Evolving indications for hematopoietic stem cell transplantation in multiple myeloma and other plasma cell disorders

EVOLVING INDICATIONS FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA AND OTHER PLASMA CELL DISORDERS

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BACKGROUND

Multiple myeloma is a differentiated clonal B-cell tumor, consisting in the early stages of the disease of slowly proliferating malignant plasma cells (myeloma cells). It is the second most common hematologic malignancy after non-Hodgkin's lymphoma. Normal plasma cells are very hardy cells and usually the only type of cell to survive the effects of myelosuppressive chemotherapy and radiation. The plasma cells have abnormal cytogenetics even at the stage of monoclonal gammopathy of undetermined significance, as evidenced by fluorescence in-situ hybridization (FISH) or cIg/DNA content (1-3). Before it transforms to an aggressive disease, which is typically associated with extramedullary disease, immature morphology of the myeloma cells, rapid proliferation and increase in LDH, the disease is entirely bone marrow stroma-dependent and, therefore, contained within the active hematopoietic bone marrow, although breakout lesions from the bone can be seen. The myeloma cell displays on its membrane a multitude of receptors, the ligands of which are present in the micro-environment. Such receptors are the IL-6 receptor, the IL-15 receptor, the IGF (insulin-like growth factor) receptor, CD38 and Notch (4-7). Binding of these receptors by their ligands results in the activation of four major pathways : STAT-3, RAS, Akt and NF-kB, which promote growth and survival of the myeloma cells and also result in the secretion by the plasma cells of angiogenic factors (8-11) . There is a tremendous redundancy in this system so that blocking one of these pathways will have little effect on the survival and growth of myeloma cells. This is in contrast to chronic myeloid leukemia where blocking of a single

pathway (BCR-ABL) will have a major effect on the disease. In addition to supporting growth and survival, the micro-environment also places most of myeloma cells in a deep G1 phase by up-regulation of p21 and p27. This is accomplished by binding of fibronectin and V-CAM present in the micro-environment to VLA-4 (CD49d) expressed on the membrane of myeloma cells, as well as by Jagged-1 induced Notch signaling (12,13). Cells in the G1 phase of the cell cycle are very poor targets for conventional dose chemotherapy.

It is very likely that in myeloma just as many other cancers, a cancer stem cell population exists. It is estimated that one in 10,000 to one in 20,000 malignant cells is a cancer stem cell. Based on the extensive somatic mutations in the complementarity regions of the gene coding for the heavy chain, it is very likely that this cancer stem cell arises from a B-cell that has had extensive exposure to antigen in the germinal center and therefore most likely is either a memory B-cell or a plasmablast (14,15). It has been proposed that the myeloma cancer stem cell is CD138-negative and CD19-positive, based on the observation that this population, whether derived from myeloma cell lines or primary myeloma samples has an increased clonogenic potential (16). The clonogenic potential of myeloma cells is increased by dendritic cells, leading to loss of CD138 and expression of bcl-6 (17). Hedgehog signaling, which determines the fate of progenitor cells, promotes the expansion of the myeloma stem cell (18). The small subset of myeloma cells that manifests hedgehog pathway activation is markedly concentrated in the tumor stem cell compartment. If there is indeed a myeloma stem cell, such a cell will have many characteristics in common with a

hematopoietic stem cell in that it is resistant to conventional doses of chemotherapy, and that such doses of chemotherapy will be necessary, which can at least eradicate hematopoietic stem cells, and therefore such therapy will require stem cell support. The agents most toxic to hematopoietic stem cells are alkylators such as melphalan, busulfan and BCNU, agents found to be very effective in myeloma, while other alkylators such as cyclophosphamide and platinum compounds, which spare hematopoietic stem cells, are much less effective in myeloma even at higher doses. The difficulty in myeloma is not to eradicate the more differentiated myeloma compartment, which comprises more than 99.9% of the tumor mass, but to also kill the myeloma stem cells. Consequently, achieving a hematologic remission, as currently defined, will have a poor correlation with long-term outcome and should not be used as an early substitute for survival estimates.

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

AUTOLOGOUS TRANSPLANTATION FOR RECENTLY DIAGNOSED MYELOMA PATIENTS

It has been more than 25 years since the late Tim McElwain and colleagues introduced high-dose melphalan for the treatment of multiple myeloma. Administration of melphalan 100-140 mg/m^2 without stem cell support

induced biochemical and bone marrow remissions in three (all previously untreated) of the nine myeloma patients, which was much higher than the 3-5% complete response typically seen with conventional therapy (19). The efficacy of high dose melphalan in myeloma was subsequently confirmed in larger studies (20,21). However, high-dose melphalan induced prolonged aplasia of 5 to 8 weeks and was therefore associated with high morbidity and mortality rates in a disease with a median age of 67 years (19,22). This led other investigators to the concept of stem cell rescue, which allowed further dose escalation of melphalan to 200 mg/m^2. Stem cell support was initially provided with autologous bone marrow, which could contain up to 30% plasma cells (23), and subsequently with peripheral blood stem cells, containing more $CD34^+$ cells/kg and therefore resulting in more prompt bone marrow recovery and less morbidity and mortality. Indeed, with a dose of 5×10^6 $CD34^+$ cells/kg or more, the median time of severe neutropenia and thrombocytopenia is not much longer than one week (24). This made application of autologous hematopoietic stem cell transplantation (HSCT) feasible in patients 60 to 75 years old, who were in otherwise good clinical condition, and it reduced procedure-related mortality to 2-5% (25), which is not higher than that seen with 6 months of conventional chemotherapy and/ or the novel agents, such as bortezomib, thalidomide and lenalidomide (26-29). In an attempt to minimize toxicity and to maximize myeloma cell kill, the concept of tandem autologous transplantation was introduced by Barlogie and colleagues in Total Therapy I (30). The underlying hypothesis was that rather than giving a single very intensive preparative regimen prior to stem cell rescue, providing

effective, but less toxic high-dose chemotherapy twice would be better tolerated in older patients and equally effective. Total therapy I was designed to include all active agents available at that time for the treatment of myeloma to increase the complete remission rate as a first important step to improve overall survival. A total of 231 patients were enrolled from 1990 to 1994. With a median follow up of 12 years, 62 patients are still alive and 31 have not progressed. Patients still alive more likely had normal cytogenetics, a normal C-reactive protein (CRP), hemoglobin and lactate dehydrogenase (LDH) level, and had completed two transplants within a 12-month period. The 10-year event-free and overall survivals were 15% and 33%, respectively. The superiority of Total Therapy I over conventional treatment was established by using historical controls, matched for all-important available prognostic markers and who were treated on Southwest Oncology trials during the same period (31). Autotransplantation induced a higher response rate (85% vs. 52%; $p < 0.001$) and significantly extended event-free (49 vs. 22 months; $p = 0.001$) and overall survival (62+ vs. 48 months; $p = 0.01$). The superiority of autologous HSCT over conventional therapy was subsequently confirmed in prospective randomized trials. The IFM-90 study by the Intergroupe Francais Du Myelome (IFM) included 200 patients under the age of 65. Stem cell rescue was performed with bone marrow. Data were analyzed on intent to treat basis (32). More than one quarter of patients randomized to the transplant arm never received a transplant. Nevertheless, response rates (81% vs. 57%), complete remission rates (22% vs. 5%), and 5 year event-free (28% vs. 10%) and overall survival (52% vs. 12%) were significantly better in the transplant arm. This study

was criticized because of the small number of patients and the poor response rate in the control arm. In another randomized study performed in the United Kingdom, the Medical Research Council Myeloma VII Trial enrolled 407 previously untreated myeloma patients younger than 65; 401 could be evaluated. Also in this study with a median follow up of surviving patients of 42 months, the complete response rate (44% vs. 8%; $p < 0.001$), progression-free (28 vs. 20 months; $p < 0.001$) and overall survival (54 vs. 42 months; $p = 0.04$) were superior in the transplant arm (33). There was a trend toward a greater survival benefit of HSCT in patients with poor prognosis, defined as a high β2 microglobulin level of greater than 8 mg/L. On the other hand, three studies using either high-dose total body irradiation (TBI) or oral busulfan, failed to show a benefit for the transplant arm when compared to standard chemotherapy (34-36). In one of these studies (35) patients failing to respond to induction treatment were excluded from randomization, although it is especially in this group of patients that autologous HSCT shows the most benefit compared to conventional chemotherapy (see below). Excellent outcomes were also reported by the Royal Marsden Hospital group. A total of 451 myeloma patients, 51% previously untreated, received a single autotransplant between 1985 and 2001 (37). The treatment-related mortality was 6%, which is somewhat higher than in most other studies. Fifty-nine percent of the patients achieved a complete or near-complete remission. The 10-year progression-free and overall survivals were 16.5% and 31.4%, respectively. Better overall survival was seen in patients with low β2-microglobulin, age less than 60 years and normal albumin levels. In its evidence-

based review, the American Society for Blood and Marrow Transplantation concluded that autologous HSCT is the preferred treatment modality for myeloma and that its application is recommended as *de novo* rather than as salvage therapy (38). Yet, less than half of the patients aged 65 or less with myeloma actually proceeds to transplantation (39). Between October 1998 and February 2004, 668 newly diagnosed myeloma patients were randomized upfront to intensive therapy including tandem autologous transplants with or without thalidomide during the whole treatment. With a median of 42 months of follow-up for surviving patients, the complete remission rate (62% vs. 43%; $p < 0.001$) and the 5-year event-free survival (56% vs. 44%; $p = 0.01$) were superior in the thalidomide arm. However, the 5-year overall survival was similar, approximately 65% in both arms ($p = 0.9$). Median survival after relapse was significantly shorter in the thalidomide arm (1.1 vs. 2.7 years; $p = 0.001$). Toxicity was also higher in the thalidomide arm, especially deep vein thrombosis and peripheral neuropathy (40). When comparing the non-thalidomide arm of Total Therapy 2 (more intensive induction, consolidation and maintenance therapy) to Total therapy 1, the complete remission rates were similar (43% vs. 41%). However, the 5-year event-free survival was better on Total Therapy 2 (43% vs. 28%; $p < 0.001$) with also a trend for better overall survival (62% vs. 57%; $p = 0.11$). Superior event-free and overall survivals were seen in the two-thirds of patients with normal metaphase cytogenetics (40).

