Biology of Blood and Marrow Transplantation

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Brincidofovir for Asymptomatic Adenovirus Viremia in Pediatric and Adult Allogeneic Hematopoietic Cell Transplant Recipients: A Randomized Placebo-Controlled Phase II Trial

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A B S T R A C T

Adenovirus infection in immunocompromised patients contributes to significant morbidity and mortality, especially after allogeneic hematopoietic cell transplantation (HCT). Brincidofovir (BCV, CMX001) is an orally bioavailable lipid conjugate of cidofovir that has in vitro activity against adenoviruses and other double-stranded DNA viruses. This randomized placebo-controlled phase II trial evaluated pre-emptive treatment with BCV for the prevention of adenovirus disease in pediatric and adult allogeneic HCT recipients with asymptomatic adenovirus viremia. Allogeneic HCT recipients with adenovirus viremia were randomized 1:1:1 to receive oral BCV 100 mg (2 mg/kg if <50 kg) twice weekly (BIW), BCV 200 mg (4 mg/kg if <50 kg) once weekly (QW), or placebo for 6 to 12 weeks, followed by 4 weeks of post-treatment follow-up. For randomization, subjects were stratified by screening absolute lymphocyte count (<300 cells/mm^3 versus ≥300 cells/mm^3). Assignment to BCV or placebo was double blinded; dose frequency was unblinded. The primary endpoint was the proportion of subjects experiencing *treatment failure*, defined as either progression to probable or definitive adenovirus disease or confirmed increasing adenovirus viremia (≥1 log$_{10}$ copies/mL) during randomized therapy. Between June 2011 and December 2012, 48 subjects were randomized to the BCV BIW (n = 14), BCV QW (n = 16), or placebo (n = 18) groups. The proportion of subjects with treatment failure in the BCV BIW group was 21% (odds ratio, .53; 95% confidence interval [CI], .11 to 2.71; P = .45), 38% (odds ratio, 1.23; 95% CI, .30 to 5.05, P = .779) in the BCV QW group, and 33% in the placebo group. All-cause mortality was lower in the BCV BIW (14%) and BCV QW groups (31%) relative to the placebo group (39%), but these differences were not statistically significant. After 1 week of therapy, 8 of 12 subjects (67%) randomized to BCV BIW had undetectable adenovirus viremia (<100 copies/mL), compared with 4 of 14 subjects (29%) randomized to BCV QW and 5 of 15 subjects (33%) randomized to placebo. In a post hoc analysis of subjects with viremia ≥1000 copies/mL at baseline, 6 of 7 BCV BIW subjects (86%) achieved undetectable viremia compared with 2 of 8 placebo subjects (25%; P = .04). Early treatment discontinuation because of adverse events was more common in subjects treated with BCV than with placebo. Diarrhea was the most common event in all groups (57% BCV BIW, 38% BCV QW, 28% placebo), but it led to treatment discontinuation in only 1 subject receiving BCV QW. Events diagnosed as acute graft-versus-host disease, primarily of the gastrointestinal tract, were more frequent in the BCV BIW group (50%) than in the BCV QW (25%) and placebo (17%) groups. There was no evidence of myelotoxicity or nephrotoxicity in BCV-treated subjects. The results of this trial confirm the antiviral activity of BCV against adenoviruses. Further investigation is ongoing to define the optimal treatment strategy for HCT recipients with serious adenovirus infection and disease.

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INTRODUCTION

Adenoviruses cause a wide spectrum of disease in immunocompromised patients, ranging from asymptomatic viremia to severe disseminated disease, particularly before immune reconstitution in recipients of allogeneic hematopoietic stem cell transplantation (HCT). Although post-transplantation infection with adenoviruses, as detected in stool or nasopharyngeal wash, may remain asymptomatic in patients with rapid immune reconstitution, an array of factors can increase the risk for developing disseminated disease and mortality. The most consistent risk factors are frequently associated with delayed immune reconstitution, including cord blood and other unrelated donors, in vivo or ex vivo T cell depletion, graft-versus-host disease (GVHD), young recipient age, and higher adenovirus viremia in plasma [1-3]. The incidence of infection with adenoviruses in patients undergoing allogeneic HCT is estimated to be between 5% and 47% [4-11], with higher incidence in pediatric patients; most cases of serious disease occur in the first 100 days after transplantation [5,6,11]. Mortality rates of up to 26% are reported for untreated HCT recipients with symptomatic localized infection, and mortality rates of 80% or greater are reported for lower respiratory tract infections associated with disseminated disease [3,5,10-14].

There is no approved antiviral agent for the treatment of adenovirus infection. Current treatment strategies may include a reduction in immune suppression, if clinically possible, and/or the off-label use of intravenous gamma globulins or intravenous cidofovir (CDV) [10,15-17]. However, CDV is associated with significant dose-limiting nephrotoxicity in up to 50% of patients [12,18] as well as myelotoxicity. Brincidofovir (BCV, CMX001) is an orally bioavailable lipid conjugate of CDV that has more potent in vitro activity against adenoviruses and demonstrated reduction of viral replication in an established animal model, along with substantial reduction of adenovirus-related mortality [19]. In patients who were treated for serious infections with adenoviruses through emergency investigational new drug regulations in the United States, BCV reduced adenovirus viral load and reported cases had significant reductions in adenovirus-related symptoms and associated mortality [20,21]. Unlike CDV, BCV has not been associated with overt drug-related myelotoxicity or nephrotoxicity [22].

The pre-emptive use of appropriate antiviral therapy before symptomatic disease has demonstrated a substantial reduction in cytomegalovirus (CMV)-related disease and associated mortality in patients after HCT; such an approach could theoretically also improve outcomes in patients at risk for adenovirus disease. In this report, we describe the results from the AdV HALT trial (CMX001-202; NCT01241344), a randomized, placebo-controlled, multicenter, phase II study to evaluate the safety and efficacy of pre-emptive treatment with BCV for the prevention of adenovirus disease in pediatric and adult allogeneic HCT recipients.

