Biology of Blood and Marrow Transplantation
journal homepage: www.bbmt.org
ASBMT American Society for Blood and Marrow Transplantation

Cannabidiol for the Prevention of Graft-versus-Host-Disease after Allogeneic Hematopoietic Cell Transplantation: Results of a Phase II Study

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Article history:
Received 31 March 2015
Accepted 21 May 2015

Key Words:
Allogeneic hematopoietic cell transplantation
Graft-versus-host disease
Prophylaxis
Cannabis sativa
Cannabidiol

A B S T R A C T

Graft-versus-host-disease (GVHD) is a major obstacle to successful allogeneic hematopoietic cell transplantation (alloHCT). Cannabidiol (CBD), a nonpsychotropic ingredient of *Cannabis sativa*, possesses potent anti-inflammatory and immunosuppressive properties. We hypothesized that CBD may decrease GVHD incidence and severity after alloHCT. We conducted a phase II study. GVHD prophylaxis consisted of cyclosporine and a short course of methotrexate. Patients transplanted from an unrelated donor were given low-dose anti−T cell globulin. CBD 300 mg/day was given orally starting 7 days before transplantation until day 30. Forty-eight consecutive adult patients undergoing alloHCT were enrolled. Thirty-eight patients (79%) had acute leukemia or myelodysplastic syndrome and 35 patients (73%) were given myeloablative conditioning. The donor was either an HLA-identical sibling (n = 28), a 10/10 matched unrelated donor (n = 16), or a 1-antigen-mismatched unrelated donor (n = 4). The median follow-up was 16 months (range, 7 to 23). No grades 3 to 4 toxicities were attributed to CBD. None of the patients developed acute GVHD while consuming CBD. In an intention-to-treat analysis, we found that the cumulative incidence rates of grades II to IV and grades III to IV acute GVHD by day 100 were 12.1% and 5%, respectively. Compared with 101 historical control subjects given standard GVHD prophylaxis, the hazard ratio of developing grades II to IV acute GVHD among subjects treated with CBD plus standard GVHD prophylaxis was .3 ($P = .0002$). Rates of nonrelapse mortality at 100 days and at 1 year after transplantation were 8.6% and 13.4%, respectively. Among patients surviving more than 100 days, the cumulative incidences of moderate-to-severe chronic GVHD at 12 and 18 months were 20% and 33%, respectively. The combination of CBD with standard GVHD prophylaxis is a safe and promising strategy to reduce the incidence of acute GVHD. A randomized double-blind controlled study is warranted. (clinicaltrials.gov: NCT01385124)

INTRODUCTION

Despite prophylactic immunosuppressive treatment, graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (alloHCT), affecting 30% to 50% of patients transplanted from an HLA-matched sibling donor and 50% to 70% of patients transplanted from an HLA-matched unrelated donor [1-4]. The ability to prevent GVHD is of utmost importance, because treatment for established GVHD remains suboptimal. In a survey, GVHD and its consequences were the most important reason physicians were reluctant to use transplantation [5]. Thus, developing innovative strategies to prevent and treat GVHD is a major unmet need.

Cannabis sativa, commonly known as marijuana, possesses a wide range of potent anti-inflammatory and

Financial disclosure: See Acknowledgments on page 1775.
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http://dx.doi.org/10.1016/j.bbmt.2015.05.018
1083-8791/

immunosuppressive properties. Cannabis use in healthy subjects has been associated with a decrease in lymphocyte proliferative response to mitogenic stimulation and IL-2 levels and an increase in IL-10 and transforming growth factor-β1 levels [6]. In a recent prospective placebo-controlled study, cannabis smoking induced a significant clinical response in patients with refractory Crohn's disease [7]. Cannabis contains more than 60 chemical compounds classified as cannabinoids [8]. Two cannabinoids in particular have been subjects of most studies examining medical uses: Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). In an experimental murine model, THC, the main psychoactive ingredient of marijuana, was shown to be effective in both the prevention and treatment of GVHD [9]. Its administration led to reduced tissue injury to the liver and intestine, to early recovery from body weight loss associated with GVHD, and to increased mice survival. THC-treated GVHD mice had significantly decreased levels of IL-2 and INF-γ. On a cellular level, THC treatment reduced the expansion of donor-derived effector T cells and increased the number of Foxp3+ regulatory T cells. Nevertheless, despite its promising therapeutic potential, the unwanted psychoactive effects of THC limit its consideration as a potential medication for GVHD prophylaxis.

