Letters to *Blood*

To the editor:

A pharmacogenetic analysis of the Canadian Cancer Trials Group MY.10 clinical trial of maintenance therapy for multiple myeloma

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Multiple myeloma is often treated with high-dose melphalan chemotherapy followed by maintenance therapy with immunomodulatory agents aimed to delay disease progression and prolong survival. Although it is routinely offered to many patients, randomized trials of maintenance therapy using thalidomide or lenalidomide only demonstrated improvement in progression-free survival (PFS), with inconsistent overall survival (OS) benefit.[1-4] Genetic biomarkers predictive of the efficacy and toxicity of immunomodulatory drug maintenance therapy, as well as prognosis following front-line high-dose melphalan therapy, are of potential value in identifying patients best suited for maintenance therapy. Cibeira et al found that single-nucleotide polymorphisms (SNPs) in DNA repair genes were associated with thalidomide response and survival outcome.[5] Johnson et al found several SNP associations with risk of venous thrombosis[6] and thalidomide-related peripheral neuropathy,[7] respectively. Dumontet et al found that SNPs in drug metabolism and DNA repair genes were associated with response to high-dose melphalan and survival.[8] Fernández de Larrea et al found that SNPs in *FAM179B* and *MIR196A* were associated with survival after lenalidomide salvage therapy.[9] Well-designed replication studies with independent patient cohorts are crucial in pharmacogenetics to facilitate clinical uptake of predictive biomarkers. In addition, randomized trial design is required for rigorous evaluation of predictive markers associated with benefit or harm from therapy, where the interaction between treatment assignment and outcome can be examined; otherwise, a prognostic marker can be mistakenly identified as predictive.[10]

In our study, 44 validation SNPs were selected from previous studies with significant associations with myeloma prognosis and/or

thalidomide and melphalan treatment efficacy and toxicity[5-9,11] for validation in the Canadian Cancer Trials Group (formerly named the National Cancer Institute of Canada Clinical Trials Group [NCIC CTG]) MY.10 randomized trial. Fourteen exploratory SNPs in genes shown to be involved in myeloma disease pathways as well as the CRBN-IKZF1/IKZF3-IRF4 pathway,[12-19] which is now known to be central to the mechanism of action of immunomodulatory drugs, were also investigated (the full list of candidate SNPs can be found in supplemental Table 1, see supplemental Data available at the *Blood* Web site). Exploratory SNPs were chosen due to known functional significance or had clinically significant impact in other disease settings. The MY.10 randomized trial compared thalidomide-prednisone maintenance therapy with observation post high-dose melphalan chemotherapy and autologous stem cell transplant (ASCT).[20] Bone marrow aspirates (n = 86 treatment arm; n = 101 observation arm) were obtained (for patient demographic details, see supplemental Table 2). Genomic DNA were extracted and SNP genotypes were assessed by allelic discrimination using TaqMan assays on the ViiA7 platform. To rule out potential discrepancies in SNP genotype between tumor and germ line DNA in our candidate SNPs, concordance assays between tumor and germ line DNA extracted from paired bone marrow and blood samples, respectively, from 9 patients in a separate cohort were conducted and found to be completely concordant for all 58 SNPs. Cox regression models were performed using SAS (version 9.3) for prognostic impact of a single SNP on PFS and OS, with and without adjusted covariates (age, stage, performance status, and response to ASCT). In treatment benefit analysis, PFS and OS were modeled with genotype, treatment assignment, and their interaction

Table 1. SNPs with significant prognostic impact for PFS and OS

SNP (rs#)	Gene	Location	Base change	Genotype and distribution	Association	HR	95% CI	*P*
rs1672753	*CRBN*	5′UTR	A>G	GG/GA/AA (4/44/137)	PFS	0.59	0.40-0.86	.006
rs1672753	*CRBN*	5′UTR	A>G	GG/GA/AA (4/44/137)	OS	0.55	0.33-0.92	.023
rs2440	*XRCC5*	3′UTR	G>A	AA/AG/GG (32/79/76)	OS	1.70	1.11-2.61	.015
rs4646903	*CYP1A1*	3′UTR	A>G	GG/GA/AA (1/49/137)	OS	1.59	1.04-2.43	.033
rs363717	*ABCA1*	3′UTR	T>C	CC/CT/TT (7/49/131)	PFS	1.44	1.03-2.02	.033
rs361525	*TNF-α*	5′UTR	G>A	AA/AG/GG (0/18/169)	PFS	1.70	1.03-2.81	.037
rs1056836	*CYP1B1*	Exon	G>C	CC/CG/GG (42/77/68)	PFS	0.72	0.52-0.99	.043