MATERIALS AND METHODS
Subjects

Allogeneic HCT recipients who were between the ages of 3 months and 75 years, inclusive, and who had asymptomatic adenovirus viremia in plasma based on polymerase chain reaction (PCR) testing performed at the local virology laboratory and confirmed as ≥100 copies/mL by the designated central virology laboratory were enrolled into the study. To identify prospective subjects at centers that did not routinely conduct screening assessments for adenovirus viremia, subjects could be enrolled in a companion screening protocol, in which blood samples were collected weekly for the first 100 days after transplantation and were analyzed for adenovirus by PCR by the central virology laboratory. Subjects with possible, probable, or definitive adenovirus disease per protocol definitions (see Supplementary Table S1) were ineligible for the study. Additional exclusion criteria included elevated aminotransferases (>5 times upper limit of normal [ULN]), total bilirubin (≥2 times ULN), or conjugated bilirubin (≥1.5 times ULN); treatment with CDV, ribavirin, or leflunomide within 14 days of enrollment; human immunodeficiency virus infection; and suspected gastrointestinal (GI) GVHD that was not validated by a biopsy (ie, subjects with biopsy-proven GI GVHD could be enrolled). Each subject or legal guardian provided consent before participation in the study; assent was obtained from minors where required by institutional practices.

Study Design and Endpoints

The study protocol was approved by the institutional review board at each participating center. The study was conducted in accordance with recognized international scientific and ethical standards including but not limited to the International Conference on Harmonisation guideline for Good Clinical Practice and the original principles embodied in the Declaration of Helsinki. An independent data and safety monitoring board was convened to review unblinded study data on a periodic basis and to provide recommendations regarding the continued enrollment of the study.

Subjects were randomized 1:1:1 to BCV twice weekly (BIW), BCV once weekly (QW), or placebo. Assignment to BCV or placebo was double blinded; dose frequency was not blinded. The randomization schedule included stratification by the subject's absolute lymphocyte count (ALC) at screening (<300 cells/mm^3 or ≥300 cells/mm^3) and was implemented using an integrated voice/web response system. (The randomization schema is summarized in Supplementary Figure S1.) Adult subjects (≥18 years of age) randomized to a BCV cohort received either 100 mg BIW or 200 mg QW. Pediatric subjects (age <18 years) randomized to a BCV cohort received either 2 mg/kg BIW (maximum dose of 100 mg BIW) or 4 mg/kg QW (maximum dose 200 mg QW) as solution or suspension.

Subjects underwent safety and virology assessments on days 0, 3, 7, 10, 14, and 21 and then once each week thereafter for the duration of treatment. Subjects were on study treatment for at least 6 weeks but no more than 12 weeks, followed by 4 weeks of post-treatment follow-up. A *complete virologic response* was defined as confirmed adenovirus DNA PCR values <100 copies/mL (the limit of detection) reported by the central virology laboratory for 4 consecutive weeks. From week 6 and thereafter, subjects who had a negative or undetectable adenovirus viremia for 4 consecutive weeks were determined to have completed the treatment phase of the study. These subjects were discontinued from treatment and began 4 weeks of treatment-free follow-up.

Subjects who experienced an increased adenovirus viral load (≥1 log$_{10}$ increase from baseline confirmed by 2 measurements performed 1 week apart) or who were considered to have developed probable or definitive adenovirus disease (see Supplementary Table S1 for detailed descriptions) at any time after initiating randomized treatment were offered open-label BCV 100 mg BIW (or 2 mg/kg BIW if <50 kg), regardless of how long they had been on randomized (blinded) study drug. In addition, after the implementation of a protocol amendment (in July 2012), open-label BCV BIW was also offered to subjects who developed probable or definitive adenovirus disease during prescreening or screening before initiating the randomized treatment. Subjects could receive up to 12 weeks of open-label BCV.

The primary efficacy endpoint was the proportion of subjects experiencing *treatment failure*, defined as progression to probable or definitive adenovirus disease (see Supplementary Table S1) or *increasing adenovirus viremia* during randomized therapy, defined as an increase from baseline by ≥1 log$_{10}$, confirmed by a second measurement at least 1 week later and requiring discontinuation from randomized therapy. Secondary endpoints included the incidence of and time to progression to probable or definitive adenovirus disease and the incidence of and time to death (all cause) on treatment and to the end of the study.

Safety monitoring procedures, including physical examination, vital signs, the collection of blood and urine for clinic laboratory testing, and the recording of adverse events (AEs) and concomitant medications were performed before first study drug administration and at periodic intervals after the initiation of dosing. AE severity was graded according to the National Institutes of Health/National Cancer Institute Common Terminology Criteria for Adverse Events version 4.02. A program-wide safety monitoring and management plan developed following completion of a randomized, placebo-controlled study of BCV for the prevention of CMV in CMV-seropositive allogeneic HCT recipients [22] was implemented throughout the conduct of this study. The safety monitoring and management plan provided detailed methods for monitoring, characterizing, and managing GI symptoms or hepatic laboratory abnormalities that occurred while receiving BCV. For subjects with more than 1 GI AE or grade 2 diarrhea for more than 3 consecutive days, temporary interruption of study drug was considered. For subjects with grade 3 or higher GI AEs, study drug dosing interruption was mandated. After improvement of the grade 3 or higher signs and symptoms, study drug dosing was able to be resumed.

The use of leflunomide, ribavirin, intravenous CDV, and other investigational drugs with antiviral activity against double-stranded DNA viruses was prohibited throughout the treatment (blinded and open-label) and follow-up phases of the study. The use of intravenous gamma globulins was not prohibited during the study.

Viral DNA Analysis and Resistance

Adenovirus DNA in plasma and other fluids were analyzed using a 7500 PCR-based assay by a central virology laboratory (Viracor-IBT Laboratories, Lee's Summit, MO). Plasma samples were analyzed at the central laboratory for all subject visits (except day 3 and day 10).

Adenovirus hexon typing and genotyping for viral resistance evaluation was performed by the Chimerix Virology Laboratory (Durham, NC). Adenovirus species and type were determined by hexon gene sequencing. Typing was attempted on specimens from all subjects at the earliest time point with sufficient viral DNA and on therapy for subjects with a mixed type at baseline [23-25].