CBD, in contrast, which contributes up to 40% of cannabis extract, does not produce psychoactive effects and is well tolerated by humans even when taken over extended periods of time and thus has the potential for both clinical research and therapeutic use [10-17]. Similar to THC, CBD possesses potent anti-inflammatory and immunosuppressive properties. Its administration results in attenuation of clinical disease in animal models of various inflammatory diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and diabetes mellitus [18-23]. These effects are mediated by T cell attrition and by inhibition of pro-inflammatory cytokine release (tumor necrosis factor-α, INF-γ, IL-1β, IL-6, and IL-17) and stimulation of anti-inflammatory cytokine production (IL-4, IL-5, IL-10, and IL-13) [19-25]. Furthermore, cannabinoids have recently been shown to reduce the capacity of dendritic cells to migrate to secondary lymphoid organs and activate naive T cells [26]. Based on these experimental and clinical observations, we conducted a phase II study to assess the safety and efficacy of CBD in the prevention of acute GVHD.

METHODS

This prospective, single-center phase II clinical trial was approved by the institutional review board of the Rabin Medical Center (RMC). Written informed consent was obtained from patients before enrollment.

Inclusion and Exclusion Criteria

Patients were eligible for the study if they were 18 years and older and had an available 10/10 HLA-matched or 1-antigen-mismatched related or unrelated donor. Patients were excluded if they had a mental disorder, were actively consuming illicit drugs, or were consuming cannabis during the 3 months before transplantation. Patients receiving cord blood or haploidentical transplantations were excluded.

Conditioning Regimens

Myeloablative conditioning included total body irradiation 12 Gy plus i.v. cyclophosphamide 120 mg/kg or i.v. busulfan 12.8 mg/kg plus either i.v. cyclophosphamide 120 mg/kg or i.v. fludarabine 160 mg/m^2. Reduced-intensity conditioning was composed of i.v. fludarabine 150 mg/m^2 with either i.v. busulfan 6.4 mg/kg or i.v. melphalan 100 mg/m^2. Non-myeloablative conditioning comprised total body irradiation 2 Gy plus i.v. fludarabine 90 mg/m^2. All patients received granulocyte colony-stimulating-factor mobilized peripheral blood stem cell grafts.

GVHD Prophylaxis

All patients received standard GVHD prophylaxis consisting of cyclosporine twice daily starting on day −1 with target trough levels of 200 to 400 ng/mL and a short course of methotrexate (MTX) (15 mg/m^2 on day 1 and 10 mg m^2 on days 3 and 6). Patients transplanted from unrelated donors received anti–T cell globulin (Fresenius, Germany) at a low dose of 5 mg/kg on days −3 to −1 [27].

The investigational agent, CBD (STI Pharmaceuticals, Essex, UK), was dissolved in olive oil at a concentration of 2.5% and orally administered at a fixed dose of 150 mg twice daily, starting 7 days before transplantation until day 30. Dose and duration of CBD administration were based on doses previously safely used in several clinical trials in the nontransplant setting [10-17].

Supportive Care

All patients received filgrastim (granulocyte colony-stimulating factor) at a dose of 5 μg/kg/day from day 7 until neutrophil engraftment, infection prophylaxis according to institutional guidelines [28], and ursodeoxycholic for sinusoidal obstruction syndrome prevention.

Endpoints

Endpoints were assessed based on intention-to-treat analysis. Primary endpoints were safety and cumulative incidence rates of grades II to IV and grades III to IV acute GVHD by day 100. Secondary endpoints were cumulative incidence rates of acute GVHD by day 200, overall and moderate to severe chronic GVHD, nonrelapse mortality (NRM), relapse incidence, and overall survival.

Acute GVHD was assessed by using the consensus grading system, and suspected cases of GVHD were histologically confirmed [29]. Chronic GVHD was assessed according to the National Institutes of Health consensus criteria [30]. Donor chimerism in bone marrow was measured by XY-fluorescence in situ hybridization analysis in sex-mismatched pairs and by DNA amplification of polymorphic microsatellite regions in sex-matched pairs as previously described [31,32].

We compared the outcomes of the study group with a control group of 101 adult patients who would have met the protocol inclusion and exclusion criteria and consecutively underwent alloHCT and were treated by the same medical team at the RMC between May 2007 and August 2012. This historical cohort received the same standard GVHD prophylaxis consisting of cyclosporine and a short course of MTX without CBD. Side effects possibly attributed to CBD were graded according to the Common Terminology Criteria for Adverse Events (CTCAE v4.0) classification [33].

Statistical Considerations

Cohort sample size (n = 48) was calculated to demonstrate a reduction of 50% in the occurrence of grades II to IV acute GVHD by day +100 with a power of 80% and a Type I error of .05. Neutrophil and platelet recovery were defined as the first of 3 days with absolute neutrophil counts ≥ 500/μL and the first of 7 days with an unsupported platelet count ≥ 20,000/μL, respectively.

