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ORIGINAL ARTICLE

The polymorphism *IL-1β* T-31C is associated with a longer overall survival in patients with multiple myeloma undergoing auto-SCT

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Proinflammatory cytokines are suspected to play a role in the pathogenesis of multiple myeloma (MM). Therefore, it is possible that inborn genetic variations leading to a modified expression of these cytokines will influence the outcome for these patients. We investigated 348 MM patients undergoing high-dose melphalan treatment followed by Auto-SCT and examined the influence of single nucleotide polymorphisms (SNPs) in genes involved in the inflammatory response. We found that the polymorphism *IL-1β* T-31C significantly influenced overall survival (OS; $P = 0.02$) and that carriers of the variant C-allele had a significantly longer survival than homozygous wild-type allele TT-carriers (relative risk 0.6 (95% CI = 0.5–0.9); $P = 0.008$). The polymorphisms *IL-6* G-174C, *IL-10* C592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G, *COX-2* T8473C and *NFKB1* ins/del did not influence the OS in this group of patients. Furthermore, homozygous carriers of the variant allele of *IL-1β* T-31C were at 1.37-fold (CI = 1.05–1.80) increased risk of MM as compared with population-based controls ($P = 0.02$). Our results indicate that IL-1β is involved in the pathogenesis of MM.
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Introduction

The interaction between the myeloma cell and the BM microenvironment is central to the growth and survival of myeloma cells. The myeloma cell adheres to the BM microenvironment, and thereby stimulates angiogenesis and enhances the stimulation of cytokines such as IL-1β, IL-6 and IL-10. The proinflammatory cytokines released from the BM microenvironment activate the NF-κB (nuclear factor-κB) through the classical pathway in both the malignant myeloma cells and the innate immune system. This activation leads to further growth, adhesion and survival of myeloma cells and production of inflammatory mediators, such as IL-1β, IL-6 and cyclooxygenase 2 (COX-2).[1,2]

Another nuclear activation factor involved in the inflammatory response has been found in B-cell lymphomas and multiple myeloma (MM) cells.[3] The nuclear receptor, peroxisome proliferator-activated receptor-γ (PPARγ), is an important transcription factor and member of the nuclear hormone receptor superfamily. PPARγ regulates the expression of *COX-2,* and prostaglandin 15d-PGJ_2 is a natural ligand for the PPARγ that induces apoptosis in myeloma cell lines.[3]

During the last decade, new treatments with immunomodulatory drugs and proteosome inhibitors have been introduced and have led to improved survival of the patients. These drugs induce apoptosis of myeloma cells, interrupt the interaction between myeloma cells and stromal cells in the BM, inhibit angiogenesis and inhibit the secretion of IL-1β and IL-6. The treatment effects of the new drugs support the importance of these key regulators of the immune response in the pathogenesis of MM. Inborn genetic variation in these genes may therefore be important for the risk, prognosis and treatment outcome of MM.

Proinflammatory cytokines, COX-2, NF-κB and PPARγ are important for the regulation of inflammatory response as well as cancer cell growth. Several studies have shown a link between polymorphisms in *IL-1β*, *IL-6*, *IL-10*, *COX-2*,

NF-κB and *PPARγ* and cancer risk.[4–8] They may also be central to the pathogenesis of MM. New treatment strategies directed against these targets are being developed, and inborn variations in these genes may therefore become essential for the effect of such targeted therapy. In this study, we address the importance of the polymorphisms *IL-1β* T-31C, *IL-6* G-174C, *IL-10* C-592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G, *COX-2* T8473C and *NF-KB1*-94ins/delATTG, which are known to affect the transcription levels of the genes and are also known to be important for cancer cell growth, inflammatory response and cancer risk, and the response to high-dose melphalan treatment.

Materials and methods

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

Patients, clinical data, response criteria and eligibility criteria have been described in detail earlier.[9] Briefly, patients diagnosed with MM and treated with high-dose melphalan and auto-SCT from August 1994 to August 2004 were recruited from four participating centers in Denmark. A total of 348 patients were included in the study. Of these, 185 patients were included in the high-dose treatment protocols including Auto-SCT administrated by the Nordic Myeloma Study Group (nos. 5/94, 7/98 and 11/00),[10–12] whereas the remaining 163 patients were treated with similar regimens, but not registered in these protocols. Staging was performed according to Durie and Salmon. Time to treatment failure (TTF) and overall survival (OS) were calculated from the date of transplantation to the date of progression or death. Treatment-related mortality was 3%. Two patients died from malignancies other than MM, and two patients died because of other 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 the time of death in the analysis of TTF. Time to treatment failure in 68 patients, including those who died during the transplantation procedure, was followed for less than 2 years. Induction therapy was three series VAD (vincristine, doxorubicin and dexamethasone) or 2–3 series of CY 1 g/m^2 once daily 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 series). Peripheral blood stem cells were harvested at regeneration after CY priming, and the patients thereafter underwent high-dose chemotherapy with melphalan (200 mg/m^2) followed by Auto-SCT and maintenance therapy with interferon. A random sample of 800 individuals from the Danish Diet, Cancer and Health cohort was used for comparing the allele frequencies of the examined MM patients with the corresponding frequencies observed earlier.[13] The study was approved by the Danish Ethical Committee (01-158/03).

