ORIGINAL ARTICLE

CMX001 to Prevent Cytomegalovirus Disease in Hematopoietic-Cell Transplantation

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ABSTRACT

BACKGROUND

The use of available antiviral agents for the prevention of cytomegalovirus (CMV) disease is limited by frequent toxic effects and the emergence of resistance. CMX001 has potent in vitro activity against CMV and other double-stranded DNA viruses. We evaluated the safety and anti-CMV activity of CMX001 in patients who had undergone allogeneic hematopoietic-cell transplantation.

METHODS

From December 2009 through June 2011, a total of 230 patients with data that could be evaluated were enrolled in the study. We randomly assigned these adult CMV-seropositive transplant recipients from 27 centers to oral administration of CMX001 or placebo. Patients were assigned in a 3:1 ratio to five sequential study cohorts according to a dose-escalating, double-blind design. Randomization was stratified according to the presence or absence of acute graft-versus-host disease and CMV DNA in plasma. Patients received the study drug after engraftment for 9 to 11 weeks, until week 13 after transplantation. Polymerase-chain-reaction analysis of CMV DNA in plasma was performed weekly. Patients in whom CMV DNA was detected at a level that required treatment discontinued the study drug and received preemptive treatment against CMV infection. The primary end point was a CMV event, defined as CMV disease or a plasma CMV DNA level greater than 200 copies per milliliter when the study drug was discontinued. The analysis was conducted in the intention-to-treat population.

RESULTS

The incidence of CMV events was significantly lower among patients who received CMX001 at a dose of 100 mg twice weekly than among patients who received placebo (10% vs. 37%; risk difference, −27 percentage points; 95% confidence interval, −42 to −12; P=0.002). Diarrhea was the most common adverse event in patients receiving CMX001 at doses of 200 mg weekly or higher and was dose-limiting at 200 mg twice weekly. Myelosuppression and nephrotoxicity were not observed.

CONCLUSIONS

Treatment with oral CMX001 at a dose of 100 mg twice weekly significantly reduced the incidence of CMV events in recipients of hematopoietic-cell transplants. Diarrhea was dose-limiting in this population at a dose of 200 mg twice weekly. (Funded by Chimerix; CMX001-201 ClinicalTrials.gov number, NCT00942305.)

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N Engl J Med 2013;369:1227-36.
DOI: 10.1056/NEJMoa1303688

Cytomegalovirus (CMV) infection is a common cause of illness after allogeneic hematopoietic-cell transplantation.[1,2] CMV seropositivity in transplant recipients is also associated with an increased risk of death after transplantation, despite preemptive and prophylactic strategies with available antiviral agents.[3-5] Although valganciclovir is approved for prophylaxis against CMV infection after solid-organ transplantation, its use is limited by myelosuppression, particularly after hematopoietic-cell transplantation.[3,6-8] Thus, there is an unmet need for effective drugs against CMV infection that have a better safety profile.

CMX001 is an orally bioavailable lipid acyclic nucleoside phosphonate that is absorbed in the small intestine and transported throughout the body as a phospholipid. It crosses target cell membranes by means of facilitated and passive diffusion and has a long intracellular half-life.[9-11] CMX001 is converted intracellularly to cidofovir diphosphate after cleavage of its lipid moiety and phosphorylation by intracellular kinases.[9,10] Unlike cidofovir, CMX001 is not a substrate of organic ion transporter 1, is not concentrated in renal proximal tubules, and is unlikely to have renal toxicity.[9-12] CMX001 was originally synthesized and evaluated as an oral treatment for smallpox for biodefense initiatives,[9,13,14] but it also has potent antiviral activity against herpesviruses,[15,16] polyomaviruses,[17-19] adenoviruses,[20] and other orthopoxviruses[21,22] in vitro and in animal models. CMX001 is approximately 400 times more potent than cidofovir in vitro against CMV, including ganciclovir-resistant strains,[23] and is effective as treatment for CMV disease in animal models.[24,25]

In this study, we evaluated the safety, side-effect profile, and effectiveness of various doses of CMX001 for the prevention or control of CMV infection in CMV-seropositive hematopoietic-cell transplant recipients.