Genotyping of baseline and on-therapy samples was used to explore selection for resistance-associated mutations. Full-length amino acid adenovirus DNA polymerase gene sequences were aligned against the adenovirus species C reference sequences C5 (GenBank: AAW65499), C1 (GenBank: AF534906), C2 (GenBank: J01917), and C6 (GenBank: Q413315) to identify amino acid differences. Resistance testing was performed at baseline on specimens from all subjects and from specimens collected at the relevant time point after baseline for the following: (1) subjects with detectable plasma adenovirus DNA at the last on-therapy measurement; (2) subjects who had *virologic breakthrough*, defined as confirmed detectable plasma adenovirus DNA after confirmed adenovirus below the limit of detection, or confirmed increase in plasma adenovirus DNA by ≥1 $\log_{10}$ after experiencing at least a 1 $\log_{10}$ decrease from baseline; and (3) subjects who had adenovirus relapse after completion of treatment.

Statistical Analysis

The primary efficacy endpoint was the proportion of subjects experiencing treatment failure using the intent-to-treat (ITT) analysis set, which included all randomized subjects who took at least 1 dose of study drug (BCV or placebo). The BCV groups were compared with the placebo group using a logistic regression model with the randomization stratum (ALC <300 cells/ mm^3 versus ≥300 cells/mm^3) and dosing frequency (QW, BIW) in the model as covariates. The odds ratio for treatment and corresponding 95% confidence intervals (CIs) were calculated. Effect estimates were determined for each BCV group and placebo. Secondary endpoints were summarized using the ITT analysis set by treatment group. Mortality and other "time to" endpoints were analyzed using Kaplan-Meier time-to-event methodology. Treatment group comparisons with placebo were made in a pairwise fashion using a log-rank test. For dichotomous secondary endpoints, comparisons between BCV treatment groups and placebo were made using logistic regression models with treatment as the independent variable. Odds ratios and corresponding 95% CIs were presented.

RESULTS

Subject Disposition and Demographics

Between June 2011 and December 2012, more than 700 subjects were prescreened for participation in this study. Of these, 83 subjects were found to have adenovirus viremia and underwent further screening. Of these, 48 eligible subjects were determined to have asymptomatic adenovirus viremia and were eligible for randomization to blinded therapy. The remaining 35 subjects were ineligible for randomization; 4 were enrolled directly to open-label BCV after developing symptomatic adenovirus disease and 31 were considered screen failures (Figure 1). In 15 of the 31 screen failures, adenovirus viremia spontaneously resolved between prescreening and screening. Four of the screen failures developed symptomatic disease before the option to enroll directly to open-label BCV. The ITT population consisted of 14 subjects randomized to BCV BIW, 16 randomized to BCV QW, and 18 randomized to placebo; in each of these groups, respectively, 4 (29%), 6 (38%), and 9 (50%) of subjects received complete treatment courses. Five (2 receiving BCV BIW, 1 receiving BCV QW, and 2 receiving placebo) of 48 (10%) subjects with central laboratory–confirmed adenovirus viremia had undetectable viremia on the first day of dosing. Two sub-

jects from the BCV BIW group were discontinued from treatment prematurely because of site error during weeks 5 and 6, after recording 4 consecutive weeks of undetectable adenovirus viremia. Probable or definitive adenovirus disease developed in 10 subjects (21%), while 6 subjects (13%) had increasing adenovirus viremia.

Thirty-five randomized subjects were children <18 years of age and 13 were adults. Though the numbers are low, proportionally more adult subjects discontinued treatment in the BCV groups because of AEs (2 of 3 adults [67%] versus 0 of 11 pediatric subjects in the BCV BIW group; 1 of 4 adults [25%] versus 1 of 12 pediatric subjects [8%] in the BCV QW group; and 1 of 6 adults [17%] versus 2 of 12 pediatric subjects [17%] in the placebo group).

Baseline demographics and transplantation characteristics are shown in Table 1. The treatment groups were generally well balanced with respect to age, baseline adenovirus viremia, baseline ALC, and conditioning regimens. Baseline adenovirus viremia for individual subjects ranged from <2.0 $\log_{10}$ copies/mL to 6.4 $\log_{10}$ copies/mL, with no significant differences among the 3 groups ($P = .12$). Notably, 6 of the 14 subjects (43%) randomized to BCV BIW and 9 of the 18 subjects (50%) randomized to placebo had adenovirus viremia at baseline of ≤2.5 $\log_{10}$ copies/mL. The majority of grafts were from a matched, unrelated donor and approximately one-half of the subjects in each treatment group had received a myeloablative conditioning regimen. Of note, cord blood grafts were more frequent and bone marrow grafts were less frequent in the BCV BIW group than in the BCV QW and placebo groups ($P = .049$). Although there were no other statistically significant differences between the groups in baseline demographics and transplantation characteristics, an imbalance between the groups with respect to high-risk characteristics, such as haploidentical transplantation and cord blood transplantation (both greatest in the BCV BIW group) and matched related donor (greatest in placebo group), was observed. Four of 5 subjects who received a matched sibling transplant were randomized to placebo and were, thus, considered at lower risk of progression to adenovirus disease. Subjects who were classified as high risk for rapid disease progression (ie, haploidentical or cord blood transplantation, total or partial T cell depletion, or baseline grade III/IV GVHD, and within the first 6 months after transplantation) were over-represented in the BCV BIW cohort compared with the QW and placebo cohorts.