NRM was defined as death in remission. Death was treated as a competing risk in the analyses of relapse and progression and acute and chronic GVHD. Relapse and progression were treated as a competing risk when analyzing NRM. Probabilities of overall survival and disease-free survival were calculated using the Kaplan-Meier estimates. The log-rank test was used for univariate comparisons. Categorical variables of study and historical cohorts were compared using the chi-square or Fisher Exact test, as appropriate. All *P* values are 2-sided. Statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA) and Prism version 5.0 (GraphPad, San Diego, CA, USA).

RESULTS

Patients

From September 2012 through January 2014, 50 consecutive unselected adult patients were evaluated for enrollment. Two patients refused informed consent. Among 48 enrolled patients, 1 patient with acute leukemia declined informed consent 2 days after starting CBD; a second patient with aplastic anemia had primary graft failure and received a second transplant for which CBD was not administered. These 2 patients were included in the intention-to-treat analyses. Baseline characteristics and transplantation parameters of the 48 patients are depicted in Table 1.

Median patient age was 56 years (range, 22 to 73), and 19 patients (40%) were 60 years or older. Most patients (n = 38, 79%) had acute leukemia or myelodysplastic syndrome.

Table 1
Patient and Transplantation Characteristics

Characteristic	All Cohort (N = 48)	Historical Cohort (N = 101)	*P*
Patient characteristics			
Age, yr, median (range)	56 (22-73)	51 (19-71)	.07
Female (%)	17 (35)	40 (40)	.7
HCT-CI > 2 (%)	10 (21)	12* (39)	.13
CMV +/+ (%)	35 (73)	84 (84)	.8
Disease (%)			.9
Acute leukemia	33 (69)	74 (74)	
AML	26	63	
ALL	7	11	
MDS	5 (10.5)	6 (6)	
LPD	7 (14.5)	12 (12)	
Other	3 (6)	9 (9)	
Disease risk according to the refined DRI (%)			.19
Low	2 (4)	10 (10)	
Intermediate	24 (51)	38 (38)	
High	15 (32)	45 (45)	
Very high	6 (13)	8 (8)	
Donor characteristics			
Matched sibling	28 (58)	58 (58)	1
Full matched unrelated	16 (33)	41 (41)	1
One-allele/antigen-mismatched unrelated (%)	4 (9)	2 (2)	.18
Female to male (%)	7 (15)	18 (18)	.5
Preparative regimen			.6
Myeloablative (TBI/Cy or Bu/Cy)	11 (23)	26 (26)	
Myeloablative reduced toxicity	24 (50)	42 (42)	
Reduced intensity	13 (27)	33 (33)	
Antithymocyte globulin	20 (42)	43 (43)	1
Transfused CD34 × 10^6/kg, median (range)	6 (2.7-13)	6.8 (2.1-9)	.8

HCT-CI indicates hematopoietic cell transplantation comorbidity index; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LPD, lymphoproliferative disease; TBI, total body irradiation; Cy, cyclophosphamide; Bu, busulfan.

* Of 31 patients with available data.

Among 47 patients with hematological malignancies, 4% (n = 2), 51% (n = 24), 32% (n = 15), and 13% (n = 6) had low-, intermediate-, high-, and very-high-risk disease, respectively, according to the refined Disease Risk Index (DRI) for alloHCT (Table 1) [34]. Four patients underwent a prior autologous stem cell transplantation (multiple myeloma, n = 1; non-Hodgkin lymphoma, n = 2; Hodgkin lymphoma, n = 1). Thirty-five patients (73%) received a myeloablative conditioning and 20 patients (42%) received allografts from unrelated donors (10/10 match, n = 16; 9/10 match, n = 4). Seven male patients (15%) received allografts from a female donor (Table 1). The median follow-up of survivors was 16 months (range, 7 to 23).

Compliance and Toxicity

The overall compliance with the protocol was good, with 32 patients (70%) taking 100% of doses. Median dose compliance among noncompliant patients was 86% (range, 43% to 96%). Noncompliance with study medication was mainly due to mucositis and nausea. No grades 3 to 4 nonhematological toxicities related to CBD were observed.

Engraftment and Chimerism

In all, 47 patients (98%) achieved primary engraftment. One female patient with aplastic anemia had a primary graft failure. This patient subsequently had a second transplant from the same donor without reconditioning and engrafted properly. She did not receive MTX and CBD during the second transplant.

The median time to absolute neutrophil counts ≥ 500/μL was 11 days (range, 10 to 22). The median time to unsupported platelet counts ≥ 20,000/μL was 16 days (range, 13 to 33). None of the patients experienced secondary graft failure. Excluding the patient with primary graft failure, the median percentage of donor whole marrow chimerism was 99% (range, 91% to 100%) on day 30 and 100% (range, 95% to 100%) on day 200.

