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Clinical Trials: Targeted Therapy

Clinical Cancer Research

Vitamin D Modifies the Incidence of Graft-versus-Host Disease after Allogeneic Stem Cell Transplantation Depending on the Vitamin D Receptor (VDR) Polymorphisms

Estrella Carrillo-Cruz[1], José R. García-Lozano[2], Francisco J. Márquez-Malaver[1], Fermín M. Sánchez-Guijo[3], Isabel Montero Cuadrado[1], Christelle Ferra i Coll[4], David Valcárcel[5], Oriana López-Godino[3], Marian Cuesta[6], Rocío Parody[7], Lucía López-Corral[3], Miguel Alcoceba[3], Teresa Caballero-Velázquez[1,8], Alfonso Rodríguez-Gil[8], José A. Bejarano-García[8], Teresa Lopes Ramos[8], and José A. Pérez-Simón[1,8]

Abstract

Purpose: The biologically active metabolite of vitamin D3, 1,25-dihydroxyvitamin D3 (vit D), has immunoregulatory properties via binding vitamin D receptor (VDR). In a prospective trial, we previously reported a reduction in the incidence of chronic GvHD (cGvHD) among patients who received vit D after allogeneic stem cell transplantation (allo-HSCT; Clinical Trials.gov: NCT02600988). Here we analyze the role of patients and donors' VDR SNPs on the immunomodulatory effect of vit D.

Patients and Methods: Patients undergoing allo-HSCT were included in a prospective phase I/II clinical trial (Alovita) in three consecutive cohorts: control (without vit D), low-dose (1,000 IU/day), and high-dose (5,000 IU/day) groups. Vit D was given from day −5 until +100 after transplant. Genotyping of four SNPs of the VDR gene, FokI, BsmI, ApaI, and TaqI, were performed using TaqMan SNP genotyping assays.

Results: We observed a decrease in the incidence of overall cGvHD at 1 year after allo-HSCT depending on the use or not of vit D among patients with FokI CT genotype (22.5% vs 80%, $P = 0.0004$) and among those patients without BsmI/ApaI/TaqI ATC haplotype (22.2% vs 68.8%, $P = 0.0005$). In a multivariate analysis, FokI CT genotype significantly influenced the risk of cGvHD in patients treated with vit D as compared with the control group (HR 0.143, $P_{interaction} < 0.001$).

Conclusions: Our results show that the immunomodulatory effect of vit D depends on the VDR SNPs, and patients carrying the FokI CT genotype display the highest benefit from receiving vit D after allo-HSCT.

Introduction

Vitamin D_3 is a fat-soluble steroid that is synthesized in the skin from 7-dehydrocholesterol in the presence of ultraviolet irradiation. 25-hydroxylase (CYP27A1) in the liver and 1alpha-hydroxylase (CYP27B1) in the kidney are responsible for the conversion in 1,25-dihydroxyvitamin D_3 or $1,25(OH)_2D_3$ (vit D), which is the most biologically active metabolite. Even though vit D is primarily known for its involvement in calcium and phosphate homeostasis, it has other physiologic functions such as the regulation of the immune response through several mechanisms such as modulating cell proliferation and differentiation, inhibiting dendritic cell maturation, downregulating T-cell activation, increasing regulatory T cells, and decreasing the production of proinflammatory cytokines (1–5).

Vit D operates via binding vitamin D receptor (VDR), which is expressed in osteoblasts, intestinal mucosa cells, and immune cells, including macrophages, dendritic cells, mature $CD8^+$ T and B cells and immature T cells in the thymus (6). Vit D-VDR heterodimerize with retinoid-X-receptor (RXR) within the cell nucleus where it binds to vitamin D responsive elements to serve as a transcription factor for numerous target genes. Depending on the target gene either coactivators or corepressors are attracted to the VDR/RXR complexes to induce or repress gene transcription (7, 8).

In humans, more than 200 polymorphisms of the gene encoding VDR have been reported. However, only a few of them have

[1]Department of Hematology, Hospital Universitario Virgen del Rocío/Instituto de Biomedicina de Sevilla (IBIS)/CSIC/CIBERONC, Universidad de Sevilla, Seville, Spain. [2]Department of Immunology, Hospital Universitario Virgen del Rocío, Seville, Spain [3]IBSAL-Hospital Universitario de Salamanca/CIBERONC, CIC-Universidad de Salamanca, Salamanca, Spain. [4]Institut Catalad'Oncologia, Hospital Germans Trias i Pujol, Barcelona, Spain. [5]Hospital Vall d'Hebron, Barcelona, Spain. [6]Hospital Carlos Haya, Málaga, Spain. [7]Institut Catala d'Oncologia, Hospital Duran i Reynals, Barcelona, Spain. [8]Instituto de Biomedicina de Sevilla (IBIS)/ CSIC/ CIBERONC, Universidad de Sevilla, Seville, Spain.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: José A. Pérez-Simón, University Hospital Virgen del Rocio, Instituto de Biomedicina de Sevilla (IBIS / CSIC / CIBERONC), Universidad de Sevilla, Av Manuel Siurot s/n Seville 41013, Spain. Phone: 349-5501-3260; Fax: 349-5501-3265; E-mail: josea.perez.simon.sspa@juntadeandalucia.es

