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The Effect of Cidofovir on Adenovirus Plasma DNA Levels in Stem Cell Transplantation Recipients without T Cell Reconstitution

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A B S T R A C T

Cidofovir is frequently used to treat life-threatening human adenovirus (HAdV) infections in immunocompromised children after hematopoietic stem cell transplantation (HSCT). However, the antiviral effect irrespective of T cell reconstitution remains unresolved. Plasma HAdV DNA levels were monitored by real-time quantitative PCR during 42 cidofovir treatment episodes for HAdV viremia in 36 pediatric allogeneic HSCT recipients. HAdV load dynamics were related to T and natural killer (NK) cell reconstitution measured by flow cytometry. To evaluate the in vivo antiadenoviral effect of cidofovir, we focused on 20 cidofovir treatment episodes lacking concurrent T cell reconstitution. During 2 to 10 weeks of follow-up in the absence of T cells, HAdV load reduction (n = 7) or stabilization (n = 8) was observed in 15 of 20 treatments. Although HAdV load reduction was always accompanied by NK cell expansion, HAdV load stabilization was measured in 2 children lacking both T and NK cell reconstitution. In cases with T cell reconstitution, rapid HAdV load reduction (n = 14) or stabilization (n = 6) was observed in 20 of 22 treatments. In the absence of T cells, cidofovir treatment was associated with HAdV viremia control in the majority of cases. Although the contribution of NK cells cannot be excluded, cidofovir has the potential to mediate HAdV load stabilization in the time pending T cell reconstitution.

INTRODUCTION

Human adenoviruses (HAdV) are nonenveloped double-stranded DNA viruses. Currently, more than 50 serotypes have been described. In healthy individuals, HAdV infections cause self-limiting infections, such as conjunctivitis, upper respiratory tract, urinary tract, or gastrointestinal infections [1]. In pediatric hematopoietic stem cell transplantation (HSCT) recipients, HAdV reactivations or primary infections can progress to viremia and disseminated disease. It is broadly accepted that T cells are essential for the protection from and clearance of HAdV viremia [2-4]. However, in the absence of T cell surveillance, the mortality of HAdV viremia is high because of progression to HAdV-related multiorgan failure [4-9].

The adoptive transfer of donor-derived adenovirus-specific T cells is a promising treatment [3-10], but it is not available in all transplantation centers. Therefore, preemptive pharmacological treatment is of great importance for the majority of patients with HAdV viremia. To this end, ribavirin and cidofovir have been explored. The evidence for a beneficial effect of ribavirin is limited to case reports and ribavirin could not prevent the progression of HAdV viremia in the absence of lymphocyte reconstitution [11]. Cidofovir (Vistide, Gilead Sciences Inc, Foster City, CA), a monophosphate nucleotide analogue with in vitro antiviral activity against different HAdV strains [12-15], is a widely used antiviral agent for HAdV infections after HSCT.

A number of studies have addressed the antiadenoviral effect of cidofovir, but results are highly variable, with treatment successes ranging from 24% to 98% [16-22]. The discrepancies in cidofovir's effectiveness might be related to the fact that HAdV viremia and cidofovir treatment generally occur during the critical phase of lymphocyte reconstitution after HSCT [4-7]. When T cell reconstitution is not taken into

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account, this will result in a biased evaluation of the effect of cidofovir. An unbiased evaluation of the in vivo anti-adenoviral effect of cidofovir is required because the use of cidofovir is associated with considerable nephrotoxicity [21-24].

In this study, we aimed to evaluate the in vivo antiviral effect of cidofovir in patients with HAdV viremia after pediatric HSCT without the confounding effect of concomitant T cell reconstitution. Hereto, we monitored the change of plasma HAdV DNA levels during cidofovir treatment, focusing on cidofovir treatments in the absence of T cell reconstitution.