Graft-versus-Host-Disease

All 48 patients were assessable for acute GVHD. None of the patients developed acute GVHD while consuming CBD (ie, before day 30). One patient developed grade I acute GVHD, and 7 patients developed grades II to IV acute GVHD after CBD discontinuation. Two patients had involvement of the gastrointestinal tract (GIT) only, 3 patients had involvement of the GIT and skin, 1 patient had involvement of the GIT and liver, and 1 patient had involvement of the GIT, skin, and liver. Among the 7 patients with GIT involvement, 2 patients had only involvement of the lower GIT and 5 patients had involvement of both the upper and lower GIT. Organ-specific stages of acute GVHD are depicted in Table 2.

Median time to onset of grades II to IV acute GVHD was 60 days (range, 41 to 150). Based on an intention-to-treat analysis, cumulative incidence rates of grades II to IV acute GVHD by days 100 and 200 were 12.1% (95% confidence interval [CI], 1% to 41%) and 14.8% (95% CI, 1% to 45%), respectively (Figure 1). Four patients developed grades III to IV acute GVHD. Among them, 1 patient developed steroid-refractory grade III acute GVHD on day 46, which was subsequently treated with extracorporal photopheresis and gradually improved. A second patient developed grade III acute GVHD on day 71 from which he recovered. This patient subsequently had a relapse of leukemia and died on day 186 after alloHCT. A third patient with aplastic anemia stopped CBD on day 8 because of severe mucositis, had primary graft failure, and hence received a second transplant on day 32 for which MTX and CBD were not administered. The patient developed grade IV acute GVHD on day 106 and died from sepsis on day 138 after alloHCT. The fourth patient developed grade IV acute GVHD on day 150, upon cessation of cyclosporine, and died of sepsis on day 179 after alloHCT.

Table 2
Organ-Specific Involvement by Acute GVHD

	(%) Patients in the CBD Cohort (N = 48)	(%) Patients in the Control Cohort (N = 101)	*P*
Skin			.0033
Stage 1	0	12	
Stage 2	6	17	
Stage 3	6	5	
Stage 4	0	1	
Upper GIT	12	33	.0057
Lower GIT			<.0001
Stage 1	4	34	
Stage 2	0	4	
Stage 3	4	2	
Stage 4	4	4	
Liver			.34
Stage 1	2	3	
Stage 2	0	3	
Stage 3	0	1	
Stage 4	2	3	

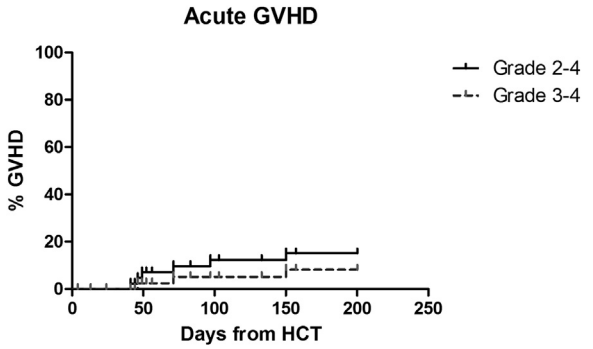

Figure 1. Cumulative incidence of acute GVHD.

Cumulative incidence rates of grades III to IV acute GVHD by days 100 and 200 were 5% (95% CI, 1% to 45%) and 8% (95% CI, 2% to 51%), respectively (Figure 1).

Among patients surviving more than 100 days after alloHCT (n = 41), chronic GVHD occurred in 15 patients (overlap, n = 3; classic, n = 12), with a median time to onset of 159 days (range, 110 to 486). Chronic GVHD was classified as mild, moderate, and severe in 7, 1, and 7 patients, respectively. Notably, 6 of 7 patients with severe chronic GVHD had moderate (score 2) lung involvement.

The cumulative incidence rates of overall and moderate-to-severe chronic GVHD at 1 year were 49.7% (95% CI, 26% to 65%) and 20% (95% CI, 5% to 52%), respectively (Figure 2). The cumulative incidence rates of overall and moderate-to-severe chronic GVHD at 18 months were 58% (95% CI, 36% to 69%) and 33% (95% CI, 12% to 57%), respectively (Figure 2).