THE PREPARATIVE REGIMEN FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Most preparative regimens are based on either melphalan alone or a combination of melphalan and TBI. Other alkylating agents such as busulfan, carmustine, thiotepa and cyclophosphamide have been used less often. The IFM study 9502, which included 282 newly diagnosed and evaluable myeloma patients under the age of 65, compared in a prospective randomized trial, melphalan 140 mg/m^2 with 8 Gy of TBI to melphalan 200 mg/m^2 (41). Patients randomized to the melphalan arm only, showed significantly faster recovery of neutrophils and platelets, required less transfusions and the median duration of hospitalization was significantly shorter. There was a trend towards a better complete and very good partial remission rate in the melphalan only arm (55% vs. 43 %; $p = 0.06$). The median event-free survival was identical, but the 45-month overall survival was superior in the melphalan only arm (65.8% vs. 45.5%; $p = 0.05$), probably due to more effective salvage treatment in the melphalan only patients. Two additional non-randomized studies confirm the superiority of melphalan 200 mg/m^2 over TBI-containing preparative regimens. In the University of Arkansas experience, TBI-containing regimens were associated with a higher treatment-related mortality and inferior event-free and overall survival, despite similar complete remission rates (42). These investigators speculated that a more profound and prolonged immunosuppression after TBI was responsible for the inferior outcome. The European Group for Blood and Marrow Transplantation (EBMT) analysis on

prognostic factors for outcome after autologous transplantation in myeloma also demonstrated that non-TBI preparative regimens were independently associated with a superior outcome (43). In a study from the M.D. Anderson Cancer Center, which included 186 newly diagnosed patients, a preparative regimen with thiotepa, busulfan and cyclophosphamide was compared to melphalan 200 mg/m^2 in a retrospective analysis. The response rate (66% vs. 69%), progression-free (21 vs. 20 months) and overall survival (46 months vs. not reached) were similar in both groups. The authors concluded that a more intensive regimen did not improve outcome and that melphalan 200 mg/m^2 should be the standard preparative regimen (44). Based on all these data, melphalan 200 mg/m^2 has become the preferred preparative regimen.

GRAFT CONTAMINATION WITH MYELOMA CELLS

There is ample and convincing evidence that not only bone marrow, but also peripheral blood stem cells are contaminated with myeloma cells (45,46). Applying quantitative PCR amplification assays of patient-specific CDR3 DNA sequences on peripheral blood mononuclear cell samples, myeloma cells can be detected in virtually all myeloma patients (46,47). The re-infusion of contaminated peripheral blood stem cell collections may contribute to disease relapse as has been demonstrated for other malignancies such as acute and chronic myeloid leukemia, and neuroblastoma (48,49). Additionally, an inverse correlation has been established between plasma cell contamination of the

peripheral blood stem cell product and disease-free survival, although this was a small study which include only 33 patients (50). This may be more a reflection of the higher tumor burden than contribution of contaminated myeloma cells to relapse. Indirect evidence of the potential importance of a clean graft comes from an EBMT study, comparing outcomes of 25 myeloma patients, receiving bone marrow or peripheral blood stem cells from an identical twin to 125 case-matched controls who received autologous and thus contaminated transplants (51). The overall survival tended to be better (73 vs. 44 months; $p = 0.1$) and progression-free survival was significantly better (72 vs. 25 months; $p = 0.009$) for the syngeneic transplants. The risk of relapse at 48 months was significantly lower (36% vs. 78%; $p = 0.009$). Different *ex vivo* purging techniques, based on chemical and immunologic approaches have been applied to obtain "tumor-free" grafts. Delayed hematologic recovery and increased infectious complications have compromised the applicability of these strategies, despite their success in substantially reducing myeloma cell contamination of the graft. In a multi-center Phase III randomized trial, hematologic recovery and toxicity after autologous transplantation were compared between patients receiving $CD34^+$ cell-selected grafts versus unselected grafts (52). Time to platelet recovery was slightly delayed in patients receiving $CD34^+$ cell-selected grafts with less than 2×10^6 $CD34^+$ cells/kg. There was no difference in event-free and overall survival between the two arms. Moreover, salvage therapy may be more difficult in patients who have received $CD34^+$ cell-selected grafts. Because of the high cost and the lack of benefit of tumor cell-reduced grafts, this area of research is no longer pursued.

SINGLE VERSUS TANDEM AUTOLOGOUS TRANSPLANTS

Despite the superiority of autologous transplantation over conventional chemotherapy, the 7-year event-free survival in the IFM 90 trial was only 16% with no plateau on the survival curve. Achievement of a very good partial response (VGPR; i.e. more than 90% reduction in M-protein) or better was associated with a significantly better overall survival. Therefore, the same French group tested in a prospective randomized trial (IFM 94) whether outcome could be improved by the application of tandem transplants (53). In that study 399 newly diagnosed myeloma patients under the age of 60 years were randomly assigned to a single versus tandem transplants. Patients in the single transplant arm received melphalan 140 mg/m^2 with 8 Gy of TBI, while those in the tandem transplant arm received a first transplant with melphalan 140 mg/m^2, followed by melphalan 140 mg/m^2 with 8 Gy of TBI for the second transplant. No difference was observed in the VGPR or better rate between the two arms (50% vs. 42%; $p = 0.1$). However, the event-free (20% vs. 10%; $p = 0.03$) and overall survival at 7 years (42% vs. 21%; $p = 0.01$) was superior in the tandem transplant arm. For patients in the single transplant arm who did not achieve at least a VGPR within 3 months after transplant the 7-year survival was only 11% versus 43% in the tandem transplant arm ($p < 0.001$). However, patients achieving at least a VGPR after a single transplant did not appear to benefit significantly from a second transplant ($p = 0.7$). On multivariate analysis, β2 microglobulin, LDH and the

treatment arm were all independent prognostic markers associated with survival (all $p < 0.01$). In another prospective randomized study, the Bologna 96 clinical study of single versus double autologous HSCT for myeloma, 321 patients were randomly assigned to receive either melphalan 200 mg/m^2 alone or melphalan 200 mg/m^2 followed by melphalan 120 mg/m^2 plus busulfan 12 mg/kg (54). A higher percentage of patients in the double transplant arm achieved at least a near complete remission in the tandem transplant arm (47% vs. 33%; $p = 0.008$) and these patients had a longer relapse-free (42 vs. 28 months; $p < 0.001$) and event-free survival (35 vs. 23 months; $p = 0.001$). Benefits offered by tandem transplantation were particularly evident among patients failing to achieve at least a near complete remission after the first transplant. Transplant-related mortality was 3% in the single and 4% in the tandem transplant arm. The administration of a second transplant and the introduction of novel agents in the treatment of myeloma for patients relapsing in the single transplant arm resulted in a failure to see a benefit in overall survival for the double transplant group. Therefore, the available data favor tandem transplants in younger myeloma patients at least in those not achieving an excellent response after the first transplant. The issue of benefit and timing of a second autologous HSCT has been addressed in a retrospective analysis of the EBMT, which included approximately 7,500 patients (55). Since this was not a prospective randomized study, there may be major biases related to differences in prognostic factors and to the multiple centers who had contributed to the patient database. On the other hand, the large number of patients analyzed probably compensated for many of these biases. In this study,

the hazard ratio (HR) of relapse was clearly lower if a second transplant was performed within 12 months after the first transplant (HR compared to no second transplant was 0.43 for second transplants performed < 6 months after the first and 0.51 between 6 and 12 months). A second transplant more than 12 months after the first transplant still had a significantly lower relapse rate compared to no second transplant before relapse (HR = 0.64), but its benefit was not as pronounced as with a second transplant within 12 months. Moreover, the transplant-related mortality was clearly higher if the second transplant was performed more than 12 months after the first. When in this retrospective analysis an elective second transplant was compared to a second transplant at relapse, an elective second transplant clearly improved overall survival (HR for survival 1.7; $p < 0.0001$), while a second transplant at relapse did not confer any survival benefit over salvage treatment with non-transplant modalities (HR = 1.06; $p = 0.55$).