Methods

Patients

Recipients of allogeneic hematopoietic-cell transplants who were 18 years of age or older and who were seropositive for CMV were eligible for the study if they had evidence of engraftment and were able to swallow tablets. Study-drug administration was to be initiated 14 to 30 days after transplantation. Exclusion criteria included treatment with anti-CMV agents after transplantation, severe renal or hepatic dysfunction, and severe, acute gastrointestinal graft-versus-host disease (GVHD) that would preclude oral drug administration (for detailed criteria, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Within 7 days before the first dose of the study drug was administered, patients were screened for plasma CMV DNA levels by means of a polymerase-chain-reaction (PCR) assay; patients were eligible to participate in the study if they had no CMV DNA in plasma or if they had a low level that did not require treatment according to the site investigator's criteria. Patients were recruited from 27 centers in the United States.

Study Design

In each dose cohort, eligible patients were randomly assigned in a 3:1 ratio to receive CMX001 or matching placebo. Randomization was performed with the use of centralized 24-hour automated telephone voice-response and Web-based response systems. Randomization was stratified studywide according to the presence or absence of plasma CMV DNA within 7 days before dosing and according to the presence or absence of acute GVHD requiring glucocorticoid treatment at randomization. Study personnel, site pharmacists, and patients were unaware of the drug assignments throughout the study.

Three sequential cohorts were originally planned to test weekly doses of 40, 100, or 200 mg of CMX001. Given the initial findings with CMX001 under the individual patient investigational new drug emergency use program, in which the Food and Drug Administration (FDA) had authorized doses of CMX001 up to 350 mg twice weekly,[26,27] the protocol was amended in August 2010 to include two additional sequential cohorts in order to evaluate doses of 200 mg and 300 mg of CMX001 twice weekly. The amendment required further approval from the FDA and an independent data and safety monitoring board to proceed with each of these cohorts on the basis of available safety data; the data and safety monitoring board approved each dose escalation and could propose alternative dosing regimens. The protocol specified a subsequent expansion cohort to further evaluate a particular dose on the basis of safety and efficacy.

Patients received the study drug for 9 to 11 weeks, depending on the day of randomization after transplantation, such that the study drug was discontinued on week 13 after transplantation in patients who completed the study drug. Weekly measurement of plasma CMV DNA levels by means of a PCR assay was performed by a central laboratory while patients were receiving the study drug. If CMV disease developed or if patients required treatment with a drug against CMV infection because CMV DNA was detected in plasma or for another reason, the study drug was discontinued and patients were treated according to study-site practices. Patients had follow-up assessments 1, 2, and 4 weeks after discontinuing the study drug; patients who completed the full course of the study drug had an additional follow-up visit at week 8.

STUDY OVERSIGHT

The sponsor, Chimerix, designed the protocol with input from the first two authors and the last author. All investigators and central laboratories provided the study data. The first author and employees of the sponsor performed the study analyses and vouch for their integrity and validity, and they affirm that the study was conducted as specified in the protocol (available at NEJM.org). The first author wrote the manuscript with critical input from the other authors; all authors agreed to submit the manuscript for publication. The institutional review board at each center approved the study. All patients provided written informed consent before screening.

STUDY MONITORING AND ASSESSMENT OF OUTCOMES

Laboratory procedures are described in the Supplementary Appendix. Safety was assessed at least weekly by a review of adverse events graded according to the Common Terminology Criteria for Adverse Events,[28] physical-examination findings, and results of laboratory tests. Investigators used established criteria to diagnose and grade acute GVHD.[29] CMV disease was diagnosed according to published definitions.[30]

Once 16 patients in each cohort had received four doses of the study drug, an independent statistician who was aware of the study-drug assignments reviewed safety data twice monthly. Meetings of the data and safety monitoring board were held quarterly, after enrollment of 28 patients in a cohort, at the completion of drug administration and follow-up in each cohort, and if a safety signal was detected. On an ongoing basis, the chair of the data and safety monitoring board reviewed all serious adverse events that were considered to be possibly related to the study drug and was able to unblind the patient data to determine a potential relationship. Detailed safety, dose-escalation, and stopping rules are provided in the Supplementary Appendix. Since gastropathy and enteropathy were dose-limiting effects of CMX001 in toxicologic studies in animals,[11,15] the protocol provided guidance for monitoring and managing gastrointestinal adverse events.

