REGULAR ARTICLE

blood advances

Phase 2 study of natalizumab plus standard corticosteroid treatment for high-risk acute graft-versus-host disease

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Key Points

- Biomarker scores are a feasible eligibility criterion for high-risk GVHD in which the prompt initiation of treatment is a priority.
- The combination of natalizumab with corticosteroids was not effective in improving outcomes for patients with high-risk GVHD.

Graft-versus-host disease (GVHD) of the gastrointestinal (GI) tract is the main cause of nonrelapse mortality (NRM) after allogeneic hematopoietic cell transplantation. Ann Arbor (AA) scores derived from serum biomarkers at onset of GVHD quantify GI crypt damage; AA2/3 scores correlate with resistance to treatment and higher NRM. We conducted a multicenter, phase 2 study using natalizumab, a humanized monoclonal antibody that blocks T-cell trafficking to the GI tract through the α4 subunit of α4β7 integrin, combined with corticosteroids as primary treatment for patients with new onset AA2/3 GVHD. Seventy-five patients who were evaluable were enrolled and treated; 81% received natalizumab within 2 days of starting corticosteroids. Therapy was well tolerated with no treatment emergent adverse events in >10% of patients. Outcomes for patients treated with natalizumab plus corticosteroids were compared with 150 well-matched controls from the MAGIC database whose primary treatment was corticosteroids alone. There were no significant differences in overall or complete response between patients treated with natalizumab plus corticosteroids and those treated with corticosteroids alone (60% vs 58%; P = .67% and 48% vs 48%; P = 1.0, respectively) including relevant subgroups. There were also no significant differences in NRM or overall survival at 12 months in patients treated with natalizumab plus corticosteroids compared with controls treated with corticosteroids

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The full-text version of this article contains a data supplement.

alone (38% vs 39%; P = .80% and 46% vs 54%; P = .48, respectively). In this multicenter biomarker–based phase 2 study, natalizumab combined with corticosteroids failed to improve outcome of patients with newly diagnosed high-risk GVHD. This trial was registered at www.clinicaltrials.gov as # NCT02133924.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) cures hematologic malignancies through the graft-versus-leukemia (GVL) effect mediated by alloreactive T lymphocytes in the donor graft. However, the therapeutic benefit of GVL can be offset by acute graft-versus-host disease (GVHD), the principal toxicity of allogeneic HCT, which affects the skin, liver, and gastrointestinal (GI) tract.[1] Acute GVHD of the GI tract is the most resistant to treatment with systemic corticosteroids and causes the majority of nonrelapse mortality (NRM) after allogeneic HCT.[2] Symptom severity at acute GVHD onset is only a modest predictor of response to treatment and long-term outcomes[3] and treatment is usually not escalated until after systemic corticosteroids have failed, when severe organ damage is more difficult to reverse. We designed a clinical trial to treat patients with significant GI GVHD based on biomarker assays.

The Mount Sinai Acute GVHD International Consortium (MAGIC) has previously validated that an algorithm derived from measurement of 2 serum biomarkers (suppressor of tumorigenesis [ST2] and regenerating islet–derived 3α [REG3α]) that measure the damage to GI crypts and serves as a liquid biopsy of the intestine. The MAGIC algorithm probabilities (MAPs) predict response to treatment and 6-month NRM more accurately than clinical symptoms.[4,5] Two thresholds divide MAPs at the onset of GVHD into 3 Ann Arbor (AA1-3) scores that correspond to risks of 6-month NRM of 10%, 25%, and 40%, respectively.[4] Our trial design excluded patients with low-risk (AA1) GVHD who have little to no GI crypt damage.

After encountering antigen in the intestine, T cells travel to secondary lymphoid organs and then recirculate back to the GI epithelium in which they cause inflammation leading to direct organ damage.[1,6-11] Disruption of T-cell trafficking to and from the GI tract is therefore an attractive therapeutic strategy that has proven efficacious in other inflammatory conditions. Anecdotal reports of small numbers of patients suggest that such a strategy may be an effective treatment for GVHD.[12-15] The α4β7 integrin is crucial for T-cell trafficking to the GI tract[16,17] and the number of T cells that express α4β7 increases before, and at, the onset of GI GVHD symptoms.[18,19] Natalizumab is a humanized monoclonal antibody that binds the α4 subunit of α4β7 integrins and blocks their adhesion to their counter receptors on endothelial cells.[20] We hypothesized that early treatment with the combination of corticosteroids and natalizumab at the onset of high-risk GVHD would prevent T-cell recirculation to the GI tract and, thus, control symptoms better than corticosteroid treatment alone.

