

# Mechanisms of peripheral neuropathy associated with bortezomib and vincristine in patients with newly diagnosed multiple myeloma: a prospective analysis of data from the HOVON-65/GMMG-HD4 trial



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## Summary

**Background** Bortezomib-induced peripheral neuropathy is a dose-limiting toxicity in patients with multiple myeloma, often requiring adjustment of treatment and affecting quality of life. We investigated the molecular profiles of early-onset (within one treatment cycle) versus late-onset (after two or three treatment cycles) bortezomib-induced peripheral neuropathy and compared them with those of vincristine-induced peripheral neuropathy during the induction phase of a prospective phase 3 trial.

**Methods** In the induction phase of the HOVON-65/GMMG-HD4 trial, patients (aged 18–65 years) with newly diagnosed Salmon and Durie stage 2 or 3 multiple myeloma were randomly assigned to three cycles of bortezomib-based or vincristine-based induction treatment. We analysed the gene expression profiles and single-nucleotide polymorphisms (SNPs) of pretreatment samples of myeloma plasma cells and peripheral blood, respectively. This study is registered, number ISRCTN64455289.

**Findings** We analysed gene expression profiles of myeloma plasma cells from 329 (39%) of 833 patients at diagnosis, and SNPs in DNA samples from 369 (44%) patients. Early-onset bortezomib-induced peripheral neuropathy was noted in 20 (8%) patients, and 63 (25%) developed the late-onset type. Early-onset and late-onset vincristine-induced peripheral neuropathy was noted in 11 (4%) and 17 (7%) patients, respectively. Significant genes in myeloma plasma cells from patients that were associated with early-onset bortezomib-induced peripheral neuropathy were the enzyme coding genes *RHOBTB2* (upregulated by 1.59 times;  $p=4.5 \times 10^{-5}$ ), involved in drug-induced apoptosis, *CPT1C* (1.44 times;  $p=2.9 \times 10^{-7}$ ), involved in mitochondrial dysfunction, and *SOX8* (1.68 times;  $p=4.28 \times 10^{-13}$ ), involved in development of peripheral nervous system. Significant SNPs in the same patients included those located in the apoptosis gene *caspace 9* (odds ratio [OR] 3.59, 95% CI 1.59–8.14;  $p=2.9 \times 10^{-3}$ ), *ALOX12* (3.50, 1.47–8.32;  $p=3.8 \times 10^{-3}$ ), and *IGF1R* (0.22, 0.07–0.77;  $p=8.3 \times 10^{-3}$ ). In late-onset bortezomib-induced peripheral neuropathy, the significant genes were *SOD2* (upregulated by 1.18 times;  $p=9.6 \times 10^{-3}$ ) and *MYO5A* (1.93 times;  $p=3.2 \times 10^{-2}$ ), involved in development and function of the nervous system. Significant SNPs were noted in inflammatory genes *MBL2* (OR 0.49, 95% CI 0.26–0.94;  $p=3.0 \times 10^{-2}$ ) and *PPARD* (0.35, 0.15–0.83;  $p=9.1 \times 10^{-3}$ ), and DNA repair genes *ERCC4* (2.74, 1.56–4.84;  $p=1.0 \times 10^{-3}$ ) and *ERCC3* (1.26, 0.75–2.12;  $p=3.3 \times 10^{-3}$ ). By contrast, early-onset vincristine-induced peripheral neuropathy was characterised by upregulation of genes involved in cell cycle and proliferation, including *AURKA* (3.31 times;  $p=1.04 \times 10^{-2}$ ) and *MKI67* (3.66 times;  $p=1.82 \times 10^{-3}$ ), and the presence of SNPs in genes involved in these processes—eg, *GLI1* (rs2228224 [0.13, 0.02–0.97,  $p=1.18 \times 10^{-2}$ ] and rs2242578 [0.14, 0.02–1.12,  $p=3.00 \times 10^{-2}$ ]). Late-onset vincristine-induced peripheral neuropathy was associated with the presence of SNPs in genes involved in absorption, distribution, metabolism, and excretion—eg, rs1413239 in *DPYD* (3.29, 1.47–7.37,  $5.40 \times 10^{-3}$ ) and rs3887412 in *ABCC1* (3.36, 1.47–7.67,  $p=5.70 \times 10^{-3}$ ).

**Interpretation** Our results strongly suggest an interaction between myeloma-related factors and the patient's genetic background in the development of treatment-induced peripheral neuropathy, with different molecular pathways being implicated in bortezomib-induced and vincristine-induced peripheral neuropathy.

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## Introduction

Bortezomib (Millennium Pharmaceuticals, Cambridge, MA, USA) is a boronic acid dipeptide, which specifically inhibits the chymotryptic site of the 26S proteasome. In patients with newly diagnosed myeloma, bortezomib

in combination with conventional drugs resulted in high rates of complete response and very good partial response.<sup>1–4</sup> This drug is generally well tolerated; however, one of its most frequent and potentially disabling side-effects is the development of a painful,

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sensory peripheral neuropathy,<sup>5-7</sup> often requiring dose modification or discontinuation of bortezomib, which negatively affects clinical endpoints and quality of life.<sup>8</sup> Grade 1 and 2 bortezomib-induced peripheral neuropathy can arise in 27–75% of patients with recurrent multiple myeloma and in 25–33% of those with newly diagnosed multiple myeloma, whereas grade 3 and 4 peripheral neuropathy might affect 0–30% of patients with recurrent disease and 0–18% of those with newly diagnosed disease.<sup>9</sup> In most patients, this side-effect is reversible and does not seem to be affected by the number or type of previous treatments.<sup>7</sup> Bortezomib-induced peripheral neuropathy results from axonal degeneration,<sup>10,11</sup> often occurring within the first cycles of treatment, and does not seem to increase after the fifth cycle of bortezomib.<sup>7</sup>