The primary efficacy end point was the failure to prevent progressive CMV infection, defined as CMV disease or a plasma CMV DNA level greater than 200 copies per milliliter, detected at a central laboratory within 1 week after the last dose of the study drug. Study treatment (with either CMX001 or placebo) was considered to be successful if patients had an end-of-study plasma CMV DNA level of 200 copies per milliliter or less and did not have confirmed CMV disease, even if a particular weekly measurement was greater than 200 copies per milliliter during the study-drug administration and then decreased again. If patients discontinued the study drug to start treatment for CMV infection or for other reasons, but the plasma CMV DNA level was 200 copies per milliliter or less and CMV disease was not confirmed, treatment with CMX001 was considered to be successful.

Prespecified secondary end points included the occurrence of CMV infection or an increase in the plasma CMV DNA level in patients who were negative or positive for CMV DNA at baseline (either at screening or on the first day of study-drug administration), rates of and reasons for discontinuation of the study drug, the use of antiviral agents to treat CMV events, and trough levels of CMX001 and cidofovir. Safety end points included all adverse events, changes in laboratory values and electrocardiographic assessments, and death from any cause.

STATISTICAL ANALYSIS

Assuming a cumulative incidence of CMV events of 45 to 70%, a 50% reduction in events among patients exposed to CMX001, and a 15% dropout rate, we calculated that we would need to enroll at least 32 patients in each cohort, with 24 pa-

tients randomly assigned to CMX001 and 8 to placebo, to ensure a sufficient number of patients with data that could be evaluated. Once the 32nd patient had been enrolled and had started to receive the assigned study drug, any additional patients who had already provided informed consent could be enrolled in the same cohort until the next cohort opened for enrollment.

The primary efficacy and safety analyses were performed in the intention-to-treat population (all patients who underwent randomization, with the exception of nine patients from one study site who were excluded because of data-quality issues). The primary end point was analyzed with the use of Fisher's exact test. Patients who did not undergo clinical assessments or measurement of CMV DNA levels at the end of study-drug administration were considered to have had study-treatment failure (with either CMX001 or placebo) for the primary end-point analysis. Data on patients from all cohorts who were randomly assigned to placebo were pooled for comparisons with each CMX001 dose group.

RESULTS

STUDY POPULATION

From December 11, 2009, to June 20, 2011, a total of 305 patients consented to participate in the study in five sequential cohorts (Fig. S1 in the Supplementary Appendix). Sixty-six of these patients were found to be ineligible before randomization. One study site and its 9 patients who underwent randomization (in cohorts 1 and 2) were excluded from all analyses because of data-quality issues.

Deviations from eligibility criteria were documented in 17 of 230 patients (7%): the first dose of the study drug was administered more than 30 days after transplantation in 8 patients (3%) (7 patients assigned to CMX001 and 1 patient assigned to placebo); 7 patients (3%) had a body-mass index (the weight in kilograms divided by the square of the height in meters) higher than 35 (3 patients assigned to CMX001 and 4 patients assigned to placebo) (this criterion was removed with the August 2010 protocol amendment); and 2 patients (1%), both of whom were assigned to CMX001, had serum aminotransferase levels that were higher than the maximum value specified for eligibility. All 17 patients were included in the analyses.

Reviews of data in the first three cohorts, performed by the data and safety monitoring board, were uneventful. A significant increase in serious adverse events of diarrhea and reported gastrointestinal acute GVHD in cohort 4, which consisted of patients who received CMX001 at a dose of 200 mg twice weekly or placebo, led to a prompt review by the data and safety monitoring board. Enrollment in this cohort was terminated, and the dose was decreased to 200 mg once weekly for the nine patients in the cohort who continued to receive the study drug. The data and safety monitoring board recommended that an additional cohort be assessed to compare CMX001 at a dose of 100 mg twice weekly with placebo. Investigators were provided with additional guidance regarding documentation and management of gastrointestinal adverse effects (see the Supplementary Appendix).