AUTOLOGOUS TRANSPLANTATION FOR PRIMARY REFRACTORY MYELOMA

High dose therapy has consistently increased tumor cytoreduction and has extended event-free and overall survival in patients with primary refractory myeloma (<50% reduction in M-protein). Alexanian and colleagues reported on 27 patients with primary refractory myeloma who received an autologous transplant and compared their outcome to 60 control patients receiving

conventional chemotherapy (56). The transplanted patients had a median survival of 83 months compared to 38 months for patients receiving standard treatments for primary refractory myeloma ($p = 0.03$). Autologous transplantation for primary refractory myeloma later in the disease (> 1 year), resulted in significantly lower response rates and shorter progression-free survival. In a study from the Royal Marsden Hospital, Surrey, UK, patients with primary refractory myeloma to induction therapy had a similar event-free survival compared to those with chemotherapy-sensitive patients ($p = 0.2$) with an early difference in outcome in favor of the chemotherapy-sensitive patients, mainly due to the higher transplant-related mortality in the primary refractory patients (57). The time to relapse was also identical in the two groups ($p = 0.6$). The authors concluded that myeloma patients should not be excluded from autologous HSCT based upon lack of response to induction chemotherapy. The Mayo Clinic reported their experience with outcome of stem cell transplantation in 50 patients with primary refractory myeloma to induction therapy and compared it to that of 101 patients with chemotherapy-sensitive disease (58). The one year progression-free survival for refractory patients was 70% compared to 83% for chemotherapy-sensitive patients ($p = 0.65$). The authors recommended early stem cell transplantation for patients with primary refractory myeloma.

AUTOTRANSPLANTATION FOR ELDERLY PATIENTS

Most high dose therapy trials have only included relatively young patients with good organ function. However, the median age of myeloma patients is 67 years. If a major impact of autologous HSCT on outcome is to be achieved, it will have to be performed also in patients over the age of 65. Age per se should not be a contra-indication for transplantation, but co-morbidities can be. It is obvious that co-morbidities increase in older patients, and therefore, more elderly myeloma patients may not be candidates for autologous HSCT. Several studies have reported contradictory findings on the impact of age on the ability to collect stem cells. In a retrospective analysis including 984 patients with 106 over the age of 70 years, increasing age correlated inversely with $CD34^+$ cell yield (59). However, the overwhelming majority (85%) of elderly patients were able to collect greater than 4×10^6 $CD34^+$ cells/kg provided that the duration of preceding therapy was 12 months or less and the platelet count was 200,000/uL or more. With the introduction of peripheral blood stem cell transplants, shortening the duration of severe cytopenias, and the improved supportive care, the toxicity of autologous HSCT has clearly decreased. The Arkansas group has compared the outcome of 49 previously treated and untreated myeloma patients over the age of 65 to that of pair mates matched for important prognostic factors (60). No significant difference was seen in percentage of patients completing two transplants (65% vs. 76%; $p = 0.3$). Time to hematopoietic recovery after first and second transplant was comparable in both groups. Treatment-related mortality was 8% in the older and 2% in the younger patients. The frequency of complete remission was lower in older patients (20% vs. 43%; $p = 0.02$). Median durations

of event-free (p = 0.2) and overall survival (p = 0.4) were not significantly different. The safety of autologous HSCT in older patients was subsequently confirmed in other studies (61, 62). The role of autologous HSCT in older patients has recently been challenged by the IFM 99-06 trial comparing melphalan-prednisone to melphalan-prednisone plus thalidomide and to twice melphalan 100 mg/m^2 with stem cell support (63). A total of 436 patients between the ages of 65 and 75 were enrolled. Median follow-up was 32 months. Progression-free and overall survivals were significantly better for patients randomized to the melphalan-prednisone plus thalidomide. The authors concluded that melphalan-prednisone plus thalidomide is effective treatment for older myeloma patients, probably superior to autologous HSCT. It should be noted, however, that 30% of patients randomized to transplantation never received a transplant and that the dose of melphalan was suboptimal even for older patients. In older patients in good general health, tandem autotransplants probably still is the preferred treatment, but the dose of melphalan should be reduced to 140 mg/m2 instead of 200 mg/m2 to minimize toxicity and to maximize the chance that a second transplant can be administered in a timely fashion.

AUTOTRANSPLANTATION IN PATIENTS WITH RENAL FAILURE

Pharmacokinetic studies performed in 20 patients, including six with severe renal failure, five of which were on dialysis, showed that melphalan levels and metabolism were not different in patients with renal failure (64). However,

high dose melphalan was associated with more toxicity ($p = 0.0005$) and longer hospitalizations ($p = 0.004$) in renal failure patients. The Arkansas group reported data on 81 consecutive myeloma patients with renal failure (creatinine of > 2 mg/dL or >176.8 umol/L) at the time of transplantation (65). Thirty-eight patients were dialysis dependent. The median age was 53 years and one quarter had received > 12 months of preceding therapy. The first 60 patients received melphalan 200 mg/m^2; the dose of melphalan was reduced to 140 mg/m^2 for the last 21 patients. A complete remission of 26% and 38%, respectively, was observed after the first and second transplant. Median overall survival was 52+ months. Melphalan 140 mg/m^2 was better tolerated and appeared equally effective as melphalan 200 mg/m^2. It should be mentioned that only 40% received their planned second transplant. In another study from the same group of investigators, the outcome of 59 patients on dialysis at the time of first transplant was of 4 months after autologous HSCT (66); 37 had been on dialysis for 6 months or less. The 5-year event-free and overall survivals were 24% and 36%, respectively. One quarter of patients became dialysis-independent. Shorter duration of dialysis-dependency and a pre-transplant creatinine clearance of greater than 10 mL/min predicted for a significantly higher probability of becoming dialysis-independent post-transplantation. These data suggest that autologous HSCT should be performed early in the disease course to maximize the probability of reversing end-stage renal failure. Raab et al compared outcome of 17 patients with dialysis-dependent renal failure, who received melphalan 100 mg/m^2, to that of 17 matched pairs without renal failure, treated with melphalan 200 mg/m^2 (67). No

significant difference in hematologic toxicity, transplant-related mortality or disease response was observed, and event-free and overall survivals were comparable. However, dialysis-dependent patients required more extensive intravenous antibiotic administration and longer hospitalizations. Similar observations were made by Knudsen et al (68). They did observe a significantly higher transplant-related mortality (17% vs. 1%) in patients with severe renal failure. The dose of melphalan in renal failure patients varied between 100-200 mg/m^2. These data clearly indicate that autologous HSCT in patients with renal failure is feasible and can reverse dialysis-dependency. However, it is more toxic and requires better supportive care skills. The dose of melphalan should be reduced to 100-140mg/m^2 dependent on age and co-morbidities.

PROGNOSTIC FACTORS WITH AUTOTRANSPLANTATION

Myeloma is a highly heterogeneous disease with survival ranging from a few months to more than 15 years. The Durie-Salmon staging system, which is based on renal function and estimates of tumor burden, clearly has prognostic significance and has allowed better interpretation of clinical trials, but has major shortcomings such as assessment of lytic lesions and the lack of attention to proliferation characteristics (69). The International Staging System was introduced recently and is based on data of more than 10,000 patients (70). It only uses albumin and β2-microglobulin levels, which are readily available to practicing oncologists. It clearly separates patients into good, intermediate and

high risk myeloma. Although this classification is now widely applied, it does not include any genetic information about the cancer cells. The importance of cytogenetics was first demonstrated by metaphase cytogenetics and subsequently by FISH. Patients with cytogenetic abnormalities on metaphase analysis have an inferior prognosis (71,72). Finding abnormal metaphase cytogenetics is probably the best surrogate marker available at this time for stroma-independent and, therefore, aggressive myeloma (73). The worst outcome is seen in patients with a hypodiploid karyotype and/or complete deletion of chromosome 13 or partial deletion of its long arm (74). Patients with a hyperdiploid karyotype and no deletion 13 have a somewhat better outcome. Chromosome 14q32 translocations, involving the gene coding for the immunoglobulin heavy chain are frequent in myeloma and probably represent an important early event in its pathogenesis, since these translocations are found with almost the same frequency in monoclonal gammopathy of undetermined significance. Multiple partners have been identified for the 14q32 translocations, some associated with good, others with poor prognosis. The t(11;14) (q13;q32), which results in a high expression of cyclin D1 and present in 20% of myeloma patients, is associated with a good prognosis (75,76). These patients have myeloma cells with more lymphoplasmacytic morphology and a pseudo-diploid karyotype. The plasma cells are often CD20-positive. This translocation is also common in primary amyloidosis (77). Although many patients with this translocation relapse, they remain relatively sensitive to therapy. The t(6;14)(p21;q32), present in 5% of myeloma patients, results in over-expression of cyclin D3 (78). It shares the same

good prognosis with the t(11;14). On the other hand, the t(14;16)(q32;q23), present in 5% of patients and resulting in over-expression of *c-MAF,* t(14;20)(q32;q11), present in <5% and resulting in high *MAF-B* and the t(4;14)(p16;q32), present in 15% and resulting in high expression of *FGFR3* in the majority of patients, are associated with a poor prognosis also with stem cell transplantation (79-82). These poor prognosis translocations can only be detected by FISH and not by conventional cytogenetics. They are often associated with deletion 13 on conventional cytogenetics. Deletion of 17p, involving the p53 gene is usually a mono-allelic deletion. It also has a poor prognosis and is present in 10-33% of myeloma patients (83,84). Metaphase and FISH chromosomal analysis represent only a crude way to assess DNA changes and provide no clues of which genes are either over- or under-expressed. Gene expression profiling permits quantitation of RNA expression of more than 30,000 genes with many of those related to cancer biology such as proliferation, apoptosis, DNA repair and drug resistance. Applying unsupervised hierarchical clustering to highly purified plasma cells of newly diagnosed patients, seven subgroups of myeloma have been identified, based on either spiked gene expression as a consequence of a translocation involving 14q32, hyperdiploidy or proliferation characteristics (82). This biological classification also had major prognostic significance with inferior outcome for patients with a proliferative signature or with spikes of *MMSET*, *c-MAF* or *MAF-B,* thus confirming and adding to the FISH data. To molecularly define high risk myeloma, 70 either highly over- or under-expressed genes were identified, that were linked to early myeloma–related death (85). A high