METHODS

Ethics Statement

Transplantations were performed according to European Society for Blood and Marrow Transplantation guidelines. Peripheral blood samples were routinely obtained. Data were analyzed after approval by the institutional review board (protocol P01.028 and P02.099). Informed consent was provided by the patient and/or a parent or guardian.

Patients and Cidofovir Treatment

Between January 2003 and December 2012, 321 children received 363 transplantations at the pediatric HSCT unit of the Leiden University Medical Center. Thirty-nine HSCT recipients were treated with cidofovir for HAdV viremia. One patient was not evaluable, as she died from pre-existent neurodegenerative disease within the first 2 weeks of treatment. From 2 other patients, no follow-up of the plasma HAdV DNA levels (HAdV load) was available, leaving 36 evaluable HSCT recipients. Six patients received 2 separate cidofovir treatment episodes for the same HAdV viremia and were analyzed twice. In total, 42 cidofovir treatment episodes were analyzed (Table 1).

In general, cidofovir treatment was initiated preemptively when the HAdV load was 3 log (1000) viral DNA copies/mL (c/mL) at 2 consecutive time points [6]. Reasons to stop treatment were the following: >1 log HAdV load reduction accompanied by a dynamic increase in lymphocyte numbers, HAdV load stabilization at levels below 3 log c/mL, nephrotoxicity, or treatment failure. Cidofovir was administered intravenously thrice weekly at 1 mg/kg body weight [18]. Supportive care consisted of hyperhydration with intravenous saline (3 L/m^2 body surface per 24 hours) and oral probenecid (25 mg/kg) at −3, +1, and +8 hours from the start of cidofovir infusion [23]. Other antiviral drugs were discontinued during cidofovir treatment. See Supplemental Methods and Supplemental Table S1 for a detailed description of patient inclusion and HSCT characteristics.

Monitoring of HAdV Plasma DNA Levels and Lymphocyte Reconstitution

HAdV reactivations were routinely monitored through twice weekly plasma screening for viral DNA by real time quantitative PCR as described previously [25]. The lower level of detection of this assay was 1.7 log viral DNA c/mL. Monitoring was initiated at day +3 after HSCT and continued until T cells reached 300 cells/μL of peripheral blood. To monitor immune reconstitution, peripheral blood white blood cell counts, including full leukocyte differentiations, were performed 2 to 3 times per week. The lower level of detection of lymphocytes was 10 to 20 cells/μL. Flow cytometric analysis was performed weekly to quantify (natural killer) NK and T cell reconstitution as described in the Supplemental Methods and Supplemental Table S2. T cells were defined as CD3$^+$ cells in the CD45$^+$ CD33/CD235a/CD14$^-$ lymphocyte gate and NK cells were defined as CD3$^-$ CD56$^+$ cells in the lymphocyte gate.

Definitions of HAdV Load Dynamics and Lymphocyte Reconstitution during Cidofovir Treatment

HAdV load dynamics was evaluated during cidofovir treatment. Reduction and increase were defined as a ≥ 1 log (10-fold) change of the HAdV load. Stabilization was defined as a <1 log change in HAdV load. Both reduction and stabilization of the HAdV load were regarded as viremia control. To discriminate between the (potential) effect of T cell reconstitution and cidofovir treatment on HAdV load dynamics, we applied the low threshold of 50 T cells/μL of peripheral blood to define T cell reconstitution.

We first analyzed HAdV load dynamics in 42 cidofovir treatments without (n = 20) and with (n = 22) T cell reconstitution in the first 2 weeks after treatment initiation. Subsequently, we focused on the 20 cidofovir treatments in the absence of concomitant T cell reconstitution. In this group, the HAdV load change was evaluated between treatment initiation and 1 week after the last dose of cidofovir. In cases with T cell reconstitution before the end of treatment (n = 6), follow-up was stopped 1 week before T cell numbers reached 50 cells/μL to exclude the confounding effect of T cells on the HAdV load.

