Molecular monitoring of minimal residual disease in patients in long-term complete remission after allogeneic stem cell transplantation for multiple myeloma

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In the present study, we used a polymerase chain reaction–based (PCR-based) strategy to retrospectively analyze the presence of residual myeloma cells in serial posttransplant bone marrow samples obtained from 13 patients in remission after allogeneic hemopoietic stem cell transplantation (allo SCT). For this purpose, patient-specific primers were generated from complementarity determining regions 2 and 3 of the rearranged IgH gene. The level of sensitivity of the PCR-based assay ranged from 1 in 10^5 to 1 in 10^6 normal marrow cells. Following transplantation, 9 of 12 patients who attained stringently defined complete remission (CR) remained persistently PCR^- for a median of 36 months, and 4 of the patients remained PCR^- up to the latest analysis, which was performed at 48, 72, 72, and 120 months, respectively, after allo SCT. None of the patients in the PCR^- subgroup experienced a disease relapse, and only 1 of 4 PCR^+ patients experienced a relapse. It is concluded that allo SCT has the potential ability to induce sustained serological and molecular CR in selected patients with multiple myeloma. (Blood. 2000;96:355-357)

Introduction

During the last decade, pilot reports by our group[1] and other groups[2] demonstrating the efficacy of allogeneic bone marrow transplantation (BMT) for selected patients with multiple myeloma (MM) has progressively created interest in the use of this treatment strategy.[3] In a previously reported case-matched analysis comparing autologous and allogeneic BMT,[4] the latter did not result in a consistent improvement in the complete remission (CR) rate and produced fatal complications in approximately 40% of the patients. Moreover, the risk of relapse, even for those patients who attained CR, was unfortunately high,[5,6] a finding which raises the possibility that the myeloma clone cannot be eradicated.

To address this issue, analysis of minimal residual disease (MRD) below the detection limit of methods conventionally employed to define CR may be of clinical relevance.[7] In the present study, we used a polymerase chain reaction–based (PCR-based) strategy that employed patient-specific primers designed from tumor complementarity determining regions (CDRs) 2 and 3 to retrospectively analyze MRD in serial bone marrow samples. The samples were obtained from 13 MM patients who had been in remission after allogeneic hemopoietic stem cell transplantation (allo SCT). Of these patients, 5 survived 82 to more than 180 months (median, 84 months) after transplantation.

Study design

Patient and treatment characteristics

The patients included in the present study were part of a larger series of 68 MM patients who received allo SCT from HLA-identical sibling donors at the Institute of Hematology and Medical Oncology ''Seràgnoli,'' University of Bologna, Bologna, Italy. Of these patients, 26 (38% of the total population) attained remission following transplantation, as assessed according to the criteria listed below, and 13 patients could be retrospectively evaluated for the presence of MRD by the PCR-based assay. Their clinical and treatment characteristics are summarized in Table 1.

Remission criteria

Criteria for remission following allo SCT included a decrease in bone marrow plasma cell infiltration to less than 3% on bone marrow aspirate, and the disappearance of serum and/or urine monoclonal (M) protein by standard agarose gel electrophoresis. Patients were considered to be in CR if they did not have a detectable M component by immunofixation analysis.

PCR amplification and sequencing of the myeloma VDJ

Bone marrow samples for the detection and sequencing of clonal immunoglobulin heavy chain (IgH) gene–CDRs (IgH-CDRs) 2 and 3 were available from the time of diagnosis in 10 patients and before allo SCT in the remaining 3 patients. The degree of bone marrow plasma cell infiltration ranged between 10% and 30%. Rearrangement amplification of the VDJ region was performed with a panel of VH family-specific primers together with a JH consensus primer, as previously described.[8,9] Tumor-specific primers were designed from CDRs 2 and 3 of the VDJ region.[10]

Molecular monitoring of MRD

Follow-up studies for the detection of MRD were performed on bone marrow specimens taken every 6 months during the first year after allo SCT and every year thereafter. Clonal myeloma cells were detected by amplifying 1 μg DNA, using the internal patient-specific primers designed from CDR 2 (sense) and CDR 3 (antisense). After 2 rounds of amplification, 15

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Table 1. Patient and treatment characteristics