Human tissue samples

Peripheral blood mononuclear cells (PBMCs) were purified from 292 leukapheresis products by buffy coat preparation. From 56 patients, 10 times 10 μm sections were collected from paraffin-embedded BM samples. Material was not available for 19 patients undergoing Auto-SCT, and therefore these patients were not included in the study.

DNA purification

DNA for analysis was purified from PBMCs by the salting out method[14] or from paraffin-embedded tissue by phenol extraction as described.[15]

Detection of single nucleotide polymorphism (SNPs)

Genotypes were determined on an ABI 7500 using end point readings. Reactions of 5 μl contained 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 repeated 10% subset yielded 100% identical genotypes. Moreover, for 10 persons, DNA from both BM and leukapheresis products was genotyped with identical results.

IL-1β T-31C (rs1143627), *IL6* G-174C (rs1800795), *IL10* C-592T (rs1800872), *PPARγ2*Pro[12]Ala (rs1801282), *COX-2* C8473T (rs5275) and *COX-2* A-1195G (rs689466) were genotyped as described earlier.[13] For NF-κB ins/del (rs28362491), the primer sequences were F: 5′-CTATGG ACCGCATGACTCTATCAG-3′ and R: 5′-GGGCTC TGGCTTCCTAGCA-3′. Probe sequences were NFKB1 INS: 5′-FAM-ACCATTGATTGGGCCCGG-BHQ-3′ and NFKB1-DEL: 5′-Yakima Yellow-CCGACCATTGGGC CCG-BHQ-3′.

Statistical methods

SPSS statistical software was used for all calculations (SPSS for Windows, Rel. 14.0.0.2005, Chicago: SPSS Inc.). All tests were two-sided, and P-values <0.05 were regarded as significant.

Fisher's exact test was used for comparing categorical variables and the Mann–Whitney test was used for comparing 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, log-likelihood statistics, was applied for univariate analyses of covariates and for multivariate analysis. Significant variables with a P-value <0.05 by univariate analysis were included in the multivariate Cox analyses to identify variables of independent significance. Analysis of risk was performed as a case–control study. The frequencies of the different genotypes were compared in the myeloma patients and in a control group. For significance testing, Fisher's exact test was used. The comparison group was described in a recent publication by Vogel *et al*.[13]

Results

SNP

Genotypes of *IL-1β* T-31C, *IL-6* G-174C, *IL-10* C592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G, *COX-2* T8473C and *NF-KB1*-94ins/del were determined. There was no difference in the allele frequencies among patients from different participating centers. The allele frequencies of the examined MM patients were compared with the corresponding

frequencies observed earlier in a random control sample. No difference was found in the distribution of the genotypes *IL-6* G-174C, *IL-10* C592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G and *COX-2* T8473C when compared with the comparison group. Heterozygous carriers of the C-allele of *IL-1*β T-31C were at 1.15-fold higher risk of MM (CI = 0.94–1.41), and homozygous carriers were at 1.37-fold increased risk of MM (CI = 1.05–1.80) ($P = 0.02$) than homozygous carriers of the wild-type allele when compared with the random sample of 753 persons from the Diet, Cancer and Health cohort. The effect of the genotypes on TTF and OS was tested in univariate analysis, and the results are presented in Table 1. TTF data adjusted for β2-microglobulin and OS data adjusted for all other survival-related factors (β2-microglobulin, creatinine and Durie–Salmon stage) are shown in parentheses.

