transplant did not influence NK cell numbers in our cohort and this is most probably due to the cross-sectional nature of the study. Having said this, our observational results indicate a potentially novel role for NK cells in HSCT for non-malignant disease and give an indication for further research in a larger cohort, minimizing heterogeneity.

**Disclosure of conflicts of interest**

The authors have no conflicts of interest to disclose.

Ursalan A. Khan
Judith A. Davidson
Kay V. Poulton
Robert F. Wynn
James E. Fildes

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**A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with zoledronic acid-related osteonecrosis of the jaw in multiple myeloma patients: analysis by DMET microarray profiling**

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The aminobisphosphonate zoledronic acid (ZA) is the most important antiresorptive agent for the treatment of multiple myeloma (MM)-related bone disease (BD). Osteonecrosis of the jaw (ONJ) is an important complication of ZA-treated MM patients (Vannucchi *et al*, 2005; Filleul *et al*, 2010). So far, the mechanism of ONJ pathogenesis has not been clearly understood. A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with ZA-related ONJ in patients with MM. The investigators conducted a study to evaluate whether polymorphisms in PPARG are associated with ZA-related ONJ in patients with MM. They performed DMET microarray profiling and identified a SNV in PPARG that was significantly associated with ZA-related ONJ in patients with MM. This finding provides evidence for the involvement of PPARG in the pathogenesis of ZA-related ONJ in patients with MM. Further research is needed to further investigate the role of PPARG in the pathogenesis of ONJ.
elucidated. Recently, a genetic susceptibility to ONJ has been suggested and a polymorphism of the cytochrome P450 CYP2C8 has been associated with ZA-related ONJ in MM (Sarasquete et al., 2008).

To further investigate the genetic bases of ONJ, we genotyped in a case-control study a cohort of 19 MM patients treated with ZA who developed [nine cases, median age 66 years (range: 63–79)] or not [10 matched controls, median age 69 years (range: 63–84)] ONJ. We used the novel Affymetrix DMET™ plus platform (Affymetrix, Santa Clara, CA, USA), which interrogates 1936 genetic variations in 225 genes associated with phase I–II drug metabolism, disposition and transport (Deeken, 2009). The study protocol was approved by our University Hospital Bioethical Committee and informed consent was obtained from each patient. All patients received ZA according to the conventional dose and administration schedule; the ONJ group received 20 ± standard deviation (SD) 5-1 treatment courses and the control group underwent 15 ± 4.2 courses. MM patients were homogeneous on clinical and pathological characteristics at diagnosis and on their response to treatment. ONJ was diagnosed by clinical examination and imaging, including radiographs and/or computed tomography or magnetic resonance imaging. Peripheral blood was collected and used for DNA extraction. Genotypes were determined for each single nucleotide polymorphism (SNP) site of the 1931 of all SNPs interrogated SNPs and for the five Copy Number Variations (CNVs) included in DMET™ Plus GeneChip. Pharmacogenomic profiles were generated by Affymetrix DMET™ Console software®. Statistical analysis was performed by two-tailed Fisher’s exact test. No correction for multiple comparisons was performed. Results are therefore to be interpreted as hypothesis generating.

Eight SNPs were significantly ($p < 0.05$) associated with ONJ occurrence. Table I shows these SNPs, the reference and variant allele and the genotype and allele frequencies. All alleles were in Hardy-Weinberg equilibrium. The four genes correlated to the eight statistically relevant SNPs were PPARG (peroxisome proliferator-activated receptor gamma), ABP1 [amiloride binding protein 1 [amine oxidase (copper-containing)]], CHST11 [carbohydrate (chondroitin 4) sulfotransferase 11] and CROT [carnitine O-octanoyltransferase]. The different distribution of SNP alleles and genotypes between ONJ patients and control cases are reported in Table II. The SNP rs1152003, mapping in PPARG, at position 12477055 of chromosome 3 (Genome Build 37.1). Although no clinical correlation has been reported for the rs1152003 variant, polymorphisms in PPARG have been associated with increased risk of a variety of diseases (Dallongeville et al., 2009). PPARG is located in the human chromosome 3, band 3p25. Chromosomal abnormalities, such as 3p deletion, have been identified in several hematologic malignancies. PPARG is involved in adipocyte differentiation and in angiogenesis (Rosen & Spiegelman, 2001). Recently, the PPARG pathway has been recognized as key mechanism for bone remodelling. It acts on mesenchymal stem cell differentiation by increasing adipogenesis but also inhibiting osteoblast and osteoclast formation. Moreover, PPARG polymorphisms correlate with the bone mass density (Ackert-Bicknell et al., 2008). However, a recent study on a wide cohort of Korean individuals, with idiopathic, steroid-induced or alcohol-induced osteonecrosis of the femoral head, failed to demonstrate a significant correlation with three common PPARG polymorphisms (Kim et al., 2007). Interestingly, modulation of PPARG activity within the bone marrow microenvironment has been recently shown to interfere with cytokines such as IL6, which is involved with a central role in the pathogenesis of MM (Wang et al., 2004), suggesting also that PPARG may represent a valuable therapeutic target in MM (García-Bates et al., 2008).