UPN	Age/sex	Status at allo SCT	β2M (mg/L)	Conditioning	GVHD prophylaxis	Source of SC	GVHD		Time to CR, mo	Survival, mo	Latest disease status
							Acute	Chronic			
15	35/F	Resp	1.7	TBI + Cy + MEL	CsA	BM	1	limited	12	188+	CR
95	49/F	Resp	1.9	BU-Cy 4	T depletion	BM	0	none	3	98+	CR
116	37/F	Resp	2.1	BU-Cy 4	CsA + MTX	BM	1	limited	3	84+	CR
142	36/F	Resp	1.4	BU-Cy 4	CsA + MTX	BM	0	none	—	84+	Stable
168	46/F	Resp	1.5	BU-Cy 4	CsA + MTX	BM	0	none	6	82+	CR
215	53/M	Refr	3.4	TBI + Cy + MEL	T depletion	BM	0	none	5	37	Relapse
231	30/F	Resp	1.3	TBI + Cy + MEL	T depletion	BM	0	none	3	46+	CR
239	47/F	Resp	1.6	TBI + Cy + MEL	CsA + MTX	PB	0	limited	2	45+	CR
254	47/M	Refr	3.1	TBI + Cy + MEL	CsA + MTX	PB	0	limited	3	36+	CR
260	43/F	Resp	1.1	TBI + Cy + MEL	CsA + MTX	PB	1	none	Already in CR	36+	CR
275	47/M	Refr	2.9	TBI + Cy + MEL	CsA + MTX	PB	0	extensive	6	30+	CR
304	33/F	Resp	1.2	TBI + Cy + MEL	CsA + MTX	BM	0	none	Already in CR	18+	CR
306	54/F	Resp	1.4	TBI + Cy + MEL	CsA + MTX	PB	2	extensive	2	12	CR

SC indicates stem cells; Resp, responsive; Refr, refractory; TBI, total body irradiation; Cy, cyclophosphamiole; MEL, high-dose melphalan; BU, busulfan; CsA, cyclosporine A; MTX, methotrexate; BM, bone marrow; PB, peripheral blood.

μL PCR product was analyzed by 3% agarose gel. A rearranged band of approximately 150–base pair (150-bp) was obtained from each patient. The sensitivity level of the PCR-based assay ranged from 1 in 10^5 to 1 in 10^6 normal marrow cells. Sensitivity experiments were performed by diluting patient tumor cells with normal bone marrow cells (data not shown).

Results and discussion

After allo SCT, stringently defined CR was documented in 12 patients (Table 1). At the censoring date of September 1999, the median follow-up time for all patients was 45 months, and the longest follow-up extended to 188 months after transplantation. In September 1999, 11 patients were alive; 10 patients were in continued CR, and 1 patient had a stable residual M component. Two patients died: one had chronic graft-versus-host disease (GVHD) while in CR, and the other experienced cholangiocarcinoma while in relapse.

Detailed analysis of transplantation outcome in the subgroup of 5 patients who survived more than 6 years revealed that 4 of the patients remained in CR up to the latest follow-up at 82, 84, 98, and 188 months, respectively, from transplantation (Table 1). No M component was detected at routine electrophoresis in the fifth patient, however, serial immunofixation analyses performed at time-points ranging from 6-84 months after transplantation showed the persistence of a small, albeit stable, M protein of the same isotype that was detected at diagnosis.

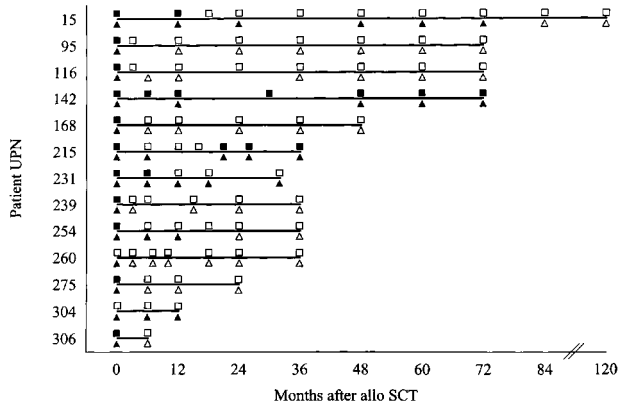

Figure 1. Results of PCR analysis for MRD detection on serial bone marrow samples taken before and after allo SCT. The figure represents positive (■) and negative (□) results of immunofixation analysis and positive (▲) and negative (△) results of the PCR-based assay.