The baseline characteristics of the patients are shown in Table S2 in the Supplementary Appendix. The study-dose groups were similar with respect to age, sex, race and ethnic group, weight, underlying disease, donor type and hematopoietic-cell source, and the proportion of patients who received antithymocyte globulin. There were some imbalances among the groups with respect to the proportion of patients who underwent myeloablative conditioning (which was higher in the placebo group and in the groups that received 200 mg of CMX001 weekly and 100 mg of CMX001 twice weekly), the proportion of adult HLA-mismatched donors (which was higher in the groups that received CMX001 at a dose of either 100 mg or 200 mg twice weekly), donor CMV seropositivity (which ranged from 33 to 56%), and the prophylactic use of tacrolimus (which ranged from 64 to 93%).

DRUG EXPOSURE AND PLASMA CONCENTRATIONS

Patients began to receive the study drug at a median of 24 days (range, 14 to 36) after transplantation and continued to receive the study drug for a median of 9 weeks (range, 1 to 11). For most of the patients in each CMX001 group, weekly trough concentrations of CMX001 in plasma were below or near the lower limit of quantification for the assay, without evidence of accumulation. Trough concentrations of cidofovir in plasma were detectable, but they remained low in patients receiving once-weekly CMX001. Higher weekly trough concentrations of cidofovir were

observed after twice-weekly administration of CMX001; mean weekly trough values were between 10 and 17 ng per milliliter after receipt of 100 mg twice weekly and between 10 and 30 ng per milliliter after receipt of 200 mg twice weekly (see the Supplementary Appendix).

PRIMARY END POINT

Patients who received CMX001 at doses of 100 mg weekly or higher had fewer CMV events than patients who received placebo (Table 1); the number of CMV events was significantly reduced with CMX001 at a dose of 100 mg twice weekly, as compared with placebo (10% vs. 37%; risk difference, −27 percentage points; 95% confidence interval [CI], −42 to −12; P=0.002). The results of analyses according to stratification criteria (the presence or absence of acute GVHD and CMV DNA in plasma at baseline) were consistent with the results of the primary analysis (see the Supplementary Appendix). On the basis of these results, evaluation of a planned expansion cohort was not conducted.

SECONDARY EFFICACY END POINTS

CMV disease was reported in nine patients (4%): two (3%) in the placebo group and seven in the CMX001 groups (three received 40 mg per week [12%], three received 100 mg per week [11%], and one received 100 mg twice weekly [2%]). In six of these nine patients, CMV DNA was detected in plasma at baseline (range, 200 to 5300 copies per milliliter). When CMV disease or the occurrence or progression of CMV infection (defined as a plasma CMV DNA level >1000 copies per milliliter) was evaluated as a secondary end point, a dose–response effect with CMX001 administration was observed (Table 2). The results were similar when a time-to-event analysis was performed (Fig. S2 in the Supplementary Appendix). Among patients who had CMV DNA in plasma at baseline, CMX001 at a dose of 200 mg per week or higher controlled plasma levels of CMV DNA in some patients. In patients who did not have CMV DNA in plasma at baseline, CMX001 at a dose of 100 mg per week or higher significantly reduced the likelihood of the development of a plasma CMV DNA level higher than 1000 copies per milliliter; this level of CMV DNA was not reported in any patient who received CMX001 at a dose of 100 mg twice weekly during study-drug administration.

Table 1. Primary Efficacy End Point in the CMX001 Dose Groups as Compared with the Placebo Group (Intention-to-Treat Population).*

Study Group	Patients with CMV Events	Absolute Risk Difference	P Value†
	no./total no. (%)	*percentage points (95% CI)*	
Placebo	22/59 (37)	—	—
CMX001			
40 mg weekly	13/25 (52)	15 (−8 to 38)	0.23
100 mg weekly	6/27 (22)	−15 (−35 to 5)	0.22
200 mg weekly	12/39 (31)	−6 (−26 to 13)	0.53
200 mg twice weekly	7/30 (23)	−14 (−34 to 6)	0.24
100 mg twice weekly	5/50 (10)	−27 (−42 to −12)	0.002

* The primary efficacy end point was a cytomegalovirus (CMV) event, defined as CMV disease or a level of CMV DNA greater than 200 copies per milliliter at the end of treatment assessment. If a CMV DNA result obtained by means of a polymerase-chain-reaction assay was missing at the end-of-treatment assessment, the patient was considered to have had treatment failure. CI denotes confidence interval.

