Clinical Medicine Insights: Oncology

ORIGINAL RESEARCH

Combination of IFN-α/Gm-CSF as a Maintenance Therapy for Multiple Myeloma Patients After Autologous Stem Cell Transplantation (ASCT): A Prospective Phase II Study

Donya Salmasinia[1], Myron Chang[2], John R. Wingard[1], Wei Hou[2] and Jan S. Moreb[1]

[1]Department of Medicine, [2]Department of Epidemiology and Health Policy Research, University of Florida, Gainesville, Florida, USA. Corresponding author email: morebjs@medicine.ufl.edu

Abstract: Interferon alpha (IFN-α) has been used as a maintenance therapy after autologous stem cell transplantation (ASCT) for multiple myeloma (MM) patients. In this study, we combined GM-CSF with IFN-α in order to improve IFN tolerance in post-ASCT myeloma patients. Primary aims were to evaluate myelotoxicity and effectiveness of this maintenance therapy. The treatment included 4×10^6 units of IFN-α and 125 μg/m^2 of GM-CSF given three times a week for twelve months. Twenty seven patients were enrolled within 120 days after ASCT. One patient discontinued treatment due to thrombocytopenia and seven others were taken off study due to flu-like symptoms and/or increase in liver enzymes. With a median follow-up of 45.5 months, the median overall survival was not reached while the median progression-free survival was 28 months. Eleven patients (42%) have remained in very good partial remission or complete remission since ASCT. In conclusion, our results demonstrate that maintenance with GM-CSF and IFN-α is safe and effective.

Keywords: autologous stem cell transplant, interferon alpha, GM-CSF, multiple myeloma, maintenance therapy, toxicity

Clinical Medicine Insights: Oncology 2010:4 117–125

doi: 10.4137/CMO.S6161

This article is available from http://www.la-press.com.

Introduction

The standard treatment of newly diagnosed multiple myeloma (MM) consists of induction therapy followed by single or tandem autologous stem cell transplant (ASCT).[1–4] However, although survival is improved, the majority of these patients still relapse despite achieving good initial responses and very few are cured from MM. Post ASCT interventions have been sought to delay relapses and possibly increase the number of cured patients. Post ASCT maintenance regimens have been studied with usually some benefits. Until recently, interferon-α (IFN-α) with or without steroids was the main maintenance regimen.[5,6]

Since the first report by Mandelli et al[7] on the usefulness of IFN-α as a maintenance drug for MM patients, multiple studies, including randomized and meta-analysis studies, have demonstrated conflicting results in regards to the effects of IFN-α maintenance, after conventional or high-dose chemotherapy, on progression-free survival (PFS) and/or overall survival (OS).[8–14] These results, the toxicity profile, and the availability of new effective novel agents have resulted in the decline in using IFN-α for maintenance in MM patients. On the other hand, long-term therapy with interferon in other diseases, such as CML and hairy cell leukemia,[15,16] has been well tolerated and resulted in long-term disease-free survival. A hint of long-term effect has been noted in data published by the Italian Multiple Myeloma Study Group on 10 year follow up observations of 9 patients still alive in the interferon-maintained group versus 2 in the unmaintained group.[17]

Although IFN-α maintenance post autologous transplant has been shown to be beneficial, not all MM patients can start this treatment due to delayed recovery of blood counts and many more will stop therapy due to significant myelosuppression. Our unpublished data on 33 MM patients who underwent ASCT revealed that 5 of these patients were unable to start IFN-α due to slow hematopoietic recovery (historical control), while 7 more had to discontinue IFN-α due to significant drop in blood counts with grade ≥IV toxicity. Thus, a significant proportion of patients are not able to benefit from IFN-α due to low blood counts after ASCT or IFN-α-induced myelosuppression. These results are consistent with findings reported in other published studies.[13,18,19]

GM-CSF has been shown to shorten duration of chemotherapy-induced myelosuppression therapy.[20–24] Furthermore, GM-CSF activates macrophages, increases functional capacity of monocytes, mediates proliferation, maturation and migration of dendritic cells, and increases the production of angiostatin by macrophages that inhibit angiogenesis.[25–27] Because of these multiple functions, some studies have suggested possible GM-CSF anti-tumor activity in patients with melanoma and advanced prostate cancer as well as other tumors.[28–31] The use of GM-CSF/IFN-α combination has been reported in patients with CML in chronic phase and melanoma.[32–34] The addition of GM-CSF allowed the escalation of IFN-α dose in half the patients with CML and showed improvement in overall response rate and the complete cytogenetic responses. No toxicity was attributed to the addition of GM-CSF.[32]

In this study, our aim was to determine whether the addition of GM-CSF to IFN-α would allow the use of IFN-α maintenance in higher proportion of MM patients and thus possibly improve its direct anti-myeloma effect and prolong survival.

