating the apheresis might explain the PCR negativity after PBPC reinfusion, as all available molecular methods fail to detect residual clonal cells at a concentration below 1×10^{-6}. Furthermore when the tumor burden is significantly reduced by therapy (including HDCY), immunosurveillance mechanisms may regain activity. For this reason the survival of reinfused clonal cells, whenever clonogenic, might not be able to cause disease relapse. However, our method is only qualitative and can neither detect clonal cells below certain levels (1×10^{-6}) nor define their exact number in the apheresis product and in successive biological samples after PBPC reinfusion or during follow-up. For this reason we are convinced that only high sensitivity molecular analysis performed after HDT has prognostic relevance, since the number of clonal cells after HDT (both reinfused with the apheresis product and persisting after chemotherapy) seems crucial for the outcome of patients. Novel quantitative and more sensitive methods might increase the prognostic significance of molecular analysis.

In conclusion, with the limitation of the low number of the studied cases, we can outline some points:

a) Our method allows the analysis of MRD in patients with intermediate and high-grade NHL undergoing HDT. It is more easily performed and cheaper than techniques based on sequencing of specific IgH gene rearrangements and might be proposed as the standard method for MRD analysis;

b) In our experience the detection of a clonal Jh rearranged band in the apheretic harvest does not indicate bad prognosis and does not predict molecular or clinical relapse;

c) The persistence of a clonal rearranged band in patients reaching CR after HDT and PBPC reinfusion may predict disease recurrence.

However our results need to be confirmed on larger series of patients with high-risk aggressive NHLs with bone marrow infiltration receiving HDT.

Contributions and Acknowledgments

MM and GS designed the study and wrote the manuscript. MM, RG, DP and GP were responsible for the molecular analyses. SN and GS were involved in the patients'care. MC, IP, RV, FB and LC contributed critically to the drafting of the article. MG gave his final approval to the work.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Anne Kessinger, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Kessinger and the Editors. Manuscript received March 9, 2001; accepted June 6, 2001.

Potential implications for clinical practice

Highly sensitive methods are required for the analysis of MDR after HDT in NHL (see also recent papers appeared in this journal on high-dose therapy with peripheral blood progenitor cell rescue for non-Hodgkin's lymhoma.[47-50]

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