Table 1
Patient and Cidofovir Treatment Characteristics

Characteristics	Value
Patient characteristics (n = 36)	
Age, median (range), yr	4.5 (.5-18)
HSCT indication	
Primary immunodeficiency	8 (22%)
Benign hematological disorder	12 (33%)
Hematological malignancy	16 (44%)
Conditioning	
Reduced intensity	6 (17%)
Myeloablative	30 (83%)
Donor type	
Identical related donor	2 (6%)
Other related donor	7 (19%)
Matched unrelated donor	27 (75%)
Graft source	
Bone marrow, T cell−replete	16 (44%)
Bone marrow, T cell−depleted	1 (3%)
PBSC, T cell−replete	2 (6%)
PBSC, T cell−depleted	8 (22%)
Cord blood	9 (25%)
Serotherapy	
Antithymocyte globulin	23 (64%)
Alemtuzumab	13 (36%)
GVHD prophylaxis	
None	4 (11%)
CsA	3 (8%)
CsA + methotrexate	18 (50%)
CsA + methylprednisolone	9 (25%)
CsA + MMF	2 (6%)
Acute GVHD ≥ grade II	5 (14%)
Cidofovir treatment	
1 Episode	30 (83%)
2 Episodes	6 (17%)
First day plasma HAdV DNA level >1.7 log c/mL*, median (range)	20 (3-96)
First day plasma HAdV DNA level 2x > 3 log c/mL*, median (range)	28 (8-117)
First day of first cidofovir treatment episode*, median (range)	29 (7-121)
Final outcome	
HAdV clearance	27 (75%)
Death from HAdV/MOF	6 (17%)
Death from other cause	3 (8%)
Cidofovir-treatment episodes (n = 42)	
Day start cidofovir*, median (range)	31 (7-214)
Plasma HAdV DNA level at start, median (range), log c/mL	4.1 (1.7-6.5)
Treatment duration, median (range), d	16 (1-99)

PBSC indicates peripheral blood stem cells; GVHD, graft-versus-host disease; CsA, cyclosporin A; MMF, mycophenolate mofetil; HAdV/MOF, HAdV-related multiorgan failure.
Data presented are n (%) unless otherwise indicated.
* Day after HSCT.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 20 (IBM SPSS Inc., Chicago, IL). GraphPad Prism 6.00 (GraphPad Software, San Diego, CA) was used to construct figures. Because data did not follow Gaussian distribution, the Mann-Whitney U test was used for the analysis of numerical parameters. Pearson's chi-square tests were used for analysis of categorical parameters.

RESULTS

Characteristics of Cidofovir-treated Patients

The effect of cidofovir treatment on the HAdV load was evaluated in 36 HSCT recipients, of whom the characteristics are summarized in Table 1 and Supplemental Table S1. Six patients received a second cidofovir treatment episode

Table 2
Details of human adenovirus viremia, cidofovir treatment, lymphocyte reconstitution and HAdV load dynamics

