A polymorphism in *NFKB1* is associated with improved effect of interferon-α maintenance treatment of patients with multiple myeloma after high-dose treatment with stem cell support

Annette J. Vangsted,[1,2] Tobias W. Klausen,[2] Peter Gimsing,[3] Niels F. Andersen,[4] Niels Abildgaard,[5] Henrik Gregersen,[6] and Ulla Vogel[7]

[1]Dept. of Oncology and Haematology, Roskilde Hospital, Copenhagen Univesity, Roskilde; [2]Dept. of Haematology, University Hospital of Copenhagen at Herlev; [3]Dept. of Haematology, University Hospital of Copenhagen at Rigshospitalet, Copenhagen; [4]Dept. of Haematology, Aarhus University Hospital, Aarhus; [5]Dept. of Haematology, Odense University Hospital, Odense; [6]Dept. of Haematology, Aalborg University Hospital, Aalborg, and [7]National Food Institute, Technical University of Denmark, Copenhagen and Institute for Science, Systems and Models, Roskilde University, Roskilde, Denmark

ABSTRACT

Background

Maintenance therapy with interferon-α after high-dose treatment with stem cell support in multiple myeloma has been intensively debated. In this study, we evaluated the response to treatment with interferon-α in relation to genetic variation in genes related to inflammation.

Design and Methods

In a retrospective study of 296 patients with multiple myelom undergoing high-dose therapy between 1994 and 2004, 146 patients were treated with interferon-α as maintenance therapy. We tested the polymorphisms *IL1B* T-31C, *IL6* G-174C, *NFKB1*-94ins/delATTG, *CD3EAP* G-21A and *PPP1R13L* IVS1 A4364G for associations with time to treatment failure and overall survival with and without interferon-α treatment.

Results

The wild type ins-allele of polymorphism *NFKB1*-94 ins/delATTG was, by multivariate Cox analysis, associated with longer time to treatment failure (p=0.01) and overall survival (p=0.0084) when tested between treatment arms and in the subgroup of patients treated with interferon-α the wild type ins-allele was associated with longer overall survival (p=0.002). In the absence of interferon-α treatment, there was no association between the polymorphisms and treatment outcome, except for patients homozygous for the wild type G allele of *IL6* G-174C who survived longer (p= 0.0074) than variant allele carriers. There was no association between the polymorphisms *IL1B* T-31C, *CD3EAP* G-21A and *PPP1R13L* IVS1 A4364G and treatment outcome for interferon-α.

Conclusions

Patients who are homozygous carriers of the wild type ins-allele of the *NFKB1* -94ins/delATTG polymorphism may benefit from treatment with interferon-α, in contrast to patients carrying the variant allele. This result may indicate that the effect of interferon-α treatment is dependent on the availability of nuclear factor-κB and the polymorphism in *NFKB1* may, therefore, be a good prognostic marker for multiple myeloma patients on maintenance treatment with interferon-α after high-dose therapy. A prospective study of interferon-α treatment in relation to *NFKB1* -94ins/delATTG is highly warranted.

Key words: polymorphism, multiple myeloma, interferon, NF-κB, treatment outcome.

Citation: Vangsted AJ, Klausen TW, Gimsing P, Andersen NF, Abildgaard N, Gregersen H, and Vogel U. A polymorphism in NFKB1 is associated with improved effect of interferon-α maintenance treatment of patients with multiple myeloma after high-dose treatment with stem cell support. Haematologica 2009;94:1274-1281.doi:10.3324/haematol.2008.004572

Funding: this work was supported by grants from the Kong Christian den Tiendes Fond and the Research Fund at Herlev Hospital. Participants of NMSG.

Manuscript received on December 7, 2008. Revised version arrived on March 25, 2009. Manuscript accepted on April 3, 2009.

Correspondence:
Annette Vangsted, MD, Department of Oncology and Haematology, Roskilde Hospital, Copenhagen University, Køgevej 9-13, 4000 Roskilde.
E-mail: avag@regionsjaelland.dk

Introduction

Interferon-alpha (INF-α) as maintenance therapy after high-dose therapy (HDT) for multiple myeloma (MM) has been intensively debated during the last 30 years because several clinical studies have been published with conflicting results. Barlogie *et al.* randomized responders after HDT to INF-α or observation, and found no benefit of IFN-α treatment.[1] In another randomized trial of maintenance treatment with IFN-α following high-dose chemotherapy in MM, progression-free survival and overall survival (OS) were significantly prolonged in the treated group in comparison to the control group.[2] These results were later confirmed by the Myeloma Trialists' Collaborative Group in a meta-analysis of 1543 patients treated with IFN-α as maintenance treatment. They found an increase in progression-free survival of 6 months and a small, but significant, improvement in OS.[3]

IFN-α is an antiviral and immunomodulating drug which, in a complex manner, interferes with both the innate and adaptive immune systems.[4] Furthermore, IFN-α induces apoptosis or growth arrest in myeloma cells. Survival of myeloma cells is dependent on interactions with bone marrow stromal cells. In the bone marrow, the myeloma cells stimulate both the production of the pro-inflammatory cytokines interleukin-1beta (IL-1β) and interleukin-6 (IL-6) and angiogenesis and thereby potentiate their own growth in autocrine and paracrine fashions. In this process, nuclear factor-κB (NF-κB) is a key regulator of the inflammatory response and NF-κB has been shown to link inflammation with cancer.[5]