† P values were calculated with the use of Fisher's exact test.

At randomization, 15 patients (9 patients in the CMX001 groups and 6 patients in the placebo group) had acute GVHD requiring systemic glucocorticoids. Among 7 patients in this randomization stratum who received CMX001 at doses of 100 mg per week or higher, only 1 (14%) had a CMV event, as compared with 4 of 6 patients who received placebo (67%).

SAFETY ANALYSES

In the pooled group of patients who received placebo and the groups of patients who received CMX001 at doses of either 40 mg or 100 mg once weekly, plasma CMV DNA levels requiring treatment were the most frequent reason for discontinuing the study drug, whereas adverse events were the primary reason for discontinuing the study drug in the other dose groups (Fig. S1 in the Supplementary Appendix). Common clinical adverse events (Table 3) were gastrointestinal in nature and similar in the placebo group and the cohorts of patients who received either 40, 100, or 200 mg of CMX001 weekly, with the exception of an increased incidence of nausea in the group that received 200 mg of CMX001 once weekly (28%, vs. 10% in the placebo group). In the group that received 200 mg of CMX001 twice weekly, diarrhea was very common (reported in 70% of patients) and was often serious (in 33%) (Table 4). Diarrhea and other gastrointestinal adverse events

Table 2. Effect of Various Doses of CMX001 versus Placebo on Plasma CMV DNA Levels during Study-Drug Administration.

Study Group	All Patients*			Patients with Detectable CMV DNA at Baseline			Patients without Detectable CMV DNA at Baseline		
	Positive for CMV DNA during Drug Administration†	Absolute Risk Difference	P Value‡	Positive for CMV DNA during Drug Administration†	Absolute Risk Difference	P Value‡	Positive for CMV DNA during Drug Administration†	Absolute Risk Difference	P Value‡
	no./total no. (%)	*percentage points (95% CI)*		*no./total no. (%)*	*percentage points (95% CI)*		*no./total no. (%)*	*percentage points (95% CI)*	
Placebo	25/59 (42)	—	—	10/12 (83)	—	—	15/47 (32)	—	—
CMX001									
40 mg weekly	10/25 (40)	−2 (−25 to 21)	1.0	6/7 (86)	3 (−31 to 36)	1.0	4/18 (22)	−10 (−33 to 14)	0.55
100 mg weekly	6/27 (22)	−20 (−40 to 0)	0.09	4/4 (100)	17 (−4 to 38)	1.0	2/23 (9)	−23 (−41 to −6)	0.04
200 mg weekly	7/39 (18)	−24 (−42 to −7)	0.02	5/10 (50)	−33 (−71 to 4)	0.17	2/29 (7)	−25 (−41 to −9)	0.01
200 mg twice weekly	2/30 (7)	−35 (−51 to −20)	<0.001	2/8 (25)	−58 (−95 to −22)	0.02	0/22	−32 (−45 to −19)	0.002
100 mg twice weekly	4/50 (8)	−34 (−49 to −20)	<0.001	4/9 (44)	−39 (−78 to 0)	0.16	0/41	−32 (−45 to −19)	<0.001

* The number of patients with CMV DNA in plasma differed from the number in the analysis of the primary end point because some patients had CMV DNA levels greater than 1000 copies per milliliter while the study drug was being administered that resolved by the end of study-drug administration, including some patients who received placebo. Patients with missing values at the end of study-drug administration were not considered to have had treatment failure and were not excluded from this analysis.

† Positivity for CMV DNA in plasma was defined as a plasma level of CMV DNA greater than 1000 copies per milliliter. Patients with CMV disease were classified as positive for CMV DNA in plasma, regardless of the level.

