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Long-term follow up of tandem autologous-allogeneic hematopoietic cell transplantation for multiple myeloma

Enrico Maffini,[1] Barry E. Storer,[1,2] Brenda M. Sandmaier,[1,3] Benedetto Bruno,[4] Firoozeh Sahebi,[5] Judith A. Shizuru,[6] Thomas R. Chauncey,[1,3,7] Parameswaran Hari,[8] Thoralf Lange,[9] Michael A.Pulsipher,[10] Peter A. McSweeney,[11] Leona Holmberg,[1,2] Pamela S. Becker,[1,3] Damian J. Green,[1,3] Marco Mielcarek,[1,3] David G. Maloney,[1,3*] and Rainer Storb[1,3*]

[1]Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, WA, USA; [2]University of Washington School of Public Health, Seattle, WA, USA and [3]Department of Medicine, Seattle, WA, USA; [4]University of Turin, Department of Molecular Biotechnology and Health Sciences, Turin, Italy; [5]City of Hope National Medical Center/Southern California Kaiser Permanente Medical Group, Duarte, CA, USA; [6]Stanford University, CA, USA; [7]VA Puget Sound Medical Health Care System, Seattle, WA, USA; [8]Medical College of Wisconsin, Milwaukee, USA; [9]University of Leipzig, Germany; [10]Children's Hospital of Los Angeles, CA, USA and [11]Colorado Blood Cancer Institute, Denver, CO, USA

**Co-senior authors.*

ABSTRACT

We previously reported initial results in 102 multiple myeloma (MM) patients treated with sequential high-dose melphalan and autologous hematopoietic cell transplantation followed by 200 cGy total body irradiation with or without fludarabine 90 mg/m^2 and allogeneic hematopoietic cell transplantation. Here we present long-term clinical outcomes among the 102 initial patients and among 142 additional patients, with a median follow up of 8.3 (range 1.0-18.1) years. Donors included human leukocyte antigen identical siblings (n=179) and HLA-matched unrelated donors (n=65). A total of 209 patients (86%) received tandem autologous-allogeneic upfront, while thirty-five patients (14%) had failed a previous autologous hematopoietic cell transplantation before the planned autologous-allogeneic transplantation. Thirty-one patients received maintenance treatment at a median of 86 days (range, 61-150) after allogeneic transplantation. Five-year rates of overall survival (OS) and progression-free survival (PFS) were 54% and 31%, respectively. Ten-year OS and PFS were 41% and 19%, respectively. Overall non-relapse mortality was 2% at 100 days and 14% at five years. Patients with induction-refractory disease and those with high-risk biological features experienced shorter OS and PFS. A total of 152 patients experienced disease relapse and 117 of those received salvage treatment. Eighty-three of the 117 patients achieved a clinical response, and for those, the median duration of survival after relapse was 7.8 years. Moreover, a subset of patients who became negative for minimal residual disease (MRD) by flow cytometry experienced a significantly lower relapse rate as compared with MRD-positive patients (P=0.03). Our study showed that the graft-*versus*-myeloma effect after non-myeloablative allografting allowed long-term disease control in standard and high-risk patient subsets. Ultra-high-risk patients did not appear to benefit from tandem autologous/allogeneic hematopoietic cell transplantation because of early disease relapse. Incorporation of newer anti-MM agents into the initial induction treatments before tandem hematopoietic cell transplantation and during maintenance might improve outcomes of ultra-high-risk patients. Clinical trials included in this study are registered at: *clinicaltrials.gov identifiers: 00075478, 00005799, 01251575, 00078858, 00105001, 00027820, 00089011, 00003196, 00006251, 00793572, 00054353, 00014235, 00003954.*

Correspondence:

rstorb@fredhutch.org

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Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for multiple myeloma (MM) but its role is controversial. The first clinical experience with myeloablative regimens proved to be curative for a small proportion of patients but was accompanied by unacceptably high non-relapse mortality (NRM) rates.[1,2] The introduction of less intensive conditioning regimens for allogeneic HCT, which relied on graft-*versus*-tumor (GvT) effects for tumor eradication, lowered NRM but at the expense of higher disease relapse rates.[3,4] In the late 1990s, combining cytoreductive high-dose chemotherapy before autologous HCT with subsequent minimal intensity conditioning allogeneic HCT, an approach aimed at inducing GvT effects, proved to be less toxic than myeloablative allogeneic HCT and was well tolerated.[5,6] Seven prospective trials compared clinical outcomes of autologous HCT *versus* tandem autologous/minimal intensity allogeneic HCT in newly diagnosed MM patients and yielded discordant results regarding depth of response, overall survival (OS), and progression-free survival (PFS). Differences in conditioning regimens, as well as graft-*versus*-host disease (GvHD) prophylaxis, including ATG use, patient selection, definition of MM risk profiles, and duration of follow up, made meaningful comparisons between trials difficult.[7-15] We previously reported initial results in 102 MM patients given tandem high-dose melphalan and autologous HCT followed by 200 cGy total body irradiation (TBI) with or without fludarabine 90mg/m^2 and HLA-matched HCT from related or unrelated donors.[16] Here we update the early observations and add results from 142 additional patients treated with the same approach for a total of 244 patients with a median follow up of 8.3 years (range, 1.0-18.1).

Methods

Patients

From August 1998 to January 2016, 244 MM patients completed sequential treatment with high-dose melphalan and autologous HCT followed by 200 cGy TBI ± fludarabine and allogeneic, granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cell (PBMC) infusion. One hundred and sixty-four (67%) patients were transplanted at the Fred Hutchinson Cancer Research Center (Fred Hutch), Seattle, WA, USA, and 80 (33%) patients received their transplant at eight other institutions. All patients included in the analysis were treated under eighteen clinical trials that were co-ordinated by Fred Hutch, approved by each institution's review board, and registered at "*clinicaltrials.gov*". All patients and donors signed written informed consent in accordance with the Declaration of Helsinki. The nature of the analysis is retrospective, and we present clinical data for those 244 patients who received both autologous and allogeneic HCT. Patients' characteristics are detailed in Table 1. Median age at diagnosis was 51 years (range, 25-67). Ninety-seven (42%) patients had high-risk cytogenetics. Fifty-seven (25%) had high-risk disease according to International Staging System (ISS) stage III and 36 (16%) according to Revised ISS (R-ISS) stage. Ninety-one (37%) had received more than one induction therapy line for unresponsive disease. A total of 209 patients (86%) received tandem autologous-allogeneic upfront while 35 patients (14%) had failed a previous autologous HCT before the planned autologous-allogeneic HCT.