Genes activated by the NF-κB pathway include many pro-inflammatory cytokines, such as *IL1B* and *IL6*. It is, therefore, possible that the outcome of IFN-α maintenance treatment is related to inborn variation in genes involved in inflammation. Previously, we have shown that the polymorphism *IL1B* T-31C is associated with the both risk of MM and survival after HDT.[6] Furthermore, we have shown that genetic variation in the DNA repair genes *ERCC2* (excision-repair cross-complementing 2), *XRCC3* (X-ray repair cross-complementing group 3) and *CD3EAP* (CD3e molecule, epsilon associated protein) influence treatment outcome in MM patients undergoing HDT.[7]

In the present study, we addressed the question of whether inborn variation in genes involved in inflammation influence outcome of IFN-α maintenance treatment of patients with MM after HDT. We selected polymorphisms known to be functionally important. The C allele of the promoter polymorphism *IL1B* T-31C results in increased IL-1β secretion upon stimulation with lipopolysaccharide.[8,9] The polymorphism *IL6* G-174C is also located in the promoter region of the gene. The wild type G allele is found to have a higher transcriptional level than the variant C-allele and the presence of the C-allele in healthy individuals results in low IL-6 levels and low lymphocyte levels upon stimulation.[10] The *NFKB1* -94ins/delATTG promoter polymorphism has been suspected to be involved in susceptibility to ulcerative colitis, but the relevance of this polymorphism in MM is unknown.[11] The polymorphism is an insertion/deletion of four bases in the promoter region of the *NFKB1* gene encoding both of the NF-κB transcription factors, p50 and p105. The allele containing the deletion is less able to bind transcription factors and produces lower transcript levels in luciferase reporter systems. Consequently, carriers of the del-allele have lower levels of NF-κB.[11] The gene *CD3EAP*, also named *CAST* and *ASE-1* (anti-sense to excision-repair cross-completing 1, ERCC1) is of interest, since this gene may be involved in the regulation of T-cell function.[12] The variant A-allele has been associated with increased risks of basal cell carcinoma, breast cancer and lung cancer.[13-15] *PPP1R13L* (protein phosphate 1, regulatory (inhibitory) subunit 13 like, also named RAI or iASPP) encodes an inhibitor of the RelA subunit of NF-κB.[16] It is, therefore, possible that polymorphisms affecting the activity of *PPP1R13L* could influence the activity of NK-κB.

In this study we tested whether the polymorphisms *IL1B* T-31C, *IL6* G-174C, *NFKB1* -94ins/delATTG, *CD3EAP* G-21A and *PPP1R13L* IVS1 A4364G influence the efficacy of IFN-α maintenance treatment after HDT in patients with MM. In order to do this, we retrospectively studied a population of 296 patients with MM who underwent HDT in Denmark between 1994 and 2004.

Design and Methods

Subjects, clinical data, response criteria, eligibility criteria, and treatment

Subjects, clinical data, response criteria, and eligibility criteria have previously been described in detail.[7] Briefly, patients diagnosed with MM and treated with high-dose melphalan and stem cell support from August 1994 to August 2004 were recruited from four participating centers in Denmark. Three hundred forty-eight patients were included in the study. Of these, 185 patients were included in the high-dose treatment protocols organized by the Nordic Myeloma Study Group (NMSG no. 5/94, 7/98 and 11/00),[17-19] whereas the remaining 163 patients were treated with similar treatment regimens, but not registered in these protocols. The patients' were staged according to Durie and Salmon and the International Staging System (ISS). Time-to-treatment failure (TTF) and OS were calculated from date of stem cell infusion to date of progression or death. Progression was defined by a more than 25% increase in serum M-protein or 25% increase in immunoglobulin levels above upper normal levels, confirmed by two separate measurements within a 1-month interval. An increase in bone marrow infiltration of plasma cells by 25%, an increase of Bence-Jones proteinuria to more than 1.0 g/24 h or other signs of progression such as hypercalcemia, progressive skeletal disease or soft-tissue plasmacytoma were also considered as progression. Eleven patients died during HDT. Two patients died from malignancies other than MM, and two patients died due to other non-MM causes. The occurrence of other malignancies and death without progression was regarded as events not related to progression. These patients were included in the analysis of OS, but they were excluded at time of death from the analy-

sis of TTF. The median follow-up after HDT was 54.5 months, (range, 0.4 to 163 months). The median survival after HDT was 70.1 months.

Induction therapy consisted of three courses of VAD (vincristine, doxorubicin and dexamethasone) or two or three courses of cyclophosphamide 1 g/m^2 i.v. on day 1 combined with dexamethasone 40 mg daily p.o., on days 1-4 and days 9-11 (total dose 320 mg for each course) repeated every 3 weeks. Peripheral blood stem cells were harvested at regeneration after cyclophosphamide priming, and the patients thereafter underwent HDT with melphalan (200 mg/m^2) followed by stem cell support. Fifty-three patients received a second HDT up-front. Data on maintenance therapy were collected retrospectively from the medical records. Maintenance therapy with IFN-α was implemented 3 months after HDT according to standard treatment pratice in the particular region (median 105 days). The percentage of patients treated with IFN-α varied from 12% in one region to 71% in another. More than 3 months of treatment with IFN-α was required for inclusion in the treatment group. Twenty patients were treated with IFN-α for less than 3 months and were included in the control group. Medical information on IFN-α was unknown for six patients, and these patients were excluded from the study. Forty-six patients suffered from recurrence of disease within 6 month after HDT and were, therefore, excluded from the study. The median duration of IFN-α treatment was 425 days (range, 162-914). The planned IFN-α therapy was a dose of 5×10^6 U s.c. 3 times a week, but the dose was adjusted to levels tolerated by the patient. Two hundred and ninety-six patients were included in the study and of these, 146 patients were treated with IFN-α. The study was approved by the Danish Ethical Committee (01-158/03).