We conducted a multicenter phase 2 clinical trial with 75 patients with high-risk GVHD (AA2 or 3) and compared their outcomes with a well-matched synthetic control cohort of 150 patients from the MAGIC database.

Methods

This phase 2 study was approved by the institutional review board at each of the participating centers and was coordinated by the MAGIC Data Coordinating Center at the Icahn School of Medicine at Mount Sinai. Patients who were eligible were recruited and enrolled between August 2016 and November 2020. Informed consent was obtained from all study participants. Participating centers are listed in supplemental Table 1. This trial was registered at www.clinicaltrials.gov as #NCT02133924.

Patients

Patients were recruited from MAGIC centers at which procedures for obtaining and screening serum samples for MAP scoring have previously been established.[21] Patients who were eligible had new onset acute GVHD that was high risk by an AA score of 2 or 3 with any clinical severity. Biopsy confirmation was not required. All patients were aged ≥18 years and had received an allogeneic HCT from a related (including haploidentical) or unrelated donor. Inclusion criteria permitted any conditioning regimen, donor, HLA-match, stem cell source, and GVHD prophylaxis. No previous systemic treatment for acute GVHD was allowed before enrollment except for topical therapies, and a maximum of 3 days of systemic corticosteroids treatment. Exclusion criteria included relapse of underlying malignancy, uncontrolled infection, abnormal liver function tests (direct bilirubin ≥2 mg/dL, and aspartate aminotransferase or alanine transaminase levels of ≥5 times the upper limit of normal), hemodialysis, ventilator support, chronic GVHD, a history of allergic reaction to natalizumab, or history of or current diagnosis of progressive multifocal leukoencephalopathy (PML).

AA scoring

Screening serum samples were shipped overnight to the central laboratory at Mount Sinai. ST2 and REG3α were analyzed by enzyme-linked immunosorbent assay, as previously described.[22] MAP was categorized as follows: AA1, ≤0.140; AA2, 0.141 to 0.290; AA3, >0.29. Results were provided to the site within 30 hours of shipment. Patients who did not meet all inclusion and exclusion criteria were deemed screen failures and treated using the best available therapy per physician discretion.

Treatment

Treatment with natalizumab (Tysabri; Biogen, Cambridge, MA) was initiated within 3 days of systemic corticosteroid treatment for acute GVHD. Natalizumab was administered IV at a dose of 300 mg. A second dose was administered after 14 days (±2 days) to maximize the potential benefit before assessment of the primary end point at day 28 of treatment. Treatment with prednisone 2 mg/kg (or its IV equivalent) could begin anytime between obtaining the screening and initiation of natalizumab but was required to continue for at least 3 days after the first dose of natalizumab. Afterward local institutional tapering practices could be followed.

GVHD data collection

Acute GVHD was staged per published MAGIC guidelines.[23] GVHD staging, toxicity, and management were recorded weekly for the first 4 weeks and subsequently on a reduced schedule until 1 year from the start of treatment for study patients and controls. Research staff at each site demonstrated proficiency in applying MAGIC guidelines for the staging of GVHD before entering data for the clinical trial. All GVHD data were centrally reviewed.

Control cohort

The MAGIC database and biorepository contains detailed longitudinal biomarker and clinical data prospectively obtained from patients at centers in North America, Europe, and Asia. This observational trial used a prospective-specimen-collection, retrospective-blinded-evaluation (ie, a PRoBE) design[24] and was approved by the institutional review board at each center. Serum samples were collected at the initiation of GVHD treatment (±3 days) and AA scores were determined retrospectively. We identified 326 patients at 17 MAGIC centers who received an allogeneic HCT between January 2015 and February 2021 and who were treated with corticosteroids alone for GVHD categorized as AA2 or AA3. We created a control cohort of 150 patients (2:1 ratio of controls to cases) from this group such that (1) each control patient met all criteria required for trial participation (eg, no uncontrolled infections and no severe organ dysfunction) and (2) the control cohort proportionally matched the case population for both clinical and biomarker characteristics. The matching process prioritized target-organ involvement, AA score, GVHD prophylaxis, and donor type. Nearest neighbor optimal matching with Mahalanobis distance as a metric of distance between cases and controls was used to finalize the control cohort.[25] The control cohort included 92 patients from MAGIC centers that participated in the clinical trial who developed GVHD before or after the trial enrollment period and 58 patients from MAGIC centers that did not accrue patients to the interventional trial. Participating centers at which control patients were recruited are listed in supplemental Table 1.