Little is known about the mechanism of bortezomib-induced peripheral neuropathy, but a multifactorial pathogenesis seems likely. Damage to mitochondria and endoplasmic reticulum through activation of apoptosis has been seen in dorsal root ganglia of mice given bortezomib.<sup>11</sup> Additionally, mechanisms such as dysregulation of mitochondrial calcium homeostasis,<sup>12</sup> autoimmune factors and inflammation,<sup>13</sup> and blockade of nerve-growth-factor-mediated neuronal survival through inhibition of the activation of nuclear factor  $\kappa$ B (NF $\kappa$ B)<sup>6</sup> could contribute to bortezomib-induced peripheral neuropathy. Evidence that multiple myeloma is also implicated in peripheral neuropathy was described by Ropper and Gorson<sup>14</sup> in 1998. Baseline neuropathy is present in 15–20% of patients with newly diagnosed myeloma,<sup>15,16</sup> which might be of both axonal and demyelinating subtypes.<sup>14</sup> The role of myeloma-related factors in peripheral neuropathy related to treatment is not clear. Bortezomib-induced peripheral neuropathy was noted at higher frequencies in patients with multiple myeloma than in those with solid tumours.<sup>17</sup> Richardson and colleagues<sup>16</sup> characterised the possible role of myeloma-related factors in bortezomib-induced peripheral neuropathy using plasma cells from patients with multiple myeloma. Additionally, we have noted that inherited single-nucleotide polymorphisms (SNPs) are associated with a higher probability of developing thalidomide-induced or bortezomib-induced peripheral neuropathy (Corthals SL, unpublished data). We therefore analysed myeloma-related gene expression and inherited patient variations as indicators of the potential risk of developing treatment-related peripheral neuropathy. We investigated whether particular molecular profiles were specific for early-onset versus late-onset bortezomib-induced peripheral neuropathy and compared these with genetic profiles associated with early-onset versus late-onset vincristine-induced peripheral neuropathy to elucidate molecular differences associated with the development of peripheral neuropathy after the different treatments.

## Methods

### Patients

833 patients (aged 18–65 years) with newly diagnosed Salmon and Durie stage 2–3 multiple myeloma were enrolled in a prospective, randomised phase 3 trial (HOVON-65/GMMG-HD4; EudraCTnr2004-000944-26) in 75 centres in the Netherlands, Germany, and Belgium.<sup>3</sup> Patients were excluded if they had amyloidosis or monoclonal gammopathy of unknown significance, and baseline peripheral neuropathy of grade 2 or more.

The trial was done in accordance with the Declaration of Helsinki, and was approved by a medical ethics review committee. We obtained written informed consent from the patients for treatment and sample procurement.

### Procedures

Patients were randomly assigned to three cycles of induction treatment with vincristine 0.4 mg intravenously on days 1–4, doxorubicin 9 mg/m<sup>2</sup> intravenously on days 1–4, and dexamethasone 40 mg orally on days 1–4, 9–12, and 17–20 or bortezomib 1.3 mg/m<sup>2</sup> intravenously on days 1, 4, 8, and 11, doxorubicin 9 mg/m<sup>2</sup> intravenously on days 1–4, and dexamethasone 40 mg orally on days 1–4, 9–12, and 17–20. Stem-cells were mobilised by use of cyclophosphamide 1000 mg/m<sup>2</sup> intravenously on day 1, doxorubicin 15 mg/m<sup>2</sup> intravenously on days 1–4, dexamethasone 40 mg orally on days 1–4, and granulocyte colony-stimulating factor (filgrastim) 10  $\mu$ g/kg per day subcutaneously, divided in two doses per day, from day 5 until last stem cell collection. After induction therapy, patients received one or two cycles of high-dose melphalan (200 mg/m<sup>2</sup> intravenously) with autologous stem-cell rescue followed by maintenance treatment with thalidomide (50 mg per day orally; group assigned to vincristine-based induction treatment) or bortezomib (1.3 mg/m<sup>2</sup> intravenously once every 2 weeks; group assigned to bortezomib-based induction treatment) for 2 years. Treatment was not masked for physicians and patients.

Severity of neuropathy was graded at baseline and after each treatment cycle by use of the National Cancer Institute's Common Toxicity Criteria for Adverse Events criteria (version 3.0).<sup>18</sup> All data were analysed centrally. No neurological assessment was undertaken to objectify peripheral neuropathy. Since grade 1 peripheral neuropathy could easily be missed or misinterpreted, and because it does not include pain or interfere with the activities of daily life, we decided that grade 1 peripheral neuropathy was not clinically significant enough for the molecular analysis and therefore cases of this grade were excluded. Furthermore, the dose-modification guidelines established during the SUMMIT,<sup>6</sup> CREST,<sup>5</sup> and APEX<sup>19</sup> trials did not recommend discontinuation of bortezomib or dose modifications when grade 1 bortezomib-induced peripheral neuropathy occurred. We did not routinely assess data for diabetes and vascular disease. Development

of peripheral neuropathy after the first cycle of induction treatment is described as early onset, and after two to three cycles of induction treatment as late onset. Vincristine-induced peripheral neuropathy was used as a reference when we assessed the incidence and severity of bortezomib-induced peripheral neuropathy.