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Translational Relevance

In a previous prospective trial, we reported a very low toxicity profile and a lower incidence of chronic GvHD among patients who received 1,25-dihydroxyvitamin D3 (vit D) after allogeneic stem cell transplantation (allo-HSCT), without a significant increase in relapses or infections. In this study, we analyze the impact of vitamin D receptor (VDR) polymorphisms on the immunomodulatory effect of vit D. We observed that patients carrying the *FokI* CT genotype display the highest benefit from receiving vit D. These data suggest a higher sensitivity to vit D in this specific form of VDR and might allow us to identify those patients who are the best candidates to receive this supplement after allo-HSCT. These findings should be considered for future studies.

been studied and associated to diseases such as osteoporosis, cancer, and immune disorders (9).

Four common SNPs in the VDR gene have been extensively investigated: *FokI* (rs2228570 T/C), *BsmI* (rs1544410 A/G), *ApaI* (rs7975232 C/A), and *TaqI* (rs731236 T/C). *FokI*, located in exon 2, is a missense polymorphism, which produces a longer protein due to an alternative initiating codon in the T genotype. *ApaI*, *BsmI*, and *TaqI* are in strong linkage disequilibrium. *ApaI* and *BsmI* are both located in intron 8 and their variants might affect the mRNA stability (10). *TaqI*, in exon 9, is a synonymous polymorphism in which TT genotype has been associated to higher levels of VDR mRNA and protein (11).

Some retrospective studies have been performed to elucidate the role of VDR SNPs on the outcome of patients undergoing allogeneic stem cell transplantation (allo-HSCT). Specific genotypes have been associated to the likelihood of developing grades 2–4 acute GvHD (aGvHD) or infections. Moreover, an impact on survival has been reported in several retrospective studies (12–14). In a study by Middleton and colleagues, *ApaI* G allele (formerly A allele) was related to a higher frequency of aGvHD and a worse survival (14). Also *FokI* CC genotype (formerly FF) has been related to an increased risk of aGvHD according to Bogunia and colleagues (13).

In addition, vit D deficiency has been related to a worse outcome after allo-HSCT, which might be related to an increased risk of relapse among patients diagnosed with acute myeloid leukemia (15) or to a higher risk of mortality (16). Interestingly, it has also been described that the administration of vit D or analogs induces an immunomodulatory effect, as demonstrated in animal models after solid organ or bone marrow transplantation (17, 18).

Finally, we have previously described in a phase I/II prospective multicenter trial that the administration of vit D in the post-transplant setting reduces the risk of chronic GvHD (cGvHD; Alovita trial). Also, vit D modified the immune response after transplantation, decreasing the number of B cells and naïve CD8 T cells, with a lower expression of CD40L as an activation marker in T cells (19).

There are no studies analyzing the role of VDR SNPs on the effect of vit D as an immunomodulatory drug after transplantation. Therefore, in this study, we analyzed the effect of the *FokI*, *BsmI*, *TaqI*, and *ApaI* SNPs on the incidence of GvHD and on the outcome of patients undergoing allo-HSCT treated or not with vit D within the Alovita Trial.

Patients and Methods

Study population

Patients undergoing allo-HSCT were included in a multicenter and prospective phase I/II clinical trial (Alovita) from May 2011 to February 2014 in three consecutive cohorts: control group (without vit D), low-dose group (1,000 IU/day), and high-dose group (5,000 IU/day). Vit D was given from day −5 until +100 after transplantation. Patients with either a related or unrelated donor with a maximum of one HLA allele mismatch of eight were allowed to be included into the study. Exclusion criteria were: hypercalcemia (calcium level in blood, 10.5 mg/dL), serum creatinine equal or higher than twice the upper normal limit, and use of any *ex vivo* or *in vivo* procedure of T-cell depletion as GVHD prophylaxis. Because a similar effect on cGvHD incidence between the low- and the high-dose group was observed in the original study, both groups were merged and compared with the control group in this study.

Data from patients and their respective donors were considered for analysis if genomic DNA stored before transplant was available. Among patients selected for this study, 71 patients received 1,000 IU/day or 5,000 IU/day of vit D, and 36 belonged to the control group. Overall, 107 patients and 102 donors from six Spanish centers were included.

The institutional ethics committees of all participating centers approved the study. The trial was registered at www.clinicaltrialsregister.eu as EudraCT: 2010-023279-25 and Identifier of ClinicalTrials.gov: NCT02600988. All patients signed written informed consent.

VDR genotyping

Genomic DNA was obtained from peripheral blood or bone marrow using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's recommendations and stored at −20°C. Genotyping of four SNPs of the VDR gene, rs2228570C/T (*FokI*), rs1544410A/G (*BsmI*), rs7975232T/G (*ApaI*), and rs731236 T/C (*TaqI*), were performed using TaqMan SNP Genotyping Assays (Applied Biosystems) in a LightCycler 480 (Roche).