There was no difference in TTF for any of the polymorphisms studied. Carriers of the variant C-allele of *IL-1*β T-31C had a median OS of 80.1 months when compared with a median OS of 48.5 months for the homozygous carriers of the wild-type T-allele ($P = 0.008$; Figure 1 and Table 1). The correlation was still statistically significant after adjustment for all other prognostic factors known to influence OS (β2-microglobulin, creatinine and Durie–Salmon stage). The correlation of polymorphism in *IL-1*β T-31C with OS was even more marked when analyzed after treatment failure. Analyzed from the time of progression, carriers of the variant C-allele of *IL-1*β T -31C had a median OS of 37.1 months when compared with a median OS of 16.2 months for the homozygous carriers of the wild-type T-allele ($P = 0.002$). There was no correlation between the allele distribution of polymorphism *IL-1*β T-31C and treatment outcome either after induction treatment or after Auto-SCT (Table 2). There was no effect on patient survival of the polymorphisms *IL-6* G-174C, *IL-10* C-592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G, *COX-2* T8473C and *NF-KB1*-94ins/del. There was no interaction between sex and genotypes in relation to TTF and survival.

Combination analyses of SNPs

The proinflammatory cytokines studied are known stimulators of myeloma cell growth. The three polymorphisms *IL-1*β T-31C, *IL-6* G-174C and *IL-10* C592A were combined, and the effect on TTF and OS of these combinations were investigated in a univariate analysis (Table 3).

There was no association between *IL-1*β T-31C, *IL-6* G-174C and *IL-10* C592A polymorphisms. No additive effect on survival was seen when the variant genotype of *IL-1*β was combined with the genotypes of *IL-6* or *IL-10*. The variant alleles of the two *COX-2* polymorphisms, *COX-2* A-1195G and *COX-2* T8473C, are not part of the same haplotype.[16] Consequently, we tested combinations of these polymorphisms, but no additional effects on TTF or OS were seen (Table 3).

Multivariate analysis of prognostic markers

The *IL-1*β T-31C polymorphism was compared with known prognostic factors (β2-microglobulin, creatinine, albumin, sex, age and Durie–Salmon stage). No significant covariance was found (Table 4).

The polymorphism *IL-1*β T-31C was examined in a multivariate analysis to see whether it had independent prognostic value. *IL-1*β T-31C was tested separately against the statistically significant parameters from the univariate analyses to calculate the adjusted hazard ratios and *P*-values. In one center, β2-microglobulin was not analyzed routinely. Therefore, only 240 patients were available in the multivariate analysis with *IL-1*β T-31C. The adjusted hazard ratios and *P*-values were similar to the unadjusted hazard ratios (Table 5).

Using a wider range of the commonly used prognostic variables (age, sex, albumin, creatinine, β2-microglobulin and Durie–Salmon stage) and a backward stepwise method, the *IL-1*β T-31C polymorphism stayed in the model as a statistically significant prognostic factor ($P = 0.04$) together with age ($P = 0.02$), sex ($P = 0.04$) and β2-microglobulin ($P = 0.0002$).

Discussion

Immunological and inflammatory response genes are of particular interest in hematological malignancies because these genes both participate in the normal recruitment and differentiation of hematopoietic cells, the normal immune response and may be of importance in carcinogenesis. Several cytokines function as paracrine and autocrine growth factors and are expressed in both myeloma cells and in BM stromal cells. In contrast to myeloma cells, BM stromal cells are expected to have normal regulatory functions. The myeloma cells home in the BM, where the interaction with BM stromal cells may be crucial for further development, growth and resistance to chemotherapy.[17] In this study, we have addressed the question how inborn variations in genes involved in the immune and inflammatory responses influence the outcome of patients with MM treated with high-dose melphalan and Auto-SCT. Furthermore, we have analyzed whether any of the examined polymorphisms are associated with the risk for development of MM.

The polymorphism *IL-1*β T-31C influenced OS. We found that patients carrying the variant C-allele (CT + CC, 59% of patients) had an increased OS of 80.1 months compared with 48.5 months for the homozygous carriers of the wild-type T-allele (TT, 41% of patients) ($P = 0.008$). The effect on OS was more pronounced when examined after TTF. Furthermore, *IL-1*β T-31C was found to be a statistically significant prognostic marker for prolonged OS in a Cox multivariate analysis.