‡ P values were calculated with the use of Fisher's exact test for the comparison with the pooled results in patients who received placebo.

were frequently reported in the group that received 100 mg of CMX001 twice weekly, but these events were milder in nature and did not lead to an increased rate of discontinuation of the study drug; 16 patients (32%) did not receive a median of 2 doses of CMX001 (range, 1 to 3) because of gastrointestinal symptoms, and 13 patients were able to resume the drug at the same dose. The frequency of relapse of hematologic disease and overall mortality were similar among all dose groups.

The overall incidence of reported acute GVHD was also increased in the groups of patients that received 100 or 200 mg of CMX001 twice weekly, but this was due to an increased incidence of gastrointestinal acute GVHD without appreciable proportional increases in the frequency or severity of acute skin or liver GVHD (Table 3). These patients received systemic glucocorticoids more frequently than patients in the other CMX001 dose groups and the placebo group (see the Supplementary Appendix), which may explain the increased frequency of oral candidiasis (Table 3). As part of the review by the data and safety monitoring board after the discontinuation of the cohort for the evaluation of 200 mg of CMX001 twice weekly, two pathologists with expertise in gastrointestinal GVHD, who were unaware of the study-group assignments, reassessed gastrointestinal-biopsy specimens obtained from 25 patients. The histopathological findings in these biopsy specimens were compatible with gastrointestinal acute GVHD, with no differences in the distribution of severity among the various CMX001 dose groups. Individual investigators reported that the clinical severity of diarrhea was higher than the histopathological degree of acute gastrointestinal GVHD in several patients who received 200 mg of CMX001 twice weekly.

Elevated alanine aminotransferase levels were more common among patients who received more than 200 mg of CMX001 per week than among those who received lower doses or placebo, but these elevations were not associated with increases in levels of bilirubin or aspartate aminotransferase. There were no consistent differences in the frequency of anemia, thrombocytopenia, or neutropenia during study-drug administration among the various dose groups (Table 3). There was no evidence of increased nephrotoxicity or ocular toxicity with increasing doses of CMX001.

ANALYSIS OF ANTIVIRAL RESISTANCE

Plasma CMV DNA was detected during study-drug administration in 30 patients who received

Table 3. Adverse Events (Reported in ≥10% of Patients in the Intention-to-Treat Population).*

Variable	Placebo (N=59)	CMX001, 40 mg Weekly (N=25)	CMX001, 100 mg Weekly (N=27)	CMX001, 200 mg Weekly (N=39)	CMX001, 200 mg Twice Weekly (N=30)	CMX001, 100 mg Twice Weekly (N=50)
			number of patients (percent)			
Patients with ≥1 adverse event	58 (98)	25 (100)	27 (100)	39 (100)	30 (100)	50 (100)
Clinical adverse events†						
Diarrhea	16 (27)	3 (12)	8 (30)	13 (33)	21 (70)	26 (52)
Nausea	6 (10)	6 (24)	5 (19)	11 (28)	11 (37)	17 (34)
Vomiting	11 (19)	2 (8)	6 (22)	6 (15)	8 (27)	22 (44)
Abdominal pain	4 (7)	4 (16)	2 (7)	5 (13)	11 (37)	13 (26)
Gastroesophageal reflux	1 (2)	2 (8)	4 (15)	2 (5)	2 (7)	0
Acute GVHD	17 (29)	8 (32)	9 (33)	14 (36)	24 (80)	33 (66)
Gastrointestinal‡	9 (15)	5 (20)	10 (37)	13 (33)	24 (80)	28 (56)
Cutaneous	17 (29)	8 (32)	13 (48)§	15 (38)	14 (47)	14 (28)
Hepatic	0	1 (4)	1 (4)	1 (3)	8 (27)	6 (12)
Peripheral edema	6 (10)	3 (12)	5 (19)	3 (8)	3 (10)	8 (16)
Staphylococcal bacteremia	2 (3)	0	1 (4)	1 (3)	1 (3)	6 (12)
Oral candidiasis	1 (2)	0	0	2 (5)	1 (3)	6 (12)
Fatigue	9 (15)	1 (4)	3 (11)	5 (13)	0	9 (18)
Insomnia	1 (2)	2 (8)	2 (7)	5 (13)	2 (7)	9 (18)
Anxiety	3 (5)	0	3 (11)	3 (8)	2 (7)	3 (6)
Disease relapse	8 (14)	5 (20)	1 (4)	3 (8)	1 (3)	5 (10)
Laboratory adverse events¶						
ALT >3× upper limit of normal range	9 (15)	3 (12)	2 (7)	10 (26)	12 (40)	16 (32)
Bilirubin >1.5× upper limit of normal range	0	1 (4)	1 (4)	3 (8)	2 (7)	4 (8)
Creatinine >1.0 × upper limit of normal range	27 (46)	10 (40)	21 (78)	18 (46)	12 (40)	26 (52)
Absolute neutrophil count <1000/mm^3	6 (10)	2 (8)	5 (19)	9 (23)	4 (13)	13 (26)
Platelet count <50,000/mm^3	18 (31)	13 (52)	10 (37)	20 (51)	16 (53)	13 (26)
Hemoglobin <8 g/dl	6 (10)	1 (4)	2 (7)	5 (13)	2 (7)	5 (10)
Patients with adverse events leading to withdrawal of study drug	27 (46)	15 (60)	9 (33)	15 (38)	18 (60)	18 (36)