RNA isolation and microarray processing was done as previously described.<sup>20</sup> Microarray data presented in this report have been stored in the Gene Expression Omnibus database (National Center for Biotechnology Information, Bethesda MD, USA), accession number GSE19784. Gene expression arrays were done with RNA extracted from myeloma plasma cells that were purified ( $\geq 80\%$ ) from the extra bone marrow aspiration taken at diagnosis and met the criteria for quality.<sup>20</sup>

DNA was extracted from peripheral blood nucleated cells or CD138-negative bone marrow cells and quantified by use of the Nanodrop Spectrophotometer (Nanodrop, Wilmington, DE, USA). Samples were genotyped by use of the Affymetrix Targeted Genotyping (Affymetrix, Santa Clara, CA, USA) custom built panel, with 3404 SNPs, selected with a hypothesis-driven strategy, targeting genes and SNPs for which associations or putative functional effects have been noted (Corthals SL, unpublished data).

### Statistical analysis

For differences in incidence of baseline and grade 2–4 peripheral neuropathy after one cycle and after two to three cycles of bortezomib-based and vincristine-based treatment,  $\chi^2$  analysis was done with a two-sided p value of 0.05. For gene expression data, class comparison of groups of arrays was done with one-way ANOVA in Partek Genomics Suite (version 6.4), followed by multiple-test correction with a false discovery rate of less than 0.05.

For SNP genotyping data, deviations from Fisher's exact *t* test for Hardy-Weinberg equilibrium at  $p < 0.00001$  and bias in missing data were controlled for each SNP. SNPs with a minor allele frequency of less than 5% and a call rate of less than 80% were removed from further analysis. To assess SNP associations with treatment-related peripheral neuropathy and calculation of odds ratios (ORs), a Cochran-Armitage trend test and a Fisher's *t* exact test were done. We assessed the genomic inflation factor  $\lambda$  based on the median  $\chi^2$  for each analysis with PLINK (version 1.07).<sup>21</sup> To account for multiple testing, 10 000 permutation tests were done with the max(T) permutation procedure with PLINK. To assess the effect of non-synonymous SNPs associated with bortezomib-induced peripheral neuropathy and vincristine-induced peripheral neuropathy, SNPs were characterised by use of the prediction program Sorting Intolerant From Tolerant (version 4.0.3).

Analysis of the gene and SNP sets for peripheral neuropathy associated with bortezomib and vincristine was done by use of Ingenuity Pathway Analysis software (version 8.7).

	Bortezomib-based induction treatment (n=250)	Vincristine-based induction treatment (n=250)	p value
Baseline peripheral neuropathy	8 (3%)	13 (5%)	0.37
Peripheral neuropathy after one cycle			
Grade 2–4	20 (8%)	11 (4%)	0.27
Grade 2	10 (50%)	9 (82%)	..
Grade 3	7 (35%)	1 (9%)	0.18*
Grade 4	3 (15%)	1 (9%)	..
Peripheral neuropathy after two or three cycles			
Grade 2–4	63 (25%)	17 (7%)	<0.0001
Grade 2	31 (49%)	11 (65%)	..
Grade 3	24 (38%)	6 (35%)	0.72*
Grade 4	8 (13%)	0	..

Data are number (%), unless otherwise indicated. The denominator for calculation of the percentages of patients with grades 2, 3, and 4 drug-related peripheral neuropathy was the total number of patients presenting with drug-related peripheral neuropathy after one or two to three cycles of treatment, respectively. \*For difference in percentage of patients with grade 3 and 4 peripheral neuropathy associated with bortezomib and vincristine among the total number of patients presenting with grade 2–4 bortezomib-associated and vincristine-associated peripheral neuropathy, respectively.

**Table 1: Incidence of baseline, bortezomib-induced, and vincristine-induced peripheral neuropathy**

This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN64455289.

### Role of the funding source

The sponsors had no role in the design, gathering, analysis, and interpretation of the data, or the writing of the report. The corresponding author had full access to all the data and the final responsibility to submit for publication.

### Results

We did gene expression arrays for 329 (39%; 170 treated with bortezomib, 159 treated with vincristine) of 833 patients included in the trial, and SNP profiles for samples taken from 369 (44%; 186 treated with bortezomib, 183 treated with vincristine) patients. Simultaneous gene expression and SNP data were obtained for 185 patients; only SNP data were available for 184 patients, and only gene expression data were available for 144 patients. The baseline clinical characteristics of 513 patients included in this study were not different from the whole patient group included in the trial (webappendix p 1).

Table 1 shows the incidence of peripheral neuropathy at baseline and after treatment with bortezomib-based and vincristine-based induction treatments in 500 of 513 patients who were fully assessable and had a minimum follow-up of 40 months. The median time to development of bortezomib-induced peripheral neuropathy was 42 days (range 0–137). Cumulative dose of bortezomib given before development of peripheral neuropathy was 13 mg/m<sup>2</sup>. 52 patients (21%) developed grade 1 bortezomib-induced peripheral neuropathy, and 34 (14%) developed grade 1 peripheral neuropathy before

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progressing to a higher grade. When patients developed peripheral neuropathy, the dose of bortezomib was adjusted according to the established guidelines for dose modification.<sup>5-7,19</sup> Median time to development of vincristine-induced peripheral neuropathy was 37 days

(range 0–171). Cumulative dose of vincristine given before development of peripheral neuropathy was 4 mg. 60 (24%) patients developed vincristine-induced peripheral neuropathy, and 18 (7%) developed grade 1 peripheral neuropathy before progressing to a higher