IL-1β has several functions in man and is induced by nearly all microbial and many inflammatory substances and cytokines. It is expressed mainly by BM stromal cells, but is also expressed by myeloma cells and, to a lesser extent, by plasma cells in patients with monoclonal gammopathy of undetermined significance (MGUS).[18–20] IL-1β is important for both regulation of inflammation and of host defense in man, and it stimulates the production of IL-6, an important growth factor for MM cells. A recent study has shown that IL-1β-induced stimulation of IL-6

Table 1 Univariate analysis of the effect of genotype on TTF and OS

Gene	*Allele*	*N*	*Median TTF (months)*	P-*value*	*HR*	*Median OS (months)*	P-*value*	*HR*
IL-1β T-31C								
	TT	125	27.2	—	1	48.5	—	1
	CT	134	26.2	0.77	1.0 (0.7–1.3)[a]	75.2	0.036	0.7 (0.5–1.0)
				(0.89)	*(1.0 (0.7–1.4))*		*(0.062)*	*(0.7 (0.4–1.0))*
	CC	49	45.0	0.07	0.6 (0.4–1.0)	85.9	0.014	0.5 (0.3–0.9)
				(0.10)	*(0.6 (0.3–1.1))*		*(0.035)*	*(0.5 (0.3–1.0))*
	CT + CC	183	29.9	0.33	0.9 (0.6–1.2)	80.1	0.008	0.6 (0.5–0.9)
				(0.42)	*(0.9 (0.6–1.2))*		*(0.018)*	*(0.6 (0.4–0.9))*
IL-6 G-174C								
	GG	90	27.8	—	1	73.9	—	
	CG	167	30.3	0.19	0.8 (0.6–1.1)	68.5	0.92	1.0 (0.7–1.4)
				(0.11)	*(0.7 (0.5–1.1))*		*(0.78)*	*(1.1 (0.7–1.7))*
	CC	80	23.6	0.42	1.2 (0.8–1.7)	65.7	0.42	1.2 (0.8–1.8)
				(0.19)	*(1.4 (0.9–2.1))*		*(0.47)*	*(1.2 (0.7–2.1))*
	CG + CC	247	27.4	0.54	0.9 (0.7–1.2)	65.9	0.81	1.0 (0.7–1.5)
				(0.53)	*(0.9 (0.6–1.3))*		*(0.63)*	*(1.1 (0.7–1.7))*
IL-10 C-592A								
	CC	212	28.6	—	1	68.5	—	—
	AC	117	23.1	0.33	1.2 (0.9–1.6)	64.6	0.68	1.1 (0.8–1.5)
				(0.32)	*(1.2 (0.8–1.7))*		*(0.67)*	*(1.1 (0.7–1.6))*
	AA	18	48.6	0.33	0.7 (0.3–1.5)	—[b]	0.16	0.5 (0.2–1.3)
				(0.14)	*(0.5 (0.2–1.3))*		*(0.062)*	*(0.3 (0.1–1.1))*
	AC + AA	135	24.6	0.55	1.1 (0.8–1.4)	70.0	0.97	1.0 (0.7–1.3)
				(0.69)	*(1.1 (0.8–1.5))*		*(0.77)*	*(0.9 (0.6–1.4))*
PPARγ2 Pro^{12} Ala								
	Pro/Pro	241	27.6	—	1	65.6	—	
	Pro/Ala	102	24.6	0.70	0.9 (0.7–1.3)	74.1	0.33	0.9 (0.6–1.2)
				(0.20)	*(0.8 (0.5–1.1))*		*(0.13)*	*(0.7 (0.5–1.1))*
	Ala/Ala	2	50.5	—	—	—[b]	—	—
	Ala-carriers	104	24.6	0.71	0.9 (0.7–1.3)	74.1	0.33	0.9 (0.6–1.2)
				(0.20)	*(0.8 (0.5–1.1))*		*(0.13)*	*(0.7 (0.5–1.1))*
COX-2 A-1195G								
	AA	226	27.7	—	1	73.9	—	—
	AG	110	25.4	0.91	1.0 (0.8–1.4)	63.1	0.66	1.1 (0.8–1.5)
				(0.27)	*(0.8 (0.5–1.2))*		*(0.29)*	*(0.8 (0.5–1.2))*
	GG	12	27.2	0.23	1.5 (0.8–3.0)	45.2	0.13	1.7 (0.9–3.3)
				(0.20)	*(1.7 (0.8–3.6))*		*(0.035)*	*(2.2 (1.1–4.6))*
	AG + GG	122	26.6	0.69	1.1 (0.8–1.4)	59.5	0.43	1.1 (0.8–1.5)
				(0.49)	*(0.9 (0.6–1.3))*		*(0.68)*	*(0.9 (0.6–1.4))*
COX-2 T8473C								
	TT	121	25.4	—	1	63.1	—	1
	TC	128	27.6	0.11	0.8 (0.5–1.1)	73.9	0.29	0.8 (0.6–1.2)
				(0.27)	*(0.8 (0.5–1.2))*		*(0.20)*	*(0.8 (0.5–1.2))*
	CC	36	27.8	0.46	0.8 (0.5–1.3)	74.1	0.64	0.9 (0.5–1.5)
				(0.86)	*(1.0 (0.5–1.7))*		*(0.25)*	*(0.7 (0.4–1.3))*
	TC + CC	164	27.8	0.12	0.8 (0.6–1.1)	73.9	0.31	0.8 (0.6–1.2)
				(0.34)	*(0.8 (0.6–1.2))*		*(0.14)*	*(0.7 (0.5–1.1))*
NF-KB1-94 INS/DEL								
	INS	110	28.4	—	1	—[b]	—	1
	INS/DEL	163	26.4	0.79	1.0 (0.8–1.4)	64.6	0.31	1.2 (0.8–1.7)
				(0.66)	*(1.1 (0.7–1.6))*		*(0.57)*	*(1.1 (0.7–1.7))*
	DEL/DEL	55	22.5	0.17	1.3 (0.9–2.0)	65.6	0.10	1.5 (0.9–2.3)
				(0.12)	*(1.5 (0.9–2.4))*		*(0.39)*	*(1.3 (0.7–2.2))*
	DEL carriers	218	26.4	0.50	1.1 (0.8–1.5)	64.6	0.17	1.3 (0.9–1.8)
				(0.37)	*(1.2 (0.8–1.7))*		*(0.44)*	*(1.2 (0.8–1.7))*