Statistical methods

The primary study end point was day-28 complete response (CR) rate. Death, lack of CR at day 28, or the initiation of a second-line treatment for GVHD were considered failures with respect to this end point. A CR was defined as complete resolution in all target organs (the skin, liver, and GI tract). Partial response (PR) was defined as an improvement in stage in ≥1 organs with GVHD involvement without worsening in other organs. The overall response rate (CR + PR) (ORR) at day 28 was a key secondary end point. Additional secondary study end points included NRM and overall survival (OS) at 1 year. Relapse and second HCT were considered competing risks for NRM. All cumulative incidences were compared using the Gray test.[26] OS was calculated using the Kaplan-Meier method and compared using the log-rank test. MAP scores were compared using the Mann-Whitney *U* Test.

Adverse events (AEs) were reported through study day 42 and graded per the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Patients with severe GVHD have a high incidence of CTCAE AEs, and therefore only AEs grade ≥3 that were deemed related to treatment were collected. AE relatedness was centrally adjudicated by a committee of the study site principal investigators. Severe infections were defined as grade 3 by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) criteria[27] and monitored until study-day 180.

Initially, only patients with AA3 GVHD were allowed to participate for safety reasons. A sample size of 90 patients provided 85% power to detect a 15% improvement (30% to 45%) in the CR rate at day 28 compared with the historical control rate[3] with a type 1 error rate of 0.05. After sufficient experience with natalizumab had been obtained in 35 patients and given a lower than expected incidence of AA3 GVHD, eligibility criteria were broadened to include patients with MAPs of AA2 and the sample size was recalculated to reflect the inclusion of patients who were more likely to achieve a CR at day 28 of treatment. A sample size of 84 patients divided equally between AA2 and AA3 GVHD was calculated to provide 80% power to detect a 15% improvement (42.5% to 57.5%) in the CR rate at day 28 compared with the historical CR rate at day 28 with a type 1 error rate of 0.05.

The study included stopping rules for safety. PML is a potential serious complication in patients treated with natalizumab,[28] and therefore enrollment was required to pause if 1 case of PML developed. A second stopping rule required a pause in enrollment if the incidence of severe (BMT CTN grade 3) infections exceeded a prespecified threshold of 24%, based on historical data in this patient population. This rule was triggered on 11 September 2017 and enrollment was paused. A safety review provided by 3 independent experts in allogeneic HCT who were not participating in the trial determined that a causal relationship between severe infections and natalizumab was unlikely given the nature and timing of the infections in these patients with severe GI GVHD. Gram negative enteric bacteria were the predominant organisms, and the study was amended to mandate antibiotic prophylaxis with either a fluoroquinolone or institutional preference for at least 42 days and accrual resumed. A third stopping rule monitored patients by AA strata for day-100 NRM. The rule was triggered on 20 November 2020 for patients in the AA2 strata when the observed day-100 NRM (15%; 5 of 33) exceeded the historical threshold of 13% and the study was paused a second time. Although a safety review found that the nonrelapse deaths were because of uncontrolled GVHD and were not related to natalizumab, an ad hoc futility analysis found that the trial would not be able to meet the primary end point if the remaining patients all achieved a CR at day 28, prompting permanent closure of the trial on 6 December 2020.

Results

Patient characteristics

We screened 574 patients for this study. Patients were ineligible for enrollment for the following reasons: AA1 score (n = 387), AA2 score before expanded eligibility (n = 67), AA3 score after strata fully enrolled (n = 31), other exclusion criteria (n = 13). The final distribution of AA scores from the 561 patients who had scores determined (AA1, 69%; AA2, 18%; and AA3, 13%) trended toward less-severe GVHD compared with the previously reported distribution of scores[4] (AA1, 45%; AA2, 28%; and AA3, 27%). In total, 26 patients were enrolled and treated on this protocol. One patient with AA3 GVHD withdrew consent on day 14 and was replaced, resulting in 75 patients who were evaluable (AA2, n = 33; and AA3, n = 42) (Table 1). GVHD was confirmed by