Treatment Duration

During randomized (blinded) treatment, the median treatment duration was 19 (range, 1 to 49) days for the BCV BIW group, 32 (range, 1 to 78) days for the BCV QW group, and 36 (range, 1 to 81) days for the placebo group. Three of 14 (21%) subjects in the BCV BIW group received the protocol-recommended minimum of 6 weeks of blinded treatment, compared with 4 of 16 (25%) BCV QW subjects and 6 of 18 (33%) placebo subjects. However, 2 subjects were discontinued from the BCV BIW group before week 6 because of site error after recording 4 consecutive weeks of undetectable adenovirus viremia. For those subjects who received open-label BCV BIW treatment, the median treatment duration was 49 (range, 1 to 82) days. Median BCV exposures were similar between pediatric and adult subjects in the BCV BIW group (19 versus 18 days) but were shorter in adults in both the BCV QW (35 versus 25 days) and placebo groups (38 versus 23 days) during the blinded phase and during BCV BIW dosing during open-label treatment (57 versus 22 days).
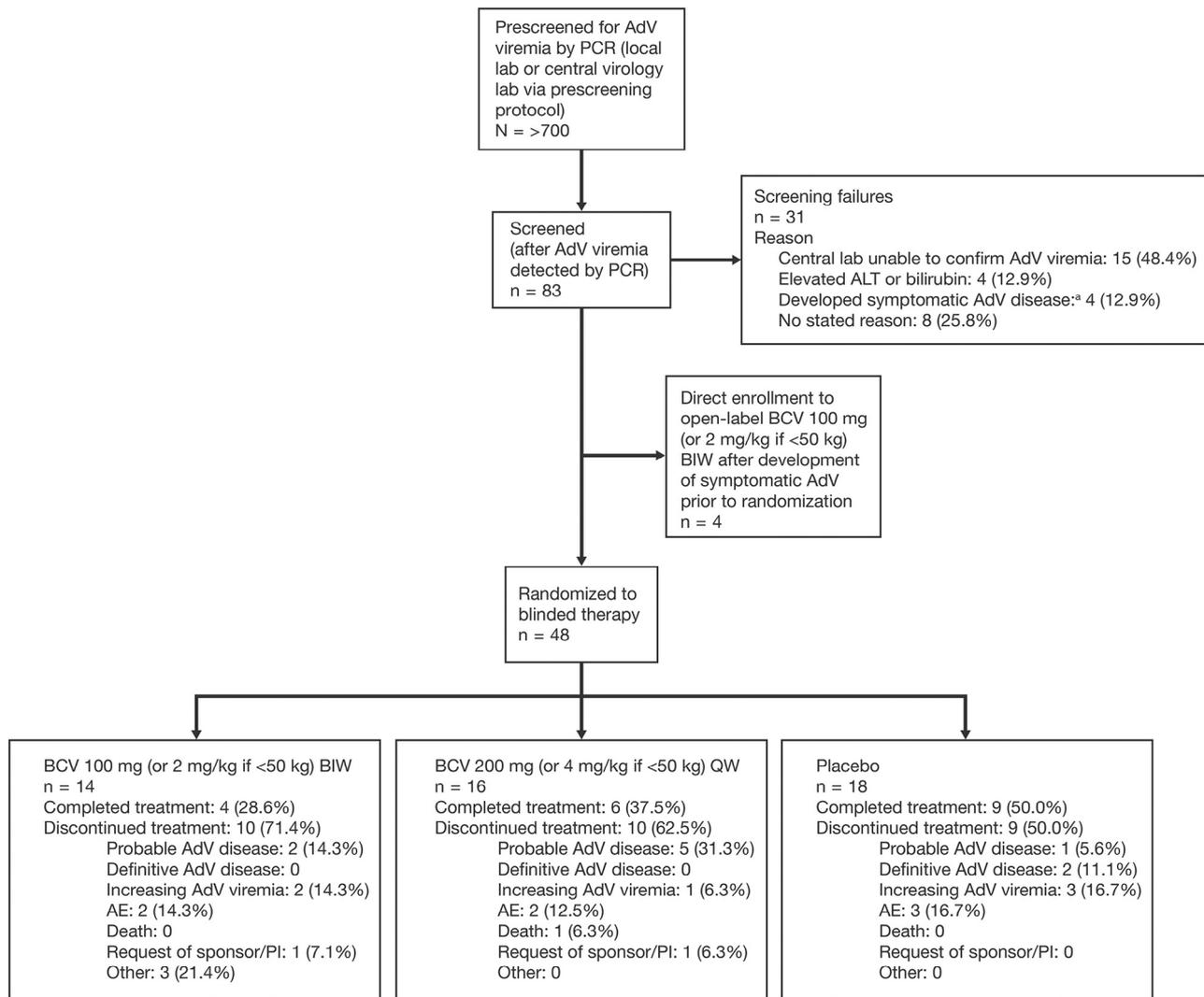
Figure 1. Patient flow diagram. AdV, adenovirus; AE, adverse event; ALT, alanine aminotransferase; BCV, brincidofovir; BIW, twice weekly; PI, principal investigator; QW, once weekly. [a]Before direct enrollment to open-label BCV was allowed.

Primary, Secondary, and Post Hoc Endpoints

Results for the primary, secondary, and post hoc endpoints are shown in Table 2. For subjects randomized to BCV BIW, the treatment failure rate was 21% compared with the placebo group failure rate of 33%.

Among pediatric subjects, 3 of 11 subjects (27%) in the BCV BIW group were treatment failures, compared with 5 of 12 subjects (42%) in the BCV QW group and 3 of 12 subjects (25%) in the placebo group. Among adult subjects, there were no treatment failures in the 3 subjects in the BCV BIW group, compared with 1 of 4 (25%) in the BCV QW group and 3 of 6 (50%) in the placebo group. There were no statistically significant differences for adults and pediatric subjects between the BCV treatment groups and placebo.

A post hoc analysis of only high-risk patients (ie, those who received a haploidentical or cord blood transplant, total or partial T cell depletion, or baseline grade III/IV GVHD, and who were enrolled within the first 6 months after transplantation) revealed treatment failure in 0 of 10 subjects receiving BCV BIW, 3 of 7 subjects (43%) receiving BCV QW, and 4 of 10 subjects (40%) receiving placebo, which trended towards clinical significance ($P = .087$ for BCV BIW versus placebo).

Secondary endpoints of all-cause mortality at the end of the study or end of treatment, graft survival, and development of probable or definitive adenovirus viremia, while showing numerical benefits for the BCV BIW group relative to placebo, also did not achieve statistical significance (Table 2). Two (14%) deaths in the BCV BIW group occurred during the treatment-free follow-up period (25 days and 43 days after the last dose of study drug) and were not adenovirus related; 1 was due to *Staphylococcus pneumonia* and 1 to fungal and human herpesvirus 6 meningitis (see Supplementary Table S2). Of the 5 (31%) deaths in the BCV QW group, 2 occurred during randomized treatment, 2 during rescue open-label BCV BIW treatment, and 1 during the treatment-free follow-up period. Of the 7 (39%) deaths in the placebo group, 1 occurred during randomized placebo treatment, 2 during rescue open-label BCV BIW treatment, and 4 during the treatment-free follow-up period. None of the deaths in any treatment group was considered by investigators to be

Table 1
Baseline Demographics and Transplantation Characteristics (ITT Analysis Set)