Definitions and risk assessment

Beta-2 (β2)-microglobulin and serum albumin values at diagnosis were available for 225 (92%) patients and were used to calculate risk according to the ISS.[17] The R-ISS was introduced in 2015,[18] and we calculated it retrospectively for 217 (89%) patients. Lactate dehydrogenase (LDH) serum levels[19] at diagnosis were available in 216 patients. Conventional cytogenetics and/or fluorescence *in situ* hybridization (FISH) studies at diagnosis and at any time before allogeneic HCT were available for 232 patients. High-risk cytogenetics were defined as follows: t(4;14);[20] t(14;16);[21] t(14;20)[22] by FISH; del (17/17p),[23] 1q21 amplifications[24] both by FISH and conventional karyotyping; and non-hyperdiploid karyotype[25] by conventional cytogenetics. Plasma cell leukemia included circulating plasma cells ≥ 20% of complete blood count or ≥2000 plasma cells per microliter.[26] Extramedullary disease at diagnosis was defined as extramedullary plasmacytomas.[27] Patients were considered high risk if they had one of the following: ISS stage III, high-risk genetic lesions, extramedullary disease presentation, plasma cell leukemia, LDH levels ≥ 2 upper normal limits or failed previous autologous HCT. Ultra-high-risk was defined as having ≥ 2 adverse factors.[23,28] All patients not meeting previous criteria were considered standard risk.

HLA-typing

Patients and donors were matched for HLA-A, HLA-B and HLA-C by at least intermediate resolution DNA typing and for HLA-DRB1 and DQB1 by high-resolution techniques, as previously described.[29] Donors were HLA-identical siblings in 179 cases and HLA-matched unrelated in 65 cases; 11 unrelated donors were mismatched with their recipients for a single HLA allele (n=7) or antigen (n=4).

Autologous hematopoietic cell transplantation

After induction treatment, patients proceeded to mobilization and collection of PBMC. Mobilization regimens included: cyclophosphamide plus dexamethasone (35% of patients), cyclophosphamide plus etoposide and dexamethasone (CED) (24%), cyclophosphamide plus paclitaxel (16%), VTD-PACE (bortezomib-thalidomide-dexamethasone-cisplatin-doxorubicin-cyclophosphamide-etoposide) (8%), VRD-PACE (bortezomib-lenalidomide-dexamethasone-cisplatin-doxorubicin-cyclophosphamide-etoposide) (5%), carfilzomib plus RD-PACE (lenalidomide-dexamethasone-cisplatin-doxorubicin-cyclophosphamide-etoposide) (1%), cyclophosphamide plus etoposide and carboplatinum (CEP) (2%), bendamustine plus etoposide and dexamethasone (BED) (1%), Hyper-CVAD (cyclophosphamide-vincristine-doxorubicine-dexamethasone-adenosine arabinoside-mesna-methotrexate) (1%), or G-CSF (10 μg/kg) alone in 7% of the patients. After PBMC collection, patients received melphalan at 200 mg/m^2 intravenously (N.B. 3 patients received melphalan 140 mg/m^2 because of impaired renal function) before autologous PBMC infusion, with a median of 7.8 (range, 2.1-30.4) × 10^6 CD34$^+$ cells/kg actual body weight.

Allogeneic hematopoietic cell transplantation

After complete recovery from autologous HCT, patients proceeded to allogeneic HCT at a median of 75 days (range, 40-281). No further therapy was given between autologous and allogeneic HCT. The conditioning regimen for allogeneic HCT consisted of 200 cGy TBI at 7 cGy/minute from a linear accelerator (n=163) or two opposing Cobalt-60 sources (n=81). Recipients of unrelated grafts (n=65) received in addition three daily doses of fludarabine for a total of 90 mg/m^2. PBMC grafts contained a median of 9.0 (range, 1.7-24.0) x 10^6 CD34$^+$ cells/kg actual body weight. Post-grafting immunosuppression included mycophenolate mofetil

Table 1. Patients' characteristics.

Characteristics	Total (n)	%
Baseline characteristics at diagnosis	**244**	
Median age, years (range)	51 (25–67)	
Male: female	143–101	59/41
Isotype		
IgG	151	62
IgA	51	22
Light chains only	30	12
Non-secretory	3	1
IgD	3	1
Plasma cell leukemia	6	2
Renal failure (serum creatinine > 2 mg/dL)	39	18
LDH values ≥ 2 ULN	60/216	28
ISS	225	
Stage I	72	32
Stage II	96	43
Stage III	57	25
R-ISS	217	89
Stage I	38	18
Stage II	143	66
Stage III	36	16
Cytogenetics, high-risk	97/232	42
≥ 2 high-risk cytogenetic abnormalities	31	32
del(17p)	28	29
t (4;14)	22	23
amp1q	9	9
Others [hypoploidy; t (14;16); t (14;20)]	7	7
Extramedullary disease (plasmacytomas)	50	20
Disease risk	214	88
Standard risk	62	28
High risk	73	35
Ultra-high risk	79	37
Characteristics at autologous HCT	**244**	
Median time from diagnosis to autologous HCT, years (range)	0.8 (0.2–18.1)	
Failed previous autograft	35	14
Induction regimens		
VAD-based	125	51
IMiDs-based	30	12
PIs-based	15	6
IMiDs + PIs	56	24
Other (MP; HD-Dex; Dex-Cy)	18	7
Median induction lines of therapy, n (range)	1 (1–5)	
Characteristics at allogeneic HCT	**244**	
Median age, years (range)	53 (25–71)	
Patients > 60 years old	50	20
Median time from autograft to allogeneic HCT, days (range)	75 (40–281)	
Patients with induction therapy-refractory disease	42	17
Sibling, unrelated donor	179 – 65	73/27
Median $CD34^+$/kg infused, n (range)	9.00 x 10^6 (1.7–24.0)	
Median $CD3^+$/kg infused, n (range)	3.28 x 10^8 (0.4–11.7)	