Human tissue samples

Peripheral blood mononuclear cells were purified from 292 leukapheresis products by buffy coat preparation. From 56 patients ten 10 μm sections were collected from paraffin-embedded bone marrow samples. Material was not available for 19 patients undergoing HDT and these patients were not included in the study.

DNA purification

DNA for analysis was purified from peripheral blood mononuclear cells by the salting out method[20] or from paraffin-embedded tissue by phenol extraction as described elsewhere.[21]

Genotyping of single nucleotide polymorphisms

Genotypes were determined on an ABI 7500 analyzer using endpoint readings. Reactions were performed on 5 μL containing approximately 50 ng DNA, 2.5 μL mastermix (Applied Biosystems, Birkerød, Denmark), 100 nM of each probe and 900 nM primers. Controls were included in each run, and a repeat analysis of a 10% subset of samples yielded 100% identical genotypes. Moreover, for ten persons, DNA from both bone marrow and leukapheresis products was typed with identical results.

IL1B T-31C (rs1143627), *IL6* G-174C (rs1800795) *CD3EAP* G-21A (rs967591), and *PPP1R13L* IVS1 A4364G (rs1970764) were genotyped as previously described.[22] For *NFKB1* -94ins/delATTG (rs28362491) the primer sequences were: F: 5'-CTA TGG ACC GCA TGA CTC TAT CAG-3' R: 5'-GGG CTC TGG CTT CCT AGC A-3'. The probe sequences were: NFKB –INS: 5'-FAM-ACC ATT GAT TGG GCC CGG-BHQ-3', NFKB-DEL: 5'-Yakima Yellow-CCG ACC ATT GGG CCC G-BHQ-3'.

Statistical methods

SPSS statistical software was used for all calculations (SPSS for Windows, Rel. 14.0.0. 2005, Chicago: SPSS Inc., USA). All tests were two-sided and *p* values less than 0.05 were regarded as statistically significant. Fisher's exact test was used to compare categorical variables and the Mann-Whitney test was used to compare continuous and categorical variables. The Kaplan-Meier method and the log rank test were used to compare TTF and OS between groups. The Cox proportional hazards model and log-likelihood statistics were applied for univariate analyses of covariates and for multivariate analysis. Variables with a *p* value less than 0.05 by univariate analysis were included in the multivariate Cox analyses to identify variables of independent significance.

Results

Study population

Two hundred and ninety-six patients were included in the study from a population of 348 patients with MM who underwent HDT in Denmark between 1994 and 2004 as described earlier.[7] The demographic and clinical data of the patients included in this study are presented in Table 1 and Figure 1. There were no statistically significant differences between the group treated with IFN-α and the group not treated with IFN-α in relation to levels of β2-microglobulin, creatinine, albumin and sex. However, patients treated with IFN-α were younger than controls and had higher Durie-Salmon stages of disease. There was no difference in the ISS criteria.

Table 1. Patients' characteristics.

	N. of patients treated with IFN-α	N. of patients not treated with IFN-α	*p* value
Total	146	150	0.8
Sex			
Male	87 (60%)	87 (58%)	
Female	59 (40%)	63 (42%)	
Age	54 (31-68)	57 (37-69)	0.007
β2-microglobulin (mg/dL)	3.9 (1.3-33)	3.5 (1.2-48)	0.3
Creatinine (μmol/L)	1.1 (0.5-6.9)	1.1 (0.6-9.4)	0.4
Albumin (g/dL)	3.5 (0.3-5.3)	3.4 (0.3-5.3)	0.1
Durie-Salmon Stage			0.02
I	8 (6%)	23 (16%)	
II	33 (23%)	34 (24%)	
III	103 (71%)	87 (60%)	
International Staging System			0.4
I	25 (22%)	27 (31%)	
II	51 (45%)	34 (39%)	
III	38 (33%)	27 (31%)	

Single nucleotide polymorphisms

Genotypes of *IL1B* T-31C, *IL6* G-174C, *NFKB1* –94ins/delATTG, *CD3EAP* G-21A and *PPP1R13L* IVS1 A4364G were determined. There were no differences in genotype distributions among patients from different participating centers. Table 2 shows the genotype distributions of *IL1B* T-31C, *IL6* G-174C, *NFKB1* –94ins/delATTG, *CD3EAP* G-21A and *PPP1R13L* IVS1 A4364G among patients who did or did not receive IFN-α maintenance treatment. TTF and OS were compared in groups subdivided by genotypes and IFN-α treatment (Table 2 and Figure 2A, B, C and D). We compared genotypes within treatment arms with homozygous carriers of the wild type allele serving as the reference and we compared carriers of the same genotype in the presence and absence of IFN-α treatment, untreated patients serving as the reference. To test whether the effect of INF-α treatment was dependent on genotype, we performed a Cox proportional hazard analysis including an interaction term between INF-α treatment and the genotypes. If this term has a p value of less than 0.05, the effect of INF-α treatment is significantly different between genotypes (Table 3, only p values for interaction term shown corrected for other prognostic markers are shown).