Patients and Methods

Patients

This trial was a prospective phase II study evaluating the myelotoxicity and effectiveness of combined maintenance with GM-CSF and IFN-α in post-transplant MM patients. The study was approved by the institutional review board. Between January 2003 and June 2007, 27 patients were enrolled after signing informed consent. Eligibility for enrollment to the study included >15 year of age, no more than 120 days from last ASCT, ECOG performance status ≤2.0, transfusion independence, platelet count above 75,000/mm^3, absolute granulocyte count (AGC) of ≥1500/mm^3, satisfactory vital organ function, and no serious active infections. Patients with slow count recovery were allowed to start with single agent GM-CSF in order to improve their count recovery and thus meet the above requirements. Patients with progressive disease were excluded from study.

Clinical data were collected on the stage of disease, time from diagnosis to transplant, the number of prior treatments including radiation therapy,

β2-microglobulin (β2M) and results of cytogenetics/FISH analysis. Disease status was evaluated before transplant, at the time of study treatment (IFN-α + GM-CSF) and at the completion of study treatment and response was classified according to the International Myeloma Working Group uniform response criteria.[35] At the time of treatment, toxicity was evaluated as an effect of combined GM-CSF with IFN-α, and classified according to the NCI Common Toxicity Criteria (CTC version 3).

Treatment plan

All patients underwent ASCT according to standard practice at our institution as published before.[36] Within 120 days of completion of ASCT/induction treatment, all eligible patients were started on IFN-α and GM-CSF simultaneously Monday, Wednesday, and Friday each week. IFN-α was given subcutaneously with an initial dose of 1×10^6 units with weekly increase up to maximal dose of 4×10^6 if tolerated. GM-CSF was given subcutaneously at 125 μg/m^2 on the same days as the IFN-α. GM-CSF was provided to patients free of charge for maximum of one year by the sponsor (Immunex/Berlex). After one year, patients could continue on IFN-α with/without GM-CSF off study at the discretion of the treating physician. If at any time while on GM-CSF, the WBC exceeded 20,000/mm^3, then the GM-CSF dose would be decreased by 50%. If at the time of enrollment AGC was <1500/mm^3, the patient would start GM-CSF daily until AGC $\geq$ 1500/mm^3 before IFN-α was started. IFN-α treatment was stopped if the PLT count dropped $<50{,}000$/mm^3 and restarted at a lower dose when the PLT count $\geq 75{,}000$/mm^3. Study drugs were to be stopped for persistent constitutional symptoms such as fever/flu like symptoms or significant abnormalities in liver function tests beyond the first two weeks of treatment or later if related to higher IFN dose. Restarting IFN treatment at a lower dose was allowed within two weeks and if the symptoms or abnormalities recurred, then the patient was taken off study.

Statistical analysis

In this study, myelotoxicity was assessed using the NCI Common Toxicity Criteria (CTC) version 3 and reported using descriptive statistics.

Effectiveness of maintenance was assessed by PFS. In this intent to treat analysis, the PFS and OS were estimated by Kaplan-Meier method. PFS is the time from first ASCT to either confirmed 25% increase/recurrence of paraprotein, doubling the percentage of plasmacytosis in BM, appearance of new lytic lesions or any combination. Patients were censored at the last follow up. OS is the time from first ASCT to death from any reason. All statistical analyses were performed using the GraphPad software Prism 4 (San Diego, CA).

Results

Patient characteristics

Total of 27 patients signed informed consents; however one patient did not start treatment and was not considered for the analysis. The patient characteristics are shown in Table 1 and reflect a representative group of MM patients.

Among the 26 study participating patients, 19 were white, six African American, and one Asian. At diagnosis, one patient had stage IA, 11 were stage IIA, one stage IIB, 10 IIIA and three were stage IIIB. The cytogenetics and FISH performed at the time of diagnosis showed that 18 patients had normal karyotype, two had complex abnormalities, 3 with deletion of chromosome 13 and 2 of them showed both deletion of chromosome 13 and complex abnormalities, and two more had other cytogenetic abnormalities. Twenty patients had β2M at diagnosis with a median value of 2.3 mg/L, and 6 of them had values greater than 4 mg/L.