proportion of up-regulated genes mapped to chromosome 1q, while a high proportion of down-regulated genes mapped to chromosome 1p. The ratio of mean expression levels of up-regulated to down-regulated genes defined a group of high-risk patients, which constituted 13% of the entire myeloma population with a short event-free and overall survival. Multivariate discriminant analysis showed that a subset of only 17 genes predicted outcome equally well as the 70 gene model. On multivariate analysis of outcomes of 220 newly diagnosed myeloma patients entered on Total Therapy 2, including standard prognostic variables, metaphase cytogenetics, magnetic resonance imaging, FISH and gene expression profiling (GEP), the hazard ratio for overall survival was highest for GEP (3.07; $p < 0.001$), followed by amplification of 1q21 (1.71; $p = 0.05$) (see below). The 3-year survival decreased progressively from 92% to 78% to 43% according to the presence of none (49% of patients), one (35%) or both (16%) of these unfavorable variables (86). One of the genes mapping to 1q21 is *CKS1B.* Over-expression of this gene is associated by itself with a poor prognosis (87). Over-expression of 1q21 can also be assessed by FISH. Amplification of 1q21 (amp1q21) heralds a poor prognosis and remains an independent factor on multivariate analysis with an inferior event-free and overall survival (88). In the absence of GEP, much of the prognostic value can be assessed by combining metaphase cytogenetics with FISH for t(11;14) and amp1q21 with a significantly inferior event-free survival for patients with cytogenetic abnormalities and amp1q21 but no t(11;14) (Figure 1).

AUTOTRANSPLANTATION FOR RELAPSED/REFRACTORY MYELOMA

Although curative in only a minority of myeloma patients, the introduction of high-dose treatment (HDT) with autologous HSCT has led to a significantly longer event-free and overall survival, and to a better quality of life when compared to standard-dose therapy (SDT).

The optimal time of the application of HDT in MM patients i.e. early in the course of the disease versus at relapse following conventional chemotherapy, is still controversial. Fermand et al compared, in a randomized fashion, the outcome of the disease in two groups of relatively young (<56 years) patients; 91 patients received HDT and autologous HSCT after a short induction treatment (early transplant) and 94 patients received HDT and HDT and autologous HSCT as rescue procedure (i.e. in case of primary resistance to conventional chemotherapy or at relapse; late transplant) (89). With a median follow-up of 58 months, the overall survival was identical (64.6 vs. 64 months) in the two groups. In 1993, three North American cooperative groups launched a prospective randomized trial (S9321) comparing HDT (melphalan 140 mg/m^2 plus total-body irradiation 12 Gy) with SDT using the vincristine, carmustine, melphalan, cyclophosphamide, and prednisone (VBMCP) regimen. Responders on both arms ($\geq$75%) were randomly assigned to interferon (IFN) or no maintenance treatment. After induction therapy with four cycles of vincristine, adriamycin and dexamethasone (VAD), patients were randomly assigned to either HDT with melphalan plus TBI or to SDT with VBMCP for one year; all patients received

high-dose cyclophosphamide, and except for allogeneic HSCT candidates, proceeded with peripheral blood stem cell collection. Patients were stratified according to Durie-Salmon stage, β2-microglobulin serum level, and response to VAD induction. Responding patients (≥75% M-protein reduction) were randomly assigned to 4 years of maintenance therapy with interferon versus observation. Patients treated on the VBMCP arm were offered the option of salvage autologous HSCT at the time of disease progression or relapse. In the VBMCP arm, 87 of 157 patients with follow-up after relapse received a salvage autotransplant, resulting in a median survival time of 30 months (Fig. 2); this was slightly higher than the survival time of 23 months noted among the remaining patients receiving non-transplantation based salvage therapies (*P*=.13) (36).

Patients with primary refractory, progressive disease, or not achieving 50% monoclonal protein reduction with the initial standard-dose regimen respond differently to HDT than patients who relapse either while on conventional chemotherapy or after discontinuation of such treatment (see above). Vesole et al reported the effect of HDT on 135 patients with refractory myeloma (90). Either melphalan 100 mg/m^2 (47 patients), TBI with melphalan or thiotepa (21 patients), melphalan 200 mg/m^2 x 1 (25 patients) and melphalan 200 mg/m^2 x 2 (45 patients) were applied as preparative regimens. When compared to historic controls, even among patients with resistant relapse and high β2-microglobulin levels, more intensive treatment resulted in superior event-free and overall survival durations. Primary refractory patients treated with TBI experienced significantly longer event-free and overall survival durations (32 and 66 months,

respectively) than those with resistant relapse (4 and 7 months, respectively; p = 0.007, 0.007 respectively). Similar results were observed in melphalan 200 mg/m^2 recipients. Primary refractory patients experienced longer event-free and overall survival durations (4 and 7 months, respectively) than those with resistant relapse (17 and 21 months, respectively; P=0.006, 0.01 respectively). In a subset analysis reported by the University of Arkansas, primary refractory status was also associated to superior event-free survival (23 vs. 14 months; p = 0.002) and overall survival (39 vs. 25 months; p = 0.08) compared to patients with resistant relapse (22). The effectiveness of HDT in refractory MM (relapsed and primary refractory) was also evident in an Intergroup trial (91). On an intent-to-treat basis from transplant registration, the median progression-free survival and overall survival duration in a group of 66 patients with refractory myeloma to alkylating agents, dexamethasone or VAD was 11 and 19 months, respectively. Rajkumar et al reported on 75 patients who received transplantation for relapsed or primary refractory myeloma; the OS for conventionally treated patients, relapsed myeloma after conventional therapy, and primary refractory individuals, differed significantly with median survivals of 12, 21 and 30 months, respectively (92). Plasma cell labeling index was significantly lower in patients with primary refractory disease when compared with relapsed cases, suggesting that the low proliferative activity of the disease in the former group might partly explain the resistance to conventional chemotherapy. In contrast to the experience in other B-cell malignancies, primary refractory status does not negatively affect the anti-myeloma effect of HDT with autologous HSCT.

Pineda et al recently reported on the effect of high dose melphalan-based autotransplant for multiple myeloma (93). A total of 1064 previously treated patients enrolled on different HDT protocols were examined. Trials included, for previously treated patients, induction with DT-PACE (dexamethasone, thalidomide, cisplatin, adriamycin, cyclophosphamide, and etoposide) followed by intended tandem transplants either with MEL200 (melphalan 200 mg/m^2) or MEL140 (melphalan 140 mg/m^2) in case of renal insufficiency with creatinine greater than 3 mg/dL or advanced age greater than 70 years (94). Others received a MEL-DT-PACE hybrid regimen (MEL140 plus DT-PACE). In case partial response (PR) was not achieved after the first transplant, second transplant regimens used MEL140 plus either TBI or high-dose cyclophosphamide or the BEAM regimen (carmustine [BCNU], etoposide, arabinosyl cytosine, melphalan) (95). Myeloma responses were reported according to the Blade criteria. Median follow-up was 38 months (range, 0.6 -201). The Kaplan-Meier method was applied to estimate event-free and overall survival, and comparisons between different arms were made using the log-rank test. Event-free survival (median = 24 months) and overall survival (median = 44 months) were measured from the first day of melphalan administration until disease recurrence or death. Overall survival according to the number of favorable pre-transplant parameters for all enrolled patients is shown in Figure 4.

Significant differences were observed between the 5 subgroups: outcomes worsened progressively as the number of good-risk features declined from 5 to 4 to 3 to 2 to less than 2. Ten-year overall survival was 25% with the best and 2% in

the worst constellation of prognostic factors. As most patients eventually relapse after one or two cycles of HDT, it is important to consider the issue of further treatment intensification as salvage approach. Resistance of the malignant clone and subclinical toxicity to vital organs may compromise long-term survival. Mansi et al were the first to prove "continuing chemosensitivity" by reporting a response rate of 93% and a response duration of 17 months in a group of 29 patients who, after relapsing following a single course of melphalan 140 mg/m^2, were treated with re-induction therapy followed by melphalan 200 mg/m^2 and autologous bone marrow transplant (96). At the University of Arkansas, the outcome of 196 patients who relapsed after a single or double transplant was evaluated (97). Patients received standard-dose treatment or a further transplant. Multivariate analysis showed that patients who relapsed late (>1 year) after the previous HDT and had low β2-microglobulin levels at the time of relapse were the best candidates for further autotransplantation. Among a total of 1358 patients receiving two prior autologous transplants (median interval from first to second transplant = 4 months), 98 received a further autologous transplant at relapse. In a search for favorable features associated with post-third autotransplant survival, pre-transplant 1, 2 and 3 features, especially the presence of metaphase cytogenetic abnormalities (CA) and standard laboratory features such as, β2-microglobulin, CRP, LDH, albumin, hemoglobin and creatinine were examined as well as the time interval from second to third transplant and whether a third transplant was applied to rescue Total Therapy 1 or 2 (newly diagnosed) or other patients receiving tandem transplants after more extensive prior treatment (98,40).