Table 1. Clinical characteristics

Patient characteristics	NATA (n = 75)	Controls (n = 150)	*P* value
Recipient median age, y (range)	59 (23-74)	57 (18-78)	.65
Recipient age (y), % (n)			
≤60	57 (43)	60 (90)	.77
>60	43 (32)	40 (60)	
HCT CI			
<3	61 (46)	63 (94)	.89
≥3	39 (29)	37 (56)	
Donor type, % (n)			
Related	31 (23)	29 (43)	.91
Unrelated	59 (44)	61 (92)	
Haploidentical	11 (8)	10 (15)	
Donor cell source, % (n)			
Marrow	20 (15)	17 (25)	.81
Peripheral blood	75 (56)	77 (116)	
Cord	5 (4)	6 (9)	
Indication, % (n)			
Acute leukemia	48 (36)	53 (79)	.62
MDS/MPN	33 (25)	27 (40)	
Lymphoma	11 (8)	14 (21)	
Multiple myeloma	3 (2)	4 (6)	
Nonmalignant	5 (4)	3 (4)	
Conditioning regimen intensity, % (n)			
Full	43 (32)	53 (79)	.20
Reduced	57 (43)	47 (71)	
GVHD prophylaxis, % (n)			
CNI based	82 (61)	85 (128)	.10
Cyclophosphamide based	13 (10)	14 (21)	
T-cell depletion	5 (4)	1 (1)	
GVHD serotherapy prophylaxis, % (n)			
ATG	9 (7)	32 (48)	**<.001**
No ATG	91 (68)	68 (102)	
GVHD characteristics			
Median onset day (range)	36 (10-231)	29 (5-174)	**.002**
Minnesota risk, % (n)			
Standard	48 (36)	51 (77)	.67
High	52 (39)	49 (73)	
Tx GVHD grade, % (n)			
Grade 1	8 (6)	10 (15)	.98
Grade 2	35 (26)	34 (51)	
Grade 3	45 (34)	43 (65)	
Grade 4	12 (9)	13 (19)	
Tx organ involvement			
Skin stage 1-2 ± UGI	20 (15)	16 (25)	.37
Skin stage 3-4 ± UGI	8 (6)	13 (19)	
LGI ± other	71 (53)	71 (106)	
Liver only	1 (1)	0	

CNI, calcineurin inhibitor; CI, combordity index; LGI, lower gastrointestinal tract; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; Tx, transplant; UGI, upper gastrointestinal tract.

Significant value is indicated in bold.

target-organ biopsy for 71% (53 of 75) of study patients and 46% (69/150) of control patients. One study patient with an AA2 GVHD score and 1 control patients with an AA3 GVHD score had negative upper GI tract biopsies. There were no significant differences between study patients and controls in terms of pretransplant characteristics, including donor type and GVHD severity. The median onset of GVHD was later in study patients than in control participants (36 vs 29 days; $P = .002$) and fewer of them received antithymocyte globulin (ATG) during pretransplant conditioning (9% vs 32%; $P < .001$). Subset analyses by AA strata revealed that the differences in ATG use between study patients and controls was restricted to patients with AA3 GVHD (10% vs 37%; $P = .001$); there was also significantly greater use of reduced intensity conditioning regimens in study patients with AA2 GVHD (61% vs 35%; $P = .03$) (supplemental Table 2).

Safety

Natalizumab was not infused until all eligibility criteria had been confirmed. The first natalizumab infusion occurred within 2 days of screening in 61 (81%) patients (range, 2-5 days). The first natalizumab infusion occurred >3 days from screening in 5 patients. Both doses of natalizumab were administered in 64 (85%) patients; 11 patients did not receive the second dose because of initiation of second-line treatment for GVHD progression (n = 5), death (n = 1), pause in enrollment (n = 1), withdrawal from the study (n = 1), or physician discretion (n = 3).

Severe infections were closely monitored over the course of the study. At least 1 severe (BMT CTN grade 3) infection developed within the first 180 days in 26 (35%) patients and >1 severe infection developed in 12 (16%) patients. Fewer patients developed a grade 3 bacterial infection in the first 42 days after the amendment to mandate fluoroquinolone coverage during that period (9% [5 of 55] vs 25% [5 of 20]; $P = .07$). All grade 3 infections (n = 43) are shown in supplemental Table 3. Infections involving the GI tract were of particular interest given that natalizumab inhibits T-cell trafficking to this site and high biomarker scores (AA2 and AA3) reflect significant GI crypt damage. The GI tract was involved in 58% (25/43) of severe infections, either as the likely source of enteric bacteria (n = 19) or as the site of infection, including cytomegalovirus colitis (n = 3) and *Clostridium difficile* with toxic dilation (n = 2) or renal insufficiency (n = 1). The contribution of natalizumab toward the development of these infections is difficult to determine but was not considered to be significant in the 52% (13/25) GI tract–related infections that developed >6 weeks from the last natalizumab infusion.