The median number of pre-transplant therapies was 1 (range, 1–3), and 8 of them also received prior radiation therapy. Pre-transplant regimens used included VAD (15 patients), thalidomide/dexamethasone (9 patients), hyper CVAD (2 patients), melphalan (1 patient), and lenalidomide (2 patients). At the time of first transplant, four patients were in complete remission (CR), 10 in very good partial remission (VGPR), 11 in partial response (PR), and one had minimal response (MR).

The median follow up from the completion of IFN/GM-CSF study was 33 months (range 5–66) and that from the first ASCT was 45.5 months (range 14–73). Four patients received planned tandem transplants prior to starting IFN treatment. The conditioning regimen for ASCT was busulfan, cyclophosphamide

Table 1. Patient characteristics.

Characteristics	Study group
Number of patients	26
Age, years	59 (30–71)*
Sex, M/F	14/12
Race, W/AA/other	19/6/1
MM stage	
I A	1
II A	11
II B	1
III A	10
III B	3
Disease status prior to ASCT	
CR	4
VGPR	10
PR	11
MR	1
ASCT	
CD34+ cell dose/kg	5.1×10^6 (2.5–10)
Tandem ASCT	4
Chromosome/FISH**	
Normal	18
Complex	1
Del 13	1
Complex/del 13	2
Other	2
β2M	2.3 (0.6–9.2)
Time from diagnosis to ASCT, mo	6 (3–25)
Follow up from last ASCT, mo	45.5 (14–73)
Number of prior therapy	1 (1–3)
Patients with ≥2 regimens	6
Prior radiation therapy	8
Number of salvage transplants	
Autologous	5
Allogeneic	2

Notes: *Data reflects median, (range), otherwise number of patients in each category; **Not all patients had chromosomal studies at diagnosis.
Abbreviations: AA, African American; W, white; mo, months; M, male; F, female.

and etoposide in 16 patients as described before,[36] while the rest received melphalan 200 mg/m^2. There were 5 ASCT and 3 non-myeloablative allogeneic transplants done for relapsed disease after completion of the IFN/GM-CSF treatment.

IFN-α/GM-CSF Treatment

Table 2 summarizes the statistical analysis of the IFN-α/GM-CSF combined treatment in comparison to single agent IFN-α received by a historical control group of patients treated in our institution. In the study group, the median length of treatment with IFN-α was 11.5 months (range, 1–60), while it was only 8 months (range 0.5–54) in the control group. Two patients from the study group had a slow counts recovery after ASCT and were treated with daily GM-CSF for 2 weeks before adding the IFN. Otherwise all enrolled patients were able to start treatment on time between days 90 and 120 after ASCT. Ten patients prematurely discontinued IFN-α after a median time of 2.5 months (range 1–10 months), five of whom experienced persistent flu-like symptoms, one had elevated liver enzymes, one had both flu-like symptoms and elevated liver enzymes, two relapsed and one patient experienced grade 3 thrombocytopenia. Significant leukopenia was not seen in any of the study patients. These patients who stopped study drugs did not receive other treatments until disease progression. The expected length of treatment with GM-CSF and IFN-α was one year long. However, two patients prematurely discontinued GM-CSF due to skin rash and cellulitis. Nine patients continued on with IFN-α alone after completion of one year combined therapy, four of whom were still on IFN-α as of this analysis.

Table 2. IFN-α treatment statistics in study (IFN/GM-CSF) versus historical control (IFN only) groups.

Patient groups	Study group	Control†
IFN dose, IU (10^6)	3 (2–4)*	3 (1–4)
Length of IFN therapy, mo	11.5 (1–60)	8 (0.5–54)
Prematurely discontinued IFN	10	14
Reasons for discontinuation		
Flu like symptoms	5	3
Elevated liver enzymes	1	1
Both of the above	1	0
Relapse	2	3
Thrombocytopenia only	1	0
Pancytopenia/Leukopenia	0	7**
Prematurely discontinued GM-CSF	2	NA
Reason for discontinuation		
Skin rash/cellulitis	2	NA
Using IFN > 1 year	9	6
Currently on IFN	4	NA

Notes: *Results reflect median and (range), otherwise number of patients in each category; †33 patients were included in the historical control group, only 25 received IFN-α therapy while 8 of them were not able to start the IFN treatment due to delayed count recovery (4), thrombocytopenia (1), early relapse (2), and unknown reason (1); **Significantly different with two tailed $P = 0.0173$.