Comparison of Acute GVHD Incidence between Study and Control Groups

There was no significant difference between subjects treated with CBD and the historical control patients treated at the RMC with respect to baseline characteristics and risk factors for GVHD (Table 1). The cumulative incidence rates of grades II to IV and grades III to IV acute GVHD by day 100 among the 101 historical control patients were 46% and 10%, respectively. Compared with subjects given standard GVHD prophylaxis, the hazard ratios of developing grades II to IV and grades III to IV acute GVHD by day 100 among subjects treated with CBD plus standard GVHD prophylaxis were .3 (95% CI, .2 to .6; $P = .0002$) and .6 (95% CI, .2 to 1.8; $P = .3$), respectively. Nevertheless, this did not translate into statistically significant difference in NRM at 12 months (13.4% versus 20%, $P = .95$). Median time for developing acute GVHD in the control group was 20 days (range, 9 to 137). The median time to onset of acute GVHD was significantly shorter in the control group compared with the CBD group (20 versus 60 days, $P = .001$). Furthermore, the 2 groups differed significantly with respect to organ-specific involvement by acute GVHD as depicted in Table 2. Patients treated with CBD had less often skin ($P = .0033$), upper GIT ($P = .0057$), and lower GIT ($P < .0001$) involvement.

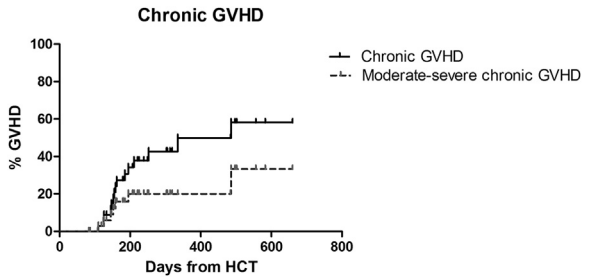

Figure 2. Cumulative incidence of chronic GVHD.

Infections and Transplantation Related Toxicities during Aplasia

Seven patients (15%) had grades 3 to 4 mucositis, mostly involving the upper GIT. Eight patients had microbiology documented infections during aplasia (*Escherichia coli* bacteremia, n = 5; *Pseudomonas* bacteremia, n = 1; *Clostridium difficile*–associated diarrhea, n = 2). Of these patients, 1 patient had also Candida glabrata candidemia. One of the patients with sepsis developed sinusoidal obstruction syndrome. Another patient had fatal cardiac arrhythmia associated with sinusoidal obstruction syndrome. By day 28, 8 of 47 patients at risk had cytomegalovirus (CMV) reactivation; all were treated with pre-emptive valganciclovir. By day 100, 12 additional patients of 44 at risk had CMV reactivation. None of the patients developed CMV disease.

Relapse and Survival

Among the 16 patients who relapsed, 11 had high- or very-high-risk disease according to the refined DRI for allogeneic stem cell transplantation [34] and 3 had acute myeloid leukemia with *FLT3-ITD* mutation [35]. The cumulative incidence of relapse at 1 year post-transplantation was 41% (95% CI, 19% to 55%) (Figure 3). Outcomes were unchanged when analyzed per-protocol. There were 3 early nonrelapse deaths by day 28: 1 patient with refractory angioimmunoblastic T cell lymphoma and ongoing infections at transplantation developed pseudomonas bacteremia, 1 patient with acute myeloid leukemia had polymicrobial sepsis associated with sinusoidal obstruction syndrome, and 1 patient with refractory blastic natural killer cell lymphoma had a fatal arrhythmia associated with sinusoidal obstruction syndrome. There were 2 nonrelapse-related late deaths on days 136 and 179, respectively, both due to sepsis associated with grade IV late-onset acute GVHD as previously described. In all, only 2 patients on an intention-to-treat analysis and 1 patient per-protocol analysis died from GVHD and its complications. Cumulative incidence rates of NRM by day 100

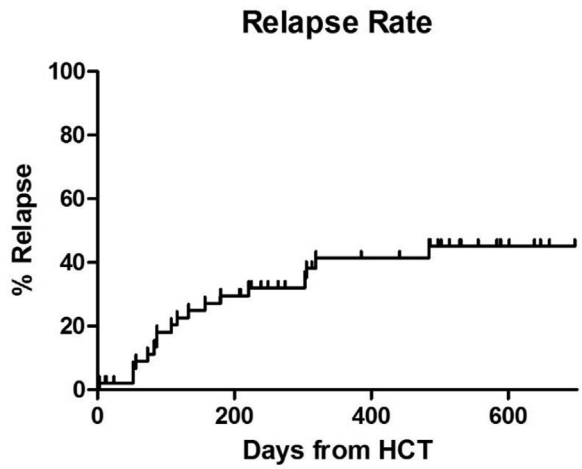

Figure 3. Cumulative incidence of relapse.

and by 1 year after transplantation were 8.6% (95% CI, 0 to 43%), and 13.4% (95% CI, 2% to 45%), respectively (Figure 4). Overall survival rates by day 100 and 1 year after transplantation were 85% (95% CI, 65% to 91%) and 68% (95% CI, 55% to 80%), respectively (Figure 5).