Natalizumab also inhibits T-cell trafficking to the central nervous system (CNS) through its blockade of $\alpha 4\beta 1$ integrins. One patient developed CNS toxoplasmosis on study day 56 that was thought to be possibly related to natalizumab. A relationship to natalizumab was considered less likely but, because of their timing relative to natalizumab administration, possible in 4 other CNS infections . One case of enterococcal meningitis that was diagnosed 111 days (>5 half-lives) from the last dose of natalizumab was considered unlikely to be related to study drug. Three cases of human herpesvirus 6 encephalitis, occurring soon after the first natalizumab infusion (0, 2, and 5 days, respectively), were confirmed by the presence of viral DNA in blood and cerebrospinal fluid, and treatment with natalizumab was discontinued.

Table 2. Treatment-related noninfectious AEs CTCAE grade 3 or higher

Type of AE	Total (n = 75)
Blood and lymphatic system disorders, % (n)	
Anemia	4 (3)
Leukopenia	4 (3)
Lymphopenia	8 (6)
Neutropenia	1 (1)
Thrombocytopenia	7 (5)
Hepatobiliary disorders	
Alanine aminotransferase increased	3 (2)
Alkaline phosphatase increased	1 (1)
Aspartate aminotransferase increased	1 (1)
Hyperbilirubinemia	9 (7)
Psychiatric disorders	
Anxiety	1 (1)
Depression	1 (1)
Vascular disorders	
Hypertension	1 (1)
Hypotension	1 (1)
Metabolism disorders	
Hypocalcemia	3 (2)
Hyponatremia	1 (1)
Nervous system disorders	
Somnolence	1 (1)
PML	0 (0)
General disorders	
Weakness/fatigue	4 (3)

Noninfectious treatment-related grade 3 to 5 CTCAE adverse effects through day 42 are summarized in Table 2. No treatment-related AE occurred in ≥10% of patients treated with natalizumab.

GVHD outcomes

There were no significant differences in response rates between study patients and controls either for the primary end point (CR rate; 45% vs 49%; $P = .23$) or secondary end point (CR + PR rate; 60% vs 56%; $P = .67$) (Figure 1). In addition, CR and CR + PR rates did not differ between study patients and controls for any subgroup of patient characteristics, such as donor type, cell source, use of ATG or GVHD prophylaxis, or GVHD target-organ severity, including in patients with lower-GI GVHD symptoms (supplemental Table 4). There was also no difference in the ORR for patients whose first infusion of natalizumab was ≤2 days from enrollment compared with patients whose first infusion was >2 days from enrollment (59% vs 64%; $P = .77$). We also confirmed that there was no difference in the ORR between controls from trial and nontrial sites (55% vs 57%; $P = 1.0$).

There was no difference in 1-year NRM or survival in patients treated with natalizumab compared with controls (38% vs 39%; $P = .80$ and 46% vs 54%; $P = .48$, respectively) (Figure 2). All causes of death are shown in supplemental Table 5. Six-month