Remarkably, the presence of CA at any of the indicated time points had no impact on survival after a third autologous HSCT, typically with a melphalan 200mg/m^2 preparative regimen. According to multivariate analysis, the second to third transplant interval (time-dependent covariate analysis) (HR 8.8, p=.002) and hypo-albuminemia < 3.0g/dL (HR 3.2, p<.0001) were independently important. Thus, post-third transplant survival was superior among the 41 patients with a post-second transplant event-free survival greater than 3 years and albumin greater than 3.0 gm/dL prior to the third transplant (median overall survival = 21 months; 5-year overall survival = 20%), with an intermediate outcome noted for the 34 patients with one of these favorable features present (median overall survival = 10 months; 3-yr overall survival = 20%) while the worst outcome was noted in the 13 patients who were both hypo-albuminemic and had received their 3rd transplant within 3 years of the second transplant (median overall survival = 3 months, no survival beyond 12 months) (99). Prolonged overall survival with second on-demand autologous transplant in multiple myeloma has been reported by Elice, et al (100). A total of 130 consecutive multiple myeloma patients were treated with autologous HSCT after conditioning with melphalan 200 mg/m^2 followed by a second autologous HSCT at relapse or disease progression. A total of 107 (82%) patients completed the first autologous HSCT. The best response obtained after autologous HSCT was complete response (CR) in 23%, very good partial response (VGPR) in 28%, partial response (PR) in 42%, and minimal response (MR) in 7%. Median overall and event-free survival were 65.4 and 27.7 months, respectively. Myeloma relapse or progression was observed in 70

patients; 26 received a second autologous HSCT (with a median time of 20.4 months from first autologous HSCT). A major response (partial remission or better) was obtained in 69% of these patients. Median overall and event-free survival rates after the second autologous HSCT were 38.1 and 14.8 months, respectively. Treatment-related mortality was 1.9% after the first autologous HSCT and no deaths occurred related to the second transplant, confirming that a second autologous HSCT at relapse or progression is a safe and effective strategy. Even in the era where new drugs such as thalidomide, lenalidomide and bortezomib alone or in combination have produced significant responses and may also have a positive effect on survival of myeloma patients, HDT remains a valuable option in the treatment of myeloma patients relapsing after transplantation, especially those with more durable responses after initial transplant(s). After the salvage transplant, a combination of newer drugs with dexamethasone can be applied to increase the response duration.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

Though new insights into its biology have identified molecular mechanisms that have become targets of recently developed agents with potent anti-tumor activity, multiple myeloma remains a fatal plasma cell malignancy (101,102). High-dose chemotherapy with autologous HSCT, after induction with chemotherapy or newer agents, is regarded as the standard of care for newly

diagnosed myeloma patients younger than 65 years (30,32,33,53,54). However, relapse is a continuous risk and only a few good-prognosis patients live disease-free for longer than 10 years (33,53). Constant recurrence following autologous HSCT is primarily due to their failure to eradicate all myeloma cells. Conversely, allogeneic HSCT remains the only potentially curative treatment for its well documented graft-versus-myeloma effects (103). Given the high transplant mortality and morbidity related to the high-dose myeloablative preparative regimens used until recently, its application has primarily been limited to younger relapsed/refractory patients (104-106). These limitations have lately been significantly reduced through reduced-intensity or non-myeloablative conditioning regimens (107). The introduction of less intense conditionings has led to at least two important clinical and biological implications: the increase of the eligible age for allogeneic HSCT up to 65-70 years even in medically unfit patients and the shift of the burden of tumor eradication from the chemotherapy of the conditioning to the immune attack of myeloma cells by donor T cells (108,109). Though the results of recent trials are promising, the subset of patients who may most benefit from an allograft remains to be defined. The clinical outcomes of myeloablative and reduce-intensity/non-myeloablative allogeneic HSCT, the biological concepts of graft-versus-myeloma effects and possible future developments are reported below.

ALLOGRAFTING AFTER MYELOABLATIVE CONDITIONING REGIMENS

High treatment-related mortality (TRM) has restricted this approach to young, medically fit patients, and even here transplant-related mortality rates are of the order of 30-60% (104-106). The recent shift from myeloablative, high-dose conditionings to reduced-intensity/non-myeloablative regimens has further limited its clinical application. Most commonly used myeloablative conditioning regimens (Table 1) have included cyclophosphamide with TBI, or busulfan with cyclophosphamide, or melphalan and TBI (104-106, 36,110-118).

Even though chemotherapy-sensitive disease has almost universally been reported as a prerequisite for higher response rates and post-transplant overall and disease-free survivals, the outcome of most trials of allogeneic HSCT for multiple myeloma has been strongly biased by patient selection and pre-transplant characteristics. Comparing results reported in different studies is therefore extremely difficult and not very helpful to establish the curative potential of the procedure. Overall, clinical complete remissions (CCR) have been observed in 20-60% of patients though variable definitions of complete remission have been used (104-106, 36,110-118). Widely used criteria for CCR require negative immunofixation of both serum and urine monoclonal paraproteins with no evidence of myeloma cells on bone marrow biopsy and bone marrow aspirate by morphology and flow cytometry (119). In most trials, approximately 50% of patients with chemotherapy-sensitive disease at the time of transplant have achieved a CCR with a median onset 3 months after transplant. Overall, despite these high rates of CCR, late relapses occur, and in most series, only 10-25% of patients remain disease-free after 10 years and possibly cured. Disease remission,

in this subset of patients, is frequently detected by molecular methods. Molecular remissions, as a prelude to tumor eradication and eventual cure, are far more frequent after myeloablative allogeneic HSCT as compared to autologous HSCT and can occur in up to 50% of patients in CCR after allografting (119). In a subset of patients, these remissions are prolonged, suggesting a complete eradication (120).

The majority of allogeneic HSCT trials have enrolled younger patients usually in their fifth decade. Despite this, the reported early TRM ranges from 20 to 50% (104-106, 36, 110-118). Causes of death are primarily regimen-related, graft-versus-host-disease (GvHD) and its treatment-related opportunistic infections. Overall, the reason of the high TRM observed in multiple myeloma rather than other hematological malignancies remains unknown. Possible explanations include myeloma effects on baseline organ functions and, importantly, a profound immunodeficiency predisposing to organ toxicities and opportunistic infections. Interestingly, the largest multi-center analysis by EBMT registry clearly suggested that survival improved in the late 90' given a remarkable reduction in TRM through better supportive care and patient selection criteria (122). In this retrospective study on 690 patients undergoing myeloablative allogeneic HSCT, patients who received a bone marrow allograft between 1983-93 were compared to those transplanted between 1994-98. In this latter cohort, a subset of patients also received granulocyte-colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC). TRM at 6 and 24 months was significantly lower in patients transplanted between 1994-1998 than

between 1983-1993 (21% vs. 38% and 30% vs. 46% respectively). However, the median age at transplant was only 44 years (range, 18-57), whereas the median age of myeloma patients at diagnosis is approximately 65 years. The reduced toxicity was associated with better clinical outcome, and median overall and progression-free survivals at 3 years increased from 35% to 55% and from 7 to 19 months for patients transplanted between 1983-1993 and 1994-1998, respectively. No differences in outcomes were reported between patients who received marrow and those who received PBSC, though a slightly higher incidence of chronic GvHD was observed with the use of PBSC. More recently, Barlogie et al reported on the randomized US Intergroup Trial S9321 (36). Initially, the study design included myeloablative allografting for patients younger than 55 years with a suitable sibling donor. This arm was prematurely closed because of 53% TRM. However, 22% of the patients enrolled remain alive and progression-free at 7 years. Importantly, the authors show that both overall and event-free survival curves remain flat with follow-up extending to 10 years, consistent with a cured subgroup of patients.

The largest single-center experience so far reported is from the Seattle group (106,110). In total, 136 patients, all younger than 60 years (median ages, 43-48 years), underwent myeloablative allogeneic HSCT between 1987 and 1999 from related (84%) and unrelated donors (16%). Most patients were heavily pre-treated, beyond first response or with chemotherapy-resistant disease, and only 21% had chemotherapy-sensitive disease. Most patients received a combination of busulfan and cyclophosphamide with or without TBI. The study reported a

currently unacceptable day-100 TRM of 48%. An additional 15% of patients died of transplant-related causes at 1 year, most commonly due to GvHD and infections. Overall, the 5-year survival was 22% with disease-free survival of 14%. However, in 34% of patients who achieved post-transplant CCR, overall and disease-free survivals at 5 years were 48% and 37%, respectively. Importantly, subgroup analyses showed that early TRM was lower, approximately 20%, for patients with responsive disease who were transplanted within one year from diagnosis.