Statistical analysis

Allele and haplotype frequencies estimation and linkage disequilibrium analysis were performed using Haploview v4.0 (20). Estimates of genetic linkage disequilibrium were made from D′ calculations as described with a coefficient below 0.5 being unlinked (in equilibrium) and a coefficient of 1.0 being in full linkage disequilibrium. Haplotypes of each individual were inferred using Famhap version 19 (21).

Comparisons of quantitative variables among independent groups were performed by Student *t* test and X2 test. Probabilities of overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method, and compared by log-rank test, while relapse, nonrelapse mortality (NRM), and GvHD probabilities were analyzed in a competing risks framework using the cumulative incidence nonparametric estimator and were compared by the Gray test. Risk factors for cGvHD that were considered for multivariate analysis included *BsmI*/*ApaI*/*TaqI* ATC haplotype, *FokI* genotype, GvHD prophylaxis, and type of donor. Once the final adjusted model was determined, tests for

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interaction among all predictors were performed. Significant interactions ($P < 0.05$) were considered as positive interactions for cGvHD between the predictors and thus retained in the model. The proportional hazards assumption was tested analytically and graphically for each variable and collinearity was checked before establishing Cox model. NRM was defined as death due to any cause (GvHD related or other), without prior relapse or progression of the underlying disease.

The relapse incidence was analyzed from transplant until the time of relapse among patients in remission. DFS was calculated from transplant until disease progression or death, and those patients who did not reach disease response any time after transplant, were considered events on day 100. OS was calculated from transplant until death from any cause, and surviving patients were censored at the last follow-up. Patients who engrafted and survived more than 100 days were evaluable for cGvHD.

Data were analyzed using SPSS.V.15, (OpenEpi v.2.3.1) and the CMPRSK package in R 2.4.1 for the analyses of cumulative incidence curves in the framework of competing risk. Differences were considered statistically significant for two-sided $P < 0.05$. Confidence intervals (CI) refer to 95% boundaries (22).

Results

Patient and donor characteristics and analysis of the linkage disequilibrium between VDR alleles

Patients' characteristics are shown in Supplementary Table S1. Median follow-up was 453 days (13–1,256 days). VDR genotype frequencies were similar to those previously described for the Spanish population (23) and are shown in Supplementary Table S2.

Among 107 patients, the genotyping analysis failed in 10 of them. Therefore, 97 patients were evaluable.

BsmI, *ApaI*, and *TaqI* alleles were in strong disequilibrium. In contrast, *FokI* did not demonstrate any association with *BsmI*, *ApaI*, or *TaqI* (Supplementary Table S3). On the basis of this data, *BsmI*, *ApaI*, and *TaqI* were evaluated as a single haplotype and *FokI* was separately considered for analysis. According to these assays, we identified several *BsmI*/*ApaI*/*TaqI* haplotypes. The most frequent patients and donors' haplotypes were GGT (46% and 52%, respectively) and ATC (33% and 35.3%, respectively), as shown in Supplementary Table S2.

VDR SNPs influence on cGvHD

Eighty-nine patients were evaluable for cGvHD. Regarding underlying diagnosis, gender and age, none of these variables significantly influenced on the risk of cGvHD Overall, there were no significant differences on the incidence of cGvHD depending on patients or donors polymorphisms (Supplementary Tables S4 and S5). In contrast, VDR genotypes significantly influenced on the impact of vit D administration on cGvHD incidence. As shown in Table 1, the administration of vit D significantly influenced on the risk of overall cGvHD among patients with *FokI*, CT [cGvHD incidence 22.5% (95% CI, 8.8–39) vs. 80% (95% CI, 30.8–95) for patients receiving or not vit D, respectively, $P = 0.0004$; Fig. 1A]. The same genotype also influenced on the risk of moderate–severe cGvHD (Fig. 1D).

We also evaluated the benefit obtained from the administration of vit D posttransplant depending on most frequent patients' *BsmI*/*ApaI*/*TaqI* genotype. In this regard, patients carrying GGT/GGT genotype had the greatest benefit from receiving vit D in terms of cGvHD incidence: overall cGvHD: 15.4% (95% CI, 1.9–35.9) versus 75% (95% CI, 20–95) for patients who did and did not receive vit D, respectively, $P = 0.008$, (Fig. 1B) and incidence of moderate–severe cGvHD: 14.3% versus 75%, respectively, $P = 0.01$ (Fig. 1E). Considering that patients carrying *BsmI*/*ApaI*/*TaqI* GGT/ATC or ATC/ATC genotypes did not benefit from receiving vit D and that there are also other patient haplotypes, we evaluated the incidence of overall and moderate–severe cGvHD depending on the presence or not of *BsmI*/*ApaI*/*TaqI* ATC haplotype. Patients who did not carry the ATC haplotype had the greatest benefit from receiving vit D: incidence of overall cGvHD 22% (95% CI, 8.8–39.5) versus 68.8% (95% CI, 37.7–86.6), $P = 0.0005$ and incidence of moderate–severe cGvHD 21.6% (95% CI, 8.5–38.6) versus 50% (95% CI, 23.1–72) for patients receiving or not vit D, respectively, $P = 0.03$ (Fig. 1C and F).