The results of MRD molecular monitoring are summarized in Figure 1. All patients were PCR^+ before allo SCT. Of 12 patients who attained stringently defined CR, 9 were PCR^- at a median of 6 months after transplantation. Of these 9 patients, 4 received bone marrow SCT, and 5 patients received peripheral blood SCT. Notably, in 1 patient after transplantation, persistence of a clonal product was demonstrated in every sample analyzed up to 72 months, after which time PCR results converted from positive to negative. All PCR^- patients were serially monitored for MRD detection for a median of 36 months, and they always tested PCR^-. In particular, in 7 of the patients, there were no residual myeloma cells detected by PCR up to 36, 36, 36, 48, 72, 72, and 120 months, respectively, after allo SCT. In contrast, in the remaining 4 patients, a clonal product was demonstrated in every sample that was analyzed up to 12, 32, 36, and 72 months, respectively, after transplantation. Three of these 4 patients were in CR, and the other did not meet the stringent CR criteria. One patient had clinical signs of relapse 20 months after allo SCT.

Results from this study add important information to the poorly defined issue of MRD detection after allo SCT for MM.[11,12] Using a sensitivity ranging from 1 in 10^5 to 1 in 10^6 normal marrow cells, we demonstrated that MRD could no longer be detected in 69% of patients who were evaluated. Obviously, these patients could still continue to harbor residual myeloma cells below the detection limit of the PCR strategy. However, if this were the case, PCR positivity and/or M protein should have appeared with time. Theoretically, 2 other factors could lead to false-negative PCR results. First, residual myeloma cells could be distributed unevenly in a patient's bone marrow,[13] in which case they might not be detected in a single marrow aspirate. However, the finding of persistent PCR negativity on serial posttransplantation bone marrow samples argues against this possibility. Second, the PCR strategy could fail to detect MRD because there were multiple clones at presentation[14] or because a modified IgH gene rearrangement emerged during the course of the disease.[15] This event, generally termed as clonal evolution, has been well documented in acute lymphoblastic leukemia but not in MM.[16]

Persistent PCR^- bone marrow samples from MM patients in long-term CR is reminiscent of data previously reported in other malignancies.[17-21] In these instances, analysis of MRD by PCR was

found to closely correlate with posttransplantation clinical outcome and to provide important information for patient management. Whether this also holds true in MM remains, as yet, unanswered. Larger molecular monitoring studies and, importantly, quantitative measures of residual tumor cells are required to further define the real value of PCR-based strategies. Results of these studies must also be compared with conventional methods of analysis to clarify the prognostic relevance of MRD detection in MM. Recently, the existence of a graft-versus-myeloma effect was formally demonstrated by several groups,[22] who reported favorable responses to donor lymphocyte infusion in patients who experienced a relapse after transplantation. This finding suggests that in MM, as in other diseases, immunological mechanisms mediated by donor leukocytes may operate in vivo and, ultimately, concur to control[23] and eventually eradicate the tumor clone. Obviously, the sample size of the presently described series is too small to allow any indirect measure of the graft-versus-myeloma effect by correlating posttransplantation outcome with acute and chronic GVHD. Studies involving larger numbers of allografted patients evaluated for the presence of the clonal IgH gene rearrangement may help clarify this clinically relevant issue in the near future.

In conclusion, the data herein reported demonstrate that allo SCT induces sustained serological and molecular remission in selected patients with MM. Although a longer follow-up is required to determine if these patients are truly cured, it is unusual for relapses to occur more than 5 years after allo BMT.[7,8,24] This finding deserves consideration and should be discussed with each patient under 50 years of age for whom a matched related and perhaps unrelated donor is available. In the meantime, efforts to reduce transplant-related mortality should continue and should focus on more careful patient selection, earlier timing of transplantation, improved supportive care, and investigational use of peripheral blood hemopoietic stem cells in an attempt to lower the risk of fatal infectious complications.[25]

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