HR represents hazard of patients with at least 1 minor allele vs patients with 2 major alleles.
CI, confidence interval.

Table 2. SNPs having significant interactions with treatment allocation in PFS and OS

SNP (rs#)	Gene	Location	Base change	Genotype and distribution	Association	2 minor alleles and heterozygote		2 major alleles		*P*-value interaction
						HR	95% CI	HR	95% CI	
rs1053667	*FAM179B*	3'UTR	T>C	CC/CT/TT (0/21/162)	PFS	2.27	0.78-6.67	0.47	0.33-0.66	.003
rs1799969	*ICAM1*	Exon	G>A	AA/AG/GG (0/33/154)	PFS	1.75	0.83-3.70	0.51	0.36-0.73	.007
rs1799969	*ICAM1*	Exon	G>A	AA/AG/GG (0/33/154)	OS	2.24	0.92-5.43	0.62	0.39-0.97	.009
rs1049216	*CASP3*	3'UTR	A>G	GG/GA/AA (19/64/103)	PFS	0.41	0.25-0.69	0.83	0.54-1.25	.029
rs6103	*SERPINB2*	Exon	C>G	GG/GC/CC (14/64/103)	OS	1.24	0.68-2.24	0.52	0.30-0.93	.041
rs17655	*ERCC5*	Exon	G>C	CC/CG/GG (12/63/112)	OS	0.46	0.25-0.87	1.09	0.65-1.83	.043

HR represents hazard of treatment vs observation for each genotype group.
Abbreviations are explained in Table 1.

term. Logistic regression models were used for analysis of thalidomide-related peripheral neuropathy with time to worsening of neuropathy by at least 1 grade using the National Cancer Institute Common Toxicity Criteria (NCI-CTC version 2.0)[21] being the outcome of interest. The dominant genetic model was used for genotype grouping (similar results with the additive model). A 2-sided *P* value of <.05 was required for statistical significance. The Research Ethics Board of University of New Brunswick, Canada provided institutional review board approval for this study (REB#03-2013).

We identified SNPs with significant prognostic impact on PFS and/or OS (Table 1). In a multivariate analysis with inclusion of known prognostic factors, all SNPs retained significant associations (supplemental Table 3). Five SNPs had significant interaction with treatment allocation in predicting PFS and/or OS (Table 2). Two SNPs were found to have significant interaction with treatment allocation in predicting time to worsening of peripheral neuropathy (Table 3).

A SNP (rs1672753) in the 5′ untranslated region (UTR) of *CRBN* had previously been associated with immunomodulatory drug (IMiD) response in myelodysplastic patients.[12] In the MY.10 cohort, this SNP had significant impact on myeloma survival outcome independent of thalidomide maintenance therapy. Patients with GG and GA genotypes had better PFS and OS compared with patients with 2 major alleles (AA) (Figure 1A-B). Although CRBN has been shown to play a central role in the mechanism of action of IMiDs,[22] the prognostic impact of this SNP regardless of treatment arm suggests that CRBN may play a role in myeloma cell biology that is independent of IMiD therapy.

SNPs in cytochrome P450 genes found to be prognostic for PFS and/or OS in our cohort (*CYP1A1* rs4646903 and *CYP1B1* rs1056836) may in fact be associated with melphalan benefit. The cytochrome P450 family of enzymes is known to mediate chemotherapeutic drug metabolism and activation.[23] We were unable to correlate these SNPs directly with melphalan benefit because all patients received melphalan as a conditioning agent. These SNPs were found previously by Dumontet et al to be associated with outcomes after high-dose melphalan,[8] with the same direction of association.