	Gene name	Gene description	Factor difference in expression	p value
<b>Grade 2–4 peripheral neuropathy (n=15) versus no peripheral neuropathy (n=134) after one cycle of bortezomib</b>				
	225189_s_at	RAPH1 Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	2.24	3.04×10 <sup>-2</sup>
	235014_at	LOC147727 Hypothetical protein LOC147727	2.15	1.91×10 <sup>-2</sup>
	1569872_a_at	LOC650392 Hypothetical protein LOC650392	1.98	9.65×10 <sup>-4</sup>
	213056_at	FRMD4B FERM domain containing 4B	1.74	8.42×10 <sup>-3</sup>
	227984_at	LOC650392 Hypothetical protein LOC650392	1.71	1.19×10 <sup>-3</sup>
	225478_at	MFHAS1 Malignant fibrous histiocytoma amplified sequence 1	1.68	5.34×10 <sup>-9</sup>
	226913_s_at	SOX8 SRY (sex determining region Y)-box 8	1.68	4.28×10 <sup>-13</sup>
	204810_s_at	CKM Creatine kinase, muscle	1.67	1.11×10 <sup>-30</sup>
	1569871_at	LOC650392 Hypothetical protein LOC650392	1.65	1.77×10 <sup>-19</sup>
	228057_at	DDIT4L DNA-damage-inducible transcript 4-like	1.59	5.59×10 <sup>-20</sup>
<b>Grade 2–4 peripheral neuropathy (n=44) versus no peripheral neuropathy (n=78) after two or three cycles of bortezomib</b>				
	205590_at	RASGRP1 RAS guanyl releasing protein 1 (calcium and DAG regulated)	2.97	2.14×10 <sup>-2</sup>
	204527_at	MYO5A Myosin VA (heavy chain 12, myosin)	1.93	3.21×10 <sup>-2</sup>
	235065_at	..	1.57	3.19×10 <sup>-2</sup>
	205422_s_at	ITGBL1 Integrin, β-like 1 (with EGF-like repeat domains)	1.44	1.35×10 <sup>-3</sup>
	228113_at	RAB37 RAB37, member of RAS oncogene family	1.41	3.69×10 <sup>-2</sup>
	210321_at	GZMH Granzyme H (cathepsin G-like 2, protein h-CCPX)	1.37	3.19×10 <sup>-2</sup>
	226969_at	MTR 5-methyltetrahydrofolate-homocysteine methyltransferase	1.34	4.26×10 <sup>-2</sup>
	204072_s_at	FRY Furry homolog ( <i>Drosophila</i> )	1.31	4.94×10 <sup>-2</sup>
	236442_at	DPF3 D4, zinc and double PHD fingers, family 3	1.30	3.38×10 <sup>-2</sup>
	243329_at	..	1.30	4.26×10 <sup>-2</sup>
<b>Grade 2–4 peripheral neuropathy (n=9) versus no peripheral neuropathy (n=129) after one cycle of vincristine</b>				
	208235_x_at	GAGE7 G antigen 7	11.55	3.21×10 <sup>-3</sup>
	206640_x_at	GAGE12I G antigen 12I	11.46	4.29×10 <sup>-3</sup>
	207739_s_at	GAGE2C G antigen 2C	7.76	1.62×10 <sup>-3</sup>
	208155_x_at	GAGE6 G antigen 6	6.88	1.06×10 <sup>-5</sup>
	206897_at	PAGE1 P antigen family, member 1 (prostate associated)	6.76	4.29×10 <sup>-2</sup>
	216063_at	HBBP1 Haemoglobin, β pseudogene 1	6.24	4.04×10 <sup>-2</sup>
	207086_x_at	GAGE4 G antigen 4	6.16	3.29×10 <sup>-5</sup>
	206626_x_at	SSX1 Synovial sarcoma, X breakpoint 1	5.93	2.61×10 <sup>-2</sup>
	207912_s_at	DAZ1 Deleted in azoospermia 1	5.86	1.06×10 <sup>-4</sup>
	214957_at	ACTL8 Actin-like 8	4.93	1.32×10 <sup>-10</sup>
<b>Grade 2–3 peripheral neuropathy (n=10) versus no peripheral neuropathy (n=103) after two or three cycles of vincristine</b>				
	210632_s_at	SGCA Sarcoglycan, alpha (50 kDa dystrophin-associated glycoprotein)	4.08	3.35×10 <sup>-2</sup>
	210992_x_at	FCGR2C Fc fragment of IgG, low affinity IIc, receptor for (CD32)	2.49	3.57×10 <sup>-2</sup>
	241991_at	..	1.80	3.35×10 <sup>-2</sup>
	206771_at	UPK3A Uroplakin 3A	1.59	2.94×10 <sup>-2</sup>
	241365_at	..	1.57	3.35×10 <sup>-2</sup>
	236266_at	RORA RAR-related orphan receptor A	1.53	2.94×10 <sup>-2</sup>
	214059_at	IFI44 Interferon-induced protein 44	1.51	4.92×10 <sup>-6</sup>
	230477_at	..	1.48	2.94×10 <sup>-2</sup>
	237322_at	MIAT Myocardial infarction associated transcript (non-protein coding)	1.45	2.94×10 <sup>-2</sup>
	239239_at	..	1.33	2.94×10 <sup>-2</sup>

First column is the probe-set identification numbers. Genes were ranked from highest to lowest change; the first ten genes with the highest changes are shown.

**Table 2: Differentially expressed genes in early-onset and late-onset bortezomib-induced and vincristine-induced peripheral neuropathy**

grade. When patients developed vincristine-induced peripheral neuropathy, vincristine was discontinued and supportive treatments such as pregabalin were used. Overall, baseline peripheral neuropathy was noted in only a small number of patients (table 1). The proportion of patients developing late-onset bortezomib-induced peripheral neuropathy was significantly higher than that of patients with late-onset vincristine-induced peripheral neuropathy (table 1).