Characteristic	BCV BIW (n = 14)	BCV QW (n = 16)	Placebo (n = 18)
Age, median (range), yr	8.5 (0-55)	9 (2-70)	11 (1-53)
Age category			
<12 yr	9 (64%)	11 (69%)	9 (50%)
12-17 yr	2 (14%)	1 (6%)	3 (17%)
>17 yr	3 (21%)	4 (25%)	6 (33%)
Female	5 (36%)	3 (19%)	7 (39%)
Weight, median (range), kg	27 (10-98)	25 (13-84)	41 (11-95)
Baseline AdV viral load, median (range), $\log_{10}$ copies/mL	3.1 (2.0-4.9)	4.0 (2.0-6.4)	2.7 (2.0-5.4)
Baseline ALC, median (range), cells/mm^3	340 (0-1650)	420 (50-2230)	290 (0-1970)
ALC (randomization strata)			
<300 cells/mm^3	8 (57%)	8 (50%)	10 (56%)
≥300 cells/mm^3	6 (43%)	8 (50%)	8 (44%)
Time from transplantation to first dose, d			
Median (range)	46 (9-208)	80 (1-928)	42 (13-265)
0-100	10 (71%)	13 (81%)	15 (83%)
100-180	3 (21%)	2 (13%)	2 (11%)
>180	1 (7%)	1 (6%)	1 (6%)
Acute GVHD at first dose			
Any	4 (29%)	5 (31%)	4 (22%)
Gut	4 (29%)	4 (25%)	3 (17%)
Liver	2 (14%)	2 (13%)	1 (6%)
Skin	2 (14%)	3 (19%)	1 (6%)
Reason for transplantation			
Malignancies	4 (29%)	5 (31%)	9 (50%)
Nonmalignant diseases	10 (71%)	11 (69%)	9 (50%)
Pretransplantation conditioning			
Myeloablative	6 (43%)	8 (50%)	10 (56%)
Reduced intensity	6 (43%)	6 (38%)	7 (39%)
Other	1 (7%)	2 (13%)	1 (6%)
None	1 (7%)	0	0
Source of graft			
Bone marrow	2 (14%)	10 (63%)	9 (50%)
Peripheral blood stem cells	6 (43%)	1 (6%)	4 (22%)
Cord blood	6 (43%)	5 (31%)	5 (28%)
Donor type			
Haploidentical	3 (21%)	0	1 (6%)
Matched related	0	1 (6%)	4 (22%)
Mismatched related	1 (7%)	0	0
Matched unrelated	9 (64%)	14 (88%)	12 (67%)
Mismatched unrelated	1 (7%)	1 (6%)	1 (6%)
CMV seropositive	8 (57%)	6 (38%)	12 (67%)
High-risk for rapid progression to AdV disease*	10 (71%)	7 (44%)	10 (56%)

Data presented are n (%) unless otherwise indicated.
AdV indicates adenovirus
  * One or more of the following characteristics: haploidentical or cord blood transplant, total or partial T cell depletion, or baseline grade III/IV GVHD, and <6 months after transplantation.

possibly or probably related to BCV. Figure 2 shows the time to death (all cause) to the end of the study in the 3 treatment groups.

Individual subject plots of adenovirus viremia during the blinded treatment period are presented in Figure 3 and show differences in change from baseline in adenovirus viral load for subjects receiving BCV BIW (Figure 3A) compared with subjects receiving BCV QW (Figure 3B) or placebo (Figure 3C). Although many subjects in the BCV BIW group had higher baseline levels of adenovirus viremia, this group nonetheless responded quickly to BCV therapy. After 1 week of therapy, 8 of 12 subjects (67%) randomized to BCV BIW had undetectable adenovirus viremia, compared with 4 of 14 subjects (29%) randomized to BCV QW and 5 of 15 subjects (33%) randomized to placebo. Of note, of the 7 subjects (50%) who received BCV BIW and had baseline adenovirus viremia of ≥3.0 $\log_{10}$ copies/mL, 6 subjects (86%) had 2 $\log_{10}$ copies/mL (lower level of detection) or undetectable viremia by day 7. However, the differences between arms were not statistically significant at any visit, as the incidence of low-level viremia (≤2.5 $\log_{10}$ copies/mL) was high at baseline (43% and 50% of sub-

jects randomized to BCV BIW and placebo, respectively), reducing differentiation between treatment groups. In subjects treated with placebo, 13 of 18 (72%) achieved undetectable adenovirus viremia during the treatment phase (Figure 3C). At the end of treatment, adenovirus viremia changed from baseline by a mean (standard deviation) of –.6 (1.09) $\log_{10}$ copies/mL in the BCV BIW group, by –.6 (1.47) $\log_{10}$ copies/mL in the BCV QW group, and by –.7 (1.27) $\log_{10}$ copies/mL in the placebo group.

One subject in each of the BCV BIW (7%) and BCV QW (6%) groups had a ≥ 1 $\log_{10}$ copies/mL increase in adenovirus viremia, compared with 4 subjects (22%) in the placebo group; the differences between the groups was not statistically significant.

Figure 4 shows adenovirus viremia over time in subjects with viremia ≥3 $\log_{10}$ copies/mL at baseline. In 6 of 7 (86%) such subjects treated with BCV BIW, adenovirus viremia decreased to ≤2 $\log_{10}$ copies/mL (ie, the lower limit of detection) by day 7 after treatment initiation. By contrast, in placebo-treated subjects with viremia ≥3 $\log_{10}$ copies/mL at baseline, viral load at day 7 had decreased to ≤2 $\log_{10}$ copies/mL in only 2 of 8 (25%) subjects (Fisher's exact test, $P = .04$).

Table 2
Primary, Secondary, and Post hoc (High-Risk Group) Endpoints (ITT Analysis Set)

Endpoint	BCV BIW (n = 14)	BCV QW (n = 16)	Placebo (n = 18)
Primary endpoint			
Treatment failure*	3 (21%)	6 (38%)	6 (33%)
P value (versus placebo)[†]	.45	.78	–
Odds ratio (95% CI) relative to placebo	.53 (.11-2.71)	1.23 (.30-5.05)	
Secondary endpoints			
All-cause mortality at end of treatment	0 (0%)	4 (25%)	3 (17%)
All-cause mortality at end of study	2 (14%)	5 (31%)	7 (39%)
Graft survival	14 (100%)	15 (94%)	15 (83%)
Probable or definitive AdV disease	2 (14%)	5 (31%)	5 (28%)
Post hoc analysis: high-risk group[‡]	n = 10	n = 7	n = 10
Treatment failure*	0 (0%)	3 (43%)	4 (40%)
P value (versus placebo)[§]	.087	1.000	
Probable or definitive AdV disease	0 (0%)	2 (29%)	3 (30%)
P value (versus placebo)[§]	.211	1.000	
Death during study	2 (20%)	3 (43%)	5 (50%)
P value (versus placebo)[§]	.350	1.000	

Data presented are n (%) unless otherwise indicated.