* ALT denotes alanine aminotransferase, and GVHD graft-versus-host disease.
† Data shown are for all clinical adverse events as reported by each site investigator.
‡ The occurrence and severity of organ-specific acute GVHD were documented on the basis of weekly assessments.
§ Reports of rashes were attributed to acute GVHD in the weekly assessments in this cohort, but they were not considered to be acute GVHD in the safety database.
¶ Abnormal values that occurred at any time during study-drug administration are shown.

CMX001 at doses of 100 mg per week or higher. Sequencing showed an R1052C mutation[31,32] in the UL54 gene in specimens obtained from 3 patients; one of these mutations was present before CMX001 exposure. No mutations in *UL97* were detected. With the exception of 1 patient, who received preemptive treatment with cidofovir, all these patients had a response to subsequent preemptive treatment against CMV disease after discontinuation of CMX001.

Table 4. Serious Adverse Events (in ≥5% of Patients in the Intention-to-Treat Population).

Variable	Placebo (N=59)	CMX001, 40 mg Weekly (N=25)	CMX001, 100 mg Weekly (N=27)	CMX001, 200 mg Weekly (N=39)	CMX001, 200 mg Twice Weekly (N=30)	CMX001, 100 mg Twice Weekly (N=50)
	number of patients (percent)					
Patients with ≥1 serious adverse event	27 (46)	12 (48)	10 (37)	19 (49)	21 (70)	30 (60)
Serious adverse event						
Acute GVHD	4 (7)	1 (4)	2 (7)	6 (15)	12 (40)	15 (30)
Diarrhea	1 (2)	0	0	1 (3)	10 (33)	5 (10)
Fever	7 (12)	0	0	3 (8)	1 (3)	2 (4)
Pneumonia	0	0	2 (7)	0	1 (3)	4 (8)
Patients with adverse event leading to death	5 (8)	2 (8)	0	3 (8)	4 (13)	5 (10)

EXPLORATORY ANALYSES

Given the range of body weights (40.6 to 146.9 kg) and doses of CMX001 (40 to 400 mg per week), we explored whether weekly weight-adjusted dosing was associated with the development of gastrointestinal adverse effects, which were defined as the composite adverse outcome of diarrhea of grade 2 or higher[28] or any degree of acute gastrointestinal GVHD.[29] CMX001 doses greater than 3.5 mg per kilogram of body weight per week were associated with significantly increased gastrointestinal adverse effects. In multivariable analyses of gastrointestinal adverse effects, the variable of the weight-adjusted CMX001 dose was stable and unconfounded by other known risk factors for acute GVHD or diarrhea after transplantation (see Section H and Table S10 in the Supplementary Appendix). Similarly, higher trough levels of cidofovir, but not CMX001, 1 and 2 weeks after the initiation of CMX001 were associated with an increased risk of subsequent gastrointestinal adverse effects (see Section I and Tables S11 and S12 in the Supplementary Appendix).