DISCUSSION

In this study, we showed that the addition of CBD to standard GVHD prophylaxis consisting of cyclosporine and MTX resulted in low cumulative incidence rates of both grades II to IV (12.1%) and grades III to IV (5%) acute GVHD by day 100. In view of patient (median age 56 years), donor (unrelated, 43%; mismatched unrelated, 9%), and transplant characteristics (myeloablative conditioning, 73%), one could anticipate significantly higher rates of both acute GVHD (more closely to 50%) and NRM (20% to 30%) [4,36,37]. Indeed, among the 101 control subjects treated by the same medical team at the RMC and given standard GVHD prophylaxis consisting of cyclosporine and MTX, the cumulative incidence rates of grades II to IV and grades III to IV acute GVHD by day 100 were 46% and 10%, respectively. Compared with control subjects, the hazard ratio of developing grades II to IV acute GVHD by day 100 among subjects given the same standard GVHD prophylaxis plus CBD was .3 ($P = .0002$). There was also a lower hazard for developing grades III to IV acute GVHD, but this did not reach statistically significance (hazard ratio .6, $P = .3$). Nevertheless, the favorable impact of CBD on acute GVHD incidence did not translate into a significantly lower NRM rate (13.4% versus. 20%, $P = .95$). Remarkably, none of the patients developed acute GVHD while consuming CBD. Moreover, the median time to onset of acute GVHD was significantly longer in the CBD group compared with the control group (60 versus 20 days, $P = .001$).

The low incidence rates of acute GVHD and moderate-to-severe chronic GVHD witnessed among patients treated with CBD in this study were of a similar magnitude to the incidence rates of GVHD revealed in recent phase I/II studies among patients treated with novel agents like maraviroc (CCR5 antagonist) [38] and bortezomib (proteasome inhibitor) [39] and compared favorably with incidence rates of GVHD displayed among patients treated with vorionostat (histone deacetylase) [40].

In view of the high-risk disease characteristics of this study population, of which 45% had a high or very-high DRI

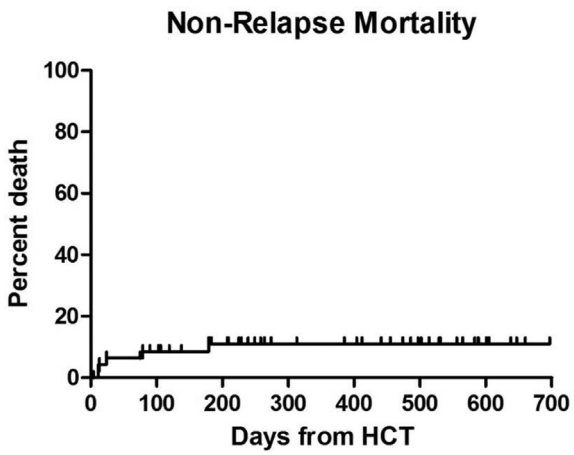

Figure 4. Cumulative incidence of NRM.

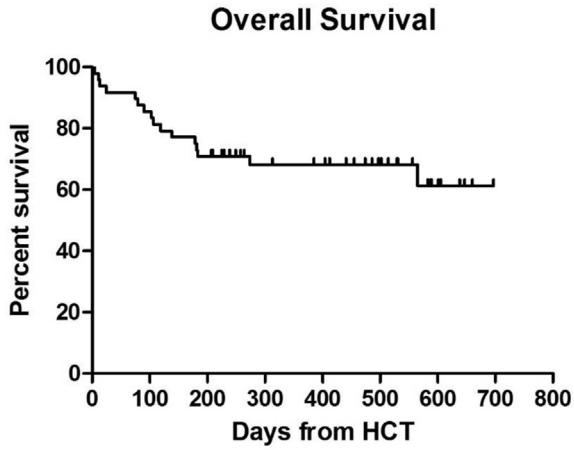

Figure 5. Kaplan Meier estimation of OS.

at transplantation with an expected 2-year survival rate of 33% and 23%, respectively, the observed 1-year cumulative incidence of relapse of 41% was not higher than expected [34]. This in line with the common notion that heightened graft-versus-tumor effect correlates more substantially with chronic GVHD than with acute GVHD [37].