* Defined as progression to probably or definitive AdV disease, or increasing AdV viremia during randomized (blinded) therapy (defined as increase from baseline in AdV viremia by ≥ 1 log$_{10}$, confirmed by a second measurement at least 1 week later and requiring discontinuation of study drug).

† Based on a logistic regression model adjusted for randomization stratum (ALC <300 cells/mm^3 versus ≥ 300 cells/mm^3) using the ITT analysis set.

‡ One or more of the following characteristics; haploidentical or cord blood transplantation, total or partial T cell depletion, or baseline grade III/IV GVHD, and <6 months after transplantation.

§ Fisher's exact test.

Two subjects in the BCV BIW group met the criteria for probable adenovirus disease; in both, viremia decreased to undetectable levels but blinded treatment was discontinued because of a diagnosis of adenovirus pneumonitis in 1 and adenovirus enterocolitis in the other.

Adenovirus Typing and Resistance

Adenovirus was typed for 42 of the 48 subjects, with species C being the most common (n = 31; 74%). Because of the high proportion of species C types in the population, it was not possible to determine if antiviral activity of BCV varies significantly based on adenovirus species or type. A mutation (V303I) previously associated with decreased in vitro sensitivity to BCV and CDV (data on file at Chimerix) was ob-

served in 1 subject in the BIW group at the time of virologic failure. The subject was randomized to blinded BCV 100 mg BIW but was transitioned to open-label BCV after receiving just 1 dose of blinded therapy. Neither resistance at baseline nor available pharmacokinetic data appear to explain treatment failure in this subject.

Safety

Overall, 56% to 71% of subjects in each treatment group reported at least 1 treatment-emergent AE (TEAE) of grade 3 or higher severity during the randomized treatment phase (Table 3). Serious TEAEs were considered by investigators to be drug-related in 1 subject in each treatment group. AEs leading to study drug discontinuation were more common
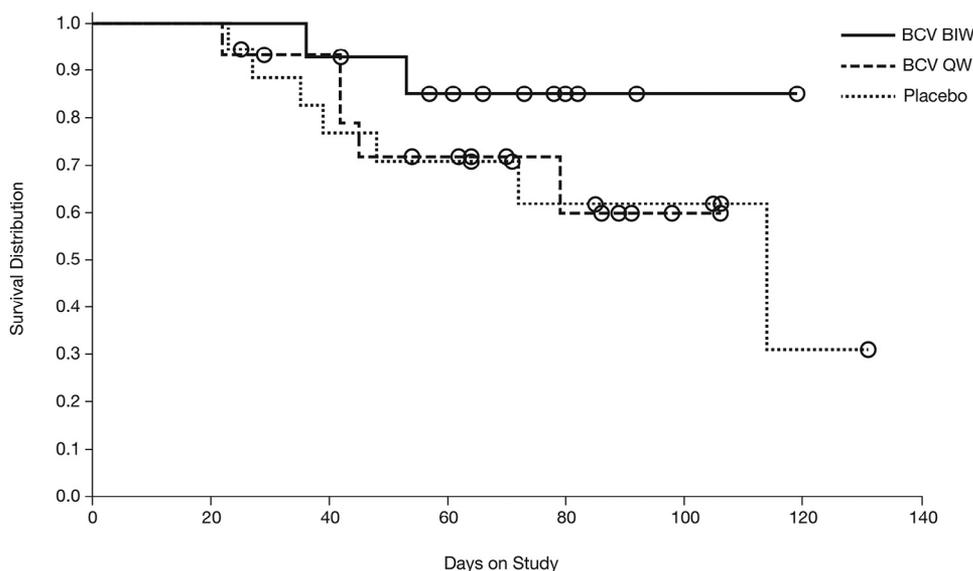

Figure 2. Kaplan-Meier estimates of all-cause mortality[a] to the end of the study (ITT analysis set) in subjects treated with BCV BIW (n = 14), BCV QW (n = 16), or placebo (n = 18). Open circles represent censoring. Blinded treatment was initiated on day 0. BCV, brincidofovir; BIW, twice weekly; CI, confidence interval; ITT, intent-to-treat; QW, once weekly. [a]No deaths were attributed to BCV; causes of death are listed by treatment arm in Supplementary Table S2.
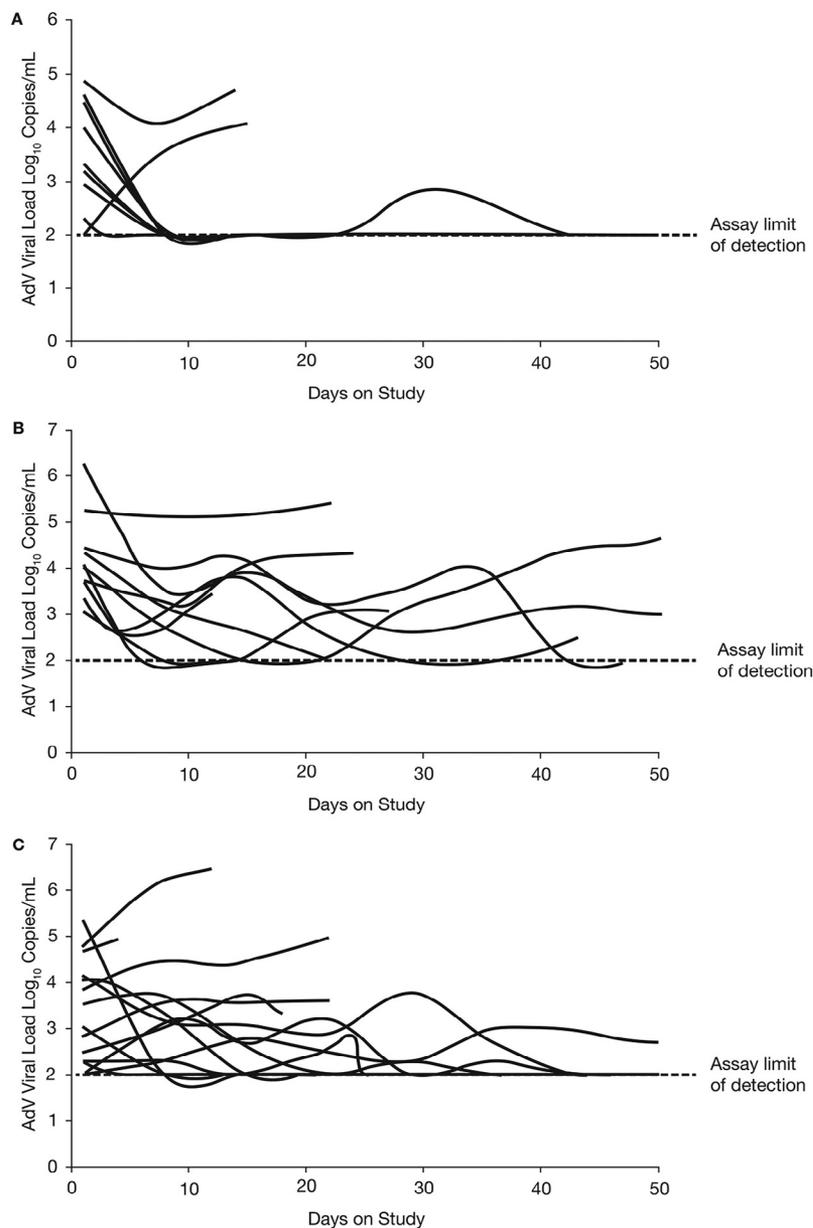
Figure 3. Adenovirus viremia during the blinded treatment period (ITT analysis set). (A) BCV BIW (n = 14); (B) BCV QW (n = 16); (C) placebo (n = 18). Each line represents an individual subject. Blinded treatment was initiated on day 0. AdV, adenovirus; BCV, brincidofovir; BIW, twice weekly; ITT, intent-to-treat; QW, once weekly.