Direct nucleotide sequencing was carried out on patient specimens to further confirm the presence of genetic variations, using an Applied Biosystems ABI 3100 Genetic Analyser. We found a concordance rate of 100% between DMET genotyping and sequence analysis (Fig 1B).

The rs1152003 SNP maps in the 3'UTR region of PPARG, at position 12477055 of chromosome 3 (Genome Build 37.1). Although no clinical correlation has been reported for the rs1152003 variant, polymorphisms in PPARG have been associated with increased risk of a variety of diseases (Dallongeville et al., 2009). PPARG is located in the human chromosome 3, band 3p25. Chromosomal abnormalities, such as 3p deletion, have been identified in several hematologic malignancies. PPARG is involved in adipocyte differentiation and in angiogenesis (Rosen & Spiegelman, 2001). Recently, the PPARG pathway has been recognized as key mechanism for bone remodelling. It acts on mesenchymal stem cell differentiation by increasing adipogenesis but also inhibiting osteoblast and osteoclast formation. Moreover, PPARG polymorphisms correlate with the bone mass density (Ackert-Bicknell et al., 2008). However, a recent study on a wide cohort of Korean individuals, with idiopathic, steroid-induced or alcohol-induced osteonecrosis of the femoral head, failed to demonstrate a significant correlation with three common PPARG polymorphisms (Kim et al., 2007). Interestingly, modulation of PPARG activity within the bone marrow microenvironment has been recently shown to interfere with cytokines such as IL6, which is involved with a central role in the pathogenesis of MM (Wang et al., 2004), suggesting also that PPARG may represent a valuable therapeutic target in MM (García-Bates et al., 2008).
glycoprotein that is expressed in many epithelial and haematopoietic tissues. Moreover, a further three ONJ-associated SNPs map to \textit{CHST11}, which was recently described as a factor required for proper chondroitin sulfation and cartilage morphogenesis. Expression of the chondroitin sulfotransferase genes is crucial for the correct mammalian bone morphogenesis. Finally, the ONJ-associated rs2097937 maps to \textit{CROT}, whose protein is involved in the trans-esterification of acyl-CoA molecules.

Our findings indicate that genetic polymorphisms are involved in the pathogenesis of ONJ in MM patients. The highly significant association of ONJ with the rs1152003 SNP polymorphism in \textit{PPARG} strongly suggests this genetic variant as candidate biomarker for the identification of MM patients at risk of ONJ if treated with ZA. In fact, the C/C genotype demonstrated an odds ratio of 31.5 (95% confidence interval, 2.35–422.32) for developing ONJ following ZA treatment. Differently from the recent report (Sarasquete et al., 2008), where the study was based on the 500K Affymetrix high density array, we used the DMET platform that interrogates only highly selective SNPs associated with drug toxicity and has the advantage of avoiding an extremely high number of comparisons, which requires statistical corrections and large patient cohorts. We propose the rs1152003 C/C genotype as a candidate genetic biomarker for ONJ, which warrants validation in larger series.

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**Table II.** Allele and genotype frequencies of polymorphisms in MM patients.

| SNP and variants | Gene      | Allele distribution | Genotype distribution | Clinical association |
|------------------|-----------|---------------------|-----------------------|---------------------|
| rs 1152003       | PPARG     | C                   | CC                    | 7/9 (77.7) 1/10 (100) 0.0055 Unknown |
|                  |           | G                   | CG                    | 2/9 (22.2) 7/10 (70)  |
|                  |           | G                   | GG                    | 0/9 (00) 2/10 (20)  |
| rs10893          | ABPI      | G                   | AA                    | 7/9 (77.7) 2/10 (200) 0.023 Unknown |
|                  |           | A                   | AG                    | 2/9 (22.2) 7/10 (70)  |
|                  |           | A                   | GG                    | 0/9 (00) 1/10 (10)  |
| rs4723573        | ABPI      | G                   | GG                    | 7/9 (77.7) 2/10 (200) 0.023 Unknown |
|                  |           | A                   | AG                    | 2/9 (22.2) 7/10 (70)  |
|                  |           | A                   | AA                    | 0/9 (00) 1/10 (10)  |
| rs1049793        | ABPI      | C                   | CC                    | 7/9 (77.7) 2/10 (200) 0.023 Unknown |
|                  |           | G                   | CG                    | 2/9 (22.2) 7/10 (70)  |
|                  |           | G                   | GG                    | 0/9 (00) 1/10 (10)  |
| rs2463437        | CHST11    | G                   | AA                    | 6/9 (66.6) 1/10 (10) 0.0198 Unknown |
|                  |           | A                   | AG                    | 3/9 (33) 8/10 (80)  |
|                  |           | G                   | GG                    | 0/9 (00) 1/10 (10)  |
| rs903247         | CHST11    | C                   | TT                    | 6/9 (66.6) 1/10 (10) 0.0198 Unknown |
|                  |           | T                   | CT                    | 3/9 (33) 7/10 (70)  |
|                  |           | T                   | CC                    | 0/9 (00) 2/10 (20)  |
| rs2468110        | CHST11    | G                   | GG                    | 6/9 (66.6) 1/10 (10) 0.0198 Unknown |
|                  |           | A                   | AG                    | 2/9 (22.2) 8/10 (80)  |
|                  |           | A                   | AA                    | 1/9 (11) 1/10 (10)  |
| rs2097937        | CROT      | G                   | AG                    | 6/9 (66.6) 1/10 (10) 0.0198 Unknown |
|                  |           | A                   | AA                    | 3/9 (33) 9/10 (90)  |
|                  |           | G                   | GG                    | 0/9 (00) 0/10 (0)  |

