Beneficial effect of high-dose chemotherapy in multiple myeloma patients with unfavorable prognostic features

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Introducing

In multiple myeloma (MM), there has been a lack of progress with standard-dose chemotherapy (SDT) since the introduction of melphalan/prednisone ~30 years ago. After SDT, objective remissions can be achieved in 50–60% of patients, with complete remission in <5% of patients and a median survival of ~3 years. Owing to this limited efficacy, extensive trials of chemotherapy combinations have been performed, but this approach has not led to major improvements in clinical outcome of MM patients [1]. Following the demonstration of a relationship between dose intensity of cytotoxic drugs and tumor response in various hematologic malignancies, high-dose melphalan has been evaluated in MM, and encouraging results were reported in patients with relapsed/refractory MM [2–5]. In the prospective, randomized trial by Attal et al. [6], the superiority of high-dose melphalan with autologous stem cell transplantation over SDT has been demonstrated. According to recent results, tandem autologous transplantation may further improve treatment results [7, 8], but as of today, long-term disease-free survival occurs only in a small fraction of MM patients after autologous transplantation.

Recent studies indicate that chromosomal abnormalities, especially partial or complete deletions of chromosome 13q (del(13q)), provide important prognostic information for patients with MM [9–17]. It was shown that the combined assessment of chromosome 13q and serum β2-microglobulin (β2M) levels at diagnosis provides a powerful prognostic model discriminating groups of MM patients with significantly different survival, and this was true for both SDT [18] and high-dose therapy (HDT) [15]. However, little information is available with respect to the question of which risk-group of MM patients may experience a particular benefit of HDT as compared with SDT [19].

To address this issue, we evaluated the outcome of 77 MM patients enrolled in HDT regimens, according to chromosome 13q status as determined by interphase fluorescence in situ hybridization (FISH) and β2M. Patients were assigned to a standard-risk group (disomic chromosome 13q14 and β2M ≤4 mg/l) or a high-risk group (del(13q14) and/or β2M >4 mg/l). Outcome of HDT patients was compared with that of similar patients (age <65 years) who would be eligible for HDT but were treated by SDT.

Key words: β2-microglobulin, chromosome 13, high-dose chemotherapy, multiple myeloma, prognostic factors, standard-dose chemotherapy

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Patients and methods

Patients

Patients treated with HDT. This analysis included all patients with newly diagnosed MM who were treated by up-front HDT and from whom a diagnostic bone marrow aspirate adequate for FISH analysis was available. There were 77 patients with a median age of 51.8 years (range 30.3–65) at diagnosis; 42 patients were male (54.6%) and 35 were female (45.4%). Distributions according to the Durie and Salmon staging system were as follows: three patients (3.9%) were at stage I; 13 patients (16.9%) were at stage II; and 61 patients (79.2%) were at stage III. The paraprotein was IgG in 47 patients (61.0%), IgA in 15 patients (19.5%) and any other subtype in 15 patients (19.5%). The induction regimen was VAD (vincristine–doxorubicin–dexamethasone) in all patients, followed by high-dose cyclophosphamide and granulocyte-colony stimulating factor (G-CSF) for stem cell mobilization, except nine patients who received IEV (ifosfamide–epirubicin–etoposide) and G-CSF for stem cell mobilization. HDT consisted of one course of high-dose melphalan in 42 patients (melphalan 200 mg/m² in 23 patients, melphalan 140 mg/m² plus total-body irradiation in 15 patients), two courses of melphalan 200 mg/m² in 16 patients, and three courses of melphalan 100 mg/m² in nine patients. There were six patients who received allogeneic bone marrow transplantation, and four patients who were treated by one course of melphalan 200 mg/m² followed by an allogeneic transplantation with a non-myeloablative conditioning regimen. Among patients receiving an allogeneic transplant, no treatment-related death was noted. Time between diagnosis and first administration of HDT was <1 year in all patients.

Patients treated with SDT. This group consisted of 64 newly diagnosed patients with a median age of 55.3 years (range 34–64.9). These patients would be candidates for HDT, but were treated with SDT, mainly because they received treatment prior to routine administration of HDT as up-front therapy. Some patients elected to receive SDT rather than HDT. Twenty-nine patients were male (45.3%) and 35 (54.7%) were female. Seven patients (10.9%) were at stage I, 12 patients (18.8%) were at stage II and 45 patients (70.3%) were at stage III. The paraprotein was IgG in 42 patients (65.6%), IgA in 17 patients (26.6%) and any other subtype in five patients (7.8%). In 55 patients, treatment consisted of an alkylating agent-containing regimen (either melphalan 100 mg/m² plus total-body irradiation in 19 patients), two courses of melphalan 200 mg/m² in 16 patients, and three courses of melphalan 100 mg/m² in nine patients. There were six patients who received allogeneic bone marrow transplantation, and four patients who were treated by one course of melphalan 200 mg/m² followed by an allogeneic transplantation with a non-myeloablative conditioning regimen. Among patients receiving an allogeneic transplant, no treatment-related death was noted. Time between diagnosis and first administration of HDT was <1 year in all patients.