In multivariate analysis a significant interaction for the risk of overall cGvHD was observed between *FokI* genotype and vit D administration. Accordingly, the risk of cGvHD of patients treated with vit D was lower among patients carrying *FokI* CT genotype [adjusted hazard ratio (aHR) 0.143; 95% CI, 0.045–0.452; $P_{\text{interaction}} < 0.001$; Table 2]. Upon analyzing patients who did not receive vit D, neither the *FokI* genotype nor the *BsmI*/*ApaI*/*TaqI* ATC haplotype had any effect on the risk of overall cGvHD. In contrast, among patients receiving vit D, patients carrying *FokI* CT genotype had a lower risk of overall cGvHD as compared with

Table 1. Effect of patient VDR polymorphisms on the impact of vit D on cGvHD incidence

		Overall cGvHD incidence (1 year)			Moderate–severe cGvHD incidence (1 year)		
		Cumulative incidence			Cumulative incidence		
		% (95% CI)			% (95% CI)		
Polymorphism	Genotype N (Vit D/no vit D)	Vit D ($n = 71$)	No vit D ($n = 36$)	P	Vit D ($n = 71$)	No vit D ($n = 36$)	P
		Incidence of cGvHD depending on the genotype					
FokI	CC (20/16)	47.6 (24–67)	53.3 (24–70)	0.7	28.6 (11.1–0.49)	33.3 (11.2–57.6)	0.6
	CT (28/10)	22.5 (8.8–39)	80 (30.8–95)	**0.0004**	11 (2.7–26)	60 (22–84.2)	**0.001**
	TT (9/6)	22 (2.8–53)	50 (5.5–84.7)	0,28	22 (2.8–0.53)	50 (2.9–58)	0.28
		Incidence of cGvHD depending on the most frequent haplotypes					
GGT/GGT	16/8	14.3 (1.9–35.9)	75 (20–95)	**0.0007**	13.3 (1.9–35.7)	75 (20–95)	**0.002**
GGT/ATC	15/6	40.4 (11–69)	33.3 (2.5–72.2)	0.8	24.9 (4.4–53.7)	16.7 (3.3–58.5)	0.8
ATC/ATC	5/6	40 (3.1–78.6)	50 (6.9–83.6)	0.9	20 (0.4–62)	33.3 (2.9–71)	0.6
		Incidence of cGvHD depending on the presence/absence of the ATC haplotype					
ATC Yes	30/15	42.2 (23.3–59.9)	53.3 (24.4–75.6)	0.5	42.2 (23.3–59.9)	53.3 (24.4–75.6)	0.5
ATC No	28/16	22.2 (8.8–39.5)	68.8 (37.7–86.6)	**0.0005**	21.6 (8.5–38.6)	50 (23.1–72)	**0.03**

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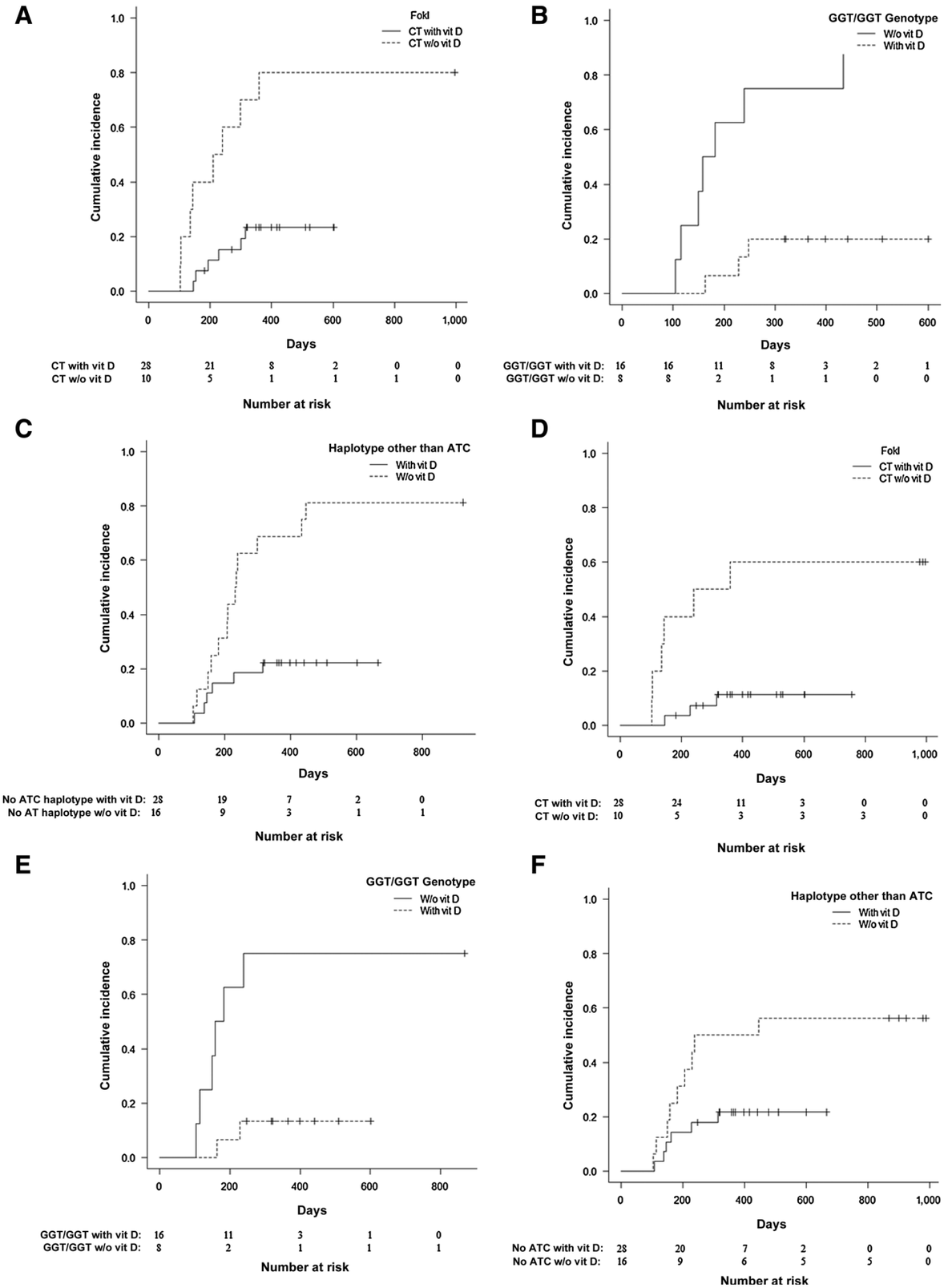