No prospective randomized trials have compared allografting after myeloablative conditioning regimen with autografting. A retrospective case-matched analysis of 189 patients who underwent myeloablative allogeneic HSCT and 189 who were treated with autologous HSCT before 1995 and reported to the EBMT registry showed superior clinical outcomes with autografting (123). A more recent single-center retrospective comparison of autologous HSCT versus allogeneic HSCT from HLA-identical siblings or unrelated donors has been reported (124). One-hundred-fifty-eight patients younger than 55 years were transplanted through the Leukemia/Bone Marrow Transplantation Program of British Columbia between 1989 and 2002. Seventy-two patients received an allograft after myeloablative conditioning, 58 from a sibling donor and 14 from an unrelated donor, whereas 86 received an autograft. After a median follow up of 88 months, 61 patients of the entire series were alive. Twenty-eight patients were alive following allogeneic HSCT after a median follow-up of 102 months and 33 following autologous HSCT after a follow-up of 87 months. Twenty-one (75%)

out of the 28 patients who received an allograft remained in continuous remission. However, no statistically significant differences were observed in either overall or event-free survivals between the two groups. Interestingly, neither acute or chronic GvHD had an impact on overall or event-free survival. As the authors state, the lack of formal inclusion criteria led to inevitable selection bias. Patients with younger age and chemo-resistant disease were more likely to be offered allogeneic HSCT. Other confounding factors were different initial therapeutic strategies, conditioning regimens, prognostic factors, and comorbidities.

Overall, in the light of the current available data, it is important to point out that the retrospective nature and the heterogeneous inclusion criteria and treatment strategies of most studies inevitably reflect a substantial selection bias that reduces the statistical power and prevents from determining the real role of myeloablative allogeneic HSCT in multiple myeloma. However, published reports almost unanimously conclude that better outcome is associated with chemo-sensitive disease at transplant and, importantly, that allografting at an earlier disease phase is associated with better clinical outcomes. Altogether, clinical trials also support the concept that, though long-term cure is possible in a subset of patients, allogeneic HSCT has so far benefited a minority of younger patients with matched sibling donors.

Acute and chronic GvHD (reviewed in Chapter 11 by Antin and Korngold and Chapter 12 by Martin and Pavletic, respectively) have been the most challenging transplant-related complication occurring up to 60% in T-replete allografts. The incidence of GvHD may further increase with patient age, with the

use of unrelated or mismatched donors and female donors, especially if multiparous (125).Though, GvHD has also been associated with decreased risk of disease relapse (110), no recent changes in its treatment have yet translated into significant survival advantages. Most early clinical trials on myeloablative allografting included bone marrow grafts. G-CSF mobilised PBSC have recently been increasingly utilized as a source of stem cells especially in patients with HLA-identical sibling donors. The biological differences of the graft composition may have an important clinical impact. In PBSC grafts, the content of T cells is significantly higher as well as their polarisation toward a Th-2 cell phenotype more typically observed during active chronic GvHD. The EBMT analysis reported a trend toward higher chronic GvHD in PBSC recipients; however, the short follow-up did not allow to draw conclusions on the ultimate impact on clinical outcome of the use of PBSC (122). Though no higher incidence and severity of acute GvHD have been reported, it is still controversial if the use of PBSC is correlated with a higher incidence of extensive GvHD that may affect quality of life and clinical outcomes (126-128). Currently, prospective randomised studies comparing bone marrow vs. PBSC grafts in hematological malignancies are in progress.

Many laboratory parameters have been used to predict prognosis in myeloma patients undergoing allogeneic HSCT. Low albumin and high β2-microglobulin have been associated with worse clinical outcome after allografting (110,122). Newer biological parameters have recently been more helpful to categorize patients. Chromosome 13 deletion [del(13)], detected by standard

cytogenetics or FISH, has been the genetic abnormality most commonly associated with worse prognosis in several studies (72,129,130). However, a recent more comprehensive analysis failed to define del(13) as an independent prognostic factor. In fact, its prognostic significance appeared to be associated with the presence of other abnormalities such as t(4;14) and del(17p) (131). In the near future, advanced technologies such as gene-expression profiling may allow to correlate the genetic constitution and the biological behavior of the disease and determine how these factors influence prognosis (85,86,132). However, these molecular technologies are not readily available at most Institutions and their translational role in clinical practice remains to be determined in future years.

ALLOGRAFTING AFTER NON-MYELOABLATIVE/REDUCED INTENSITY CONDITIONING REGIMENS

The association of allografting with long-term disease-free survival in a subset of patients suggested that graft-versus-myeloma effects may have potentially been curative for myeloma. This observation led to the exploration of less intense, highly immunosuppressive, though less myelosuppressive, conditioning regimens, aimed at establishing stable donor engraftment while drastically reducing organ toxicity. One of the most widely used conditionings was developed by the Seattle group based upon pre-clinical studies on the dog model which showed that donor engraftment could be obtained after a non-myeloablative regimen consisting of low dose of TBI of 200 cGy coupled with

potent post-transplant immunosuppression with cyclosporine and mycophenolic acid (mycophenolate mofetil) (133). This strategy was soon translated into clinical studies. However, in the first 18 myeloma patients treated with this approach, two rejections of the donor cells were observed, and only transient CCR and partial remission were achieved in two and three patients, respectively (134). These results indicated that it would be imperative to explore the effects of more effective cytoreduction before non-myeloablative allogeneic HSCT to improve responses. A new treatment modality, especially for newly diagnosed patients who had not been heavily pre-treated, involved an autologous HSCT followed, 2 to 4 months later, by a TBI-based non-myeloablative allogeneic HSCT (109). As compared to myeloablative conditioning, designed to produce simultaneous cytoreduction and adequate immunosuppression to establish stable donor engraftment, the tandem autologous-allogeneic approach allows to separate in time the high-dose cytoreduction and the graft-versus-myeloma effect with the potential of reducing treatment-related toxicity. The first multi-center experience with this approach included 54 stage II-III patients, median age 52 years (range, 27-71), half of them with refractory or relapsed myeloma (109). Fifty-two patients completed the tandem autologous-allogeneic HSCT procedure. CCR was reported in 57%, and overall TRM was 22%. Overall chronic GvHD developed in 60%. After a median follow-up of 60 months, overall and progression free survivals were 69% and 38% respectively.

Overall, in recent years, a number of reduce-intensity regimens (table 2) have been introduced into phase II clinical trials including intermediate-dose

melphalan (100-140 mg/m^2), with or without fludarabine, 200 cGy TBI 200 alone or with fludarabine, and intermediate-dose busulfan (135-143).

Anti-thymocyte globulin or the anti-CD52 antibody alemtuzumab have also been included in some studies to reduce GvHD (136,142). Even though there is no consensus on which regimen is superior in terms of toxicity and efficacy, a planned autologous HSCT followed by a non-myeloablative or reduced-intensity allogeneic HSCT with G-CSF mobilized PBSC to reduce the risk of graft rejection and, possibly, determine higher graft-versus-myeloma effects appears to be the most widely used approach (108,109).

Recently, an EBMT study has retrospectively compared the clinical outcomes of allogeneic HSCT after either reduced-intensity or myeloablative conditioning regimens in patients transplanted between 1998-2002 (144). One-hundred-ninety-six patients conditioned with myeloablative regimens were compared with 321 patients conditioned with non-myeloablative or reduced-intensity regimens between 1998-2002. Though TRM was significantly lower in the reduced-intensity group (24% vs. 37% at 2 years, $p = 0.002$), no statistical differences in overall and progression-free survivals were observed between the two groups by multivariate analysis. This finding was due to significantly higher relapse rate in the reduced-intensity group ($p = 0.0001$). The use of less intense regimens can indeed come at the cost of higher relapse rates, however, the conclusions of this study should be considered with some caution as many selection biases are evident between the two cohorts of patients. In the reduced-intensity group, there was a remarkably higher number of patients who failed one

or more autologous transplants, more patients with refractory disease, and more T-cell depleted allografts, and higher use of unrelated donors.

The concept of "Mendelian or genetic randomization" has recently been applied to assess clinical outcomes between patients with hematological malignancies treated with allografting or other therapies (145-148). Though not universally accepted, this method relies on the biological process, described by Mendel, through which offspring randomly inherit genetic traits half from the mother and half from the father. One in four siblings is then expected to have a potential HLA-identical sibling donor. The comparison by the intention-to-treat principle between patients with HLA-identical siblings, who can be assigned to allografting, and those without, who cannot receive an allograft, is used as a surrogate for an unbiased randomization. Only a formal statistical randomization, however, between patients with suitable donors could provide stronger evidence. A French study initially compared two protocols which enrolled high risk myeloma patients in the light of elevated serum β2-microglobulin and del(13) (149). All patients underwent a first autologous transplant after high dose melphalan at 200 mg/m^2. Sixty-five patients with HLA-identical sibling donors received an allograft after a reduced intensity conditioning with busulfan, fludarabine and high-dose anti-thymocyte globulin at 12.5 mg/kg. These patients were compared with 219 patients without a suitable sibling donor who were treated with a second autograft after melphalan at 220 mg/m^2. TRM and response rates were not statistically different. After a median follow-up of 2 years, overall and event free survivals were 35% and 25%, and 41% and 30% for the double