Interphase FISH studies

Bone marrow cells, which were obtained during the diagnostic work-up, were used for FISH analyses. To specifically study bone marrow plasma cells, we applied a triple-staining method for the simultaneous detection of cytoplasmatic immunoglobulins with a goat anti-human lambda light chain antibody conjugated with 7-aminocoumarin-3-acetic acid (AMCA; Vector Laboratories, Inc., Burlingame, CA, USA) and interphase FISH signals, as previously described [20]. To determine the status of chromosome 13q, we used a locus-specific DNA probe for 13q14 (RB-1) labeled with Spectrum-orange. The RB-1 probe was simultaneously hybridized with a reference probe (α-satellite probes for the pericentromeric regions of chromosomes 7 or 11) conjugated with Spectrum-green. Probes were purchased from Vysis (Downers Grove, IL, USA). Hybridizations were performed according to our previously published protocol [12, 21]. Plasma cells on slides were scored, and 200 cells were evaluated by fluorescence microscopy using an Axioplan-2 immuno-fluorescence microscope (Zeiss, Jena, Germany), equipped with appropriate filters to visualize green, red and blue immunofluorescence, either separately or simultaneously.

Statistical analysis

To estimate the significance of differences between patient groups, the proportion of patients were compared using Fisher’s exact test and z-test for independent samples. Progression-free survival (PFS) and overall survival (OS) curves were calculated from the time of initiation of chemotherapy and were plotted according to the Kaplan–Meier method. Comparisons were made by means of the Mantel–Cox test.

Results

MM features and treatment results of HDT and SDT patients

In this retrospective analysis, we compared 77 patients receiving HDT with 64 patients under the age of 65 years who had previously been treated with SDT. As shown in Table 1, patients treated with HDT had a lower age than the conventionally treated group (mean ± SD, 51.8 ± 8.4 years versus 55.3 ± 7.0 years; P = 0.01), but major MM-associated parameters were not significantly different between both patient populations. Most notably, both β2M and del(13q14), which were used as prognostic parameters for risk assessment, were similar in both groups of patients.

The comparison of the treatment outcome of both patient populations revealed that patients receiving HDT had a higher rate of complete remissions (28% versus 2%; P <0.0001) and experienced longer PFS (median 30.2 versus 21.2 months; P = 0.01) and OS (median 54.9 versus 49.4 months; P = 0.048) than patients treated with SDT. In the HDT group, there were 29 patients receiving more than one course of high-dose chemotherapy; PFS and OS times of these patients was not significantly different from those patients who were treated with only a single course of HDT (data not shown).

Survival according to chromosome 13q14 status

A del(13q14) by FISH was observed in 58 patients of the entire group of 141 previously untreated MM patients (41.1%): in 31 of the 77 patients of the HDT group (40.3%) and in 27 of the 64 patients of the SDT group (42.4%; P = 0.81). MM patients with a del(13q14) experienced shortened survival: among HDT patients, PFS and OS times were 25.5 and 34.2 months, respectively, for 13q14-deleted patients; median PFS and OS of MM patients with a disomic chromosome 13q14 was 35.6 months and has not yet been reached up to now (P = 0.06 and 0.004, respectively). Similarly, in patients belonging to the SDT group, presence of a del(13q14) was associated with shorter PFS and OS times compared with SDT patients with disomic chromosome 13q14 (median PFS 10.9 versus 27.9 months, P = 0.03; median OS 24.6 versus 74.8 months, P = 0.001). These results are in agreement with previous FISH studies [11, 12, 14–16] and confirm the negative impact of del(13q14) on survival, in both HDT- and SDT-treated MM patients.
Comparison of HDT with SDT in patients with del(13q14) indicated that PFS was significantly longer in the HDT-treated group \((P = 0.02; \text{Table 2})\). OS of patients with del(13q14) was also longer in the HDT group than in the SDT group, but the difference was not statistically significant \((P = 0.19)\). The comparison of HDT with SDT in patients with normal chromosome 13q14 suggested a trend towards longer PFS in the HDT group \((P = 0.09)\), but again no statistically significant difference with respect to OS \((P = 0.22)\) (Table 2).

### HDT versus SDT in high-risk and standard-risk patients

For a more precise discrimination of prognostic groups, chromosome 13q14 status and serum levels of \(\beta_2\)M were used to characterize MM patients at standard risk (normal chromosome 13q14 and \(\beta_2\)M \(\leq\) 4 mg/l) and at high risk [del(13q14) and/or \(\beta_2\)M \(>4\) mg/l]. Analysis of the entire population of 141 MM patients under the...
age of 65 years revealed a median PFS of 22.5 months and median OS of 34 months among patients of the high-risk group; in the standard-risk group, median PFS was 39.7 months ($P = 0.0008$) and median OS was 77.4 months ($P < 0.00001$) (Figure 1).