Gene expression arrays for 15 patients developing early-onset grade 2–4 bortezomib-induced peripheral neuropathy were compared with arrays of patients who did not develop bortezomib-induced peripheral neuropathy (table 2). Grade 2–4 early-onset bortezomib-induced peripheral neuropathy was characterised by 19 differentially expressed genes (false discovery rate <0.05). The genes showing the highest changes in the gene expression arrays are shown in table 2, and the complete number of differentially expressed probe

sets are shown in the webappendix pp 2–3. The genes showing the highest change in expression included *RAPH1* (involved in signal transduction), *FRMD4B*, *MFHAS1* (possibly an oncogene regulated by NFκB or tumour necrosis factor), and *DDIT4L* (a DNA-damage inducible transcript; table 2). Genes that might play a direct part in bortezomib-induced peripheral neuropathy are transcription regulator *SOX8* (involved in development of peripheral nervous system), and *CPTIC* and *RHOBTB2* (webappendix pp 2–3). Ingenuity pathway analyses of gene and SNP sets showed enrichment of genes implicated in the canonical pathway of signalling mediated by AMP-activated protein kinase (AMPK), including *CPTIC*, *CKM*, and *PIK3CG* (three of 156 genes involved in AMPK signalling were upregulated,  $p=7.33 \times 10^{-5}$ ).

Gene expression arrays for 44 patients with grade 2–4 late-onset bortezomib-induced peripheral neuropathy were characterised by 27 differentially expressed genes,

	Chromosome	Gene	Single-nucleotide polymorphism type	Odds ratio (95% CI)	p value	Permuted p value
<b>Grade 2–4 peripheral neuropathy (n=13) versus no peripheral neuropathy (n=147) after one cycle of bortezomib</b>						
rs2251660	17	<i>RDM1</i>	Coding non-synonymous	3.65 (1.55–8.57)	$9.06 \times 10^{-4}$	$2.40 \times 10^{-3}$
rs4646091	1	<i>CASP9</i>	Intron	3.59 (1.59–8.14)	$1.43 \times 10^{-3}$	$2.90 \times 10^{-3}$
rs1126667	17	<i>ALOX12</i>	Coding non-synonymous	3.50 (1.47–8.32)	$2.95 \times 10^{-3}$	$3.80 \times 10^{-3}$
rs4344473	17	<i>ALOX12</i>	Coding non-synonymous	3.50 (1.47–8.32)	$2.95 \times 10^{-3}$	$4.10 \times 10^{-3}$
rs7823144	8	<i>LSM1</i>	Intron	4.11 (1.48–11.39)	$2.30 \times 10^{-3}$	$7.60 \times 10^{-3}$
rs1879612	15	<i>IGF1R</i>	Intron	0.22 (0.07–0.77)	$9.42 \times 10^{-3}$	$8.30 \times 10^{-3}$
rs1029871	3	<i>NEK4</i>	Coding non-synonymous	0.30 (0.11–0.81)	$8.31 \times 10^{-3}$	$9.30 \times 10^{-3}$
<b>Grade 2–4 peripheral neuropathy versus (n=49) no peripheral neuropathy (n=80) after two or three cycles of bortezomib</b>						
rs1799800	16	<i>ERCC4</i>	Intron	2.74 (1.56–4.84)	$5.16 \times 10^{-4}$	$1.00 \times 10^{-3}$
rs1799801	16	<i>ERCC4</i>	Coding synonymous	2.48 (1.43–4.28)	$8.85 \times 10^{-4}$	$1.10 \times 10^{-3}$
rs2300697	2	<i>SRD5A2</i>	Intron	0.63 (0.37–1.05)	$4.80 \times 10^{-2}$	$2.90 \times 10^{-3}$
rs1059293	21	<i>IFNGR2</i>	Untranslated, intron	2.30 (1.37–3.87)	$8.97 \times 10^{-4}$	$3.20 \times 10^{-3}$
rs2276583	2	<i>ERCC3</i>	Locus	1.26 (0.75–2.12)	$3.87 \times 10^{-1}$	$3.30 \times 10^{-3}$
rs189037	11	<i>ATM</i>	Locus, untranslated	0.53 (0.32–0.89)	$2.32 \times 10^{-2}$	$3.60 \times 10^{-3}$
rs10501815	11	<i>MRE11A</i>	Intron, TagSNP	3.27 (1.39–7.74)	$4.41 \times 10^{-3}$	$4.20 \times 10^{-3}$
rs664677	11	<i>ATM</i>	Intron	0.57 (0.34–0.96)	$4.36 \times 10^{-2}$	$5.90 \times 10^{-3}$
rs664982	11	<i>ATM</i>	Intron	0.51 (0.30–0.85)	$1.72 \times 10^{-2}$	$6.20 \times 10^{-3}$
rs6131	1	<i>SELP</i>	Coding non-synonymous	0.43 (0.23–0.83)	$6.69 \times 10^{-3}$	$6.30 \times 10^{-3}$
rs1130499	7	<i>PTPRN2</i>	Coding non-synonymous	0.43 (0.23–0.79)	$6.23 \times 10^{-3}$	$6.60 \times 10^{-3}$
rs4722266	7	<i>STK31</i>	Coding non-synonymous	0.29 (0.12–0.74)	$5.66 \times 10^{-3}$	$8.30 \times 10^{-3}$
rs2267668	6	<i>PPARD</i>	Intron	0.35 (0.15–0.83)	$9.30 \times 10^{-3}$	$9.10 \times 10^{-3}$
<b>Grade 2–4 peripheral neuropathy versus (n=7) no peripheral neuropathy (n=151) after one cycle of vincristine</b>						
rs7739752	6	<i>PPARD</i>	Intron	13.43 (3.90–46.22)	$6.34 \times 10^{-7}$	$8.00 \times 10^{-4}$
rs2288087	9	<i>ALDH1A1</i>	Intron, TagSNP	7.62 (1.68–34.65)	$1.40 \times 10^{-3}$	$1.50 \times 10^{-3}$
rs1494961	4	<i>HEL308</i>	Coding non-synonymous	6.67 (1.47–30.32)	$2.30 \times 10^{-3}$	$2.60 \times 10^{-3}$
rs6901410	6	<i>PPARD</i>	Intron	9.67 (2.65–35.30)	$7.75 \times 10^{-5}$	$6.00 \times 10^{-3}$
rs6902123	6	<i>PPARD</i>	Intron	9.67 (2.65–35.30)	$7.75 \times 10^{-5}$	$6.00 \times 10^{-3}$
rs2274407	13	<i>ABCC4</i>	Coding non-synonymous	7.15 (2.02–25.31)	$2.94 \times 10^{-4}$	$6.10 \times 10^{-3}$
rs909253	6	<i>LTA</i>	Intron	4.67 (1.52–14.34)	$3.09 \times 10^{-3}$	$6.60 \times 10^{-3}$
rs6457816	6	<i>PPARD</i>	Intron	8.89 (2.46–32.17)	$1.40 \times 10^{-4}$	$7.30 \times 10^{-3}$
rs1041981	6	<i>LTA</i>	Coding non-synonymous	4.52 (1.47–13.88)	$3.58 \times 10^{-3}$	$7.40 \times 10^{-3}$
rs3803258	13	<i>SLC10A2</i>	Untranslated	4.30 (1.45–12.74)	$3.51 \times 10^{-3}$	$7.40 \times 10^{-3}$
rs3749442	3	<i>ABCC5</i>	Coding synonymous	4.64 (1.5–14.05)	$2.72 \times 10^{-3}$	$9.60 \times 10^{-3}$