Patients in the historical control group underwent single ASCT between November 1996 and March 2000. However, only 25 of those patients were able to start the IFN-α while 8 were not able to start the treatment. The reasons for not being able to start included delayed counts recovery (4), thrombocytopenia only (1), early relapse (2), and one unknown reason. The median length of IFN therapy was 8 months (range, 0.5–54). Fourteen patients prematurely discontinued the treatment after median of 3 months (range 0.5–8 months). The reasons for discontinuation included relapse (3), pancytopenia (5), leukopenia (2), flu like symptoms (3), and elevated liver enzymes (1). Despite obvious differences between the two groups in terms of the ability to start therapy with IFN and the number of patients who had to stop IFN, the proportion of patients who discontinued IFN therapy in each group was not significantly different using the logrank test (10 patients in the study group vs. 14 patients in the control group, $P = 0.325$). In addition, the median length of IFN-α treatment was not significantly different between the two groups (11.5 months in the study group versus 8 months in the control, $P = 0.341$). On the other hand, the frequency of myelotoxicity in the study group (1 patient with thrombocytopenia) was significantly less than that seen in the control (7 patients) group (two tailed $P = 0.0173$).

Treatment-related toxicity

The treatment related toxicity of GM-CSF/IFN-α combined therapy is given in Table 3 and was classified according to the NCI CTC (Version 3). Three patients developed persistently elevated liver enzymes that resulted in discontinuation of therapy in 2 of them. Six patients experienced skin rash/cellulitis mostly related to GM-CSF, 4 of whom had only grade I toxicity while 2 had grade IV. Eight patients developed flu-like symptoms, 3 patients with grade II and 5 patients with grade IV. Two patients had grade II and III thrombocytopenia, 2 patients had grade I fatigue, and another 2 had grade II leukocytosis. None of the patients experienced pancytopenia or leukopenia. There was no grade V toxicity resulting in death or hospitalization.

Table 3. Treatment-related (IFN+GM-CSF) toxicity using CTC version 3.

Symptoms*	0	I	II	III	IV
Liver abnormalities			1	1	1
Skin rash (cellulitis)		4			2
Flu-like symptoms			3		5
Thrombocytopenia			1	1	
Fatigue		2			
Leukocytosis			2		

Note: *Some patients had more than one side effect due to treatment.

Outcomes and survival analysis

All patients survived more than one year post-transplant and the disease status was evaluated prior to and at completion of 12-month therapy (see Table 4). Prior to treatment initiation, 9 patients were determined to be in PR, 5 in CR, and 12 in VGPR. At the completion of 12-month therapy, the number of patients with PR decreased to 3, CR increased to 8, 9 were still in VGPR while 6 patients relapsed. Three of the relapsed patients were those with chromosome 13/13q deletion and two of them died from progressive disease. Overall, eleven patients (42%) have remained in VGPR/CR since ASCT.

At a median follow up of 45.5 months (range, 14–73) from the first ASCT, 20 patients are still alive, 8 of whom are relapse-free for a minimum of 14 months, and 6 have died. Figure 1 shows the Kaplan-Meier estimates of OS and PFS. The 2-year OS probability (±SD) was 0.8 ± 0.08 and the PFS was 0.59 ± 0.1. Median OS was not reached for the study treatment group, while the median PFS was 28 mo.

A sub-analysis of the effects of IFN-α and GM-CSF combination in high-risk patients with

Table 4. Disease status at the time of initiation and completion of 12-month therapy with IFN-α + GM-CSF.

Status	Prior	At completion
PR	9*	3
CR	5	8
VGPR	12	9
Relapse	N/A	6

Note: *Results reflect number of patients in each response category.

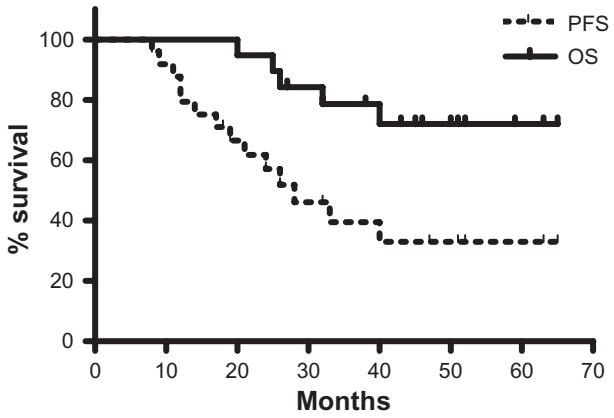

Figure 1. The Kaplan-Meier estimates of survival status of MM patients in the study group. Median OS was not reached while the median PFS was 28 mo.