When patients in the high-risk group were compared according to treatment, median PFS was 26.4 months and OS was 40 months for the HDT group in comparison to a PFS of 10.7 months ($P = 0.004$) and OS of 23 months ($P = 0.05$) for the SDT group (Table 2; Figure 2A and B). The analysis of standard-risk patients according to treatment revealed that the median PFS was 38.9 months and OS has not yet been reached in the HDT group, in comparison with a median PFS of 39.3 months ($P = 0.2$) and a median OS of 75.5 months ($P = 0.34$) in the SDT group (Table 2; Figure 2C and D).

**Discussion**

There is now a growing body of evidence that HDT with autologous stem cell transplantation can be considered as the standard of care for MM patients under the age of 65 years. Both randomized trials [6, 22] and comparisons with historical controls [23–25] have shown that HDT is superior to SDT with regard to achievement of complete remissions and prolongation of PFS and OS. Although other trials [26–28] have up to now failed to demonstrate a statistically significant survival benefit for HDT-treated patients compared with SDT, this may be related at least in part to the administration of HDT as salvage strategy after SDT induction treatment. However, limited information is available concerning the comparison of SDT and HDT in different prognostic groups of MM patients.

The aim of our present investigation was therefore to address the question of whether or not use of prognostic factors could contribute to the identification of a patient population with a particular benefit of HDT. In this analysis, we decided to focus on two major independent prognostic indicators, chromosome 13q and $\beta_2$M, based upon the experience of previous studies [15, 18]. The outcome of the patient populations reported here is in agreement with clinical results of published series. HDT and historical SDT patients were not significantly different with respect to standard laboratory and clinical MM parameters, with the exception of age. However, there is no reason to assume that the slightly younger median age of HDT patients (51.8 years as opposed to 55.3 years for the SDT patients) could explain their superior clinical course compared with the SDT group. As shown in Figure 1, MM patients of the present series could be clearly distinguished according to the prognostic factors chromosome 13q14 and $\beta_2$M leading to the definition of standard-risk and high-risk groups.

Treatment of MM patients with poor prognostic indicators remains difficult. Administration of HDT, even as tandem autologous transplantation, failed to result in durable PFS and OS, as soon as a del(13q14) was present [9, 13–15]. Also, in our present analysis there were only limited survival times of patients assigned to the high-risk group [del(13q14) and/or $\beta_2$M >4 mg/l]. However, despite the overall short survival of high-risk patients, comparison of HDT with SDT in this high-risk group revealed significant differences: compared with SDT, HDT led to a significant improvement of both median PFS (26.4 versus 10.7 months) and median OS (40 versus 23 months). From randomized trials evaluating HDT and SDT, information about outcome according to prognostic factors is only available from the preliminary report of the Medical Research Council Myeloma VII trial [22]. In this analysis, HDT was superior to SDT, and there was a trend towards greater survival benefit in the group of patients with poor prognosis defined by high $\beta_2$M.

MM patients of the standard-risk group [absence of a del(13q14), $\beta_2$M ≤4 mg/l] had favorable PFS and OS times. This was true for patients not only after HDT, but also after SDT. It is important to note that none of the SDT patients received HDT as salvage treatment, and that a comparable fraction of SDT and HDT patients received thalidomide as salvage treatment; thus, the favorable outcome of standard-risk SDT patients could indeed be attributed to chemotherapy at conventional doses. The results of our SDT patient group are comparable to those previously reported by the PETHEMA group, showing that MM patients at an age of ≤65 years and with responsive disease to SDT may experience a survival duration similar to that reported in HDT trials [29]. MM patients with del(13q14) are less likely to respond to SDT [12, 16]; thus, it can be assumed that in this Spanish analysis [29] patients with chemosensitive MM may largely correspond to a
patient population with absence of adverse prognostic features. On the other hand, these results should not lead to the conclusion that SDT and HDT are equally effective in standard-risk patients. It needs to be considered that the documentation of a potential survival difference between SDT and HDT in younger MM patients with favorable prognostic indicators would certainly require larger patient numbers than those available in our present analysis. In addition, the duration of the observation period of such patient populations could be an important issue. For example, in the IFM 90 randomized trial [6], survival curves of SDT and HDT patients were almost superimposable during the first 3 years, and only thereafter did a survival benefit of the HDT group become apparent. Further intensification of HDT by administration of tandem autologous transplantation can result in PFS beyond 7 years in a fraction of MM patients with favorable prognostic factors [13, 30]. In contrast, this may only occasionally be observed in MM patients after SDT.

In conclusion, although adverse prognostic indicators are associated with shortened survival in MM patients undergoing HDT, intensified treatment is nevertheless able to improve the outcome of such patients as compared with SDT. Thus, in order to further improve the outcome of patients with high-risk MM, it would be worth exploring the role of tandem transplantation, either as tandem autologous transplantation [8] or as sequential autologous/allogeneic transplantation with non-myeloablative conditioning [31]. Incorporation of novel agents (thalidomide and its analogs, proteasome inhibitors) may be an additional strategy to improve the still grim prognosis of MM patients with high-risk disease.

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References