autologous and the autologous-allogeneic groups, respectively. These findings may indicate that patients with poor prognostic factors such as del(13) and high β2-microglobulin may not benefit from reduced-intensity allogeneic HSCT. Though a remarkably low 7% incidence of chronic GvHD was reported, the high dose of anti-thymocyte globulin is a matter of concern as it may have highly prevented a strong graft-vs.-myeloma effect. However, another report by Kroger et al. showed that del(13) was an independent, poor-risk factor for overall and progression-free survival after reduced-intensity allogeneic HSCT, given a higher risk of relapse (150). A recent study reported on 245 consecutive myeloma patients, up to the age of 65 years, who were newly diagnosed between 1998-2004. One-hundred-sixty-two of 199 patients with at least one sibling were HLA-typed with their potential sibling donors. All patients received induction with two to three cycles of VAD-based regimens, cyclophosphamide and G-CSF for PBSC mobilization followed by autologous HSCT after melphalan at 200 mg/m^2 (151). Eighty patients with an HLA-identical sibling were offered TBI-based non-myeloablative conditioning followed by allogeneic HSCT with G-CSF mobilized PBSC, whereas 82 patients without an HLA-identical sibling were assigned to receive a second autologous transplant after high-dose (140-200 mg/m^2) or intermediate-dose (100 mg/m^2) melphalan-based conditioning regimens. The new feature of this study was the assignment of treatment in function of a single criterion: presence/absence of an HLA-identical sibling donor, regardless of disease stage and prognostic factors. By intent-to-treat analysis, after a median follow up of 45 months, overall and event-free survivals were significantly longer

in patients with donors: 80 versus 54 months (p = 0.01) and 35 versus 29 months (p = 0.02), respectively. By multivariate analysis, the presence of HLA-identical siblings was an independent variable significantly associated with longer overall and event-free survivals. Fifty-eight and 46 patients completed the tandem autologous-allogeneic and the tandem autologous HSCT programs, respectively. CCR rates were 55% and 26% with the tandem autologous-allogeneic and the tandem autologous (p = 0.004), whereas TRM was 10% and 2% respectively (p = non-significant). Median overall survival was not reached in the tandem autologous-allogeneic group and was 58 months in the tandem autologous group (p = 0.03). Event-free survival was 43 and 33 months, respectively (p=0.07). Given that cytogenetic information was available in only a third of the patients registered in the study, the impact of del(13) after the tandem autologous-allogeneic HSCT approach could not be determined. However, though exploratory with low statistical power, a stratified analysis on the intent-to-treat population that defined high risk patients in the light of high β2-microglobulin levels or del(13), reported adjusted hazard ratios of 0.34 and 0.52 for overall and event-free survivals, respectively, similar to those obtained in the whole series. This finding suggests that patients with an HLA-identical sibling have better overall and event-free survivals as compared to those without an HLA-identical sibling. Large prospective studies, based on the Mendelian randomization principle, such as the BMT-CTN-0102 trial in the U.S.A. and the Dutch-Belgian Hemato-Oncology Cooperative Group (H.O.V.O.N.) trial in Europe are currently in progress and will offer helpful information to determine the role of the tandem

autologous-allogeneic HSCT approach using a TBI-based non-myeloablative conditioning regimen in the next few years. An extended phase II trail of 106 newly diagnosed myeloma patients transplanted with the Seattle regimen was recently presented by Italian Group for Bone Marrow Transplantation (G.I.T.M.O.) (152). After a median follow-up of 54 months, overall survival was not reached and event-free survival was 35 months. Overall response, defined as combined CCR and partial remission, was 91% with 53 patients achieving CCR after allografting. Response prior to allogeneic HSCT was significantly associated with the achievement of post-transplant CCR and longer event-free survival. Interestingly, chronic GvHD was not correlated with either the achievement of CCR or response duration.

GRAFT-VERSUS-MYELOMA EFFECTS AND GRAFT-VERSUS-HOST DISEASE

The unique and potentially eradicating effect of allografting relies on the immune attack of donor T cells against disease-specific antigens capable of inducing a potent graft-versus-myeloma effect. Initial evidence for the existence of such an effect was the transfer of myeloma idiotype-specific immunity from an actively immunised marrow donor to the recipient (153). Anecdotal observations of complete responses after the infusion of donor lymphocytes or withdrawal of immunosuppression in patients with persistent or relapsed disease after allografting were further evidence (154-157). Subsequent larger studies showed

that, though donor lymphocyte infusions (DLI) could induce response rates up to 50%, durable complete responses were, however, achieved in only a minority of patients (103,158,159). Furthermore, this cell therapy was often associated with clinical GvHD. Lokhorst et al. reported on 27 relapsed patients who received 52 DLI at a median of 30 months after allogeneic HSCT (160,161). Debulking therapy was administered to 13 patients before DLI. Overall 14 patients (52%) responded, including 6 (22%) who achieved CCR. Major toxicity was acute and chronic GvHD present in 55% and 26% of patients respectively. Median overall survival was 18 months, 11 for patients who did not respond and not yet reached for responding patients. Other studies reported that the strongest predictors for response following DLI were acute and chronic GvHD (162,163). The Authors concluded that both GvHD and graft-versus-myeloma shared the same antigenic targets.

GvHD and its treatment-related complications have always been a matter of concern for clinicians. Moreover, chronic GvHD can highly affect the patient's quality of life. GvHD can indeed be almost completed eliminated by T-cell depletion of the donor graft. However, this manipulation has invariably been associated with higher risk of relapse of the underlying hematological malignancies (164). Though the experience is limited in myeloma, partial T-cell depletion to allow donor engraftment and limit the risk of GvHD has also been investigated. Alyea et al. used a myeloablative conditioning regimen followed by a CD6-depleted donor bone marrow graft. Selected $CD4^{+}$-donor lymphocytes were infused later to evoke graft-versus-myeloma effect (112). The incidence of

grade II-III GvHD was 21%, and TRM was 10%. Only one patient achieved a CCR at 6 months without the addition of donor lymphocytes. Fourteen out of 24 patients received donor lymphocytes and response was observed in 10 pateints, with 7 developing acute or chronic GvHD. Two-year overall and progression-free survivals were 55% and 42% respectively. Importantly, donor lymphocytes could not be given as scheduled to 42% of patients, either as a result of GvHD or other transplant-related complications. The use of alemtuzumab (a.k.a. Campath), a monoclonal anti-CD52 antibody, has also been explored to reduce the incidence of GvHD, either by treatment "in the bag" or by systemic infusion prior to the conditioning regimen (136,165). Though the incidence of GvHD was significantly reduced, the use of alemtuzumab clearly affected disease responses and their duration (136). All these observations clearly indicate the important role of donor T cells in providing efficient graft-versus-myeloma activity. Lokhorst et al. recently reported on a prospective phase III study by the H.O.V.O.N. group (166,167). Fifty-three patients with an HLA-identical sibling underwent a partially T-cell-depleted allograft as part of their initial treatment plan. The overall response was 89%, including 19% CCR. After a median follow-up of 38 months post-transplant, 20 patients were alive and 33 dead, 14 from progressive disease and 18 from TRM. Median overall and progression-free survivals after allografting were 17 and 25 months respectively. Only 3 patients were in continuous CCR. This prospective multi-center study did not support the use of T-cell-depleted myeloablative allogeneic HSCT in myeloma. The strategy of pre-emptive DLI after partially T-cell depleted allografting has also been evaluated.

Levenga et al. reported on 24 myeloma patients treated with a partially T-cell-depleted myeloablative allogeneic HSCT (168). Patients enrolled in the study were intended to receive subsequent pre-emptive DLI. Twenty of 24 patients responded with 10 patients (42%) reaching complete remission. One-year TRM was 29%. Overall, 13 patients (54%) received pre-emptive lymphocyte infusions. GvHD higher than grade I following the infusion developed in 4 (30%). After a median follow-up of 67 months, 11 patients (46%) were alive, 7 of whom (29%) in continuous CCR including 4 in molecular remission. All these patients had received pre-emptive lymphocytes.

Though GvHD was associated with disease response in the majority of clinical trials, it is encouraging that more recent studies employing non-myeloablative conditionings did not correlate disease response and its duration with the development of chronic GvHD (151,152). In a recent evaluation of 106 patients enrolled in a prospective phase II study, the development of both acute or chronic GvHD was not significantly associated with either the achievement of complete remission or its duration post transplant (152). This is consistent with the notion that GvHD may be not essential for graft-versus-myeloma, though the relationship between the two phenomena appeared strong in studies employing myeloablative conditioning. New methods to augment graft-versus-myeloma effects to allow long-term disease control and possibly decrease toxicity are presented below.

FUTURE DEVELOPMENTS

Tandem autologous-allogeneic HSCT approaches are currently widely used in clinical trials. The rationale of the tandem approach is to separate temporally the high-dose chemotherapy from allografting, to combine the benefits of autologous HSCT (higher disease response and prolonged survival compared to conventional chemotherapy) and allogeneic HSCT (graft-versus-myeloma effects) while reducing transplant-related toxicities. Drastic reduction in early TRM and CCR rates of over 50% including molecular remissions have been reported. However, the risk of relapse is not negligible. New methods to augment graft-versus-myeloma effects should be explored to allow better long-term disease control. For this purpose, allogeneic HSCT and new drugs with molecular targets, such as thalidomide, lenalidomide and bortezomib, should not be viewed as mutually exclusive. Bortezomib and thalidomide re-induce responses in relapsed patients following allografting and may also be employed to achieve profound cytoreduction and reduce myeloma to a minimal residual state before allografts (169-172). Thus, it is imperative to thoroughly explore their roles in increasing the efficacy of tandem autologous-allogeneic HSCT. Major improvements will also lye in the separation of the potentially eradicating graft-versus-myeloma effects from the detrimental GvHD. New insights into the pathophysiology of acute GvHD have led to the development of conditioning regimens with total-lymphoid irradiation that reduce its incidence, but appear to preserve the anti-tumor effects

of donor T cells (173). Furthermore, the identification of disease-specific antigens may trigger more potent myeloma-specific immune responses of donor cytotoxic T cells (174). The recurrent observation that allografting at an earlier phase of the disease is associated with more effective GVM may also be related to an expression profile of potential antigenic targets for T cells that varies through the various disease phases. Siegel et al. recently identified HLA-A*0201-presented T cell epitopes derived from the oncofetal antigen-immature laminin receptor protein in hematological cancers which include myeloma (175). However, the expression of these antigens on myeloma cells is lost when the disease is advanced. In conclusion, the therapeutic role of allogeneic HSCT will ultimately be determined in control studies where patients are allocated treatment in the light of prognostic factors and groups are confronted in a randomized fashion.