The ins-allele of *NFKB1* -94ins/delATTG was associated with an effect on TTF and OS (Table 2). In a multivariate Cox analysis, testing whether INF-α treatment had different effects depending on genotype, the effect of the ins-allele was related to treatment with IFN-α (p=0.01 and p=0.04) (Figure 2A and B and Table 3). Among

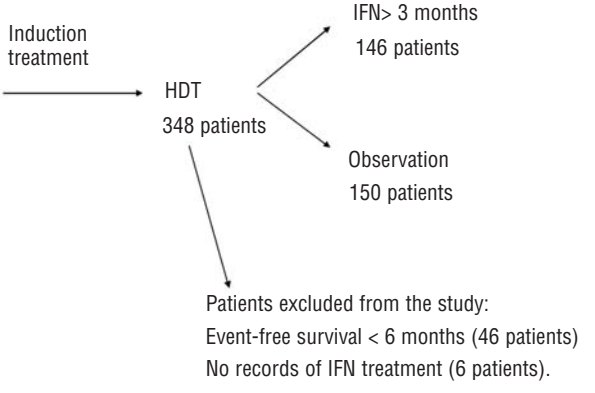

Figure 1. Flow-chart for patients with multiple myeloma undergoing HDT followed by IFN-α maintenance treatment. Patients received induction treatment with VAD or cyclophosphamide and dexamethasone. Induction treatment was followed by HDT with melphalan 200 mg/m² with stem cell support. Three months after HDT the patients were treated with IFN-α according to regional decisions.

Table 2. Univariate analysis of the effect of genetypes on time-to-treatment failure and overall survival in patients treated or not with IFN-α.

Gene	Genotypes	N.	Median TTF (months)	p^a	HR[a]	p^b	HR[b]	Median OS (months)	p^a	HR[a]	p^b	HR[b]
IL1B T-31C												
	TT	57	28.7		1		1.0	65.7		1		1.0
	CT + CC	76	26.6	0.4	1.2[c](0.8-1.7)		1.0	70.9	0.7	0.9 (0.6-1.5)		1.0
+ IFN-α	TT	49	29.9		1	0.6	0.9 (0.6-1.4)	57.7		1	0.9	1.0 (0.6-1.7)
+ IFN-α	CT + CC	82	48.0	0.2	0.8 (0.5-1.1)	0.0002	0.5 (0.3-0.7)	*	0.01	0.5 (0.3-0.9)	0.01	0.6 (0.4-0.9)
IL6 G-174C												
	GG	37	30.0		1		1.0	*		1		1.0
	CG + CC	108	26.2	0.5	1.2(0.8-1.8)		1.0	64.6	0.007	2.2 (1.2-4.0)		1.0
+ IFN-α	GG	35	30.5		1	0.4	0.8 (0.5-1.4)	73.9		1	0.1	1.7 (0.8-3.4)
+ IFN-α	CG + CC	109	42.6	0.5	0.9 (0.6-1.3)	0.001	0.6 (0.4-0.8)	103.5	0.2	0.7 (0.4-1.2)	0.0003	0.5 (0.3-0.7)
NFKB1 -94 INS/DEL					-							
	Ins	46	23.4		1		1.0	69.7		1		1.0
	del-carriers	96	29.4	0.07	0.7 (0.5-1.0)		1.0	69.9	0.5	0.8 (0.5-1.4)		1.0
+ IFN-α	Ins	45	49.9		1	0.0001	0.4 (0.2-0.6)	*		1	0.003	0.4 (0.2-0.7)
+ IFN-α	del-carriers	93	34.4	0.09	1.4 (0.9-2.1)	0.3	0.8 (0.6-1.2)	74.4	0.002	2.3 (1.3–4.2)	0.7	0.9 (0.6-1.4)
CD3EAP G-21A												
	GG	101	27.4		1		1.0	69.7		1		1.0
	AG + AA	45	32.2	0.5	0.9 (0.6-1.3)		1.0	80.1	0.8	0.9 (0.6-1.5)		1.0
+ IFN-α	GG	88	30.5		1	0.1	0.8 (0.6-1.1)	75.1		1	0.4	0.9 (0.6-1.3)
+ IFN-α	AG + AA	53	53.0	0.05	0.7 (0.5-1.0)	0.02	0.6 (0.4-0.9)	*	0.005	0.5 (0.3-0.8)	0.04	0.5 (0.3-1.0)
PPPIRI3L IVS1 A4364G												
	AA	91	27.2		1		1.0	63.0		1		1.0
	AG + GG	56	29.3	0.2	0.8 (0.6-1.2)		1.0	96.1	0.09	0.7 (0.4-1.1)		1.0
+ IFN-α	AA	91	32.9		1	0.001	0.6 (0.4-0.8)	94.0		1	0.01	0.6 (0.4-0.9)
+ IFN-α	AG + GG	43	42.7	0.8	1.0 (0.7-1.5)	0.1	0.7 (0.5-1.1)	103.9	0.6	0.9 (0.5-1.5)	0.4	0.8 (0.4-1.4)

*[a]Comparison of genotypes within treatment arms, homozygous carriers of the wild type allele serve as the reference; [b]comparison of carriers of the same genotype in the presence and absence of IFN-α treatment, untreated patients serve as the reference; [c]values in parentheses are 95% CI. CI: confidence interval; HR: hazard ratio; TTF: time-to-treatment failure; OS:overall survival; *indicates that the median survival was not reached. The allele distributions of homozygous wild type carriers are presented together with heterozygous and homozygous variant carriers not treated or treated with IFN-α.*