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CMV serostatus	212	87
Recipients positive	113	53
Recipients negative with Donor positive	30	14
Recipients and Donor negative	69	33
HCT-CI score	206/244	84
0	59	29
1 or 2	79	38
≥ 3	68	33
KPS scale < 80%	42/218	19
Allogeneic HCT conditioning		
TBI 200 cGy	164	67
TBI 200 cGy + fludarabine 90mg/m^2	80	33
GvHD prophylaxis		
MMF + CSP	176	72
MMF + TAC	56	23
MMF + CSP/TAC + SRL	12	5
Median follow up for surviving patients years (range)	8.3 (1.0–18.1)	

CMV: cytomegalovirus; CSP: cyclosporine; Dex-Cy: dexamethasone + cyclophosphamide; GvHD: graft-*versus*-host disease; HCT: hematopoietic cell transplantation; HCT-CI: hematopoietic cell transplantation-comorbidity index; HD-Dex: high-dose dexamethasone; IMiDs: immunomodulatory drugs; KPS: Karnofsky Performance Status; LDH: lactate dehydrogenase; MMF: mycophenolate mofetil; MP: melphalan + prednisone; n: number; PIs: proteasome inhibitors; R-ISS: Revised-International Staging System; SRL: sirolimus; TAC: tacrolimus; TBI: total body irradiation; ULN: upper limit of normal; VAD: vincristine + doxorubicin + dexamethasone.

Table 2. Disease response.

Disease status	Status at autologous HCT n= 244	%	Status at allogeneic HCT n= 244	%	Best response after allogeneic HCT n= 244	%
CR	12	5	62	26	111	46
VGPR	35	14	47	19	42	17
PR	106	42	93	38	49	20
RD/PD	91	39	42	17	42	17

CR: complete remission; HCT: hematopoietic cell transplantation; n: number; PD: progressive disease; PR: partial response; RD: refractory disease; VGPR: very good partial response.

(MMF) (from a minimum of 28 days for sibling recipients to a maximum of 180 days for unrelated donors) and a calcineurin inhibitor (CNI) of either cyclosporine (n=176) or tacrolimus (n=56) for a minimum of 80 days with a subsequent taper to 180 days, as previously described.[5] Twelve patients received sirolimus in addition to MMF and CNI at the dose of 2 mg orally once daily from day -3 to day +80 (n=4), day +180 (n=6), and day +365 (n=2).[30] Thirty-one patients included in the analysis also received bortezomib (n=21; either at 1.6 mg/m^2 intravenously or 2.6 mg/m^2 subcutaneously every 14 days for up to 9 months) or lenalidomide (n=10; starting dose of 10 mg per day, range: 5-25 mg per day, on days 1-21 of each 28-day cycle, for 12 cycles of planned treatment) as maintenance treatment after allogeneic HCT, per protocol, as specified in the Results section.

Chimerism evaluation

Donor chimerism was assessed at days 28, 56, 84, 180 and 365 after allogeneic HCT on peripheral blood CD3$^+$ T lymphocytes and CD33$^+$ myeloid cells, while unfractionated marrow was analyzed only on day +84. This involved FISH analyses in sex-mismatched pairs and polymerase chain reaction-based studies of polymorphic microsatellite regions in all other patients.[31]

Disease response assessment

Disease responses were based on the 2016 Uniform Response Criteria developed by the International Multiple Myeloma Working Group[32] with some minor modifications. Complete response (CR) required negative immunofixation (IFIX) on the serum and urine, disappearance of any soft tissue plasmacytomas and/or osteolytic bone lesions, <5% plasma cells in bone marrow aspirates, and no evidence of clonal disease on flow cytometry analysis; very good partial response (VGPR) was defined as serum and urine M-protein detectable by IFIX but not on electrophoresis (SPEP) or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg/24 hours (h). Partial response (PR) required ≥75% reduction of serum M-protein and reduction in 24 h urinary M-protein by ≥90% or to <200 mg/24 h and no increases in sizes or numbers of soft tissue plasmacytomas and/or lytic bone lesions; stable or progressive disease (PD) before autologous HCT was defined as chemo-refractory disease, while the achievement of at least a PR as chemotherapy-sensitivity disease. Patients were evaluated both before autologous HCT and before allogeneic HCT in order to estimate the baseline levels of disease activity before each transplantation, again on days 28, 56, 84 and 180 after allogeneic HCT, and thereafter on a clinical basis. Disease evaluation includ-

ed serum and urine SPEP and IFIX for M-protein detection and quantification, plasma cell quantification, cytogenetics and FISH studies in the marrow, and radiological imaging to assess for osteolytic lesions/plasmacytomas whenever appropriate. Six-color multi-parameter flow cytometry analysis of marrow cells for detection of minimal residual disease (MRD) was carried out for a subset of patients who achieved IFIX-negative CR after tandem autologous-allogeneic HCT and were treated at Fred Hutch (n=28). Samples were analyzed at the University of Washington Hematopathology Laboratory. The sensitivity of the flow cytometry assay for plasma cell neoplasms ranged from 0.01 to 0.001%. MRD negativity (MRD^{NEG}) status was defined as no evidence of quantifiably detectable disease.

Graft-*versus*-host disease evaluation

Grading of acute and chronic GvHD was performed according to previously described methods.[33,34] Information regarding the administration of systemic immunosuppressive treatment for GvHD was collected prospectively.

End points and statistical methods

Primary objectives of this study were OS and PFS. Secondary end points included: cumulative incidences of acute GvHD, chronic GvHD, NRM, disease response, and disease relapse. We also examined response to treatment and survival among those patients who experienced disease relapse after allogeneic HCT. OS, PFS, and NRM were defined as the times from allogeneic HCT to death, death or progression, and death without progression, respectively. Probabilities of OS and PFS were estimated using the Kaplan-Meier method; cumulative incidences of relapse, NRM, and GvHD were estimated taking competing risks into account. Cox and Fine & Gray regression models were used to

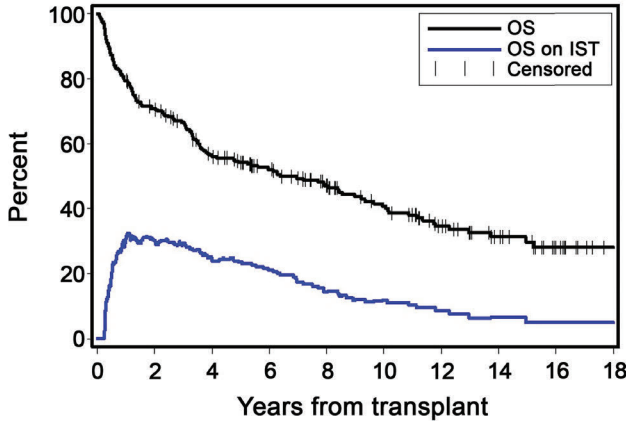

Figure 1. Prevalence curve of patients alive requiring immunosuppressive treatments (IST) for chronic graft-*versus*-host-disease (GvHD). Black line represents the standard survival curve (OS) for the entire patient cohort (n=244). Patients alive and on IST for chronic GvHD treatment are represented by the blue curve. The graphical difference between the two curves is the fraction of patients alive and off IST, during different time points.