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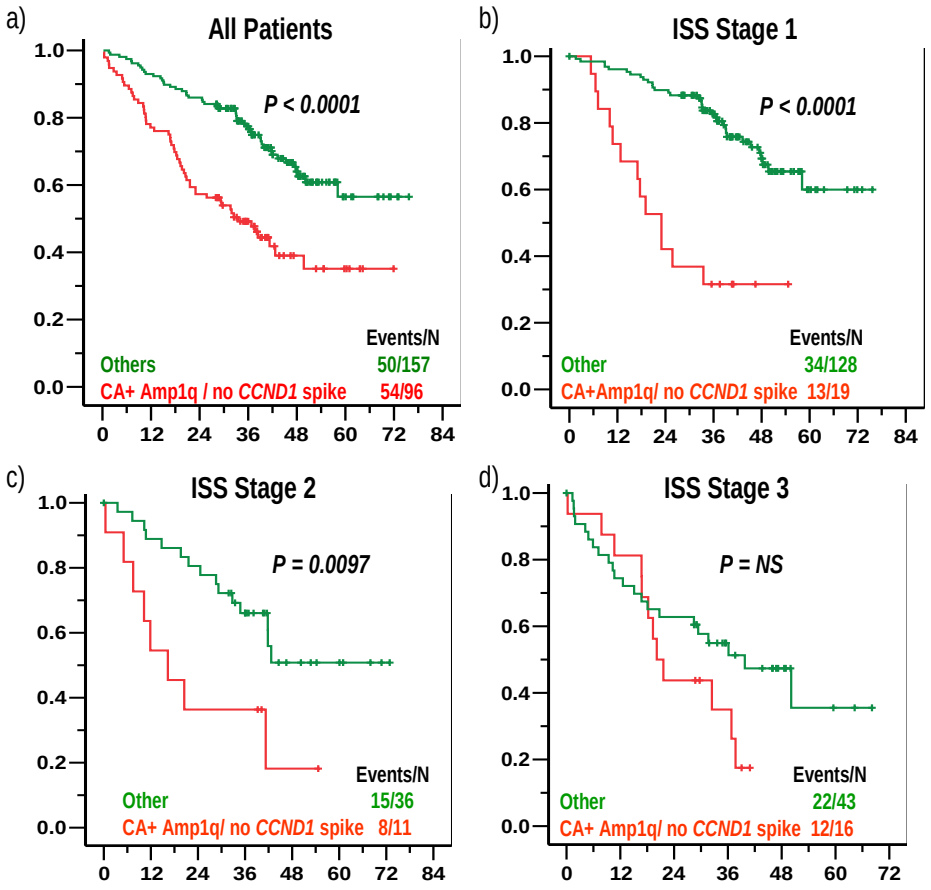
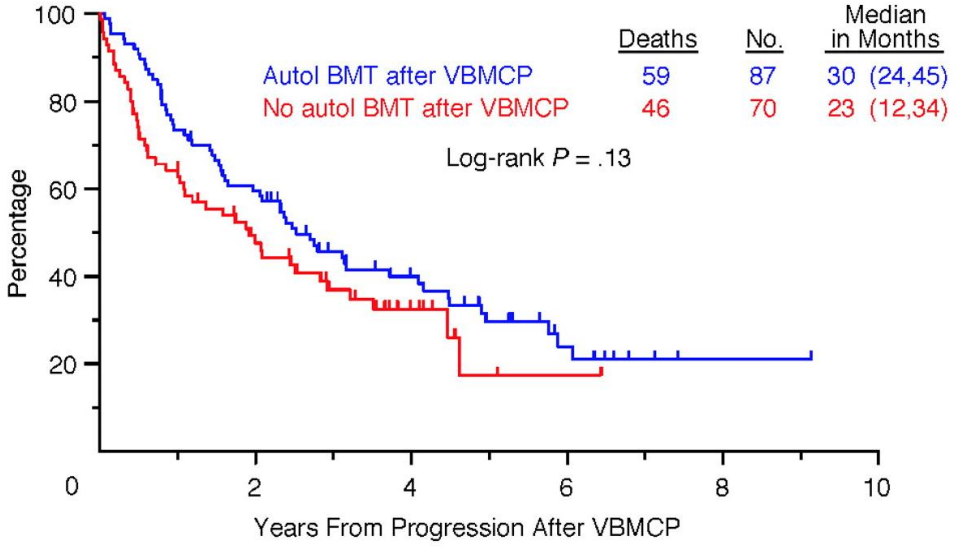


Figure 1. In a subgroup of 253/351 patients FISH analysis for amplification of 1q21 was performed. An inferior event-free survival was observed in patients with CA and amplification of 1q21 in the overall group and in ISS stages 1 and 2. Patients with *CCND1* spikes were grouped with those lacking CA or amplification of 1q21 or both.

Figure 2: Overall Survival according to salvage therapy



Errore. Il segnalibro non è definito.

Figure 3 depicts Kaplan-Meier plots of the durations of OS and EFS. Univariate and multivariate regression analyses revealed that CR, EFS and OS durations were independently favorably affected by the absence of metaphase cytogenetic abnormalities (CA) of the deletion13/hypodiploidy variety ("CA13/hypo"), low B2-microglobulin and CRP levels, and higher albumin and platelet concentrations.

Errore. Il segnalibro non è definito.

Table 1. Myeloablative Conditioning Regimens Utilized for Allogeneic HSCT in Multiple Myeloma

Author	Patients	Median Age (years)	Donor Related/Unrelated	Conditioning	Transplant-Related Mortality, %	Complete Remission, %	Overall Survival, %
Bensinger et al.[11,15]	136	43-48 (<60)	114/22	Bu, Cy, ±TBI	48 (at day 100) 63 (at 1 year)	34	22 (at 5 years)
Barlogie et al.[16]	36	≤55	36/0	Melphalan (100 mg/m^2), TBI (12Gy) Cy,TBI	53 (at 1 year)	---	39 (at 7 years)
Reece et al.[17]	26	43	22/4	Bu,Cy Melphalan (100 mg/m^2), TBI	19 (at day 100)	62	47 (at 3 years)
Alyea et al.[18]	24	46	24/0	Cy,TBI (14Gy) Bu,Cy Melphalan (110 mg/m^2), TBI	10	---	55 (at 2 years)
Kulkarni et al.[19]	33	38	29/4	(10.5Gy) Cy, TBI (9.5Gy) Cy, Melphalan Bu,Cy Cy, TBI (12Gy)	54	37	36 (at 3 years)
Le Blanc et al.[20]	37	47	37/0	Melphalan (140 mg/m^2), TBI (10.5Gy) Bu,Cy Others	22	57	32 (at 40 months)
Couban et al.[21]	22	43	22/0	Melphalan (160 mg/m^2), TBI (12Gy) Cy, TBI (12Gy) Bu,Cy	59	50	32 (at 3 years)

Varterasian et al.[22]	24	43	---	Cy, TBI Melphalan, TBI Bu,Cy, TMI Others	25	---	40 (at 3 years)

Table 1. Nonmyeloablative and Reduced Intensity Conditioning Regimens Utilized for Allogeneic HSCT in Multiple Myeloma

Author	Patients	Donor Related/Unrelated	Conditioning	Transplant-Related Mortality, %	Chronic GVHD, %	Complete Remission, %	Overall Survival, %
Mohty et al.[44]	41	41/0	Bu, Fluda, ATG	17	41	24	62 (at 2 years)
Peggs et al.[45]	20	12/8	TBI, Fluda, alemtuzumab[1]	15	---	10	71 (at 2 years)
Einsele et al.[46]	22	7/15	TBI(2Gy), Fluda, Cy	23	32	27	26 (at 2 years)
Giralt et al.[47]	22	11/9	Fluda , Melphalan (90/140 mg/m^2)	41	27	32	30 (at 2 years)
Gerull et al.[48]	52	32/20	TBI(2Gy), Fluda	17	70	27	41 (at 1.5 years)
Bruno et al.[49]	22	0/22	TBI(2Gy), Fluda	18	61	20	79$^{\phi}$/27$^{\gamma}$ (at 2 year)
Maloney et al.*[14]	54	52/0	TBI(2Gy)/(2Gy)TBI, Fluda	22	60	57	69 (at 5 years)
Lee et al.[50]	45	33/12	Melphalan (100 mg/m^2), TBI(2Gy), Fluda	38	13	64	36 (at 3 years)
Kroger et al.*[13]	17	9/8	Melphalan (100 mg/m^2), Flu, ATG	18	7	73	74 (at 2 years)
Kroger et al.[51]	21	0/21	Melphalan (100-140 mg/m^2), Flu, ATG	24	12	40	74 (at 2 years)
Galimberti et al.*[52]	20	20/0	TBI(2Gy), Fluda /Cy, Fluda	20	30	35	58 (at 2 years)