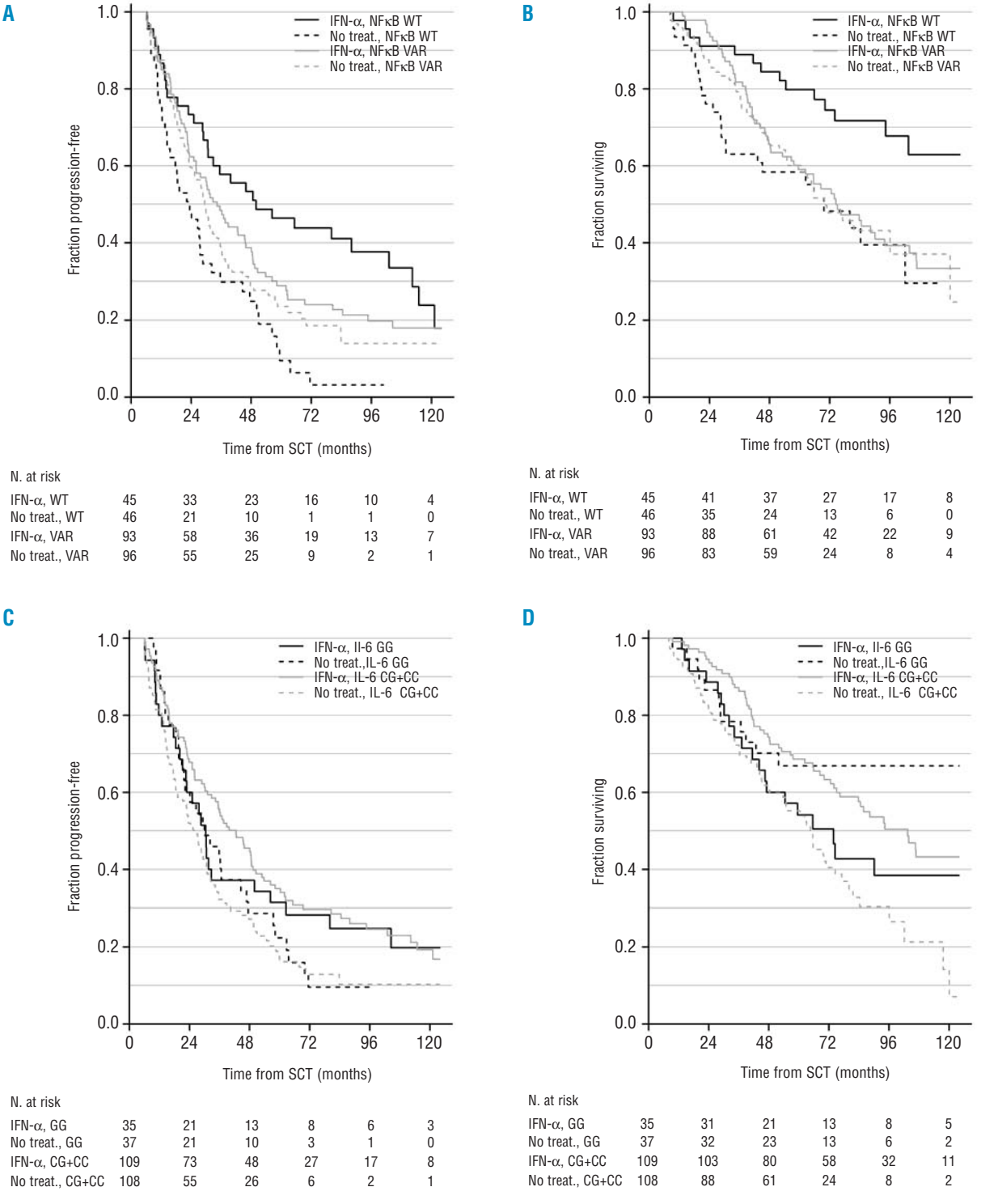

Figure 2. Kaplan-Meier plots of TTF and OS in patients treated or not with IFN-α in relation to genotypes of *NFKB1* -94ins/del and *IL6* G-174C. The numbers of patients at risk at 0, 24. 48 and 72 months are presented below the figure. (A) and (B): outcomes according to genotypes of *NFKB1* -94ins/del. (A) TTF in patients treated with IFN-α carrying the wild type allele ins (solid black line) and the variant allele del (solid gray line). TTF in patients not treated with IFN-α carrying the wild type allele ins (dashed black line) and the variant allele del (dashed gray line). (B) OS in patients treated with IFN-α carrying the wild type allele ins (solid black line) and the variant allele del (solid gray line). OS in patients not treated with IFN-α carrying the wild type allele ins (dashed black line) and the variant allele del (dashed gray line). In multivariate Cox analysis, TTF and OS of patients with the wild type ins/ins-genotype was related to treatment with IFN-α (p=0.01 and p=0.04, respectively). (C) and (D): outcomes according to genotypes of *IL6* G-174C. (C) TTF in patients treated with IFN-α carrying the wild type allele GG (solid black line) or the variant allele CG+CC (solid gray line). TTF in patients not treated with IFN-α carrying the wild type allele GG (dashed black line) or the variant allele CG+CC (dashed gray line). (D) OS in patients treated with IFN-α carrying the wild type allele GG (solid black line) or the variant allele CG+CC (solid gray line). OS in patients not treated with IFN-α carrying the wild type allele GG (dashed black line) or the variant allele CG+CC (dashed gray line). In multivariate Cox analysis, OS of patients with the wild type GG-genotype was related to treatment with IFN-α (p=0.04).

patients who were treated with IFN-α, homozygous carriers of the wild type ins-allele had a TTF of 49.9 months and median OS was not reached at a follow-up of 120 months after HDT. In contrast, carriers of the variant del-allele treated with IFN-α had a TTF of 34.4 months (p=0.09) and a median OS of 74.4 months (p=0.002). In the absence of IFN-α treatment, there was no association between the polymorphism *NFKB1* -94ins/delATTG and TTF and OS.

