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Carfilzomib and lenalidomide response related to VEGF and VEGFR2 germline polymorphisms

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Abstract

The combination of carfilzomib, lenalidomide, and dexamethasone (CRd) has induced deep responses in patients with newly diagnosed multiple myeloma. While vascular endothelial growth factor (VEGF) pathway polymorphisms have been associated with clinical outcomes for antiangiogenesis agents, we explored associations between such polymorphisms and CRd clinical response. The *VEGF-1498C>T* (rs833061) and *VEGFR2 V2971* (rs2305948) were associated with CRd response (OR : 0.10, *P*: 0.009), whereas *VEGF-1498C>T* and *VEGFR2 Q472H* (rs1870377) were associated with minimum residual disease negativity (*P*: 0.023). As these SNPs were not associated with disease parameters (e.g., plasma VEGF, albumin, or beta-2-microglobulin concentration), data suggest these SNPs may be markers of CRd response.

Keywords

Carfilzomib; Lenalidomide; Multiple myeloma; Polymorphism; Vascular endothelial growth factor; Vascular endothelial growth factor receptor; KDR

Introduction

Carfilzomib is a proteasome inhibitor that, in combination with lenalidomide (Revlimid®) and dexamethasone (CRd), induces deep responses in patients with multiple myeloma. In two recent studies [8, 13], the three-drug combination resulted in a complete response in 32–62% of patients and significant improvement in median progression-free survival (PFS) over lenalidomide and dexamethasone.

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Conflict of interest The authors have no conflicts of interest to disclose.

VEGF/VEGFR2 signaling results in increased myeloma activity and drug resistance [1, 2, 11]. Secretion of the vascular endothelial growth factor (VEGF) by myeloma cells promotes VEGF receptor (VEGFR; especially VEGFR2, KDR) signaling in bone marrow and stromal cells, which increases secretion of cytokines and growth factors that promote disease progression. Polymorphisms in the *VEGF/VEGFR2* genes have been shown to result in variation in VEGF expression and VEGFR2 signaling [7]. In myeloma, polymorphisms in these genes are related to myeloma risk, progression, toxicity, and thalidomide efficacy [2, 3]. *VEGFR2* polymorphisms have also been related to clinical outcome and toxicity of other antiangiogenesis agents, such as bevacizumab and sorafenib [6].

We hypothesized that polymorphisms in the VEGF/VEGFR2 pathway are related to treatment outcomes with the CRd combination. We aimed to compare commonly inherited *VEGF* and *VEGFR2* polymorphisms to carfilzomib/lenalidomide response and toxicity. Based on previous studies [2, 5, 6], we selected 2 polymorphisms in *VEGF* (−634G>C (rs2010963) and 1498C>T (rs833061)) and two SNPs in *VEGFR2* (V2971 (rs2305948), and Q472H (rs1870377)).

Patients and methods

Patients

Patient characteristics, response criteria, minimum residual disease (MRD) monitoring methods, and pharmacokinetics have been previously published [8]. Briefly, all patients ($n = 45$) had newly diagnosed multiple myeloma and received eight 28-day cycles of CRd.

Genotyping

DNA was extracted from whole blood using a QiaBlood DNA extraction kit following the manufacturer's instructions (Qiagen, Valencia, CA, USA) and stored at 4 °C. For *VEGFR2* SNPs, genotyping methods were previously published [6]. To ascertain genotype at *VEGF* SNPs, we used TaqMan® assays as per the manufacturer's instructions (see rs2010963 and rs833061, Life Technologies, Carlsbad, CA).

Plasma VEGF concentrations

Baseline plasma concentrations of VEGF were measured in patients just after the 30-min carfilzomib infusion on day 1, which did not differ from those collected directly after the infusion (median difference = 0.017 ng/mL; $P = 0.31$; $n = 5$). Plasma concentrations were also ascertained within 2 cycles of the best response, except for 3 patients from whom samples were obtained 3, 4, and 7 cycles after the best response. Plasma VEGF concentrations were determined by ELISA (R&D Systems, Quantikine ELISA Kit, Minneapolis, MN). Three samples were excluded due to contamination with heme, which was shown to interfere with the ELISA measurement of VEGF.

Statistical considerations

The Jonckheere-Terpstra and Cochran-Armitage tests were used to assess trends in continuous distributions and dichotomous factors, respectively, across three genotypes, while the Wilcoxon rank sum test and Fisher's exact test were used when two groups were

compared. Firth's method was applied to estimate the odds ratio when one cell count was zero. The Spearman rank correlation coefficient (r) was used to quantify associations between continuous and ordered categorical variables. Hardy-Weinberg equilibrium and consistency with previously published genotype frequencies were ascertained with the Chi squared test. Progression-free survival was measured from the on-study date to the date of progression or last follow-up. Strata were compared using the log-rank test, and hazard ratio estimates are from proportional hazards regression. Exact tests were used as appropriate to calculate P values and 95% confidence intervals (Cis). In cases where exact CI methods are not available in the software employed (SAS/STAT 12.1), large-sample Cis of test statistics may be inconsistent with exact P values. Considering the large number of tests performed (200+), only very small P values can be named statistically significant.