in subjects treated with BCV BIW and BCV QW than those treated with placebo.

The most frequently reported TEAE (of any grade) was diarrhea, which was reported more frequently in the BCV treatment groups (57% BIW, 38% QW) compared with the placebo group (28%). Diarrhea was generally considered to be of mild-to-moderate severity. Grade 3 or higher diarrhea was reported by more subjects in the BCV BIW (14%) and BCV QW (19%) groups than in the placebo (6%) group. One subject in the BCV BIW group and 1 subject in the BCV QW group required dose interruption because of diarrhea. Among all 3 treatment groups, diarrhea led to permanent study drug discontinuation in 1 subject in the BCV QW group. In the BCV BIW group, 2 study discontinuations were due to GI AEs; 1 for abdominal pain and 1 for GI hemorrhage. Notably, acute

GVHD, primarily of the GI tract, was reported in a higher proportion of subjects in the BCV BIW group than in the BCV QW and placebo groups and was the most frequently reported serious TEAE. Grade 3 or higher acute GVHD was reported for more subjects in the BCV BIW group (21%), compared with the BCV QW (6%) and placebo (11%) groups. None of the cases of acute GVHD in BCV-treated subjects was considered by the investigator to be related to study drug and none required permanent study drug discontinuation.

As the majority of subjects (35 of 48; 73%) were children, the AE profile for the pediatric subjects reflected the overall profile (see Supplementary Table S3 for a summary of TEAEs reported for the pediatric subjects). Despite the small numbers, the adult AE profile was not substantially different from that of the pediatric subjects (data not shown).
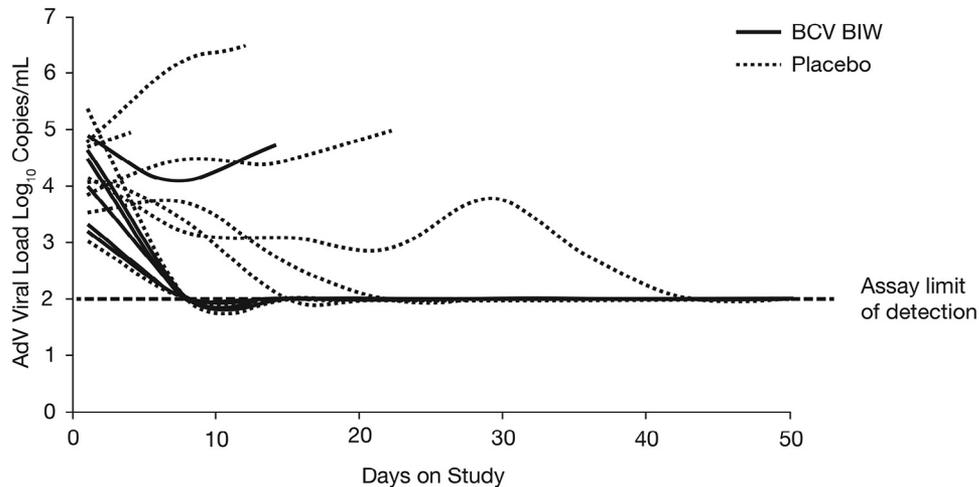
Figure 4. Adenovirus viremia during the blinded treatment period (ITT analysis set) with BCV 100 mg BIW (n = 7) versus placebo (n = 8) in subjects with viremia ≥3 $\log_{10}$ copies/mL at baseline. Each line represents an individual subject. Blinded treatment was initiated on day 0. AdV, adenovirus; BCV, brincidofovir; BIW, twice weekly; ITT, intent-to-treat.

No grade 3 or higher increases in serum creatinine occurred with BCV or placebo treatment; however, increases in alanine aminotransferase occurred more frequently in the placebo group than in the BCV BIW group.

DISCUSSION

Currently there is no approved therapy for adenovirus. This trial is the first to evaluate pre-emptive treatment with BCV in allogeneic HCT recipients with asymptomatic adenovirus viremia. Although statistically significant results were not established in this exploratory study, likely because of the small sample size and the inclusion of patients at low risk of pro-

gression to serious adenovirus disease, ITT and subset analyses of disease progression demonstrated trends favoring the BCV BIW regimen over placebo or BCV QW. Treatment with BCV BIW resulted in early and consistent declines in adenovirus viremia that were most apparent in subjects with viremia ≥1000 copies/mL at baseline.