The allele distribution of *IL6* G-174C did not influence TTF (Figure 2C) when analyzed by multivariate Cox analysis (Table 3) and among patients treated with IFN-α, there was no difference in TTF or OS in relation to the genotype distribution of *IL6* G-174C. However, among patients who did not receive treatment with IFN-α, carriers of the wild type G allele *IL6* G-174C had a longer OS and median survival was not reached at a follow-up of 120 months after HDT, whereas carriers of the variant C allele had a median survival of 64.6 months (p=0.007) (Figure 2D). In a multivariate Cox analysis, the GG-genotype of *IL6* G-174C remained significant for untreated patients when adjusted for age, sex and β2-microglobulin (Table 3; p=0.01).

The genotype distribution of *NFKB1* -94ins/delATTG was as follows: 91 ins-carriers (wild type), 143 heterozygous carriers and 46 homozygous del-carriers, and for *IL6* G-174C the genotype distribution was: 72 GG-carriers (wild type), 149 CG-carriers and 68 CC-carriers. We tested the distribution of baseline characteristics between treatment groups for *NFKB1* -94ins/delATTG and *IL6* G-174C and found no differences (Table 4). Furthermore, no difference was found in response status after HDT in relation to allele distribution and treatment status of IFN-α (Table 5). There was no difference in duration of IFN-α treatment and in time to progression after cessation of IFN-α between patients with the *IL6* G-174C and *NFKB1* -94ins/delATTG genotypes. By univariate analysis, age and sex were found to be prognostic markers for TTF, and β2-microglobulin, age and sex were found to be prognostic markers for OS in this population of patients. There was no difference in results when data were adjusted for these prognostic factors. In multivariate analysis of IFN-α treated patients, age (p=0.02), sex (p=0.04), and the wild type ins/ins-genotype of *NFKB1* -94ins/delATTG (p=0.0001), remained prognostic markers for TTF, and for OS the wild type ins-allele of *NFKB1* -94ins/delATTG (p=0.02) was found to be a prognostic marker together with age (p= 0.02), sex (p=0.04) and β2-microglobulin (p=0.001). In untreated patients the wild type GG-genotype of *IL6* G-174C remained a prognostic marker (p=0.04) for OS together with β2-microglobulin (p=0.02).

Patients treated with IFN-α and carrying the variant allele C allele *IL1B* T-31C had a prolonged TTF (29.6 months and 48.0 months) and OS (70.9 months and infinity) as compared to homozygous carriers of the T allele, but when tested in a multivariate Cox analysis, the analysis showed that the difference in TTF and OS was not related to treatment with IFN-α (Table 3; p=0.09 and p=0.21). In relation to the polymorphism *CD3EAP* G-21A, carriers of the variant G allele had significant longer TTF and OS as compared to those with the wild type A-allele. When data were correlated to effect of treatment with IFN-α in multivariate Cox analysis, there was no association. The polymorphism *PPP1R13L* IVS1 A4364G was not associated with differences in TTF and OS and no difference between treatment groups was seen when the data were analyzed by multivariate Cox analysis.

Table 3. Multivariate Cox analysis of the effect of INF-α treatment depending on genotypes. *p* values are given for the comparison of TTF and OS for carriers of difference genotypes treated or not with INF-α in a multivariate Cox analysis.

Parameter	TTF *p* value[a]	OS *p* value[b]
IIB T-31C	0.09	0.21
IL6 G-174C	0.51	0.01
NFKB1 -94ins/delATTG	0.01	0.04
CD3EAP-G21A	0.29	0.12
PPPIRI3L IVS1 A4364G	0.48	0.37

[a]*adjusted for sex, and age;* [b]*adjusted for sex, age, and β2-microglobulin level.*

Table 4. Characteristics of the patients subdivided by the *NFKB1* -94ins/delATTG and *IL6* G-174C polymorphisms.

	NFKB1 INS	*NFKB1* DEL carrier	*p* value	*IL6* G-174C *GG*	*IL6* G-174C *CG CC*	*p* value
Age	54 (37-66)	56 (31-69)	0.1	55 (32-69)	56 (31-68)	0.7
β2-microglobulin	3.8 (1.2-48)	3.7 (1.3-25)	0.7	3.7 (1.2-48)	3.7 (1.3-33)	0.8
Creatinine	1.1 (0.6-9.4)	1.1 (0.5-8.1)	0.2	1.1 (0.5-5.9)	1.1 (0.6-9.4)	0.1
Albumin	3.5 (0.3-5.3)	3.5 (0.3-4.9)	0.5	3.4 (0,3-5.3)	3.5 (0,3-5.3)	0.5
Durie-Salmon Stage			0.8			0.4
I	11 (12%)	20 (11%)		10 (14%)	20 (10%)	
II	21 (24%)	39 (21%)		18 (26%)	48 (23%)	
III	56 (64%)	126 (68%)		42 (60%)	143 (67%)	
International Staging System			1.0			0.7
I	16 (24%)	33 (26%)		13 (22%)	36 (26%)	
II	28 (42%)	54 (41%)		24 (41%)	59 (42%)	
III	22 (33%)	41 (32%)		22 (37%)	43 (31%)	
Sex			0.2			0.2
Male	49 (54%)	116 (61%)		47 (65%)	122 (56%)	
Female	42 (46%)	73 (39%)		25 (35%)	95 (44%)	