Table 3. Causes of death after allogeneic hematopoietic cell transplantation.

Events	Number	Time (months) after allografting
Disease progression	104	Median time: 36.6 (range: 1–179)
NRM	40	Median time: 10.9 (range: 2–183)
Acute GvHD	10	Median: 3.5
Gut GvHD	4	(3, 3, 3, 4)
Cerebral aspergillosis	1	(5)
Cerebral ischemia r/to septic emboli	1	(3)
Aspergillosis + TTP/HUS	1	(6)
CMV disease	2	(2, 4)
Sepsis	1	(5)
Chronic GvHD	20	Median: 12
Respiratory syncytial virus pneumonia	1	(7)
Sepsis	7	(6, 8, 18, 23, 11, 58, 15)
Bronchiolitis obliterans	6	(9, 10, 14, 16, 37, 112)
Pneumonia	5	(7, 7, 11, 13, 142)
Invasive aspergillosis	1	(11)
Second cancers	5	Median: 121
Lung cancer	2	(4, 13)
Esophageal cancer	1	(130)
Pancreatic cancer	2	(121, 182)
Other	5	Median: 45
Traumatic head injury	1	(45)
Severe grand mal seizures	1	(64)
ARDS and alveolar hemorrhage	2	(3, 3)
Congestive heart failure	1	(136)

ARDS: acute respiratory distress syndrome; CMV: cytomegalovirus; GvHD: graft-*versus*-host disease; NRM: non-relapse mortality; TTP/HUS: thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome.

determine factors influencing HCT outcomes in univariate and multivariate analyses. Multivariate risk factors analysis included only variables significant at 0.05 level in univariate analysis for any OS, PFS, and relapse incidence. Acute GvHD, chronic GvHD, and post-transplant MM maintenance treatment (administered to 31 patients) were considered as time-dependent co-variates. Since no single variable, other than grade II-IV acute GvHD, had a significant correlation with NRM by univariate analysis, NRM was not incorporated into multivariate analysis.

Results

Disease response before and after autologous hematopoietic cell transplantation

Disease responses are summarized in Table 2. At the time of autologous HCT, there were no statistical significant differences among 125 patients who received vincristine – doxorubicin – dexamethasone (VAD)-based induction, mainly between 1998 and 2006, and those treated with immunomodulatory/proteasome inhibitor (n=101) triplet regimens (2006-2016) in terms of response rate: 74 (59%) *versus* 68 (68%) achieved at least a PR, respectively, and only 12 patients were in CR before autologous HCT. Of the 18 who received other induction therapies (melphalan plus prednisone, n=6; high-dose dexamethasone only, n=11; high-dose dexamethasone plus cyclophosphamide, n=1), 11 achieved PR and 7 PD. After high-dose melphalan and autologous HCT, 62 patients (26%) were in CR, 47 (19%) VGPR, 93 (38%) PR, and 42 (17%) had PD.

Allogeneic hematopoietic cell transplantation

Sixty-seven percent of the patients had allogeneic HCT within the first 3 months after autologous HCT, 29% between 3 and 6 months and 4% beyond 6 months. Reasons for allogeneic HCT delay included: delayed hemopoietic recovery (59%), abnormal hepatic/renal function (15%), active infection requiring intravenous antibiotics (13%), persisting mucositis (4%), cytomegalovirus reactivation requiring intravenous therapy (6%), and patient choice (3%). Results after allogeneic HCT are presented with a median follow up of 8.3 (range, 1.0–18.1) years among surviving patients.

Engraftment and GvHD - All 244 patients achieved sustained engraftment after allogeneic HCT. Median values of donor chimerism on $CD3^+$ T cells in peripheral blood on days 28, 84, and 180 were 89%, 95%, and 100%, respectively, while median values of donor chimerism of $CD33^+$ myeloid cells were 96%, 100%, and 100%, respectively. The cumulative incidence of grade II-IV acute GvHD was 44% with a median onset of 39 days (range, 6-124), of which 33% was grade II, 7% grade III, and 4% grade IV. Sibling recipients (n=176) experienced less grade II-IV acute GvHD than unrelated (n=65) recipients (38% *vs.* 64%; $P<0.001$). Of the 228 patients who survived