Results and discussion

Two SNPs in *VEGF*-634C>G (rs2010963; $n = 43$) and -1498C>T (rs833061; $n = 42$), and two SNPs in *VEGFR2* (V2971 and Q472H; rs2305948 and rs1870377, respectively; $n = 44$) were detected. All genotype distributions were consistent with Hardy-Weinberg equilibrium ($P > 0.75$). Genotypes were not strongly related to demographics, disease markers, or carfilzomib clearance (Table S1). Genotypes were also only weakly related to plasma VEGF concentrations (Spearman's $|r| < 0.35$; absolute median difference < 0.035 ng/mL; Table S2). For *VEGF* 1498C>T vs. post CRd VEGF, Spearman's r (95% CI) is 0.33 (0.08–0.58; $p = 0.032$).

Baseline and post-response VEGF plasma concentrations were also not strongly related to alphaglobin, alpha-2-globin, or betaglobin concentrations at baseline (Spearman's $|r| < 0.25$, $P > 0.10$). However, pre-treatment VEGF concentrations were 0.056 ng/mL higher (95% CI 0.017, 0.128) in patients with high beta-2 microglobulin levels ($n = 35$) than in patients with normal levels ($n = 6$) ($P = 0.001$); a similar difference was observed with VEGF concentrations occurring at the cycle of best response [high levels, $n = 38$, minus normal levels, $n = 6$; median difference (95% CI): 0.056 (0.011, 0.116), $P = 0.015$]. Therefore, subsequent comparisons were made without stratifying by staging or pharmacokinetics parameters.

Several SNPs in the VEGF pathway were associated with response and outcomes (Table 1). A total of 67–79% (8/12, 15/19) of patients carrying variant alleles at *VEGF*-1498C>T had CR/nCR/sCR whereas only 27% (3/11) of patients carrying wild-type alleles experienced this outcome; the odds ratios (OR) are similar (0.10 vs. 0.19) but the OR for the heterozygous allele is less likely to equal 1 ($P = 0.009$ vs. $P = 0.10$). Those patients carrying 1498T alleles also tended to be MRD negative [CT, 84% (16/19) and TT, 91% (10/11)] when compared to those carrying CC genotypes (45% (5/11); $P = 0.023$; Table 1). All carriers of *VEGFR2* V2971 experienced only sCR (10/10), whereas only 41% (14/34) of those carrying wild-type alleles experienced sCR [OR (95% CI): 0.03 (< 0.01 , 0.30)] and only 53% (18/34) experienced sCR/nCR/CR [OR (95% CI): 0.05 (< 0.01 , 0.47); $P = 0.007$]. Finally, no carriers of *VEGFR2* 472Q alleles (0/16) had minimum residual disease while only 65% (17/26) of 472HH genotypes were MRD-free ($P = 0.011$). Progression-free survival did not appear to be related to either genotype or VEGF levels (Table 2). However,

since some subgroups had small numbers of patients and few progressions, these results should be viewed cautiously. No relationships with toxicity were detected (Table S3).

VEGF-634G is associated with greater binding of promoter elements and, therefore, a higher protein expression whereas *VEGF-1498C>T* has no available transcriptional data, but is related to various outcomes in several cancers [7, 10, 15]. These SNPs have not been compared to plasma VEGF concentration in patients with multiple myeloma [7, 12]. In our hands, only a weak association between *VEGF-1498C>T* and VEGF concentrations was observed after CRd therapy (Table S2), and no other SNPs were related to this endpoint. A previous study in myeloma has found that carriers of *VEGF-634C* and *VEGF-1498T* experienced greater benefit from thalidomide in multiple myeloma [2]. Consistently, the present results demonstrate that *VEGF-1498T* carriers experience greater benefit from the CRd combination—albeit significance was only observed in patients with heterozygous (*VEGF-1498CD*) genotypes, and findings were not strongly significant for the MRD endpoint.

VEGFR2 SNPs have been related to toxicity and clinical outcome of bevacizumab, sorafenib, and axitinib. While cell lines transfected with V2971 had lower VEGF binding affinity [14], this SNP has been associated with hypertension in one study and not another [5, 6]. *VEGFR2* 472Q carriers had longer PFS [5], and were at greater risk of hypertension and hand-foot skin reactions [6], suggesting that antiangiogenesis agents are potentially more potent in this group of patients. Polymorphic variation in *VEGFR2* has not yet been studied in thalidomide or lenalidomide. Therefore, the present data are also consistent with previous literature concerning antiangiogenesis agents, although this is the first study showing that the *VEGFR2* V2971 SNP is probably associated with response to CRd. MRD results were not significantly different even though all 2971 carriers were MRD negative.