β2M > 4 mg/L, chromosome 13/13q deletion and/or complex abnormalities was performed (Table 5). The results indicate that the combination maintenance resulted in long-term PFS and OS in 50% of patients with elevated β2M, however the median PFS for this high-risk group was much shorter (13 mo) than that for the whole study group (28 mo).

Discussion

In this study, we report the use of GM-CSF/IFN-α combination therapy as a maintenance treatment for multiple myeloma patients following single or tandem ASCT. Despite the relatively small number of patients enrolled, our results show that the addition of GM-CSF to IFN-α helps ameliorate the myelotoxicity of the latter. IFN related myelotoxicity is especially frequent and an important impediment to its use in patients after ASCT as reported by others and it can be seen in high numbers of patients.[13,18,19] Our results show that the addition of GM-CSF may allow the use of IFN maintenance in more patients and it can significantly reduce the prevalence of cytopenias in comparison to our own historical experience.

The use of the combination was associated with 28 month PFS and the OS was not reached at the time of analysis. These results compare positively with published studies reporting the use of IFN-α maintenance after ASCT.[14,38,39]

Despite overcoming the problem of IFN-α induced myelosuppression with GM-CSF, significant number of patients still could not tolerate IFN-α due to other constitutional symptoms. Ways to overcome such effects have been explored by using peginterferon with some improvement in quality of life.[40] However, it seems that some patients tolerate IFN without any side effects, while others will have symptoms even with the lowest dose possible. The mechanism for causing these side effects is not known, but the individual variability implies different pharmacodynamic responses between patients as described with other interferons[41,42] Future pharmacogenomics research in these patients may be key to improving and individualizing the use of IFN-α in the treatment of MM patients.

The main aim of maintenance therapy after ASCT in MM patients is to improve PFS and possibly OS. Achieving such positive effects depends on some known primary prognostic factors. Although our study showed significant improvement in PFS over our historical control (not published), our sub analysis results also show that some high-risk patients, especially with β2M > 4 mg/L but not with chromosome

Table 5. Effect of IFN-α/GM-CSF maintenance in high risk myeloma patients, as defined by the presence of β2M > 4 mg/L and/or del 13/13q and/or complex chromosomal abnormalities at diagnosis.

Subject	β2M; Repeat	Disease stage	PFS (mo)	OS (mo)	Cytogenetic/FISH	Status
1	9.2; 9.8	IIIB	12	32	Normal	Dead
2	4.3; 2.9	IIIA	9	12	Normal	Dead
3	7.7; 6.4	IIIB	40	58	Normal	Alive
4	5.8; 2.7	IIIB	11	25	Complex (49, –X and 13q del)	Dead
5	8.8; 2.5	IIA	58	58	Normal	Alive
6	9.6; 4.6	IIB	39	39	Normal	Alive
7	2.2; 2.4	IIA	14	20	13 del/Complex	Dead
8	2.0; 2.6	IA	12	28	13q del	Alive

Abbreviations: del, deletion; complex, multiple chromosomal abnormalities (>3).

13 deletions, may benefit from IFN-α/GM-CSF maintenance. Our study argues for the continued consideration of IFN-α especially in view of the fact that the current alternative, thalidomide, has significant toxicity and uncertainties.[43,44] Furthermore, published studies have all reported problems with tolerability and discontinuation of therapy with lenalidomide,[45,46] thalidomide[43,44,47–49] or bortezomib[50–53] due to various severe toxicities including myelosupprssion and infection, neuropathy, deep venous thrombosis and pulmonary emboli. Thus, we suggest that IFN-α is not different than any of these new novel drugs and that expanded combination maintenance therapy, including GM-CSF and IFN-α, should be further investigated. Few studies have been published on combining interferon or peginterferon with thalidomide,[54–56] which showed high rate of adverse effects and intolerance to the combination. Another study reported the use of combination of 13 cis-retinoic acid, dexamethasone and IFN-α as maintenance for MM patients post ASCT.[57] Although responses were observed, again many patients discontinued therapy due to side effects. The use of bortezomib and lenalidomide in such combinations might be less toxic and warrants investigation. Although preliminary results show promising outcomes with lenalidomide maintenance, some patients do not respond as well or may not tolerate the drug, and therefore it is important to have other options for second line maintenance such as IFN-α ± GM-CSF or in other combinations. Indeed in this study, we have seen few patients who have remained on IFN-α for many years with sustained complete remission. Perhaps, sequential cycling maintenance therapy, as reported before,[58] might also be a better way of overcoming additive toxicity and possibly avoiding/delaying the emergence of resistant myeloma clones.