Discussion

We have previously shown that inborn variations in genes involved in DNA-repair are important for TTF in MM patients treated with HDT.[7] In this study, we addressed the question of whether inborn variations in genes related to inflammation are important for the effect of IFN-α maintenance treatment after HDT. Formerly, many myeloma patients received maintenance treatment with IFN-α after HDT. Today, maintenance treatment with IFN-α is not considered the standard of care after HDT due to the uncertainty of the results from previously published clinical studies and because of side effects of the treatment. In this study, treatment with IFN-α was initiated 3 months after HDT and patients treated for less than 3 months with IFN-α were excluded from the treatment group and placed in the untreated group since no effect of IFN-α would be expected. However, re-analysis of the data excluding these patients from the untreated group did not change the results (*results not shown*). As a consequence of this design, patients with the poorest prognosis and with recurrence of disease within 6 months after HDT were not included in the study. Due to the uncontrolled retrospective design of our study, the IFN-α treatment group included younger patients (54 versus 57 years, p=0.005) with more advanced Durie -Salmon stage (p=0.02) than the group of untreated patients. This could potentially have resulted in a selection bias since less healthy patients may not be offered IFN, but the different treatment strategy between regions makes it less likely. However, this potential selection bias should not influence the association between genotype and treatment outcome within the two IFN treatment groups.

We, therefore, analyzed whether inborn variations in genes involved in the inflammatory response influenced TTF and OS in subgroups of myeloma patients treated with IFN-α. When compared within the IFN-α treatment group, patients carrying the wild type allele of *NFKB1* -94ins/delATTG did not have a significantly prolonged TTF, although this was increased from 34.4 months to 49.9 months, probably because of the low number of patients. However, the result was significant in a multivariate Cox analysis. TTF was significantly increased when the comparison was performed in relation to genotype and Figure 1A shows a clear separation of wild type ins-carriers from ins-carriers among patients not treated with IFN-α and del-carriers treated or not with IFN-α.

No difference in treatment duration of IFN-α was found in relation to genotype distribution of *NFKB1* -94ins/delATTG, which indicates that the difference in TTF is not a result of early cessation of IFN-α treatment caused by side effects.

Figure 1A also illustrates that patients not treated with IFN-α carrying the wild type ins-allele had a shorter TTF than patients not treated with IFN-α carrying the variant del-allele. These findings are not statistically significant, but may indicate that NF-κB is not the only player in the activation pathway of IFN-α.

Patients carrying the wild type ins-allele of *NFKB1* -94ins/delATTG had significantly longer OS when treated with IFN-α compared to treated variant allele carriers and also compared to all untreated patients regardless of genotype status.

Table 5. Genotype distributions of *IL6* G-174C and *NFKB1* -94 ins/del in relation to response after HDT.

Gene	Genotype	CR	PR	MR/NC	p value
IL6 G-174C					
No IFN-α treatment	GG	12 (48%)	16 (36%)	5 (15%)	0.1
	CG + CC	36 (36%)	56 (56%)	8 (8%)	-
+ IFN-α treatment	CG	15 (45%)	16 (48%)	2 (6%)	0.2
	CG + CC	45 (45%)	53 (54%)	1 (1%)	-
NFKB1 -94 ins/del					
No IFN-α treatment	INS	18 (44%)	17 (41%)	6 (15%)	0.2
	DEL-carriers	34 (38%)	49 (55%)	6 (7%)	-
+ IFN-α treatment	INS	19 (46%)	21 (51%)	1 (2%)	1
	DEL-carriers	40 (47%)	44 (51%)	2 (2%)	-

CR: complete response; PR: partial response; MR: minor response; NC: no change.

In humans, both IFN and NF-κB are central players in the regulation of apoptosis and cell growth and in the modulation of the immune system. A normal function of NF-κB is essential for the innate and adaptive immune systems and it is, therefore, interesting that IFN-α treated myeloma patients with a normal level of NF-κB had a significantly prolonged OS.[4] This may indicate that the effect of IFN-α treatment is dependent on the availability of NF-κB. This hypothesis is supported by a recent finding that the effect of IFN-γ was mediated through a JAK-STAT-NKκB signaling pathway in corticotroph tumor cells producing adrenocorticotrophin hormone.[23]

Patients who were not treated with IFN-α and carried the wild type G allele of *IL6* G-174C had a longer OS compared to patients carrying the C allele of *IL6* G-174C. The polymorphism was not associated with TTF and it is not, therefore, possible to exclude that the difference in OS was caused by later treatment modalities. IFN-α induces IL-6 and IL-6 is important for normal development of B cells and plasma cells and is known to induce proliferation of pluripotent stem cells. Our study indicates that a normal, high transcription level of the *IL6* gene gives patients with MM a survival advantage.

Patients diagnosed with MM cannot be cured in spite of intensive treatment strategies with single or tandem HDT. It is, therefore, important to establish maintenance treatment strategies that are well-tolerated and result in a good quality of life for the patients. Patients treated with IFN-α may experience side effects such as emotional and social impairment, fatigue, pain and loss of appetite. The side effects disappear when treatment is withdrawn. In a recent randomized cross-over study quality of life was considerably better in patients treated with pegylated IFN α2b than in patients treated with IFN-α.[24] New maintenance treatments for MM patients, including thalidomide, prednisone, bortezomib and lenalidomide have been proposed and studied. These treatment modalities are associated with potentially worse side effects than treatment with IFN-α. Peripheral neuropathy is a common side effect of treatment with both thalidomide and bortezomib, and may be severe and irreversible and affect daily life activities.