DISCUSSION

We found that in patients who had received allogeneic hematopoietic-cell transplants, CMX001 administered orally at doses of 100 mg once weekly or higher beginning after engraftment through week 13 after transplantation (approximately day 90) reduced the incidence of CMV events, as compared with placebo. This reduction in CMV events was significantly lower when CMX001 was administered at a dose of 100 mg twice weekly. This dose was particularly favorable in the group of patients who did not have CMV DNA in plasma at baseline: CMV disease or a plasma CMV DNA level greater than 1000 copies per milliliter did not occur in any patients (0 of 41) who received 100 mg twice weekly, whereas these events were reported in 15 of 47 patients (32%) who received placebo (risk difference, −32 percentage points; 95% CI, −45 to −19; P<0.001).

We found no evidence of increased myelosuppression or nephrotoxicity with CMX001; these are common toxic effects when patients who have undergone transplantation receive ganciclovir, valganciclovir, foscarnet, or cidofovir. The safety and side-effect profile of CMX001 in our study provides support for further development of this agent for the prevention of CMV disease in patients with hematopoietic-cell transplants, who frequently have myelosuppression and nephrotoxic effects after transplantation.

Animal models[11,15] have previously shown the potential for gastrointestinal adverse effects of CMX001; these effects are probably caused by elevated intracellular cidofovir concentrations in enterocytes.[15] However, data on the side-effect profile of CMX001 among recipients of hematopoietic-cell transplants have been lacking. In a study involving healthy volunteers, no gastrointestinal injury was noted with CMX001 at doses of up to 2 mg per kilogram.[11]

In our study, a dose of 200 mg of CMX001 twice weekly was associated with an increased frequency and severity of diarrhea and other

gastrointestinal symptoms and was dose-limiting in adults who had undergone hematopoietic-cell transplantation. Since diarrhea is also a common manifestation of acute GVHD, many patients received glucocorticoids empirically, and acute GVHD was captured as an adverse event to document the indication for glucocorticoid use. Although the proportion of patients at high risk for acute GVHD (e.g., those with HLA-mismatched unrelated donors) was higher in the cohorts that received 100 or 200 mg of CMX001 twice weekly than in the cohorts that received lower doses or placebo, the analysis of data from weekly assessments for acute GVHD showed that the excess acute GVHD was driven by an increased frequency of diarrhea in the higher-dose groups (Table 3). A review of available gastrointestinal-biopsy specimens did not show an increased proportion of patients in the 200-mg twice-weekly cohort who had findings consistent with acute GVHD. These observations suggest that the excess reports of acute GVHD were due to CMX001-related gastrointestinal adverse effects. In view of these results, a strict management plan for gastrointestinal adverse effects was instituted for the final cohort of patients who received 100 mg of CMX001 twice weekly. Although diarrhea was more frequent in these patients than in those who received placebo, it was milder, and most patients were able to complete the scheduled doses of the study drug.

The wide range of weight-based CMX001 doses administered allowed us to explore its association with gastrointestinal adverse effects. CMX001 doses higher than 3.5 mg per kilogram per week were associated with substantial rates of diarrhea, as were higher trough levels of cidofovir. Overall, CMX001 at a dose of 100 mg twice weekly was well tolerated and effective. In addition to dose interruptions, weight-based adjustments and monitoring of trough levels of cidofovir could be used as additional strategies to maximize the safety of CMX001 in the future. CMX001 at a dose of 100 mg twice weekly probably maintained cidofovir levels above CMV inhibitory concentrations more reliably than once-weekly doses and may explain its greater effectiveness than a dose of 200 mg once weekly. Our findings require confirmation in phase 3 trials involving patients undergoing hematopoietic-cell transplantation and other populations at risk for CMV disease.

Presented in part at the 2012 BMT Tandem Meetings of the Center for International Blood and Marrow Transplant Research and the American Society for Blood and Marrow Transplantation, San Diego, CA, February 1–5, 2012.

Supported by Chimerix.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the members of the data and safety monitoring board (John Zaia, M.D., chair, Andrew Badley, M.D., Sunwen Chou, M.D., Michael Ison, M.D., Saeid Khansarina, M.D., and Suzanne Hacket, M.S.) and the medical monitor, James Alexander, M.D.

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