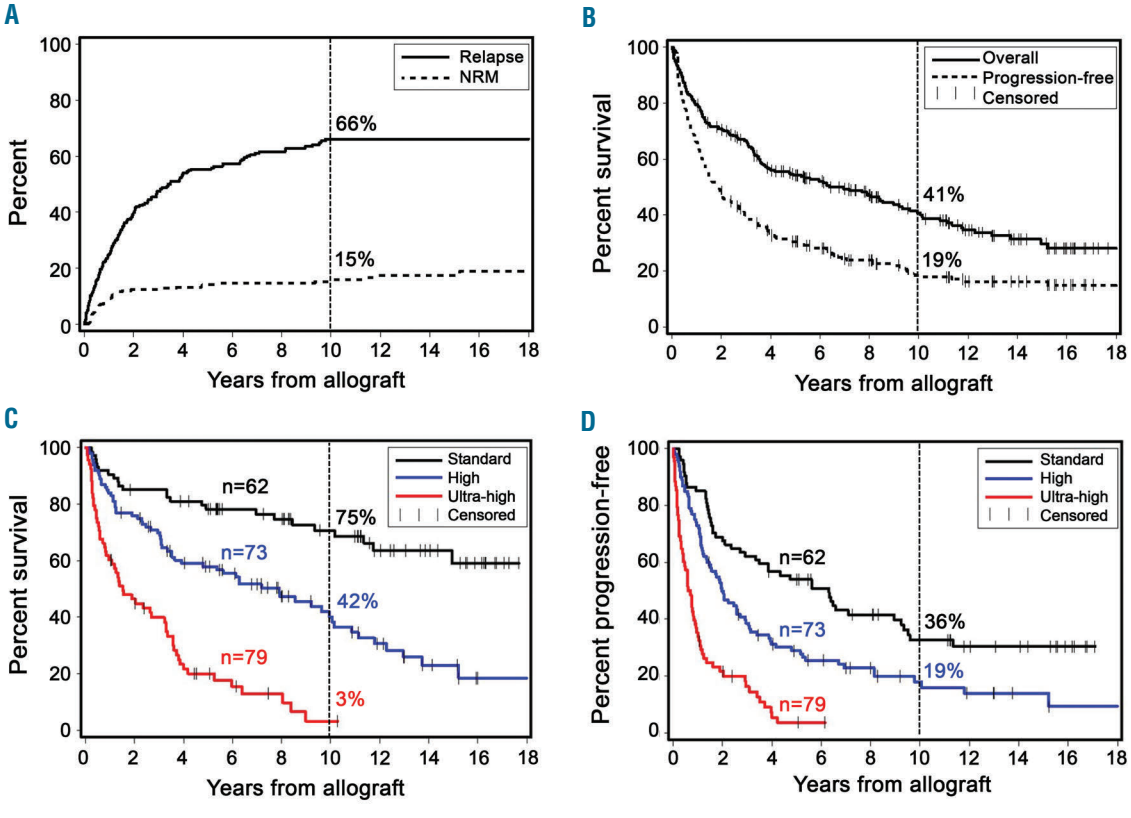

Figure 2. Long-term clinical outcomes after tandem autologous-allogeneic hematopoietic cell transplantation (HCT) for the entire population and stratified for disease-risk groups. Non-relapse mortality (NRM) and relapse incidence (A). Overall survival (OS) and progression-free survival (PFS) for the entire cohort (n=244) (B). OS (C) and PFS (D) for disease-risk stratification groups with standard-risk (n=62), high-risk (n=73), and ultra-high-risk (n=79).

longer than 100 days post-transplant, 122 developed chronic GvHD requiring treatment. The cumulative incidence of extensive chronic GvHD requiring systemic immunosuppression was 46% [95% Confidence Interval (CI): 39.5-52.4] at one year and 55% (95%CI: 47.7-60.7) at five years. No differences in chronic GvHD incidence were observed between related and unrelated recipients. Of all the surviving patients, 24% required immunosuppressive therapy for chronic GvHD at five years, 12% at ten years and 4% at 15 years, respectively (Figure 1).

Non-relapse mortality - NRM was 2% at day 100, 14% at five years and 15% at ten years after allogeneic HCT, respectively (Figure 2A). GvHD and treatment-related complications accounted for 30 of the 40 non-relapse-related deaths, while 5 patients died of secondary malignancies (Table 3) between 0.3 and 15.2 years after allografting and the remaining 5 died from other causes. Of note, no secondary hematologic cancers were observed. By univariate analysis, only the finding of grade II-IV acute GvHD [Hazard Ratio (HR) 3.12; 95%CI: 1.6-6.2; *P*=0.001] and ISS stage III at diagnosis (HR: 3.92; 95%CI: 2.0-7.6; *P*<0.001) significantly impacted NRM. Chronic GvHD, poor performance status, and comorbidities were not associated with higher risk of NRM.

Disease response and relapse - After allogeneic HCT, 111 (46%) patients achieved CR, 42 (17%) achieved VGPR, 49 achieved (20%) PR, and 42 (17%) failed to achieve a response (Table 2). Median time to best response after allografting was 6.68 months (range, 0.7-73.4). Among the 111 patients who achieved a CR as their best response, 46 remained in CR while 65 relapsed. Accordingly, the 10-year relapse incidence was 66% (95%CI: 59-72) (Figure 2A). Nineteen (12%) patients had late relapses beyond five years after allogeneic HCT (range, 5.2-9.8 years). Among patients who did not have extramedullary disease at diagnosis and who relapsed after allogeneic HCT (n=112), 28 (25%) showed extramedullary relapse (23 without evidence of marrow involvement). By multivariate analysis, ultra-high-risk patients (HR: 4.99; 95%CI: 2.9-8.7) and those with induction therapy-refractory disease before allografting (HR: 5.35; 95%CI: 3.4-8.6) had significantly higher relapse risks. The development of chronic GvHD did not protect against disease relapse (HR, 0.92; 95%CI: 0.6-1.3; *P*=0.66) (Table 4). Among patients with available marrow samples who achieved CR after allogeneic HCT (n=28), those with positive MRD (MRD^{POS}) detected by flow cytometry (n=15) experienced a higher disease relapse rate than MR^{NEG} patients (n=13) (HR: 10.4; 95%CI: 1.3-82.2; *P*=0.03).

Overall and progression-free survival - With a median follow up of 8.3 years (range, 1-18.1), median OS and PFS were 6.4 (95%CI: 3.9-9.2) years and 1.9 (95%CI: 1.4-2.6) years, respectively. Five-year OS and PFS were 54% (95%CI: 48-60) and 31% (95%CI: 25-36), respectively. Ten-year OS was 41% (95%CI: 34-48) and PFS was 19% (95%CI:13-24) (Figure 2B). By univariate analyses, ISS and R-ISS stage III, LDH >2 upper normal limits, high-risk cytogenetics, grade II-IV acute GvHD, extramedullary disease, induction-refractory disease, and a prior failed autologous HCT were all strongly associated with inferior OS and PFS. By multivariate analysis, only high and ultra-high disease risk and induction-refractory disease remained strongly associated with worsened rates of OS and shorter PFS (Table 4). Among patients with standard-risk disease (n=62), the median OS was not reached, whereas the median PFS was 6.5 (95%CI: 4.2-9.6) years. High-risk patients (n=73) experienced a median OS of 8.4 (95%CI: 3.9-10.2) years with a PFS of 2.5 (95%CI: 1.4-3.7) years, while ultra-high-risk patients (n=79) had a 2.3 (95%CI: 1.2-3.3) years median OS and 0.7 (95%CI: 0.6-0.9) year PFS (Figure 2C and D). Patients who proceeded to tandem transplantation after a previously failed autologous HCT (n=35) had poor outcomes, with a median OS of 1.2 years (95%CI: 0.6-2.0) years and a median PFS of 0.6 years (95%CI: 0.2-0.7). Similarly, patients who progressed after melphalan and autologous HCT (n=42) did poorly, having a median OS of 1.2 years (95%CI: 0.5-3.0) and median PFS of only 0.4 years (95%CI: 0.2-0.6) (Figure 3A and B); median time to relapse was 4.5 (0.1-61.9) months. Patients with extramedullary disease at diagnosis (n=50) showed median OS and PFS of 2.03 (95%CI: 1.0-3.5) and 0.95 (95%CI: 0.46-1.1) years, respectively. Patients achieving CR after allogeneic HCT displayed superior OS (median 14.9 *vs.* 3.2 years; HR: 3.2; 95%CI: 2.2-4.6), and PFS (median 6.9 *vs.* 0.9 years; HR: 4.6; 95%CI: 3.4-6.6) as compared with those who did not enter CR.