Distribution data for eight SNPs associated with ONJ in MM patients. Allele and genotype distribution between case and control groups. Polymorphisms are reported as rs number used in the human SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). The P value was calculated by two-tailed Fisher’s exact test.
**Author contributions**

M.T.D., P.S.T. and P.F.T. designed the study and performed data interpretation; M.T.D. wrote the manuscript; M.T.D. and M.A. generated datasets. M.T.D. provided statistical analysis and generated figures; P.H.G., P.V. and M.C. performed data mining; E.P., T.P., I.C., T.C. and M.R. provided blood samples and clinical data; E.L. and F.B. performed sequence analysis; P.S.T. and P.F.T. reviewed the manuscript.

**Disclosures**

The authors declare no conflicts of interest to disclose.

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Vascular endothelial growth factor (VEGF) is upregulated in multiple myeloma (MM), and circulating VEGF levels may correlate with response to therapy (Hideshima et al., 2005; Pittini et al., 2002). Thalidomide has been part of the standard treatment for MM and is thought to inhibit VEGF-associated angiogenesis (Du et al., 2004). Bevacizumab, a monoclonal antibody directed against VEGF-A, inhibits VEGF (Jenab-Wolcott & Giantonio, 2009). Accordingly, we set out to test the efficacy and safety of bevacizumab alone and in combination with thalidomide in MM patients.

A phase II prospective randomized/stratified trial led by the California Cancer Consortium, and including the University of Chicago, was approved by the Cancer Therapy Evaluation Program/National Institute of Health (N01-CM-62209). Patients with prior thalidomide exposure received bevacizumab alone (Arm A). Thalidomide-naïve patients were randomized to either Arm B (bevacizumab alone) or C (combination therapy). The study was closed early due to poor accrual, attributable to competing trials providing access to lenalidomide and bortezomib (Knight, 2005; Lu et al., 2009; Moschetta et al., 2010).

The primary objectives were response rate, event-free survival, and toxicity. The secondary objective was to measure markers of angiogenesis and assess any correlation with outcome. Immunohistochemical (IHC) staining of VEGF (VG-1; Neomarkers, Freemont, CA, USA) and its two receptors, VEGFR1/Flt-1 (AB-1; Neomarkers) and VEGFR2/KDR (AB-1; Neomarkers) was carried out on bone marrow clots or cores obtained at baseline.

The study was conducted between October 2001 and November 2004. Patients aged 18 years or older, with relapsed/progressive MM and a Karnofsky performance status (KPS) >60% were enrolled. All patients signed a voluntary informed consent form, approved by the institutional review boards of the participating institutions.

Bevacizumab was given at 10 mg/kg intravenously over a 90-min period every 14 d. Thalidomide was escalated from 100 mg/d by 100 mg/week, up to 400 mg/d. Treatment cycle length was 56 d. Treatment was discontinued due to disease progression, development of grade 3 or 4 toxicities that did not resolve to grade 1 or less (maximum 3 weeks’ delay was allowed), non-compliance, or patient request or physician discretion. The National Cancer Institute’s Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf) was used for toxicity and adverse event reporting.

Complete response was defined as disappearance of the paraprotein in the serum and/or urine by immunofixation and <5% plasma cells on bone marrow evaluation. A partial remission was defined as a ≥50% reduction but still detectable level of paraprotein, and if present, a ≥50% reduction in urine M-component. Stable disease was defined as <50% reduction in paraprotein, or if the patient had light-chain disease only, a >50% reduction in the urine M-component (Bence-Jones protein). Progressive disease was defined as a 25% increase in paraprotein from the lowest level observed, measured on at least two separate occasions 2 weeks apart. We defined event-free survival (EFS) as synonymous with time to treatment failure (TTF) to avoid reporting artificially long progression-free survival in patients who declined further protocol therapy prior to progression. TTF was therefore defined as the time from the first day of treatment to the first observation of disease progression, death, or treatment cessation due to toxicity or patient refusal.

Fourteen patients consented; one withdrew prior to initiation of treatment, and another became ineligible due to a drop in KPS. Twelve evaluable patients, eight female, four male, (median age: 58 years, range: 50–75) were enrolled; six received bevacizumab alone (Arms A or B); six received combination therapy (Arm C). Eight of the patients were enrolled with stage III disease (Durie & Salmon, 1975) and two each with stages I and II. The median β2 microglobulin was 2.7 mg/l (range 1.0–9.9 mg/l) with nine cases of IgGκ, two patients with IgGκ, and one case of non-secretory myeloma. Previous treatments

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