In summary, our data confirm that the combination of GM-CSF with IFN-α is a tolerable and effective maintenance treatment after ASCT with reduced IFN-α induced myelotoxicity and improved PFS in patients with multiple myeloma. Our results support the continued use of IFN-α as a maintenance therapy for MM patients who have responded. Future studies should investigate the addition of newer novel drugs in combination with GM-CSF and interferon.

Acknowledgements

This work was supported by Immunex and later Berlex who also provided free GM-CSF for this study.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The peer reviewers of this paper report no conflicts of interest. Funding for the study was provided to Dr Jan S Moreb by Immunex/Berlex, the makers of GM-CSF. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Gertz MA, Ghobrial I, Luc-Harousseau J. Multiple myeloma: biology, standard therapy, and transplant therapy. *Biol Blood Marrow Transplant*. 2009; 15 Suppl 1:46–52.
2. Reece DE. Management of multiple myeloma: the changing landscape. *Blood Rev*. 2007;21:301–14.
3. Attal M, Harousseau JL. The role of high-dose therapy with autologous stem cell support in the era of novel agents. *Semin Hematol*. 2009;46:127–32.
4. Kyle RA, Rajkumar SV. Treatment of multiple myeloma: A comprehensive review. *Clin Lymphoma Myeloma*. 2009;9:278–88.
5. Mihelic R, Kaufman JL, Lonial S. Maintenance therapy in multiple myeloma. *Leukemia*. 2007;21:1150–7.
6. Harousseau JL. Maintenance treatment in multiple myeloma. Maintenance treatment in multiple myeloma. *Ann Oncol*. 2008;19 Suppl 4:iv54–5.
7. Mandelli F, Avvisati G, Amadori S, et al. Maintenance treatment with recombinant interferon-alpha 2b in patients with multiple myeloma responding to convetional induction chemotherapy. *N Engl J Med*. 1990;322:1430–4.
8. Avvisati G, Mandelli F. The role of alpha-interferon in the management of myelomatosis. *Hematol Oncol Clin of North Am*. 1992;6:395–405.
9. Salmon SE, Crowley JJ, Grogan TM, et al. Combination chemotherapy, glucocorticoids, and interferon alpha in the treatment of multiple myeloma: A Southwest Oncology Group Study. *J Clin Oncol*. 1994;12:2405–14.
10. Westin J, Rödjer S, Turesson I, et al. Interferon alfa-2b versus no maintenance therapy during the plateau phase in multiple myeloma: a randomized study. Cooperative Study Group. *Br J Haematol*. 1995;89:561–8.
11. Fritz E, Ludwig H. Interferon-alpha treatment in multiple myeloma: meta-analysis of 30 randomised trials among 3948 patients. *Ann Oncol*. 2000;11: 1427–36.
12. Myeloma Trialists' Collaborative Group. Interferon as therapy for multiple myeloma: an individual patient data overview of 24 randomized trials and 4012 patients. *Br J Haematol*. 2001;113:1020–34.
13. Cunningham D, Powles R, Malpas J, et al. A randomized trial of maintenance interferon following high-dose chemotherapy in multiple myeloma: long-term follow-up results. *Br J Haematol*. 1998;102:495–502.
14. Björkstrand B, Svensson H, Goldschmidt H, et al. Alpha-interferon maintenance treatment is associated with improved survival after high-dose treatment and autologous stem cell transplantation in patients with multiple myeloma: a retrospective registry study from the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2001;27:511–5.
15. Baccarani M, Russo D, Rosti G, Martinelli G. Interferon-alpha for chronic myeloid leukemia. *Semin Hematol*. 2003;40:22–33.
16. Benz R, Siciliano RD, Stussi G, Fehr J. Long-term follow-up of interferon-alpha induction and low-dose maintenance therapy in hairy cell leukemia. *Eur J Haematol*. 2009;82:194–200.