We report that the *NFKB1* -94ins/delATTG polymorphism may select patients with MM who could benefit particularly from maintenance treatment with IFN-α after HDT. We suggest that maintenance treatment with IFN-α (maybe pegylated IFN α2b) should still be considered after HDT for patients carrying the wild type ins/ins genotype of *NFKB1* -94ins/delATTG. A prospective study of IFN-α treatment in relation to *NFKB1* -94ins/delATTG is warranted.

Authorship and Disclosures

AJV: conception and design of the study, analysis and interpretation of data, drafting the article, approval of the version to be published; TWK: conception and design of the study, analysis and interpretation of data, drafting the article, approval of the version to be published; PG: analysis and interpretation of data, revising the manuscript critically, approval of the version to be published; NFA: analysis and interpretation of data, revising the manuscript critically, approval of the version to be published; NA: analysis and interpretation of data, revising the manuscript critically, approval of the version to be published; HG: analysis and interpretation of data, revising the manuscript critically, approval of the version to be published; UV: conception and design of the study, analysis and interpretation of data, drafting the article, approval of the version to be published.

The authors reported no potential conflicts of interest.

References

1. Barlogie B, Kyle RA, Anderson KC, Greipp PR, Lazarus HM, Hurd DD, et al. Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of phase III US Intergroup Trial S9321. J Clin Oncol 2006;24:929-36.
2. Cunningham D, Powles R, Malpas J, Raje N, Milan S, Viner C, 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.
3. 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.
4. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998;67:227-64.
5. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441:431-6.
6. Vangsted AJ, Klausen TW, Ruminski W, Gimsing P, Andersen NF, Gang AO, et al. The polymorphism IL-1beta T-31C is associated with a longer overall survival in patients with multiple myeloma undergoing auto-SCT. Bone Marrow Transplant 2009;43:539-45.
7. Vangsted A, Gimsing P, Klausen TW, Nexo BA, Wallin H, Andersen P, et al. Polymorphisms in the genes ERCC2, XRCC3 and CD3EAP influence treatment outcome in multiple myeloma patients undergoing autologous bone marrow transplantation. Int J Cancer 2007;120:1036-45.
8. Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum Mol Genet 2006;15:519-29.
9. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in Helicobacter pylori infection. Gastroenterology 2002;123:1793-803.
10. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
11. Karban AS, Okazaki T, Panhuysen CI, Gallegos T, Potter JJ, Bailey-Wilson JE, et al. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. Hum Mol Genet 2004;13:35-45.
12. Yamazaki T, Hamano Y, Tashiro H, Itoh K, Nakano H, Miyatake S, et al. CAST, a novel CD3epsilon-binding protein transducing activation signal for interleukin-2 production in T cells. J Biol Chem 1999;274:18173-80.
13. Vogel U, Hedayati M, Dybdahl M, Grossman L, Nexo BA. Polymorphisms of the DNA repair gene XPD: correlations with risk of basal cell carcinoma revisited. Carcinogenesis 2001;22:899-904.
14. Nexo BA, Vogel U, Olsen A, Ketelsen T, Bukowy Z, Thomsen BL, et al. A specific haplotype of single nucleotide polymorphisms on chromosome 19q13.2-3 encompassing the gene RAI is indicative of postmenopausal breast cancer before age 55. Carcinogenesis 2003;24:899-904.
15. Vogel U, Laros I, Jacobsen NR, Thomsen BL, Bak H, Olsen A, et al. Two regions in chromosome 19q13.2-3 are associated with risk of lung cancer. Mutat Res 2004;546:65-74.
16. Yang JP, Hori M, Sanda T, Okamoto T. Identification of a novel inhibitor of nuclear factor-kappaB, RelA-associated inhibitor. J Biol Chem 1999; 274:15662-70.
17. Lenhoff S, Hjorth M, Holmberg E, Turesson I, Westin J, Nielsen JL, et al. Impact on survival of high-dose therapy with autologous stem cell support in patients younger than 60 years with newly diagnosed multiple myeloma: a population-based study. Nordic Myeloma Study Group. Blood 2000;95:7-11.
18. Lenhoff S, Hjorth M, Westin J, Brinch L, Backstrom B, Carlson K, et al. Impact of age on survival after intensive therapy for multiple myeloma: a population-based study by the Nordic Myeloma Study Group. Br J Haematol 2006;133:389-96.
19. Mellqvist UH, Lenhoff S, Johnsen HE, Hjorth M, Holmberg E, Juliusson G, et al. Cyclophosphamide plus dexamethasone is an efficient initial treatment before high-dose melphalan and autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: results of a randomized comparison with vincristine, doxorubicin, and dexamethasone. Cancer 2008;112:129-35.
20. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215.
21. Saber AT, Nielsen LR, Dictor M, Hagmar L, Mikoczy Z, Wallin H. K-ras mutations in sinonasal adenocarcinomas in patients occupationally exposed to wood or leather dust. Cancer Lett 1998;126:59-65.
22. Vogel U, Christensen J, Dybdahl M, Friis S, Hansen RD, Wallin H, et al. Prospective study of interaction between alcohol, NSAID use and polymorphisms in genes involved in the inflammatory response in relation to risk of colorectal cancer. Mutat Res 2007;624:88-100.
23. Labeur M, Refojo D, Wolfel B, Stalla J, Vargas V, Theodoropoulou M, et al. Interferon-gamma inhibits cellular proliferation and ACTH production in corticotroph tumor cells through a novel janus kinases-signal transducer and activator of transcription 1/nuclear factor-kappa B inhibitory signaling pathway. J Endocrinol 2008; 199:177-89.
24. Sirohi B, Powles R, Lawrence D, Treleaven J, Kulkarni S, Leary A, 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.