Maintenance treatments - Between May 2009 and February 2016, 31 patients received post-transplant maintenance treatment with bortezomib (n=21) or lenalidomide (n=10) starting between 61 and 150 days (median

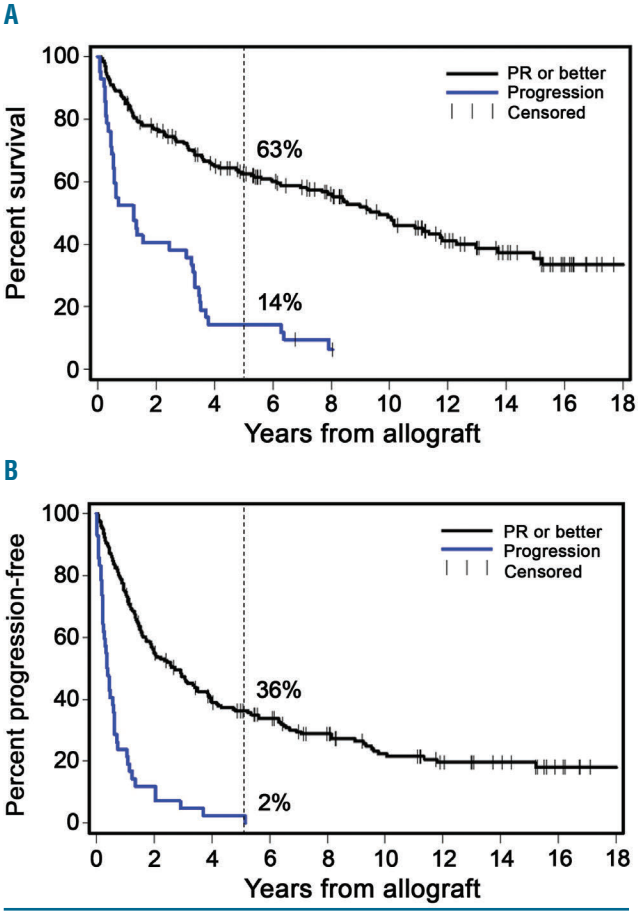

Figure 3. Kaplan-Meier estimates of overall survival (OS) and progression-free survival (PFS) stratified for disease-status after autologous hematopoietic cell transplantation (HCT). Overall survival (OS) (A) and progression-free survival (PFS) (B) among patients with progressive disease (n=42; blue line) and responders (n=202; black line).

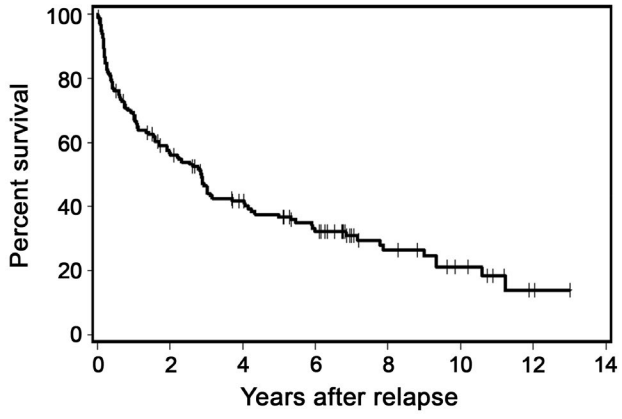

Figure 4. Survival from disease relapse/progression after allogeneic hematopoietic cell transplantation for an overall 152 relapsed patients.

86) after allogeneic HCT. Seventeen patients completed the planned treatment (lenalidomide, n=7; bortezomib, n=10). Disease progression was the reason for early treatment discontinuation in 9 of the treated patients. One patient on lenalidomide stopped the treatment due to an acute GvHD flare which was successfully treated. Other causes included: patient choice (n=1), diarrhea not GvHD-related (n=1), severe headache (n=1), and liver function abnormalities (n=1). By univariate analysis, maintenance therapy was not associated with any clinical outcome. Median OS was 6.3 (95%CI: 3.6-not reached). There was no difference in terms of median PFS between those patients who received maintenance treatment (n=31) after allogeneic HCT (2.56 years; 95%CI: 0.88-4.22) and those (n=175) who achieved a response after allogeneic HCT but did not receive maintenance (2.59 years; 95%CI: 1.86-3.50).

Survival after disease progression - One hundred and fifty-two patients (62%) experienced relapse or progression. Median survival after the first relapse/progression was 2.9 years (95%CI: 1.9-3.7) for the entire cohort (n=152) (Figure 4). Twenty-eight of the 152 received palliative best supportive care and died after a median of 2.1 months. One patient died during conditioning for a planned subsequent allogeneic HCT. Data on salvage therapy were not available for 5 patients (3%). One hundred and nineteen patients received a total of 228 lines of treatment, with a median of two lines (range, 1-9) of therapy each. Treatments included lenalidomide (n=67), bortezomib (n=54), thalidomide (n=36), alkylators/anthracyclines (n=27), pomalidomide (n=10), carfilzomib (n=9), daratumumab (n=1), and others (n=6). Twenty-seven (23%) of the 119 treated patients were unresponsive to salvage treatments and died after a median of 9.2 months (95%CI: 5.5-12.0), while the 83 patients who achieved at least a PR had a median survival of 7.8 (95%CI: 5.9-10.6) years. Disease responses for those 83 patients included: 29 CR (15 of these patients are still in CR, 4 are alive with PD, and 10 have since died), 13 VGPR (3 are still in VGPR, 2 are alive with PD, and 8 have since died), and 41 PR (9 currently with stable disease, 10 alive with PD, while 22 have since died). Data on disease response after salvage treatments were not available for 9 patients. Eighteen patients received a median of 2 donor lymphocyte infusions (range, 1-4) (preceded by chemotherapy in 9 patients) after a median of 1.4 years post-allografting (range, 0.9-8.3). Four of these achieved a partial response, while the remaining 14 did not show a response. Patients who relapsed during the first 18 months after allogeneic HCT (n=82) had a worsened prognosis (median survival after relapse/progression: 1.1 years; 95%CI: 0.6-1.9) compared to those (n=70) who relapsed beyond 18 months after allogeneic HCT (median survival after relapse/progression: 7.2 years; 95%CI: 4.3-10.6; *P*<0.0001).