Figure 1.

A, Cumulative incidence of overall chronic GvHD depending on the use or not of vit D among patients with *FokI* CT. $P = 0.0004$. **B,** Same for patients with *BsmI/ApaI/TaqI* GGT/GGT genotype. $P = 0.001$. **C,** Same for patients without ATC haplotype. $P = 0.0005$. **D,** Cumulative incidence of moderate–severe GvHD depending on the use or not of vit D among patients with *FokI* CT. $P = 0.001$. **E,** Same for patients with *BsmI/ApaI/TaqI* GGT/GGT genotype. $P = 0.002$. **F,** Same for patients without ATC haplotype. $P = 0.03$.

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Table 2. Multivariate model for the incidence of cGvHD

COX Model	HR	*P*	95% CI
Immunoprophylaxis			
CsA/MTX	1.3	0.60	0.46–3.74
TKR/MTX	0.79	0.62	0.3–1.98
TKR/RAPA	1		
Donor			
Related	0.7	0.44	0.284–1.73
Unrelated	1		
Vit D*FokI		**0.02**	
Among FokI = CC:			
Vit D versus control	1.02	0.96	0.41–2.55
Among FokI = CT			
Vit D versus control	**0.14**	**0.001**	**0.045–0.45**
Among FokI = TT			
Vit D versus control	0.2	0.096	0.034–1.32
Among vit D = control:			
CT versus CC	2.07	0.16	0.7–5.8
TT versus CC	1.75	0.43	0.4–7.2
Among vit D = vit D:			
CT versus CC	**0.3**	**0.02**	**0.1–0.8**
TT versus CC	0.3	0.19	0.08–1.7

Abbreviations: CsA, cyclosporine A; MTX, methotrexate; RAPA, rapamycin; TKR, tacrolimus.

those with CC genotype (aHR, 0.29; 95% CI, 0.105–0.802; $P_{interaction} < 0.02$), as shown in Table 2.

The same analysis was performed for donors VDR genotypes. Those patients whose donors carried *FokI* CC genotype had a significantly lower risk of cGvHD when treated with vit D [23.5% (95% CI, 9.3–41.3) vs. 33.3% (95% CI, 9.2–60.3), for patients receiving or not vit D, respectively; $P = 0.01$; Fig. 2]. Also, presence or not of *BsmI/ApaI/TaqI* ATC haplotype influenced on the risk of moderate–severe cGvHD depending on the use of vit D. These data are summarized in Table 3.

However, multivariate analysis including immunoprophylaxis, type of donor and interaction between vit D and donor *FokI* CC genotype, as well as donor *BsmI/ApaI/TaqI* ATC haplotype did not demonstrate any significant impact on the risk of cGvHD (data not shown).