(Continued on next page)

	Chromosome	Gene	Single-nucleotide polymorphism type	Odds ratio (95% CI)	p value	Permuted p value
(Continued from previous page)						
<b>Grade 2–3 peripheral neuropathy (n=14) versus no peripheral neuropathy (n=104) after two or three cycles of vincristine</b>						
rs10515114	5	CART	Locus	4.62 (1.68–12.72)	7.92×10 <sup>-4</sup>	2.90×10 <sup>-3</sup>
rs6873545	5	GHR	Intron	0.09 (0.01–0.69)	3.44×10 <sup>-3</sup>	3.60×10 <sup>-3</sup>
rs3734354	6	SIM1	Coding non-synonymous	3.30 (1.39–7.82)	2.31×10 <sup>-3</sup>	5.10×10 <sup>-3</sup>
rs11688	1	JUN	Coding synonymous	5.00 (1.80–13.91)	9.10×10 <sup>-4</sup>	5.20×10 <sup>-3</sup>
rs4129472	5	GHR	Intron	0.11 (0.01–0.80)	6.46×10 <sup>-3</sup>	5.20×10 <sup>-3</sup>
rs1413239	1	DPYD	Intron, TagSNP	3.29 (1.47–7.37)	3.03×10 <sup>-3</sup>	5.40×10 <sup>-3</sup>
rs1045020	5	SLC22A5	Untranslated	4.80 (1.83–12.61)	1.48×10 <sup>-3</sup>	5.40×10 <sup>-3</sup>
rs9885672	6	KIAA0274	Coding non-synonymous	3.89 (1.62–9.33)	2.05×10 <sup>-3</sup>	5.60×10 <sup>-3</sup>
rs3887412	16	ABCC1	Intron, TagSNP	3.36 (1.47–7.67)	3.31×10 <sup>-3</sup>	5.70×10 <sup>-3</sup>
rs6886047	5	GHR	Intron	0.10 (0.01–0.72)	3.97×10 <sup>-3</sup>	6.10×10 <sup>-3</sup>
rs1236913	9	PTGS1	Coding non-synonymous	5.40 (1.79–16.28)	1.43×10 <sup>-3</sup>	6.30×10 <sup>-3</sup>
rs2644983	16	ABCC1	Intron, TagSNP	4.22 (1.69–10.50)	2.27×10 <sup>-3</sup>	6.60×10 <sup>-3</sup>
rs1042713	5	ADRB2	Coding non-synonymous	0.23 (0.08–0.69)	5.30×10 <sup>-3</sup>	7.20×10 <sup>-3</sup>
rs1966265	5	FGFR4	Coding non-synonymous	3.47 (1.51–7.94)	3.40×10 <sup>-3</sup>	7.30×10 <sup>-3</sup>
rs2308327	10	MGMT	Coding non-synonymous	3.38 (1.33–8.58)	3.69×10 <sup>-3</sup>	7.30×10 <sup>-3</sup>
rs5759197	22	BZRP	Intron	2.93 (1.31–6.53)	6.32×10 <sup>-3</sup>	7.60×10 <sup>-3</sup>
rs1005658	22	BZRP	Locus	3.14 (1.39–7.08)	6.04×10 <sup>-3</sup>	8.50×10 <sup>-3</sup>
rs7441774	4	UGT2B7	Intron	3.60 (1.40–9.23)	6.61×10 <sup>-3</sup>	9.60×10 <sup>-3</sup>

**Table 3: Single-nucleotide polymorphisms associated with bortezomib-induced and vincristine-induced peripheral neuropathy**

using the same false discovery rate as for early onset (webappendix pp 2–3), and showed a different pattern of gene expression to that in early-onset bortezomib-induced peripheral neuropathy, without overlap (table 2). *RASGRP1* showed the highest change in patients with late-onset bortezomib-induced peripheral neuropathy compared with patients without this side-effect (table 2). Furthermore, we noted upregulation of genes involved in transcription regulation, including *TRERF1*, *TRPS1*, and *MDM2*. We noted enrichment of genes involved in the development and function of the nervous system, including *SOD2* and *MYO5A*.