The cumulative incidence rates of overall and moderate-to-severe chronic GVHD were 49% and 20%, respectively, at 12 months and 58% and 33%, respectively, at 18 months. Although mild chronic GVHD does not significantly impair daily living, moderate-to-severe chronic GVHD has profound impact on quality of life and is a major concern. Despite the potential beneficial effect of chronic GVHD on disease relapse, the favorable graft-versus-tumor effect associated with chronic GVHD had previously been shown to be offset by increased NRM [37]. Thus, future efforts should be directed toward more effective prevention of the clinically detrimental types of chronic GVHD (ie, the moderate and severe grades) yet without mitigating the graft-versus-tumor effect. Given that CBD was safe and well tolerated and in light of the encouraging results of the current trial, our institution is planning a phase II trial aiming to explore if a longer exposure to CBD may further reduce late-onset acute GVHD and/or chronic GVHD. Additionally, CBD has been shown to induce apoptosis of myeloid and lymphoid leukemic cells both in vitro and in vivo [41-43], and although never tested before in vivo in humans, prolonged exposure to CBD may potentially reveal a favorable antileukemic effect.

CBD was safely administered in combination with the standard GVHD prophylaxis composed of cyclosporine and short-course MTX. CBD was well tolerated, and no severe adverse events were attributed to its consumption. This is in accordance with safety data previously reported on CBD administered to humans, even with 3- to 4-fold higher doses and even when taken over extended periods of time [10-17]. The fact that 1 patient with aplastic anemia had primary graft failure should probably not be attributed to CBD because basically the rate of graft failure after alloHCT for aplastic anemia is in the range of 10% to 20% [44]. Furthermore, this was the only case out of 48 patients who had a graft failure.

This study has several limitations. First, the study is limited by its single-arm design, and retrospective comparisons with historical control subjects may potentially be biased. Second, despite ample data in murine models on the

mechanism of action of CBD in autoimmune and inflammatory diseases [12-17], the present study is lacking concomitant supportive cytokine and cellular data that would reinforce our clinical observations. Third, we lack pharmacokinetic and pharmacodynamic data to support adequate delivery of oral CBD in the context of transplantation. Hence, we plan to tackle these issues in a subsequent study.