VDR SNPs influence on mortality, relapse, and survival

There was no significant impact of *FokI, BsmI, ApaI,* and *TaqI* polymorphisms or haplotypes from neither the patients nor the donors on the incidence of NRM (data nor shown). In contrast, a higher incidence of relapse was observed among patients with *TaqI* CC genotype [42.9% (95% CI, 16.5–67.1)] as compared with

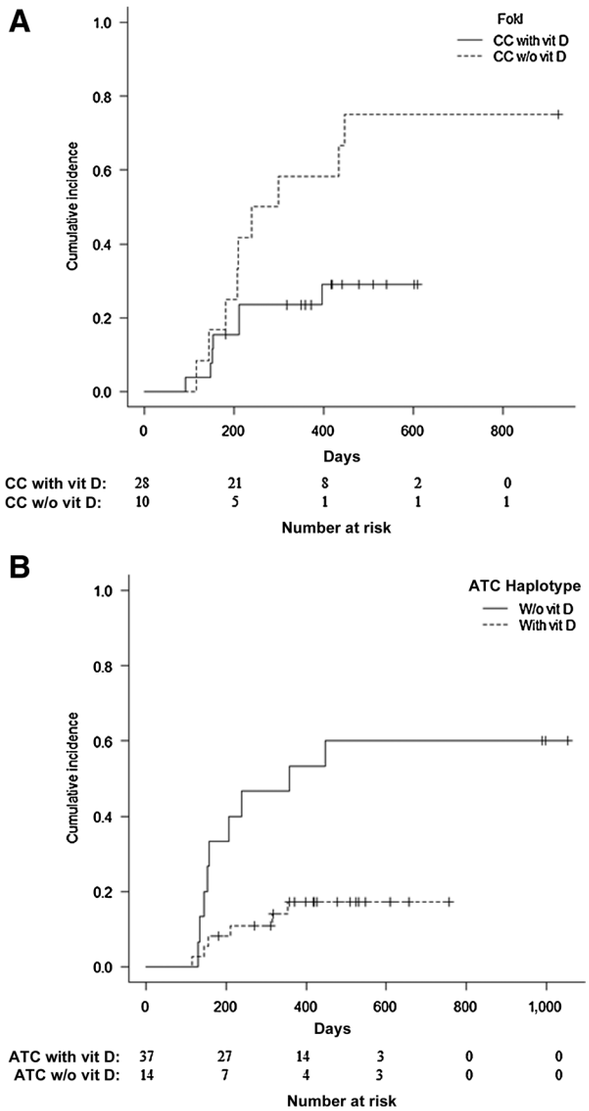

Figure 2.

A, Cumulative incidence of overall chronic GvHD depending on the use of vit D among patients with donor *FokI* CC genotype. $P = 0.01$. **B,** Cumulative incidence of moderate–severe chronic GvHD depending on the use of vit D among patients with *BsmI/ApaI/TaqI* donor ATC haplotype. $P = 0.0007$.

Table 3. Effect of donor VDR polymorphisms on the impact of vit D on cGvHD incidence

		Overall cGvHD (1 year)			Moderate–severe cGvHD (1 year)		
		Cumulative incidence % (95% CI)			Cumulative incidence % (95% CI)		
Polymorphism	Genotype *N* (Vit D/no vit D)	Vit D (*n* = 71)	No vit D (*n* = 36)	*P*	Vit D (*n* = 71)	No vit D (*n* = 36)	*P*
	Incidence of cGvHD depending on the genotype						
FokI	CC (26/12)	23.5 (9.3–41.3)	33.3 (9.2–60.3)	**0.01**	4 (0.3–17.4)	33.3 (9.2–60.3)	**0.05**
	CT (24/16)	40.5 (19.6–60.6)	62,5 (32.5–82.1)	0.2	35.2 (15.9–55.2)	43.8 (18.7–66.5)	0.5
	TT (8/6)	25 (2.9–58.3)	66.7 (9.8–93)	0.25	25 (2.9–58.3)	50 (5.5–84.7)	0.25
	Incidence of cG Immunoprophylaxis HD depending on the most frequent haplotype combinations						
GGT/GGT	16/11	26.7 (7.7–0.50)	66.7 (30.4–87.1)	0.07	25 (7.3–48)	41.7 (14–67.7)	0.3
GGT/ATC	23/7	30.8 (13–50.6)	42.9 (5.5–78.1)	0.4	28.6 (2.7–64.8)	17.8 (5.2–36.4)	0.4
ATC/ATC	9/2	33.3 (6.3–64.6)	50	0.1	—	—	
	Incidence of cGvHD depending on the presence/absence of the ATC haplotype						
ATC yes	41/14	29.2 (16–43.8)	66.7 (34.2–85.8)	**0.006**	14.8 (5.8–27.6)	53.3 (24.4–75.6)	**0.007**
ATC no	23/17	21.7 (7.6–40.5)	61.1 (33.6–80.1)	**0.01**	20.8 (7.3–39)	33.3 (13–55.4)	0.3