Patients with AA and AG genotypes for SNP rs1799969 (*ICAM1*) had inferior hazard ratio (HR) estimates for PFS and OS on maintenance therapy compared with observation, suggesting possible harm from maintenance therapy; conversely, patients with 2 major alleles (GG) had superior HR estimates for PFS and OS on maintenance compared with observation, suggesting benefit from maintenance therapy (Figure 1C-D). This SNP was found previously to be associated with decreased risk of thalidomide-related peripheral neuropathy[7]; we did not detect such an association in our study. ICAM1 is an intracellular adhesion molecule involved in myeloma cell adhesion-mediated drug resistance (CAMDR). Minor (C) allele correlates with low ICAM1 levels.[24] Thalidomide is able to downregulate ICAM1.[25] High ICAM1 levels would normally lead to CAMDR and melphalan resistance. Patients with 2 major alleles in the observation group would be melphalan resistant, leading to poorer survival outcome. If these patients receive subsequent thalidomide maintenance therapy and overcome CAMDR, this could result in an improved survival outcome compared with observation.

GLCCI1 SNP (rs37973) assayed in this study is always coinherited with SNP rs37972, which has previously been associated with corticosteroid response in asthma patients.[19] Because MY.10 patients in the maintenance arm received prednisone, it is intriguing to speculate on the potential modulation of neuropathy by corticosteroid therapy.

Due to the limited sample size and exploratory nature of this study, correction for multiple hypothesis testing was not conducted, and raises the potential for both false-negative and false-positive results. Firm conclusions cannot be drawn regarding SNPs that were significant in previous studies but were not validated in our study, and SNPs identified as significant for the first time in our study require independent validation in future patient cohorts. Functional studies will help clarify our findings.

This is the first randomized trial with a no-treatment control arm to report the impact of SNPs in predicting benefit from immunomodulatory drug maintenance therapy for myeloma. Thalidomide biomarkers discovered here may apply to lenalidomide and pomalidomide, due to their structural similarity and common mechanisms of action.

Table 3. SNPs associated with thalidomide-related peripheral neuropathy

SNP (rs#)	Gene	Location	Base change	Genotype and distribution	Treatment		Observation		*P*-value interaction
					HR	95% CI	HR	95% CI	
rs11552449	*DCLRE1B*	Exon	C>T	TT/TC/CC (5/49/133)	0.64	0.33-1.27	3.11	1.01-9.57	.010
rs37973	*GLCCI1*	5′UTR	A>G	GG/GA/AA (35/93/56)	0.51	0.28-0.91	4.03	0.52-31.10	.046