In conclusion, this prospective, single-center study showed for the first time the potential role of CBD in the prevention of GVHD. We intend to validate these promising results in a prospective, placebo-controlled, double blind, randomized study.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Weisdorf D. GVHD—the nuts and bolts. *Hematol Am Soc Hematol Educ Progr*. 2007;2007:62-67.
2. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
3. Arora M, Klein JP, Weisdorf DJ, et al. Chronic GVHD risk score: a Center for International Blood and Marrow Transplant Research analysis. *Blood*. 2011;117:6714-6720.
4. Jagasia M, Arora M, Flowers ME, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood*. 2012;119:296-307.
5. Kurosawa S, Yamaguchi T, Miyawaki S, et al. A Markov decision analysis of allogeneic hematopoietic cell transplantation versus chemotherapy in patients with acute myeloid leukemia in first remission. *Blood*. 2011;117:2113-2120.
6. Pacifici R, Zuccaro P, Pichini S, et al. Modulation of the immune system in cannabis users. *JAMA*. 2003;289:1929-1931.
7. Naftali T, Bar-Lev Schleider L, Dotan I, et al. Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled trial. *Clin Gastroenterol*. 2013;11:1276-1280.
8. Brenneisen R. Chemistry and analysis of phytocannabinoids and other cannabis constituents. In: Elsohly M, editor. *Marijuana and the cannabinoids*. Totowa, NJ: Human Press; 2007. p. 17-51.
9. Pandey R, Hegde VL, Nagarkatti M, Nagarkatti PS. Targeting cannabinoid receptors as a novel approach in the treatment of graft versus host disease: evidence from an experimental murine model. *J Pharmacol Exp Ther*. 2011;338:819-828.
10. Cunha JM, Carlini EA, Pereira AE, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology*. 1980;21:175-185.
11. Consroe P, Laguna J, Allender J, et al. Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacol Biochem Behav*. 1991;40:701-708.
12. Crippa JA, Derenusson GN, Ferrari TB, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol*. 2011;25:121-130.
13. Leweke FM, Koethe D, Pahlisch F, et al. Antipsychotic effects of cannabidiol. *Eur Psychiatry*. 2009;24:S207.
14. Bergamaschi MM, Queiroz RH, Chagas MH, et al. Cannabidiol reduces the anxiety induced by simulated public speaking in speaking treatment-naïve social phobia patients. *Neuropsychopharmacology*. 2011;36:1219-1226.
15. Hallak JE, Machado-de-Sousa JP, Crippa JA, et al. Performance of schizophrenic patients in the Stroop Color Word Test and electrodermal responsiveness after acute administration of cannabidiol (CBD). *Rev Bras Psiquiatr*. 2010;32:56-61.
16. Zuardi AW, Hallak JE, Dursun SM, et al. Cannabidiol monotherapy for treatment resistant schizophrenia. *J Psychopharmacol*. 2006;20:683-686.
17. Zuardi AW, Crippa J, Dursun SM, et al. Cannabidiol was ineffective for manic episode of bipolar affective disorder. *J Psychopharmacol*. 2010;24:135-137.
18. Kozela E, Lev N, Kaushansky N, et al. Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorated multiple sclerosis-like disease in C57BL/6 mice. *Br J Pharmacol*. 2011;163:1507-1519.
19. Malfait AM, Gallily R, Sumariwalla PF, et al. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci U S A*. 2000;97:9561-9566.
20. Borrelli F, Aviello G, Romano B, et al. Cannabidiol, a safe and non-psychotropic ingredient of marijuana plant *Cannabis sativa*, is protective in a murine model of colitis. *J Mol Med*. 2009;87:1111-1121.
21. Schicho R, Storr M. Topical and systemic cannabidiol improves trinitrobenzene sulfonic acid colitis in mice. *Pharmacology*. 2012;89:149-155.
22. De Filippis D, Esposito G, Cirillo C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. *Plos One*. 2011;6:1-8.
23. Weiss L, Zeira M, Reich S, et al. Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmunity*. 2006;39:143-151.
24. Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO. Cannabidiol-recent advances. *Chem Biodivers*. 2007;4:1678-1692.
25. Basu S, Dittel BN. Unraveling the complexities of cannabinoid receptor 2 (CB2) immune regulation in health and disease. *Immunol Res*. 2011;51:26-38.
26. Adhikary S, Kocieda VP, Yen JH, et al. Signaling through cannabinoid receptor 2 suppresses murine dendritic cell migration by inhibiting matrix metalloproteinase 9 expression. *Blood*. 2012;120:3741-3749.
27. Shimoni A, Hardan I, Shem-Tov N, et al. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia*. 2006;20:322-328.
28. Ram R, Yeshurun M, Farbman L, et al. Elevation of CRP precedes clinical suspicion of bloodstream infections in patients undergoing hematopoietic cell transplantation. *J Infect*. 2013;67:194-198.
29. Pzepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825-828.
30. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic-graft-versus-host disease. I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
31. Blume KG, Forman SJ, Appelbaum FR, et al. Documentation of engraftment and characterization of chimerism following hematopoietic cell transplantation. In: Blume KG, Forman SJ, Appelbaum FR, editors. *Thomas hematopoietic cell transplantation*. Oxford, UK: Blackwell; 2004. p. 234-243.
32. Krisst D, Israeli M, Narinski R, et al. Hematopoietic chimerism monitoring based on STRs: quantitative platform performance on sequential samples. *J Biomol Tech*. 2005;16:380-391.
33. Cancer Therapy Evaluation Program. NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0. http://www.ctep.cancer.gov. Published May 28, 2009.
34. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the disease risk index for allogeneic stem cell transplantation. *Blood*. 2014;123:3664-3671.
35. Brunet S, Labopin M, Esteve J, et al. Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult myeloid leukemia in first remission: a retrospective analysis. *J Clin Oncol*. 2012;30:735-741.
36. Saber W, Opie S, Rizzo JD, et al. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myeloid leukemia. *Blood*. 2012;119:3908-3916.
37. Storb R, Gyurkocza B, Storer BE, et al. Graft-versus-host disease and graft-versus-tumor-effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2013;31:1530-1538.
38. Reshef R, Luger SM, Hexner EO, et al. Blockade of lymphocyte chemotaxis in visceral graft-versus-host-disease. *N Engl J Med*. 2012;367:135-145.
39. Koreth J, Stevenson KE, Kim HT, et al. Bortezomib-based graft-versus-host disease prophylaxis in HLA-mismatched unrelated donor transplantation. *J Clin Oncol*. 2012;30:3202-3208.
40. Choi SW, Braun T, Chang L, et al. Vorinostat plus tacrolimus and mycophenolate to prevent graft-versus-host disease after related-donor reduced-intensity conditioning allogeneic haematopoietic stem-cell transplantation: a phase 1/2 trial. *Lancet Oncol*. 2014;15:87-95.
41. Gallily R, Even-Chen T, Katzavian G, et al. γ-Irradiation enhances apoptosis induced by cannabidiol, a non-psychotropic cannabinoid, in cultured HL-60 myeloblastic leukemia cells. *Leuk Lymph*. 2003;44:1767-1773.
42. McKallip R, Jia W, Schlomer J, et al. Cannabidiol-induced apoptosis in human leukemia cells: a novel role of cannabidiol in the regulation of $p22^{phox}$ and Nox4 expression. *Mol Pharm*. 2006;70:897-908.
43. Massi P, Solinas M, Cinquina V, Parolaro D. Cannabidiol as potential anticancer drug. *Br J Clin Pharmacol*. 2012;75:303-312.
44. Champlin RE, Perez WS, Passweg JR, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. *Blood*. 2007;109:4582-4585.