Discussion

Advances in the understanding of MM biology have led to novel treatments that have dramatically prolonged PFS and OS. First-line autologous HCT has remained standard of care for eligible patients. Three randomized trials reported significantly superior median PFS, ranging between 25 and 32 months, as compared to conventional chemotherapy.[35-37] PFS was further improved up to 43-50 months after "new drugs" were employed both in the induction and consolidation/maintenance phases.[38-40] Whether double autologous transplants are superior to a single autograft remains to be determined.[41-43] In our series, an overall median PFS of 1.9 years may appear modest. However, when applying retrospective risk stratification, median PFS was 6.5 years in standard-risk patients, 2.5 year in high-risk patients, and 0.7 years in ultra-high-risk patients. Extramedullary relapse without marrow relapse has been frequent after allografting.[44-46] Sanctuary sites may be less accessible to graft-*versus*-myeloma effects than marrow. Of note, extra-medullary relapse occurred in 25% of current patients who did not have extramedullary involvement at diagnosis. Overall NRM was low (2% at

Table 4. Multivariate† risk factors in 211 patients.

	Relapse		Progression-free survival		Overall survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
High risk	1.74 (1.1–2.9)	0.03	1.62 (1.1–2.5)	0.03	2.48 (1.4–4.3)	0.001
Ultra-high-risk	4.99 (2.9–8.7)	<0.0001	3.47 (2.1–5.7)	<0.0001	3.87 (2.1–7.2)	<0.0001
Chemorefractory	5.35 (3.4–8.6)	<0.0001	4.61 (3.0–7.1)	<0.0001	3.28 (2.1–5.2)	<0.0001
Age ≥ 60 years at allo HCT	1.16 (0.8–1.8)	0.48	1.22 (0.8–1.8)	0.29	1.33 (0.9–2.0)	0.18
Unrelated donor	1.54 (1.0–2.3)	0.04	1.47 (1.0–2.1)	0.04	1.32 (0.9–2.0)	0.20

HR: Hazard Ratio; CI: Confidence Interval; allo HCT: allogeneic hematopoietic cell transplantation. †Includes variables significant at 0.05 level in univariate analysis for any end point.

100 days and 14% at 5 years) and, with a median follow up of 8.3 years, median OS was 6.4 years. By risk stratification, median OS was not reached in standard-risk patients, while high-risk and ultra-high-risk patients had worse outcomes with median OS of 8.4 and 2.3 years, respectively. Of note, only a minority of our patients achieved a complete remission after induction treatments, and a subset of them received tandem autologous-allogeneic HCT beyond first line. Moreover, none of our patients received recently Food and Drug Administration-approved monoclonal antibodies, such as daratumumab and elotuzumab, which have been associated with remarkable response rates.

Although restricted to a small group of patients, we demonstrated that the achievement of MRD^{NEG} predicted long-term CR among patients with IFIX-negative CR after autologous HCT. Whether long-term persistence of MRD^{NEG} indicates disease eradication is unclear. Multiparameter flow cytometry and PCR-based methods are two sensitive techniques currently used to evaluate MRD in MM. Evaluation of MRD through immuno-phenotyping is more broadly available than PCR-based methods. In the present series, patients who achieved MRD^{NEG} by flow cytometry experienced a significantly lower relapse rate as compared with MRD^{POS} patients (P=0.03). These findings confirm previous observations by Giaccone *et al*. who reported on the clinical impact of immuno-phenotypic remission after allografting in 66 MM patients.[47] Conditioning was 2 Gy TBI-based in 55 of the 66 patients. After a median follow up of 7.1 years, patients who achieved conventional CR and MRD^{NEG} disease status had better clinical outcomes in terms of OS (median not reached) and PFS (median 59 months). Moreover, Ladetto *et al*. reported a PCR-based molecular analysis of MRD after minimal-intensity TBI-based conditioning in newly diagnosed patients who had not been exposed to new drugs.[48] After a median follow up of 12.1 years, the median OS and PFS were not reached in patients who achieved PCR MRD negativity. Overall, MRD studies support the hypothesis that potentially curative graft-*versus*-myeloma effects after minimal intensity conditionings allowed long-term disease control and persistent yet non-progressive MRD in a subset of MM patients.

Whether graft-*versus*-myeloma effects are associated with chronic GvHD is still a subject of debate. Ringdén *et al*. evaluated the impact of acute and chronic GvHD on relapse and survival in 177 patients transplanted from HLA-identical siblings after non-myeloablative or reduced-intensity conditioning.[49] Acute GvHD was associated with a significantly higher risk of TRM, while limited chronic GvHD significantly reduced the risk of relapse. However, the reduced relapse risk did not translate into better OS. Crawley *et al*. reported that chronic GvHD was associated with better PFS and OS after reduced-intensity conditioning.[3] In the present study, we report an incidence of chronic GvHD of 55%, which, as in other comparative prospective trials,[7,9] was not associated with better disease control. A trend towards higher chronic GvHD rates after the introduction of minimal/reduced-intensity conditioning was shown in a Center for International Blood and Marrow Transplant Research (CIBMTR) analysis on 1207 MM recipients between 1989 and 2005. Overall, 50% of the patients who survived at least five years in the 2001-2005 cohort developed chronic GvHD, 65% of whom had extensive involvement.[4] Discontinuation of all immunosuppressive agents is a surrogate for achieving immunotolerance, and is associated with improved quality of life. Importantly, only a minority of current survivors remained on immunosuppressive drugs long-term: 24%, 12%, and 4% at five, ten, and 15 years, respectively.