Abbreviations: CI = confidence interval; COX = cyclooxygenase; HR = hazard ratio; OS = overall survival; TTF = time to treatment failure.
HR is calculated by COX proportional hazards analysis.
Values in italics are adjusted for prognostic-related factors (TTF: β2-microglobulin. OS: β2-microglobulin, creatinine and Durie–Salmon stage) and are shown in parentheses.
[a]Values in parentheses are 95% CI.
[b]Median survival not reached.

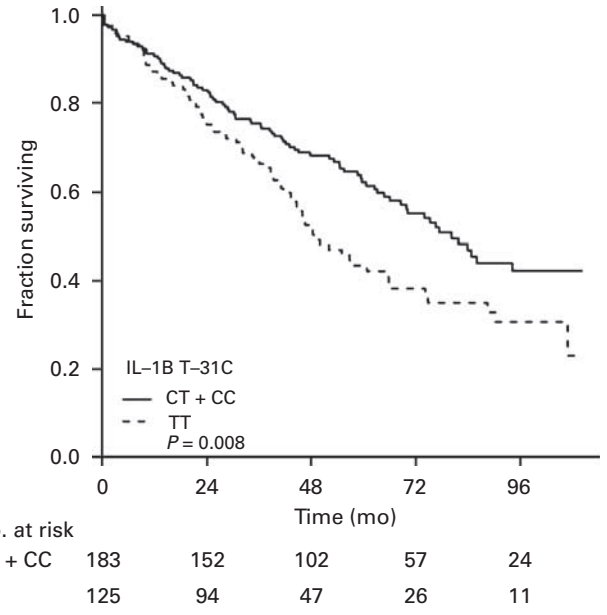

Figure 1 Effect on overall survival (OS) of polymorphisms in *IL-1β* T-31C. The variant haplotype CT + CC (full line) and the wild-type TT (dashed line) Kaplan–Meier plots of OS. The numbers at risk at 0, 24, 48 and 72 months are presented below the figure.

Table 2 *IL-1β* T-31C allele frequency in relation to response obtained after induction treatment (A) and after Auto-SCT (B)

IL-1β *T-31C*	*CR*	*PR*	*MR*	*NR*	*PD*
(A)[a]					
TT	5 (4.7%)	69 (65.1%)	20 (18.9%)	12 (11.3%)	0 (0%)
CT + CC	9 (6.0%)	92 (60.9%)	33 (21.9%)	17 (11.3%)	0 (0%)
(B)[b]					
TT	38 (34.9%)	63 (57.8%)	4 (3.7%)	4 (3.7%)	0 (0%)
CT + CC	68 (42.2%)	80 (49.7%)	7 (4.3%)	5 (3.1%)	1 (0.6%)

Abbreviations: CR = complete response; MR = minor response; NR = no response; PD = progressive disease; PR = partial response.
[a] $P = 0.9$.
[b] $P = 0.7$.

production in BM stromal cells is higher in stromal cells from patients with myeloma than in stromal cells from patients with MGUS. The effect on IL-6 production was inhibited by IL-1 antagonists.[20] This finding indicates that IL-1β is linked with the progression of MGUS to MM. We found that homozygous carriers of the variant C-allele of *IL-1β* T-31C were at increased risk of MM (relative risk of 1.37; CI = 1.05–1.80) compared with random samples of healthy Danes aged 50-64 years.[16] The finding suggests that high IL-1β levels are associated with the increased risk of MM. However, as cases and comparison groups were not matched, the observation needs further examination in a proper case–control study of patients with MGUS and MM.