All significant SNPs (permuted  $p < 0.01$ ) associated with grade 2–4 early-onset bortezomib-induced peripheral neuropathy are shown in table 3 (values of permuted  $p < 0.05$  are shown in webappendix pp 4–10). Several SNPs associated with early-onset bortezomib-induced peripheral neuropathy were located in *caspase 9* (rs4646091, rs2020895, rs2020903, rs4646032, and rs4646034). Other highly associated SNPs were located in genes *RDM1*, *ALOX12*, *IGF1R*, and *LSM1* (table 3). Pathway analysis of these associated genes showed enrichment of genes involved in cell death (14 genes,  $p = 5.25 \times 10^{-3}$ – $4.93 \times 10^{-2}$ ), DNA repair (14 genes,  $p = 5.25 \times 10^{-3}$ – $4.93 \times 10^{-2}$ ), and development and function of the nervous system (four genes,  $p = 2.01 \times 10^{-3}$ ).

The SNPs that were characteristic of late-onset bortezomib-induced peripheral neuropathy were mainly located in DNA repair genes, such as *ERCC3*, *ERCC4*, *ATM*, *BRCA1*, *EXO1*, and *MRE11A* (table 3; webappendix pp 4–10). Pathway analysis showed enrichment of

associated SNPs located in genes involved in the development and function of the nervous system (three genes,  $p = 3.35 \times 10^{-3}$ – $1.69 \times 10^{-2}$ ) and in inflammatory disease (26 genes,  $p = 2.09 \times 10^{-3}$ – $4.95 \times 10^{-3}$ ).

The genetic profile of myeloma plasma cells from nine patients who developed grade 2–4 early-onset vincristine-induced peripheral neuropathy showed overexpression of the genes for testis cancer antigens, of which the *GAGE* genes were mainly upregulated (table 2).

The gene profiles of ten patients who developed grade 2 or 3 late-onset vincristine-induced peripheral neuropathy showed only ten differentially expressed genes, including *RORA* and *IFI44* (table 2).

Table 3 shows SNPs significantly associated with early-onset vincristine-induced peripheral neuropathy. Four of the most highly associated SNPs (rs7739752, rs6901410, rs6902123, and rs6457816) were located in the transcription factor *PPARD*. Additionally, an intronic (rs909253) and a coding non-synonymous SNP (rs1041981) in *LTA* were significantly associated with early-onset vincristine-induced peripheral neuropathy. Other significant SNPs were located in genes for transporter enzymes *ABCC4*, *ABCC5*, and *SLC10A2*, oxidising enzyme *ALDH1A1*, and *GLI3* (table 3; webappendix pp 4–10). Pathway analysis showed enrichment of associated SNPs located in genes involved in cellular growth and proliferation (four genes,  $p = 1.14 \times 10^{-2}$ – $4.95 \times 10^{-2}$ ).

Some intronic SNPs in the dihydropyrimidine dehydrogenase gene *DPYD* and some in the ABC transporter gene *ABCC1* were associated with late-onset vincristine-induced peripheral neuropathy (table 3).

Pathway analysis showed that most significant SNPs (permuted  $p < 0.05$ ) were located in genes for absorption, distribution, metabolism, and excretion (six genes,  $p = 2.06 \times 10^{-2}$ – $4.18 \times 10^{-2}$ ).

## Discussion

The genetic profiles of patients with early-onset bortezomib-induced peripheral neuropathy suggest the involvement of genes involved in transcription, apoptosis, and AMPK-mediated signalling. The possible role of AMPK-mediated signalling is of particular interest because this enzyme functions by stimulating the signalling pathways that replenish cellular ATP supplies in response to low glucose, hypoxia, ischaemia, or heat shock, which might be triggered in myeloma cells in response to bortezomib. *CPT1C* codes for an enzyme found in neuron mitochondria that is involved in transport of hydrophobic fatty acid chains into mitochondria, and plays a part in mitochondrial dysfunction. It might also have an important role in bortezomib-induced peripheral neuropathy, since damage to mitochondria and endoplasmic reticulum through activation of a mitochondrial-based apoptotic pathway by bortezomib was noted in dorsal root ganglia of mice given bortezomib.<sup>11</sup> *RHOBTB2*, encodes another enzyme implicated here, has been shown to be upregulated during drug-induced apoptosis, being mainly dependent on E2F1.<sup>22</sup> Knockout of *RHOBTB2* with small interfering RNAs has been shown to delay the onset of drug-induced apoptosis.<sup>22</sup> *RASGRP1* is involved in many processes, including apoptosis and calcium-ion binding, which are potentially interesting for its role in bortezomib-induced peripheral neuropathy. The presence of polymorphisms in the apoptosis gene *caspase 9*, which plays an important part in bortezomib-induced apoptosis, suggests the possible contribution of this enzyme to early-onset peripheral neuropathy.<sup>23,24</sup> One of the most significant SNPs (rs1029871) might have a role in the splicing regulation of *NEK4*, which is involved in the regulation of cell cycle and cell division. Furthermore, SNPs in enriched pathways like DNA repair and nervous system development and function were associated with early-onset bortezomib-induced peripheral neuropathy.