Indications for allografting in MM have greatly changed over the years due to remarkable advances in the understanding of disease biology and new treatment modalities that increased median survival rates up to 8-10 years in standard-risk patients. However, relapse has remained a major issue, and poor outcomes have been observed in patients with high-risk/ultra-high-risk disease.[50] Interestingly, Sobh *et al*. described trends and clinical outcomes of allogeneic HCT for MM in Europe between 1990 and 2012.[51] The study included 7333 patients who were divided into 3 groups: 1) allogeneic HCT upfront (n=1924); 2) tandem autologous-allogeneic HCT (n=2004); and 3) allogeneic HCT as a second-line treatment or beyond (n=3405). A steady increase in numbers of allogeneic HCT over the years was observed. The use of upfront allogeneic HCT increased up to the year 2000, followed by a decline thereafter, representing 12% of allogeneic HCT performed in 2012. Tandem autologous-allogeneic HCT peaked around the year 2004 and represented 19% of allogeneic HCTs in 2012. Allogeneic HCT as salvage after at least one autograft has steadily increased over recent years and represented 69% of allogeneic HCTs in 2012. Unfortunately, only a minority of these patients were enrolled in controlled trials and remarkable heterogeneity in using allogeneic HCT was observed among different European countries.

The potential role combining "new drugs" with graft-*versus*-myeloma effects has not yet fully been explored. In a Phase II study, the feasibility of using bortezomib within a reduced-intensity conditioning regimen and as maintenance therapy post allograft was evaluated.[52] Conditioning consisted of fludarabine, melphalan, and bortezomib, while maintenance treatment consisted of 7 cycles of bortezomib. Sixteen high-risk patients who had relapsed after an autograft were prospectively enrolled. Nine of 16 patients (56%) achieved CR and 5 of 16 (31%) achieved PR after allogeneic HCT. In this heavily pretreated high-risk population, 3-year cumulative incidences of NRM, relapse, and OS were 25%, 54% and 41%, respectively. For the first time, this trial showed safety and efficacy of an intensified conditioning with a "new drug" in poor prognosis patients. Moreover, the concept of maintenance treatment after an allograft was also introduced. Our group recently published the results of a prospective Phase II single-center trial evaluating bortezomib as maintenance treatment after tandem autologous/allogeneic HCT for high-risk MM. At a median follow up of 51 months, a net benefit in terms of OS and PFS was shown among newly diagnosed patients over those with relapsed/persistent disease, suggesting that bortezomib maintenance may add a survival benefit among untreated patients. Treatment-related toxicity was limited, without any GvHD exacerbations.[53] Different observations were reported with immunomodulatory drugs. Somewhat compromised by an unacceptably high drop-out rate, the Phase III BMT CTN 0102 trial did not show a benefit of thalidomide maintenance after tandem autologous/allogeneic HCT.[13] Lenalidomide maintenance was evaluated in a study by the HOVON Group[54] where the unexpectedly high toxicity profile, mainly exacerbation of

acute GvHD, led to early discontinuation in 87% of the patients. In a Phase II CIBMTR trial on 30 patients,[55] the use of lenalidomide was feasible if given at lower doses. A lower toxicity profile of lenalidomide maintenance was also reported by Kroger *et al*. in relapsed patients after an autograft and rescued with a myeloablative allograft.[56]

Importantly, a synergy between new drugs and graft-*versus*-myeloma effects with a far safer toxicity profile has been described in the relapsed setting, suggesting that allogeneic-HCT and new drugs may be complementary. In our study, the median duration of survival of 7.8 years from the first relapse/progression among patients who achieved a response to salvage treatments supports this concept. These findings have been confirmed by two other recent reports.[57,58] An update of an Italian study focused on the role of "new drugs" in long-term clinical outcomes.[57] Median OS from first relapse was 7.5 years in the autologous/allogeneic group and two years in the tandem autologous group (P=0.01). Htut *et al*. compared the post-relapse OS after autologous/allogeneic HCT *versus* tandem autografts in patients reported to CIBMTR between 2000 and 2010.[58] Six-year post-relapse OS was significantly better in the autologous/allogeneic group as compared with the tandem autografts group, 44% *versus* 35%, respectively (P=005). Taken together, these findings suggest a synergy between new agents and the donor-derived immunological milieu.

The current role of allografting in multiple myeloma is controversial though the procedure is still used at many centers. Prospective studies were designed before agents with potent anti-myeloma activity became readily available. However, despite the recent introduction of very effective pharmacological therapies, there remains a subset of high-risk patients accounting for about 10-15% of new diagnoses, whose dismal prognosis is further compounded when early relapse (within 18 months from first-line treatment) is observed.[59,60]

The negative impact of adverse cytogenetics on clinical outcomes was not overcome by allogeneic HCT in our series. More aggressive plasma cell clones may have escaped graft-*versus*-myeloma effects after non-myeloablative 2 Gy TBI. Instead, the impact of certain high-risk cytogenetics was partly neutralized by graft-*versus*-myeloma effects in a study by Kröger *et al*.[61] on 73 patients treated with autologous HCT followed by reduced-intensity melphalan 140 mg/m^2 plus fludarabine, where no significant differences in PFS between patients with del(17p13) and/or t(4;14) and those without these abnormalities were observed after a median follow up of six years. A French trial also showed no differences in clinical outcomes between t(4;14) and non-t(4;14) patients.[62] Another study by Rasche *et al*.[63] showed no differences in survival outcomes in patients carrying del(17p), t(4;14) or amp(1q21) as compared to those with normal cytogenetics. We speculated that the incorporation of melphalan 140 mg/m^2, as employed in the studies by Kroger and Rasche, in the conditioning regimen before allogeneic HCT added more cytotoxicity and might have resulted in superior tumor cell-kill and, therefore, better survival among those with high-risk cytogenetics. Moreover, almost 50% of our patients with adverse cytogenetics did not receive "new agents" as part of induction treatment that, in part, are able to overcome certain high-risk genetic features.[64]

In summary, our study showed that tandem autologous/minimal intensity allogeneic HCT for MM was safe and characterized by low acute and long-term toxicities. Patients among standard and high-risk categories were able to achieve long-term sustained remissions, while patients with ultra-high-risk disease did not benefit from the tandem HCT. Similarly, patients with progressive disease after autologous HCT failed to respond to allogeneic HCT and succumbed to the disease, suggesting that graft-*versus*-myeloma effects alone are inadequate to control refractory and high-tumor burden disease states. Allogeneic HCT may be employed as a platform for post-transplant immune-based strategies such as novel immunomodulatory drugs or proteasome inhibitors, CAR T- and N-cell infusions, and bispecific T-cell engagers in selected high-risk populations where prognosis remains poor even in the era of new drugs.[65-67]

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