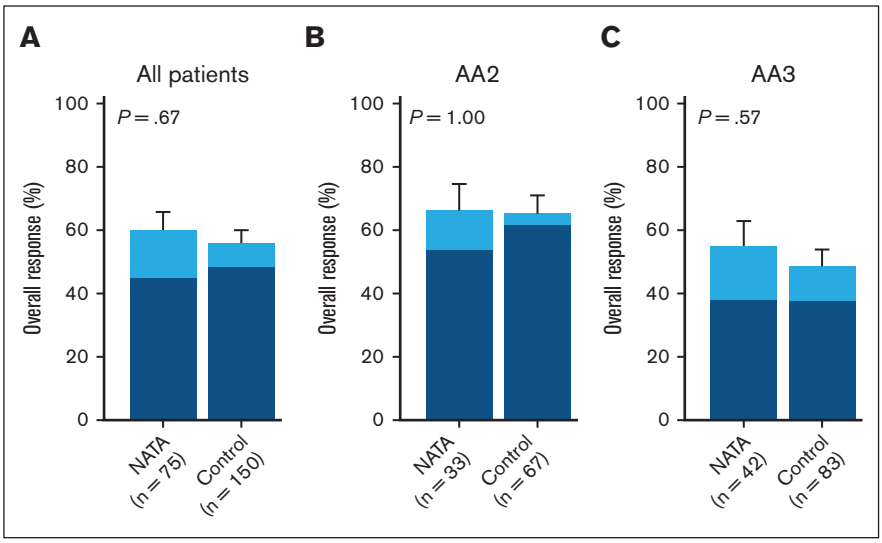

Figure 1. ORR and CR after 4 weeks of systemic therapy for GVHD. ORR was defined as CR (blue) and PR (light blue) within 28 days of treatment without additional treatment. (A) All patients; (B) patients with AA2 scores; (C) patients with AA3 scores.

NRM and OS for patients with AA2 and AA3 GVHD were consistent with previously published outcomes,[3,4] but the addition of natalizumab did not change long-term outcomes for either of these subgroups (Figure 2).

Clinical GVHD staging at time of screening was the point of reference for treatment response. In 3 cases, GVHD progressed between screening and natalizumab infusion (study day 0); all 3 cases would have been recategorized from nonresponders to partial responders if GVHD staging at day 0 was used as the baseline evaluation. This small increase in response rate for study patients does not change the results for the primary end point (CR) nor significantly improve the difference in ORR between study patients and controls (64% vs 56%; $P = .25$).

A MAP of $\leq .29$ measured at 28 days of treatment is known to correlate with a lower likelihood of NRM.[5] We measured MAPs in serum samples available on day 28 for 91% (68 of 75) of study patients and 72% (108/150) of control patients. Natalizumab treatment did not increase the proportion of patients with a low MAP (study patients vs controls, 35% vs 38%; $P = .63$). There was no statistically significant difference in the median MAP on day 28 of treatment between study and control patients consistent with the similarity of long-term outcomes in both groups (Figure 3).

Discussion

Treatment resistant GI GVHD results in high mortality[29,30] and we hypothesized that effective treatment before irreversible damage has occurred may improve survival. We used serum biomarker scores to identify patients with severe GVHD-related damage to the GI crypt who might benefit from early intensification of treatment regardless of the severity of their clinical symptoms. We then tested the hypothesis that blocking T-cell traffic to the GI tract with natalizumab would improve responses to treatment and long-term outcomes in these patients. However, in this trial, the addition of natalizumab to systemic corticosteroids did not improve upon the response rates achieved with systemic corticosteroid treatment alone, nor did it improve long-term survival. There was also no evidence that natalizumab was beneficial for any subgroup of patients across the range of biomarker and target-organ severity.

It is unclear why natalizumab did not benefit patients with GVHD given its efficacy for active Crohn disease.[31] In a separate study of 21 patients with GI GVHD, the same dose of natalizumab in combination with systemic corticosteroids produced similar responses to treatment, NRM, and survival as those reported here.[32] The dose of natalizumab used in both studies was based on the effective dose for patients with moderate to severe Crohn disease in whom >80% of the responses are evident after 1 dose.[31] Because the current trial did not include detailed pharmacokinetic or pharmacodynamic studies, it is unknown whether more intensive dosing or a more intensive dosing schedule may have produced better results. It is also possible that other drugs that inhibit T-cell trafficking may be more efficacious than natalizumab for the treatment of GVHD. It is also possible that natalizumab was ineffective because prevention of T-cell trafficking to the GI tract cannot control GVHD after severe damage has occurred.

Another monoclonal antibody, vedolizumab, that inhibits T-cell trafficking to the GI tract by selectively binding the $\alpha 4\beta 7$ heterodimer, has shown inconsistent results as a treatment for GVHD,[13,14,33-35] although a recent meta-analysis suggested that vedolizumab may be efficacious for corticosteroid-resistant GI GVHD.[36] It is possible that blockade of recirculating T cells between the secondary lymphoid organs and the GI tract may be more effective if used to prevent GVHD. A phase 1b study that investigated GVHD prevention with vedolizumab reported encouraging results[37] and this strategy is under further investigation in a randomized, placebo-controlled trial (NCT03657160).