Crosstalk between malignant plasma cells and the microenvironment, and immune evasion are the hallmarks of progression of multiple myeloma. While such crosstalk is quite complex, VEGF/*VEGFR2* promote adhesion of malignant plasma cells to bone marrow, mitogenesis and antiapoptosis, and pathogenic angiogenesis in the marrow; the latter further increases the interaction between the tumor and the microenvironment [9]. VEGF/*VEGFR2* signaling also appears to result in inter-individual differences in lenalidomide efficacy [4]. No studies have evaluated the effects of carfilzomib on VEGF pathways. The present exploratory genotyping study determined that *VEGF* and *VEGFR2* SNPs are probably markers of clinical outcome following the CRd combination. The *VEGF-1498C>T* SNP and the nonsynonymous *VEGFR2* V2971 transition were associated with response to CRd but not disease characteristics; therefore, the present data suggest that these SNPs are independent genetic markers of CRd response in patients with multiple myeloma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

VEGF/VEGFR2 genotypes versus response and minimum residual disease status

Response		MRD										
Genotype	sCR n (%)	nCR/CR n (%)	VGPR n (%)	PR/SD n (%)	P (trend) ^a	OR (95%CI) <sCR	P ^b	OR (95%CI) <CR	P ^b	MRD negative n (%)	MRD positive n (%)	P (trend) ^c
VEGF 634 C>G												
CC	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0.29	Ref.		Ref.		3 (100)	0 (0)	0.14
CG	10 (58.8)	2 (11.8)	3 (17.6)	2 (11.8)		1.40 (0.06, 94.2)	1	3.08 (0.23, 441)	0.54	14 (87.5)	2 (12.5)	
GG	11 (47.8)	1 (4.3)	9 (39.1)	2 (8.7)		2.18 (0.10, 139)	1	6.44 (0.54, 902)	0.24	15 (68.2)	7 (31.8)	
VEGF 1498 C>T												
CC	2 (18.2)	1 (9.1)	6 (54.5)	2 (18.2)	0.13	Ref.		Ref.		5 (45.5)	6 (54.5)	0.023
CT	14 (73.7)	1 (5.3)	2 (10.5)	2 (10.5)		0.08 (0.01, 0.62)	0.007	0.10 (0.01, 0.72)	0.009	16 (84.2)	3 (15.8)	
TT	6 (50.0)	2 (16.7)	3 (25.0)	1 (8.3)		0.22 (0.02, 1.94)	0.19	0.19 (0.02, 1.47)	0.1	10 (90.9)	1 (9.1)	
VEGFR2 V297I												
VV	14 (41.2)	4 (11.8)	12 (35.3)	4 (11.8)	0.002	Ref.		Ref.		23 (78.9)	9 (28.1)	0.12
VI	9 (100.0)	0 (0)	0 (0)	0 (0)		0.03 (<0.01, 0.30)	0.001	0.05 (<0.01, 0.47)	0.007	9 (100)	0 (0)	
II ^d	1 (100.0)	0 (0)	0 (0)	0 (0)						1 (100)	0 (0)	
VEGFR2 Q472H												
QQ	0 (0)	1 (100)	0 (0)	0 (0)	0.28					1 (100)	0 (0)	0.011
QH	10 (66.7)	1 (6.7)	4 (26.7)	0 (0)		Ref.		Ref.		15 (100)	0 (0)	
HH	14 (50)	2 (7.1)	8 (28.6)	4 (14.3)		1.67 (0.41, 7.18)	0.53	2.25 (0.50, 11.8)	0.33	17 (65.4)	9 (34.6)	

^aJonckheere-Terpstra trend test^bFisher's exact test^cCochran-Armitage Test^dGiven that only one patient carried a given genotype, this individual was included with the heterozygous patients for the purposes of analysis

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Table 2

VEGF/VEGFR2 genotypes versus progression-free survival on CRd

Genotype	<i>P</i> (trend)	<i>P</i> (log-rank)	HR (95% CI)	HR (95% CI)
<i>VEGF</i> 1498 C>T (CC, CT, TT)	0.78	0.84	0.64 (0.14, 2.95)	0.77 (0.15, 3.90)
<i>VEGF</i> 634 C>G (CC + CG, GG)		0.35	0.51 (0.12, 2.15)	
<i>VEGFR2</i> V2971 (VV, VI + II)		0.43	0.44 (0.05, 3.56)	
<i>VEGFR2</i> Q472H (QQ + QH, HH)		0.37	0.55 (0.15, 2.09)	