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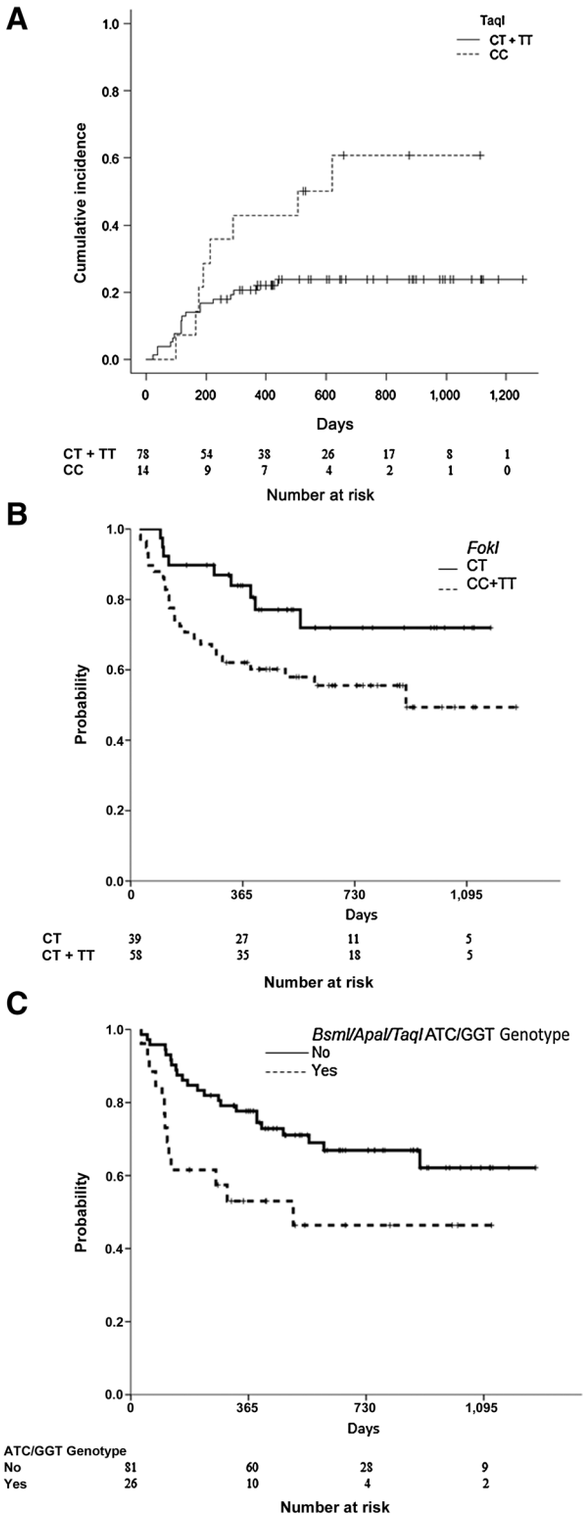

Figure 3.

A, Relapse incidence depending on *TaqI* patient genotype. $P = 0.04$. **B,** Overall survival depending on *FokI* patient genotype. $P = 0.03$. **C,** Overall survival depending on *BsmI/ApaI/TaqI* patient haplotype. $P = 0.02$.

CT or TT [20.6% (95% CI, 12.4–30.3)] genotypes, $P = 0.02$. (Fig. 3A).

There were no significant differences in DFS depending on *FokI* or *BsmI/ApaI/TaqI* genotypes from neither donors nor patients. Regarding OS, significant differences were observed for patients carrying *FokI* CT genotype (84% at 1 year) as compared with those with CC or TT (62,1%), $P = 0.03$. Patients with GGT/ATC genotype for *BsmI/ApaI/TaqI* had a worse survival at 1-year as compared with those carrying other combinations (53% vs. 77%; $P = 0.02$; Fig. 3B and C). However, none of these genotypes maintained their independent prognostic value in multivariate analysis. There were no differences in terms of survival for any donors genotypes (data nor shown).

Discussion

The effect of VDR SNPs on the outcome of patients undergoing allo-HSCT has been explored in several retrospective studies. Different genotypes have been related to a higher risk of acute GvHD, infections or even survival, suggesting that genome variations might induce differences in VDR activity. As a summary, several retrospective studies suggest that patients with *Apa-TaqI* TC haplotype have a better OS and DFS, whereas *ApaI* GG has been related to a higher frequency of infections, aGvHD, and a worse survival (13, 14). With respect to the donors, the presence of *ApaI* TT genotype (13) and *FokI* CC have been associated to a higher risk of aGvHD. According to this data, *FokI* CC and *ApaI* GG genotypes variants have been related to a more active immune response.

These data prompted several investigators to evaluate the potential impact of the levels of vit D on the outcome of patients undergoing allo-HSCT. In this regard, several studies have shown that the levels of vit D affect transplant outcomes (25–27). Glotzbecker and colleagues reported an increased risk of chronic GvHD among patients with low pretransplant levels of vit D (25). In addition, another retrospective study reported a higher incidence of CMV infection in patients with vit D deficiency (26). Interestingly, it has also been reported that low levels of vit D (25-hydroxyvitamin D3 < 20 ng/m) before transplantation have an impact on the relapse risk among patients diagnosed with myeloid malignancies (15).

On the basis of this background, we designed a prospective clinical trial to evaluate the efficacy and safety of vit D administered during and after allo-HSCT. Interestingly, we observed a decreased risk of overall and moderate–severe cGvHD among patients who received vit D (19). This study represents a unique opportunity to evaluate the potential effect of VDR SNPs on the cGvHD incidence and on transplant outcomes among patients receiving or not vit D as an immunomodulatory drug after transplantation.