17. Pulsoni A, Avvisati G, Petrucci MT, et al. The Italian experience on interferon as maintenance treatment in multiple myeloma: ten years after. *Blood*. 1998;92:2184–6.
18. Vesole DH, Crowley JJ, Catchatourian R, et al. High-dose melphalan with autotransplantation for refractory multiple myeloma: results of a Southwest Oncology Group phase II trial. *J Clin Oncol*. 1999;17:2173–9.
19. Schaar CG, Kluin-Nelemans HC, Te Marvelde C, et al; Dutch-Belgian Hemato-Oncology Cooperative Group HOVON. Interferon-alpha as maintenance therapy in patients with multiple myeloma. *Ann Oncol*. 2005;16: 634–9.
20. Nemunaitis J, Rabinowe SN, Singer JW, et al. Recombinant human-granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for lymphoid malignancy: Pooled results of a randomized, double-blind, placebo controlled trial. *N Engl J Med*. 1991;324: 1773–8.
21. Nemunaitis J, Singer JW, Buckner CD, et al. Long-term follow up of patients who received recombinant human granulocyte-macrophage colony stimulating factor after autologous bone marrow transplantation of lymphoid malignancy. *Bone Marrow Transplant*. 1991;7:49–52.
22. Büchner T, Hiddemann W, Koenigsmann M, et al. Recombinant human granulocyte-macrophage colony stimulating factor after chemotherapy in patients with acute myeloid leukemia at higher age or after relapse. *Blood*. 1991;78:1190–7.
23. Bennett CL, Stinson TJ, Tallman MS, et al. Randomized placebo-controlled Phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (>55 to 70 years of age) with acute myelogenous leukemia: A study of the Eastern Cooperative Oncology Group (E1490). *Blood*. 1995; 86:457–62.
24. Bunn PA Jr, Crowley J, Kelly K, et al. Chemoradiotherapy with or without granulocyte-macrophage colony-stimulating factor in the treatment oflLimited-stage small-cell lung cancer: A prospective phase III randomizeds Study of the Southwest Oncology Group. *J Clin Oncol*. 1995;13: 1632–41.
25. Paquette RL, Hsu NC, Kiertscher SM, et al. Interferon-alpha and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen presenting cells. *J Leuk Biol*. 1998;64:358–67.
26. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: Intermediates, effectors, and memory cells (review). *Science*. 2000;290:92–7.
27. Fattorossi A, Battaglia A, Pierelli L, et al. Effects of granulocyte-colony-stimulating factor and granulocyte/macrophage-colony-stimulating factor administration on T cell proliferation and phagocyte cell-surface molecules during hematopoietic reconstitution after autologous peripheral blood progenitor cell transplantation. *Cancer Immunol Immunother*. 2001;49: 641–8.
28. Small EJ, Reese DM, Um B, et al. Therapy of advanced prostate cancer with granulocyte macrophage colony-stimulating factor. *Clin Cancer Res*. 1999;5: 1738–44.
29. Pinedo HM, de Gruijl TD, van der Wall E, Buter J. The hidden treasures of the primary tumor: extended neoadjuvant chemotherapy plus GM-CSF in locally advanced cancer. *Clin Cancer Res*. 2000;6:4467–72.
30. Spitler LE, Grossbard ML, Ernstoff MS, et al. Adjuvant therapy of stage III and IV malignant melanoma using granulocyte-macrophage colony-stimulating factor. *J Clin Oncol*. 2000;18:1614–21.
31. Richard C, Baro J, Bello-Fernandez C, et al. Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) administration after autologous bone marrow transplantation for acute myeloblastic leukemia enhances activated killer cell function and may diminish leukemic relapse. *Bone Marrow Transplant*. 1995;15:721–6.
32. Cortes J, Kantarjian H, O'Brien S, et al. GM-CSF can improve the cytogenetic response obtained with interferon-alpha therapy in patients with chronic myelogenous leukemia. *Leukemia*. 1998;12:860–4.
33. Schachter J, Rakowsky E, Sulkes A, Adler A. A sequential four-drug chemotherapy and biotherapy with interferon alpha and GM-CSF—an innovative protocol for the treatment of metastatic melanoma. *Caner Biother Radiopharm*. 1998;13:155–64.
34. Vaughan MM, Moore J, Riches PG, et al. GM-CSF with biochemotherapy (Cisplatin, DTIC, Tamoxifen, IL-2 and interferon-alpha): A phase I trial in melanoma. *Ann Oncol*. 2000;11:1183–9.
35. Durie BG, Harousseau JL, Miguel JS, et al. International Myeloma Working Group. International uniform response criteria for multiple myeloma. *Leukemia*. 2006;20:1467–73.
36. Cogle CR, Moreb JS, Leather HL, et al. Busulfan, cyclophosphamide, and etoposide as conditioning for autologous stem cell transplantation in multiple myeloma. *Am J Hematol*. 2003;73:169–75.