Late-onset bortezomib-induced peripheral neuropathy was associated with genes involved in the development and function of the nervous system. We noted upregulation of the superoxide dismutase gene *SOD2* in myeloma plasma cells; *SOD2* is regulated by tumour necrosis factor  $\alpha$  and NF $\kappa$ B, and is known to have a role in the survival of neurons. Patients with diabetes and a polymorphism in the *SOD2* gene, leading to reduced *SOD2* activity, have been shown to be at increased risk of developing diabetic peripheral neuropathy.<sup>25</sup> The protective effect of *SOD2* might be eliminated with bortezomib-induced apoptosis, which might trigger a susceptibility to oxidative stress in treated patients. Three SNPs associated with late-onset bortezomib-induced

peripheral neuropathy were located in *SERPINB2* (plasminogen activator inhibitor-2). *SERPINB2*, with *SERPIN-1* (plasminogen activator inhibitor-1), tissue-type plasminogen activator, and urokinase-type plasminogen activator, has been shown to be induced in dorsal root ganglion neurons after peripheral axotomy in mice.<sup>26</sup> These serpins might also act as autocrine or paracrine regulators of plasminogen-activator-mediated nerve regeneration processes.<sup>26</sup> The associated SNPs might affect *SERPINB2* expression through their effect on splicing regulation. Besides genes involved in development of the nervous system, proinflammatory genes might play an important part in the pathogenesis of late-onset bortezomib-induced peripheral neuropathy, based on the presence of intronic SNPs in *MBL2* and *PPARD* (Corthals SL, unpublished data), and of about 30% of SNPs with reported inflammatory roles. The hypothesis that the DNA repair pathway is involved in bortezomib-induced peripheral neuropathy, and that this side-effect might be caused by the inability to repair neuronal damage (Corthals SL, unpublished data), could be substantiated by the presence of SNPs in *BRCA1* (rs16941 and rs799917). These non-synonymous SNPs might have an effect on the phosphorylation state of a protein, which has been shown to abolish the P871L phosphorylation site in *BRCA1*.<sup>27</sup> Therefore, early-onset and late-onset bortezomib-induced peripheral neuropathies were both associated with a myeloma genetic profile that was characterised by genes involved in the development of the nervous system; however, apoptosis was also a characteristic for the development of early-onset bortezomib-induced peripheral neuropathy. Genetic polymorphisms in genes involved in nervous system development and DNA repair play a part in both the early and late onset of this side-effect.

A comparison of the molecular profiles of bortezomib-induced peripheral neuropathy and vincristine-induced peripheral neuropathy showed no overlap in associated genes or SNPs. Genes involved in cell cycle and proliferation were mainly associated with early-onset vincristine-induced peripheral neuropathy, both in the analyses of genetic pathways and SNPs. Additionally, involvement of proinflammatory genes in early-onset vincristine-induced peripheral neuropathy was substantiated by the finding of SNPs in *PARP1* and *LTA*, and two SNPs in *GLI1* (rs2228224 and rs2228226), which both encode an amino acid change; rs2228226 has been shown to affect *GLI1* activity, thereby affecting the inflammatory response.<sup>28</sup>

Genes implicated in drug absorption, distribution, metabolism, and excretion have been shown to be involved in chemotherapy-induced peripheral neuropathy.<sup>29</sup> In accordance with this finding, an association was noted for late-onset vincristine-induced peripheral neuropathy with nine intronic SNPs in *ABCC1*; vincristine is known to be a substrate of the protein coded for by this gene.

**Panel: Research in context****Systematic review**

We did a systematic review of chemotherapy-induced peripheral neuropathy, particularly bortezomib-induced peripheral neuropathy, before doing the molecular analysis in our study.<sup>4</sup>

**Interpretation**

Our data confirm the role of myeloma-related factors in bortezomib-induced peripheral neuropathy<sup>16</sup> and indicate that both the inherited genetic constitution of the host (patient) and the tumour (myeloma) should be thought of as contributors to the risk of bortezomib-induced peripheral neuropathy.

In conclusion, this study provides the first large dataset in which the contribution of both the inherited genetic constitution of the host (patient) and the tumour (myeloma) to the development of bortezomib-induced peripheral neuropathy has been reported (panel). We identified molecular factors that are associated with bortezomib-induced peripheral neuropathy in patients with newly diagnosed multiple myeloma. Genes for apoptosis contribute to early-onset bortezomib-induced peripheral neuropathy, whereas genes that have a role in inflammatory pathways and DNA repair contribute to the development of late-onset peripheral neuropathy, indicating that distinct genetic factors are involved in the development of early-onset and late-onset forms of this side-effect. Bortezomib-induced and vincristine-induced peripheral neuropathy arise through different molecular mechanisms. Our findings strongly suggest an interaction between myeloma-related factors and the patient's genetic background in the development of bortezomib-induced peripheral neuropathy. Profiles of genetic risk might be used in future to identify patients with an increased risk of bortezomib-induced peripheral neuropathy.

**Contributors**

AB designed and participated in the research, analysed data, and wrote the report. SLC participated in the research, analysed data, and wrote the report. JLMJ is a neurologist who reviewed the report. BVDH was the statistician for the trial, gathered data, and reviewed the report. RK and MVD analysed data and reviewed the report. YDK participated in the research and reviewed the report. LEJ and UB were the trial managers, and reviewed the report. HML was the study coordinator and reviewed the report. BGD designed the research and reviewed the report. HG was the study coordinator, and designed the research and reviewed the report. PS was the principal investigator of the study, designed the research, and reviewed the report.

**Conflicts of interest**

PS has served on advisory boards for Johnson & Johnson and Millennium Pharmaceuticals. HG has served on advisory boards for Johnson & Johnson. The other authors declare that they have no conflicts of interest.

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