In this trial, patients tolerated the study treatment reasonably well with few noninfectious AEs being attributed to natalizumab. Importantly, no cases of PML developed although patients in this study were likely much more immunocompromised than patients who developed PML after treatment with natalizumab for multiple sclerosis.[28] The short duration of natalizumab treatment in this study may have been somewhat protective because risk of PML increases with duration of therapy.[28,38] Although natalizumab may have contributed to the development of severe bacterial infections,

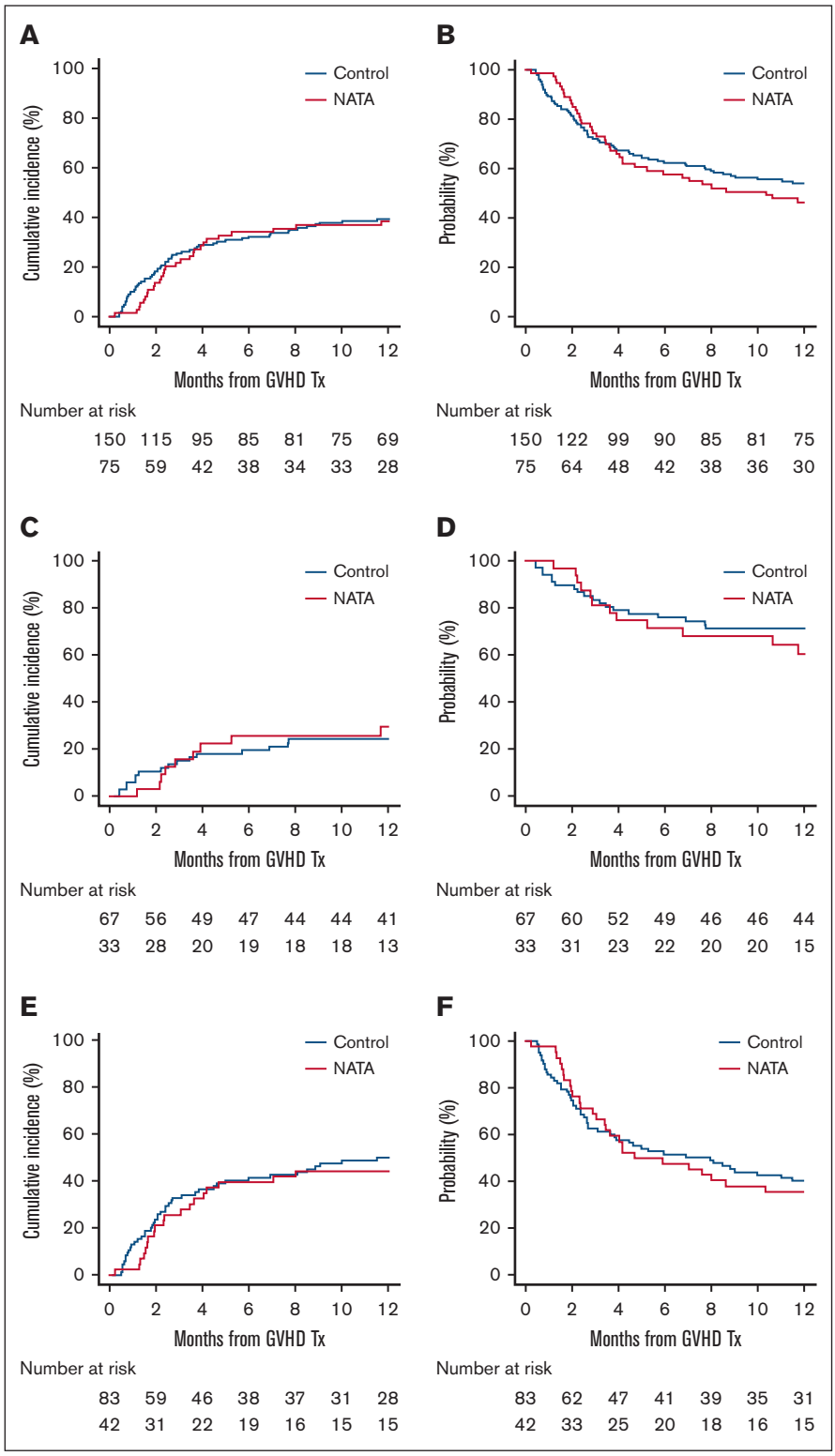

Figure 2. Long-term outcomes after systemic treatment of acute GVHD. *P* values express difference between patients treated with corticosteroids plus natalizumab and control patients treated with corticosteroids only. (A) NRM and (B) OS for all patients; (C) NRM and (D) OS for patients with AA2 scores; and (E) NRM and (F) OS for patients with AA3 scores.