The association between *IL-1β* T-31C and both OS and risk of MM emphasizes the possible biological importance of IL-1β in the pathogenesis of MM. There are several functional SNPs in the promoter region of *IL-1β*. A Japanese study of helicobacter pylori-infected patients showed that homozygous carriers of the variant allele of the polymorphism *IL-1β* C-511T had higher IL-1β mucosa levels than did carriers of the wild-type C-allele and in patients with systemic inflammatory response syndrome, high IL-6 blood levels were found in carriers of T-allele.[21] Another recent study shows that the two polymorphisms *IL-1β* T-31C and C-511T are in complete linkage equilibrium and that the two variant alleles cosegregate completely. Thus, the variant allele with the two polymorphisms is always present in the same haplotype. The study showed that although the variant C-allele of T-31C has less transcriptional activity when analyzed separately, the haplotype encompassing both variant alleles in positions −31 and −511 has a higher transcriptional response to LPS and phorbol 12-myristate 13-acetate (PMA) stimulation in the human monocytic cell line than the haplotype encompassing the wild-type alleles.[22] This result is supported by a recent study on lung epithelial cells that found that the variant allele in position −31 had a lowered transcription level when analyzed separately.[23] We observed increased survival among patients with a higher inborn IL-1β transcription level and cytokine level. We found no difference in relation to polymorphism in the *IL-1β* gene and TTF after high-dose therapy and in relation to the response to induction treatment or Auto-SCT (Table 2). This would mean that IL-1β levels do not influence the outcome of Auto-SCT, but probably influence other aspects of the disease or treatment strategy after Auto-SCT. One possibility is that the high IL-1β levels may delay the clonal expansion of myeloma cells surviving the high-dose chemotherapy by representing a biological feature of a less aggressive disease. The immune system may be a part of this protection. IL-1β has been shown to be important for the development of human T-helper cells[24] and for the generation of mature IL-12-producing dendritic cells from circulating monocytes from MM patients.[25,26] Another possibility is that IL-1β influences the treatment outcome of new immunomodulating drugs used after the recurrence of the disease.

There was no effect on TTF and patients' survival of the polymorphisms *IL-6* G-174C, *IL-10* C-592A, *PPARγ2* Pro[12]Ala, *COX-2* A-1195G, *COX-2* T8473C and *NF-KB1*-94 ins/del, and we found no correlation between the risk of MM and the polymorphisms *IL-6* G-174C and *IL-10* C592A. The results of *IL-6* G-174C and *IL-10* C592A are in accordance with other smaller studies of MM.[27,28] Polymorphisms in *PPARγ2* Pro[12]Ala, *COX-2* A-1195G and *COX-2* T8473C have not been studied in relation to the risk of MM before. In our study, the polymorphisms *COX-2* A-1195G, *COX-2* T8473C and *PPARγ2* Pro[12]Ala were not associated with the risk of MM or outcome of disease. Therefore, the polymorphisms in these genes cannot explain the discrepancy in the results of the effect of COX inhibitors in relation to the risk of disease and outcome in patients with MM.[29] However, our observations do not exclude important functions of the inflammatory-related genes in the pathogenesis of MM.

Standard treatment for younger patients with MM is high-dose melphalan treatment and autologous stem cell support. This treatment improves the survival but will not

Table 3 Univariate analysis of the effect of combinations of polymorphism on TTF and OS