HR represents hazard of patients with at least 1 minor allele vs patients with 2 major alleles.
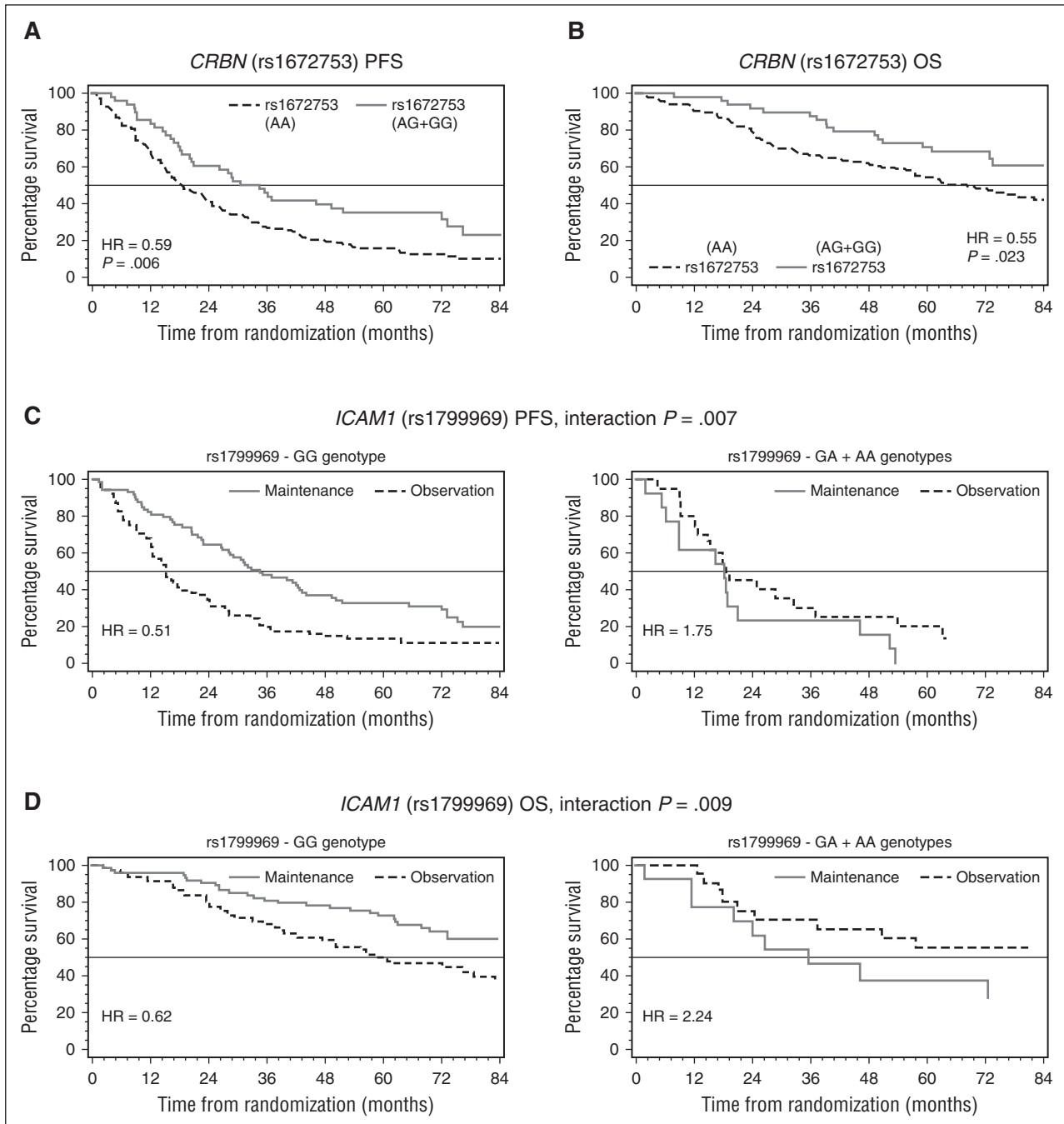Abbreviations are explained in Table 1.

Figure 1. Kaplan-Meier plot comparing survival of patients from the MY.10 clinical trial by SNP status. HR of *CRBN* (rs1672753) represent hazard of patients with at least 1 minor allele vs patients with 2 major alleles. HR of *ICAM1* (rs1799969) represents hazard of treatment (maintenance) vs observation for each genotype group.

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To the editor:

Allogeneic bone marrow transplantation for treatment of severe hemolytic anemia attributable to hexokinase deficiency

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Hexokinase (HK) deficiency is a very rare inherited disorder of the Embden-Meyerhof glycolytic pathway of erythrocytes where the predominant clinical presentation is congenital nonspherocytic hemolytic anemia. HK catalyzes the initial phosphorylation of glucose to glucose 6 phosphate and is considered 1 of the rate-limiting enzymes of red cell anaerobic glycolysis.[1] Of all of the glycolytic enzymes, HK is the most influenced by erythrocyte age. Mature erythrocytes may have no more than 2% to 3% of their HK activity originally present in the reticulocytes.[2,3] There are 4 isoenzymes of HK (HK I-IV) of which HK-I is the predominant isoenzyme in human tissues. Erythrocytes contain a specific subtype of HK (HK-R) that is encoded by the HK-I gene (*HK1*) localized on chromosome 10q22.[4] Lymphocytes, platelets, and granulocytes depend more or less heavily on energy supplied by