the nature and timing of the infections suggest that poorly controlled GVHD was the primary driver of such infections. Severe GI GVHD increases the risk for bacterial translocation, which can result in severe infections,[39-41] as occurred in this study. We cannot definitively conclude that antibiotic prophylaxis would be protective; however, the proportion of patients who developed a

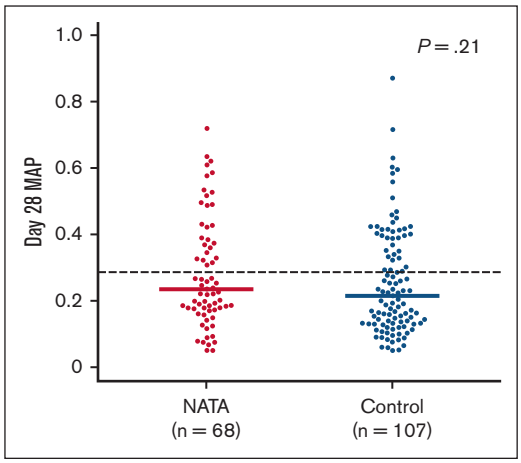

Figure 3. MAP at day 28 of treatment. MAPs in patients treated with corticosteroids plus natalizumab and control patients treated with corticosteroids only. Dashed line indicates MAP = .29 represents the threshold that discriminates between risk of NRM at day 28 of treatment. Solid line indicates median MAP.

severe bacterial infection with an enteric organism declined from 25% to 9% after the protocol was amended to mandate treatment with a fluoroquinolone (or similar antibiotic) for 6 weeks.

There were several strengths to this study despite the negative clinical results. First, this trial prospectively validates the ability of the 2-biomarker MAP to predict a response to primary GVHD therapy. Second, biomarker scores provide a method to enrich study populations for patients with high-risk GVHD who are more likely to benefit from treatment intensification. This strategy can reduce the number of patients needed to demonstrate a treatment effect, and has been promoted by the BMT CTN as an effective and efficient strategy to evaluate novel agents as a frontline therapy for acute GVHD.[42] This study has successfully demonstrated the feasibility of using biomarker scores as an eligibility criteria for high-risk GVHD in which the prompt initiation of treatment is a priority. AA scores were available the day after shipping of screening samples, and infusion of the first dose of natalizumab generally occurred within 2 days of enrollment. Future trials for high-risk GVHD by biomarker scores will enroll more efficiently if patients with AA2 and AA3 scores are included from the beginning of the trial, unlike the staggered approach used in this trial. Third, the use of a near-contemporaneous, matched control population for comparative analyses provided valuable context for the results. We also mitigated potential bias in favor of the study population by applying the study eligibility criteria to controls. Fourth, multiple centers contributed study patients and controls, lending further confidence in the results.

In conclusion, this biomarker-based multicenter GVHD therapeutic trial was feasible, and the addition of natalizumab to corticosteroids was generally well tolerated in the setting of newly diagnosed high-risk acute GVHD. However, there was no demonstrable benefit in response rate or NRM when compared with the robust contemporaneous biomarker-matched control group. New prophylaxis strategies have decreased the incidence of severe GVHD in recipients of allogeneic HCT,[43-45] but effective primary treatment remains an important unmet need because GVHD is the primary cause of NRM[46-48] and survivors can experience significant sequelae and poor quality of life.[32,49-51] Compared with single-center trials that lack contemporaneous controls, phase 2 trials that are conducted at multiple centers, use biomarkers to enrich for desired patient populations, incorporate planned interim futility analyses, and compare outcomes with well-matched control cohorts, provide an attractive method to identify the best therapies for definitive assessment in phase 3 trials.

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Authorship

Contribution: M.M.A.M, K.L., J.B., Y.A., U.Ö., N.S., J.L.M.F., and J.E.L. contributed to study concept and design, data interpretation, and wrote the manuscript; W.J.H., A.E., H.C., E.H., A.L., S.A., D.M.P., Z.D., C.L.K., K.A., R.R., A.C., G.E., I.G., S. Kasikis, S. Kowalyk, G.M., R.Y., Y.-B.C., and R.N. contributed to the conduct of the trial, performed the research, contributed to data interpretation, and contributed to writing the manuscript; F.A., C.C., M.E., S. Gleich., S. Grupp, E.H., N.R.J., and G.Y. contributed control patient data; and all authors contributed to critical revision of the manuscript for intellectual content, and read and approved the final version.

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