		N	*Median TTF (months)*	*HR (95% CI)*	P	*Median OS (months)*	*HR (95% CI)*	P
IL-1β T-31C	*IL-6* G-174C							
TT	GG	31	27.8	1	—	46.5	1	—
CT + CC	GG	51	35.8	0.7 (0.4–1.3)	0.26	73.9	0.6 (0.3–1.2)	0.14
TT	CG + CC	92	27.2	0.8 (0.5–1.4)	0.43	48.5	0.9 (0.5–1.6)	0.72
CT + CC	CG + CC	129	27.6	0.7 (0.4–1.2)	0.23	81.7	0.6 (0.3–1.0)	0.045
IL-1B T-31C	*IL-10* C-592A							
TT	CC	73	26.4	1	—	48.5	1	—
CT + CC	CC	115	35.6	0.7 (0.5–1.1)	0.14	83.8	0.6 (0.4–0.9)	0.012
TT	AC + AA	51	27.8	0.8 (0.5–1.4)	0.46	48.3	0.8 (0.5–1.3)	0.41
CT + CC	AC + AA	68	23.1	0.9 (0.6–1.4)	0.66	76.5	0.6 (0.4–1.0)	0.036
IL-6 G-174C	*IL-10* C-592A							
GG	CC	55	30.8	1	—	59.5		—
CG + CC	CC	151	28.4	0.9 (0.6–1.4)	0.67	68.5	1.0 (0.7–1.6)	0.87
GG	AC + AA	35	24.2	1.1 (0.7–2.0)	0.65	90.1	1.0 (0.5–1.9)	0.98
CG + CC	AC + AA	95	23.4	1.0 (0.6–1.5)	1.0	65.9	1.1 (0.7–1.7)	0.82
COX2 T8473C	*COX2* G-1195A							
TT	AA	61	22.5	1	—	55.4	1	
TC + CC	AA	119	27.7	0.8 (0.6–1.3)	0.41	73.9	0.8 (0.5–1.3)	0.36
TT	AG + GG	60	28.4	1.1 (0.7–1.7)	0.83	63.1	0.9 (0.6–1.5)	0.80
TC + CC	AG + GG	45	26.9	0.7 (0.4–1.2)	0.20	60.5	0.8 (0.5–1.4)	0.44

Abbreviations: CI = confidence interval; COX = cyclooxygenase; HR = hazard ratio.
HR is calculated by COX proportional hazards analysis.
Values in parentheses are 95% CI.

Table 4 Distribution of prognostic markers for MM in relation to polymorphism *IL-1β* T-31C

	IL-1β *T-31C wild-type allele TT* *Median (range) or frequency (%)*	IL-1β *T-31C variant carriers CT/CC* *Median (range) or frequency (%)*	P-*value*
Age	57 (32–69)[a]	56 (28–68)	0.7
β2-microglobulin	4.3 (1.3–57)[a]	3.9 (1.2–23)	0.7
Creatinin	1.1 (0.6–9.4)[a]	1.1 (0.5–8.6)	0.8
Albumin	3.5 (0.3–5.3)[a]	3.5 (0.3–5.3)	0.9
Durie–Salmon stage (I/II/II)	13/26/82 (11/21/68)[b]	16/44/119 (9/25/66)	0.4
ISS (I/II/III)	18/37/36 (20/41/40)[b]	26/49/47 (21/40/39)	0.9
Sex (male/female)	71/54 (57/43)[b]	105/78 (57/43)	0.9

[a]Range.
[b]Percent.

Table 5 Multivariate analysis of *IL-1*β T-31C and prognostic markers

	P-value	*HR*
β2-microglobulin[a]	0.050	1.3[b] (1.0–1.6)
Creatinine[a]	0.59	1.1 (0.8–1.4)
Stage DS	0.09	—
I vs II		2.8 (1.0–7.9)
I vs III		2.6 (0.9–7.1)
*IL-1*β	0.042	—
TT vs CT		0.7 (0.4–1.0)
TT vs CC		0.5 (0.3–1.0)

Abbreviations: CI = confidence interval; HR = hazard ratio.
These are β2-microglobulin, creatinine and Durie–Salmon stage.
[a]β2-Microglobulin and creatinine were tested as continuous variables and log transformed. The hazard ratio indicates risk when values are doubled.
[b]Values in parentheses are 95% CI.
The hazard ratios of progression for the variants were calculated with respect to having wild-type in *IL-1*β T-31C. The *P*-value is used for all combinations as a categorical variable.

cure the patients.[10] Treatment outcome for patients with MM depends on factors related to the aggressiveness of the tumor, the tumor burden at diagnosis, choice of treatment and inherited genetic variations that modify the response to treatment. We have shown earlier that the treatment outcome may be influenced by inherited variation in the individuals' capacity for DNA repair.[9] Polymorphisms in the DNA repair genes *ERCC2*, *XRCC3* and *CD3EAP* significantly influenced the treatment outcome of patients with MM undergoing high-dose treatment. There is ample evidence to show that proinflammatory cytokines are involved in the risk of cancer, but less is known about their importance for treatment outcome and survival.