The observed rapid and sustained virologic responses are consistent with a previous retrospective report of a small cohort of highly immunocompromised patients with adenovirus disease treated with BCV [20]. In a population of subjects with adenovirus disease who had failed previous CDV therapy, about 70% of patients treated with BCV achieved virologic

Table 3
Treatment-Emergent Adverse Events (ITT Analysis Set)

Adverse Event	BCV BIW (n = 14)	BCV QW (n = 16)	Placebo (n = 18)
Summary of TEAEs			
TEAE leading to study drug discontinuation	2 (14%)	2 (13%)	1 (6%)
TEAE leading to dose change or interruption	5 (36%)	1 (6%)	1 (6%)
Serious TEAE*	6 (43%)	7 (44%)	6 (33%)
≥Grade 3 TEAE	10 (71%)	9 (56%)	11 (61%)
TEAEs of interest†			
Renal			
Hematuria	0	0	2 (11%)
GI			
Diarrhea	8 (57%)	6 (38%)	5 (28%)
Nausea	2 (14%)	2 (13%)	4 (22%)
Vomiting	1 (7%)	4 (25%)	4 (22%)
Abdominal pain	2 (14%)	0	2 (11%)
Immune system			
Acute GVHD	7 (50%)	4 (25%)	3 (17%)
Gut	5 (36%)	3 (19%)	2 (11%)
Liver	0	0	1 (6%)
Skin	2 (14%)	1 (6%)	2 (11%)
Laboratory abnormalities (grades 3 and 4)			
Serum creatinine >3 x ULN	0	0	0
ALT >5 x ULN	1 (7%)	1 (7%)	4 (22%)
Bilirubin >3 x ULN	0	1 (7%)	2 (11%)
ANC <1000 cells/mm³	4 (29%)	7 (47%)	5 (28%)
Hemoglobin <8.0 g/dL	3 (21%)	6 (40%)	9 (50%)
Platelet count <50,000/mm³	6 (43%)	8 (53%)	9 (50%)

ALT indicates alanine aminotransferase.

  * TEAE that was fatal; life-threatening (subject was at immediate risk of death); required in-patient hospitalization or prolongation of existing hospitalization; resulted in congenital anomaly/birth defect; resulted in a persistent or significant disability or incapacity.

  † TEAEs of interest reported by ≥2 subjects in any treatment group.

response (≥99% decrease from baseline or undetectable adenovirus plasma viral load) by week 8, and the corresponding 8-week survival rate was 77%.

The most frequently reported AEs for BCV in this study were GI disturbances, primarily diarrhea. Although more GI AEs were reported by BCV-treated subjects than by placebo-treated subjects, few of these AEs resulted in discontinuation of study drug. Grade 3 or higher diarrhea was reported by fewer than 20% of subjects on BCV BIW or QW and led to treatment discontinuation in only 1 BCV QW subject during the randomized phase of the study. There were also more GI GVHD AEs reported in the BCV BIW group compared with in the BCW QW and placebo groups, and these events were twice as likely to be severe (grade 3 or higher).

These observations of increased diarrheal and GI GVHD events in BCV-treated subjects are consistent with the results from the recently completed phase III study of BCV for the prevention of CMV after HCT (Study CMX001-301; NCT01769170). In Study CMX001-301, BCV-related diarrheal events were often misdiagnosed as GI GVHD. Closer scrutiny of the acute GVHD data suggests that the excess GI GVHD in the BCV treatment arm was likely drug-related diarrhea mimicking the signs and symptoms of acute GI GVHD, as has been previously described for both mycophenolate mofetil and proton pump inhibitors [26,27]. In Study CMX001-301, diagnosis of many GVHD cases was based on biopsy results, and a post hoc independent pathology review concluded that GI biopsy was not able to discern between GI GVHD and BCV-related diarrhea [28,29]. Of note, subjects who interrupted BCV treatment in Study CMX001-301, allowing for gut recovery, had improved outcomes. Future studies with BCV should continue to emphasize the importance of close monitoring of diarrhea when the differential diagnosis includes GI GVHD, including the use of more conservative thresholds for interruption and, if necessary, discontinuation of BCV.

There was no evidence of renal or hematologic toxicity in subjects receiving BCV, which are well-documented toxicities associated with CDV [10,17,30]. This is attributed to the generation of significantly lower plasma concentrations of CDV in vivo after BCV administration compared with those following an intravenous dose of CDV [30,31]. In addition, BCV is not a substrate for organic anion transporter 1 in the proximal renal tubule, which significantly reduces the risk of nephrotoxicity [22,32-34].

One in vitro resistance-associated mutation (V303I) was observed in adenovirus from a subject with virologic failure in the BIW group, providing genetic evidence for an in vivo antiviral effect of BCV against adenovirus that is mediated by the viral DNA polymerase.

A number of screened subjects had evidence of adenovirus end-organ disease at the time of their first detectable adenovirus viremia. This would suggest that patients with identification of adenovirus in any organ system may be at risk of disseminated adenovirus disease and that waiting to initiate treatment until the patient has detectable viremia or viremia exceeding a predetermined threshold may not be the optimal approach, particularly for patients at higher risk for adenovirus disease.

This study has a number of limitations. In addition to the small sample size, there was heterogeneity in demographics and baseline disease and transplantation characteristics that are associated with progression to adenovirus disease and associated mortality (eg, viral load, ALC, acute GVHD, cord blood stem cell source, and recipient of haploidentical transplant) [35]. Another significant confounding factor was the rapid transition of a number of subjects to open-label treatment, based on investigator-determined progression to adenovirus disease, thereby decreasing the power to detect significant differences among treatment groups. The availability of open-label therapy with BCV may have led to bias in the determination of adenovirus disease, particularly in cases where supporting microbiologic evidence of progression was lacking. The inclusion of recipients of matched sibling transplants at low risk of progression to serious adenovirus disease may have contributed to a significant proportion of subjects spontaneously clearing their adenovirus viremia.

In future studies, the provision of rescue therapy should be strictly controlled to minimize the potential for the premature declaration of failure of blinded therapy and enrollment and stratification should be based on multiple risk factors for progression to adenovirus disease, not on ALC threshold alone, especially as adenovirus viremia may be transient for many patients, particularly those without significant risk factors for disease progression.

CONCLUSION

In this trial, BCV BIW showed antiviral activity against adenoviruses and may have the potential to improve outcomes in subjects with adenovirus infection who are at higher risk of progression to disseminated disease.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2016.12.621.

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