In this study, we did not observe significant differences in cGvHD incidence nor survival regarding the previously mentioned VDR SNPs. However, we found that the effect of the administration of vit D on overall and moderate–severe cGvHD greatly varies depending on the presence of VDR *FokI* CT genotype in the patients. In a multivariate model, this genotype decreased the risk of overall cGvHD among patients receiving vit D. Because *FokI* TT is the less frequent genotype, we cannot confirm whether or not TT genotype (the longer isoform) could also benefit from receiving vit D. Additional biological studies are required to further clarify this issue.

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Also *BsmI/ApaI/TaqI* ATC haplotype influenced on the incidence of cGvHD in patients treated with vit D, although it did not maintain its impact in multivariate analysis.

Our results suggest that genomic differences entail VDR to become more sensitive or resistant to the effect of vit D.

Interestingly, it has been described a higher amount of mRNA and VDR protein in the *TaqI* TT genotype. Accordingly, it is possible that the effect of vit D administration is influenced by the amount of VDR depending on the genotype. On the basis of this hypothesis, GGT/GGT *BsmI/ApaI/TaqI* genotype might be related to a higher amount of VDR available to interact with the vit D. Because antigen-presenting cells from the host play a key role in the pathophysiology of GvHD, it is possible that different *BsmI/ApaI/TaqI* patient genotypes modify the immune response and that this effect is modulated by the presence or not of vit D.

In this regard, it has been reported that *FokI* produces two different VDR proteins depending on the genotype. *FokI* CC genotype induces a short isoform of the VDR, which is associated with a more potent immune response than the long isoform generated by *FokI* TT genotype (28). In our study, patients carrying *FokI* CT genotype had the highest benefit from receiving vit D. The reason remains unclear, but CT genotype might comprise receptor dimers including a short and a long form which either may affect the ability of VDR to form heterodimers with other nuclear receptors such as RXR or could influence on DNA binding and transcription. Using transfection experiments, Gross and colleagues failed to demonstrate significant differences in both *FokI* isoforms regarding ligand affinity, DNA binding, or transactivation of 24-hydroxylase, osteocalcin, and osteopontin genes (29). However, another experiment carried out in human cells to investigate the functional consequences of the *FokI* polymorphism in immune cells, showed that the short isoform (*FokI* CC) resulted in higher NF-B- and NFAT-driven transcription, as well as higher IL12p40 promoter–driven transcription (28, 30). Remarkably, the effect of vit D greatly differs depending on the specific cell subpopulations analyzed. In this regard, vit D (and analogs) inhibits dendritic cell differentiation and maturation and, as a consequence, decreases alloreactive T-cell activation. Vit D also appears to upregulate tolerogenic properties selectively in myeloid dendritic cells, downregulating IL12 and the expression of the costimulatory molecules CD40, CD80, and CD86, while enhancing IL10 production, thus resulting in a decreased T-cell activation and promoting CD4+CD25+FOXP3+ T regulatory cells production. However, in T cells, vit D inhibits transcription of IL2 preventing T-cell proliferation (31) and also decreases IL17 production by blocking NFAT1 (32). Not surprisingly, in our study different VDR genotypes from either patients or donors influence on the effect of vit D administration in terms of GvHD incidence although only recipients *FokI CT* genotype was confirmed on multivariate analysis. These effects might be related to different vit D-VDR mechanisms of action in each cell subpopulation. In this regard, dendritic cells from the host play a key role in the activation of alloreactive T cells from the donor, so that different cell subsets from patient and donor are involved in the pathophysiology of GvHD.

This is the first VDR SNPs study reported among patients prospectively treated or not with vit D in the allo-HSCT setting. These results suggest that the immunomodulatory effect of vit D depends on the VDR SNPs. Accordingly, patients carrying *FokI* CT genotype display the highest benefit from receiving vit D post-transplant, thus suggesting a higher sensitivity to the vit D in this specific VDR form. These data might allow us to identify those patients who could obtain the higher benefit from receiving vit D and should be considered for future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: I. Montero Cuadrado, J.A. Pérez-Simón

Development of methodology: E. Carrillo-Cruz, J.R. García-Lozano, F.J. Márquez-Malaver, J.A. Pérez-Simón

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Carrillo-Cruz, F.M. Sánchez-Guijo, I. Montero Cuadrado, C. Ferra i Coll, D. Valcárcel, O. López-Godino, M. Cuesta, L. López-Corral, M. Alcoceba, T. Caballero-Velázquez, J.A. Bejarano-García

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Carrillo-Cruz, J.R. García-Lozano, F.J. Márquez-Malaver, D. Valcárcel, J.A. Bejarano-García, J.A. Pérez-Simón

Writing, review, and/or revision of the manuscript: E. Carrillo-Cruz, J.R. García-Lozano, F.J. Márquez-Malaver, F.M. Sánchez-Guijo, I. Montero Cuadrado, C. Ferra i Coll, D. Valcárcel, R. Parody, M. Alcoceba, A. Rodríguez-Gil, T.L. Ramos, J.A. Pérez-Simón

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Carrillo-Cruz, D. Valcárcel, J.A. Bejarano-García, T.L. Ramos

Study supervision: D. Valcárcel

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Estrella Carrillo-Cruz, José R. García-Lozano, Francisco J. Márquez-Malaver, et al.

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