In this study, we found an increased frequency of the variant allele *IL-1*β T-31C in MM patients. The *IL-1*β T-31C polymorphism did not play any role in the outcome of treatment with high-dose melphalan and Auto-SCT, but carriers of the variant C-allele had a significantly longer survival than did homozygous wild-type allele TT carriers.

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References

1 Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004; **25**: 280–288.
2 Naugler WE, Karin M. NF-kappaB and cancer-identifying targets and mechanisms. *Curr Opin Genet Dev* 2008; **18**: 19–26.
3 Ray DM, Bernstein SH, Phipps RP. Human multiple myeloma cells express peroxisome proliferator-activated receptor gamma and undergo apoptosis upon exposure to PPARgamma ligands. *Clin Immunol* 2004; **113**: 203–213.
4 El Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA *et al*. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398–402.
5 Landi S, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J *et al*. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* 2003; **63**: 3560–3566.
6 Howell WM, Rose-Zerilli MJ. Interleukin-10 polymorphisms, cancer susceptibility and prognosis. *Fam Cancer* 2006; **5**: 143–149.
7 Rothman N, Skibola CF, Wang SS, Morgan G, Lan Q, Smith MT *et al*. Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. *Lancet Oncol* 2006; **7**: 27–38.
8 Zhang X, Miao X, Tan W, Ning B, Liu Z, Hong Y *et al*. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. *Gastroenterology* 2005; **129**: 565–576.
9 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**: 1036–1045.
10 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.
11 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**: 389–396.
12 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**: 129–135.
13 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.
14 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
15 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.
16 Vogel U, Christensen J, Wallin H, Friis S, Nexo BA, Tjonneland A. Polymorphisms in COX-2, NSAID use and risk of basal cell carcinoma in a prospective study of Danes. *Mutat Res* 2007; **617**: 138–146.
17 Li ZW, Dalton WS. Tumor microenvironment and drug resistance in hematologic malignancies. *Blood Rev* 2006; **20**: 333–342.
18 Costes V, Portier M, Lu ZY, Rossi JF, Bataille R, Klein B. Interleukin-1 in multiple myeloma: producer cells and their role in the control of IL-6 production. *Br J Haematol* 1998; **103**: 1152–1160.
19 Lacy MQ, Donovan KA, Heimbach JK, Ahmann GJ, Lust JA. Comparison of interleukin-1 beta expression by *in situ* hybridization in monoclonal gammopathy of undetermined significance and multiple myeloma. *Blood* 1999; **93**: 300–305.
20 Xiong Y, Donovan KA, Kline MP, Gornet MK, Moon-Tasson LL, Lacy MQ *et al*. Identification of two groups of smoldering multiple myeloma patients who are either high or low producers of interleukin-1. *J Interferon Cytokine Res* 2006; **26**: 83–95.
21 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**: 1793–1803.
22 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**: 519–529.
23 Lind H, Haugen A, Zienolddiny S. Differential binding of proteins to the IL1B-31 T/C polymorphism in lung epithelial cells. *Cytokine* 2007; **38**: 43–48.
24 Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007; **8**: 942–949.
25 Nakahara T, Urabe K, Fukagawa S, Uchi H, Inaba K, Furue M *et al*. Engagement of human monocyte-derived dendritic cells into interleukin (IL)-12 producers by IL-1beta + interferon (IFN)-gamma. *Clin Exp Immunol* 2005; **139**: 476–482.
26 Motta MR, Castellani S, Rizzi S, Curti A, Gubinelli F, Fogli M *et al*. Generation of dendritic cells from CD14+ monocytes positively selected by immunomagnetic adsorption for multiple myeloma patients enrolled in a clinical trial of anti-idiotype vaccination. *Br J Haematol* 2003; **121**: 240–250.
27 Mazur G, Bogunia-Kubik K, Wrobel T, Karabon L, Polak M, Kuliczkowski K *et al*. IL-6 and IL-10 promoter gene polymorphisms do not associate with the susceptibility for multiple myeloma. *Immunol Lett* 2005; **96**: 241–246.
28 Zheng C, Huang DR, Bergenbrant S, Sundblad A, Osterborg A, Bjorkholm M *et al*. Interleukin 6, tumour necrosis factor alpha, interleukin 1beta and interleukin 1 receptor antagonist promoter or coding gene polymorphisms in multiple myeloma. *Br J Haematol* 2000; **109**: 39–45.
29 Prince HM, Mileshkin L, Roberts A, Ganju V, Underhill C, Catalano J *et al*. A multicenter phase II trial of thalidomide and celecoxib for patients with relapsed and refractory multiple myeloma. *Clin Cancer Res* 2005; **11**: 5504–5514.