37. Dixon DO, Simon R. Sample size considerations for studies comparing survival curves using historical controls. *J Clin Epidemiol*. 1988;41:1209–13.
38. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myélome. *N Engl J Med*. 1996;335: 91–7.
39. Barlogie B, Tricot GJ, van Rhee F, et al. Long-term outcome results of the first tandem autotransplant trial for multiple myeloma. *Br J Haematol*. 2006; 135:158–1.
40. Sirohi B, Powles R, Lawrence D, et al. An open, randomized, controlled, phase II, single centre, two-period cross-over study to compare the quality of life and toxicity experienced on PEG interferon with interferon-alpha2b in patients with multiple myeloma maintained on a steady dose of interferon-alpha2b. *Ann Oncol*. 2007;18:1388–94.
41. Satoh J, Nanri Y, Tabunoki H, Yamamura T. Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNbeta-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFN-beta-related adverse effects in multiple sclerosis. *BMC Neurol*. 2006; 6:18.
42. van Baarsen LG, Vosslamber S, Tijssen M, et al. Pharmacogenomics of interferon-beta therapy in multiple sclerosis: baseline IFN signature determines pharmacological differences between patients. *PLoS One*. 2008;3: e1927.
43. Laubach JP, Richardson PG, Anderson KC. Hematology: Thalidomide maintenance in multiple myeloma. *Nat Rev Clin Oncol*. 2009;6:565–6.
44. Cavo M, Pantani L, Tacchetti P, et al. Thalidomide maintenance in multiple myeloma: certainties and controversies. *J Clin Oncol*. 2009;27:e186–7.
45. Wang M, Dimopoulos MA, Chen C, et al. Lenalidomide plus dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma regardless of prior thalidomide exposure. *Blood*. 2008;112:4445–51.
46. Gay F, Hayman SR, Lacy MQ, et al. Lenalidomide plus dexamethasone versus thalidomide plus dexamethasone in newly diagnosed multiple myeloma: a comparative analysis of 411 patients. *Blood*. 2010;115:1343–50.
47. Sahebi F, Spielberger R, Kogut NM, et al. Maintenance thalidomide following single cycle autologous peripheral blood stem cell transplant in patients with multiple myeloma. *Bone Marrow Transplant*. 2006;37:825–9.
48. Martino M, Console G, Callea V, et al. Low tolerance and high toxicity of thalidomide as maintenance therapy after double autologous stem cell transplant in multiple myeloma patients. *Eur J Haematol*. 2007;78:35–40.
49. Chang JE, Juckett MB, Callander NS, et al. Thalidomide maintenance following high-dose melphalan with autologous stem cell support in myeloma. *Clin Lymphoma Myeloma*. 2008;8:153–8.
50. Richardson PG, Briemberg H, Jagannath S, et al. Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. *J Clin Oncol*. 2006;24:3113–20.
51. Badros A, Goloubeva O, Dalal JS, et al. Neurotoxicity of bortezomib therapy in multiple myeloma: a single-center experience and review of the literature. *Cancer*. 2007;110:1042–9.
52. Argyriou AA, Iconomou G, Kalofonos HP. Bortezomib-induced peripheral neuropathy in multiple myeloma: a comprehensive review of the literature. *Blood*. 2008;112:1593–9.
53. Corso A, Mangiacavalli S, Varettoni M, Pascutto C, Zappasodi P, Lazzarino M. Bortezomib-induced peripheral neuropathy in multiple myeloma: a comparison between previously treated and untreated patients. *Leuk Res*. 2010;34:471–4.

54. Chiou TJ, Wang TH, Chao TY, et al. Randomized Phase II trial of thalidomide alone versus thalidomide plus interferon alpha in patients with refractory multiple myeloma. *Cancer Invest*. 2007;25:140–7.
55. Kasper B, Moehler T, Neben K, Ho AD, Goldschmidt H. Combination therapy of Thalidomide and Peginterferon in patients with progressive multiple myeloma. *Ann Oncol*. 2004;15:176–7.
56. Offidani M, Corvatta L, Polloni C, et al. Thalidomide-dexamethasone versus interferon-alpha-dexamethasone as maintenance treatment after ThaDD induction for multiple myeloma: a prospective, multicentre, randomised study. *Br J Haematol*. 2009;144:653–9.
57. Friedman J, Khoury H, Adkins D, et al. Pilot study of 13cis-retinoic acid+dexamethasone+alpha interferon as maintenance therapy following high-dose chemotherapy and autologous stem cell transplant for multiple myeloma. *Bone Marrow Transplant*. 2005;35:979–84.
58. Chen CI, Nanji S, Prabhu A, et al. Sequential, cycling maintenance therapy for post transplant multiple myeloma. *Bone Marrow Transplant*. 2006;37:89–94.