	UPN	HAdV Viremia					CDV Treatment			Lymphocyte Reconst.			HAdV Load Change[#]		
		First Day PCR+[*]	Day PCR 2× >3 Log[*]	Load at Start CDV[†]	Sero-type	Species	Treatment No.	Day Start[*]	Duration[‡]	T Cell[§]	NK Cell[‖]	Steroids[¶]	Day 14[‡]	Last Day Follow-up[‡,**]	Reason Stop Follow-up[**]
T cells <50 cells/μL	543.1a	14	19	5.9	31	A	1	28	9	-	+	-	↓	↓ (14)	Stop CDV
14 days after start	549.1	19	30	3.8	2	C	1	30	7	-	+	-	↓	↓ (14)	Stop CDV
cidofovir	555.1	8	23	3.6	31	A	1	28	7	-	+	-	↓	↓ (14)	Stop CDV
	808.1	12	23	4.2	?	?	1	23	21	-	+	-	↓	↓ (28)	Stop CDV
	600.1a	26	29	3.8	1	C	1	30	49	-	+	+	=	↓ (56)	Stop CDV
	616.1	13	20	4.4	3,31	B,C	1	17	27	-	+	+	=	↓ (28)	T cell ≥50
	721.1	39	-	2.4	?	?	1	44	37	-	+	+	=	↓ (42)	Stop CDV
	514.3	20	27	4.4	2	C	1	31	14	-	+	-	=	= (21)	Stop CDV
	543.1b	n.a.	n.a.	5.7	2,31	A,C	2	55	41	-	-	-	=	= (35)	T cell ≥50
	554.2	17	53	5.4	1	C	1	53	8	-	-	-	=	= (14)	Stop CDV
	560.1	27	48	4.9	5	C	1	48	42	-	+	+	=	= (35)	T cell ≥50
	625.2	43	-	2.3	1	C	1	59	9	-	+	-	=	= (14)	Stop CDV
	648.2	70	75	4.3	31	A	1	78	50	-	-	-	=	= (14)	T cell ≥50
	681.1a	24	34	2.9	?	?	1	40	7	-	-	-	=	= (14)	Stop CDV
	545.1b	n.a.	n.a.	2.6	5,31	A,C	2	39	64	-	-	+	=	↑ (70)	Stop CDV
	551.1	19	33	2.7	31	A	1	21	47	-	+	-	↑	= (56)	Stop CDV
	613.1	40	46	4.1	5	C	1	44	17	-	-	+	↑	↑ (21)	Stop CDV
	719.1	5	8	4.0	31	A	1	7	28	-	+	-	↑	↑ (14)	T cell ≥50
	799.1	25	39	2.1	31	A	1	28	40	-	-	-	↑	↑ (14)	T cell ≥50
	804.1	7	16	3.8	31	A	1	17	11	-	+	-	↑	↑ (21)	Stop CDV
T cells ≥50 cells/μL	558.1	14	19	1.7	31	A	1	23	7	+	+	-	c	n.a.	T cell ≥50
14 days after start	586.1	61	75	4.5	1	C	1	77	12	+	-	-	c	n.a.	T cell ≥50
cidofovir	648.1	6	-	2.8	31	A	1	17	14	+	+	+	c	n.a.	T cell ≥50
	681.1b	n.a.	n.a.	2.3	?	?	2	95	3	+	-	-	c	n.a.	T cell ≥50
	697.1	5	23	4.3	?	?	1	21	12	+	-	-	c	n.a.	T cell ≥50
	732.1	10	20	4.4	?	?	1	19	1	+	+	-	c	n.a.	T cell ≥50
	787.1b	n.a.	n.a.	2.4	?	?	2	112	21	+	-	-	c	n.a.	T cell ≥50
	816.1	5	22	4.2	31	A	1	23	11	+	+	-	c	n.a.	T cell ≥50
	817.1	34	37	3.3	?	?	1	38	10	+	-	-	c	n.a.	T cell ≥50
	515.2	12	16	6.5	1,2	C	1	16	24	+	+	+	↓	n.a.	T cell ≥50
	587.1	20	-	4.1	31	A	1	31	7	+	+	-	↓	n.a.	T cell ≥50
	594.1b	n.a.	n.a.	6.0	1	C	2	214	73	+	-	-	↓	n.a.	T cell ≥50
	600.1b	n.a.	n.a.	4.9	1	C	2	100	10	+	-	-	↓	n.a.	T cell ≥50
	823.1	20	23	4.9	?	?	1	22	12	+	+	-	↓	n.a.	T cell ≥50
	515.1	65	72	4.3	2	C	1	79	48	+	-	+	=	n.a.	T cell ≥50
	545.1a	3	10	3.9	5	C	1	10	15	+	-	-	=	n.a.	T cell ≥50
	594.1a	21	28	3.9	1	C	1	32	80	+	+	+	=	n.a.	T cell ≥50
	597.1	5	12	4.2	31	A	1	8	99	+	-	+	=	n.a.	T cell ≥50
	686.2	87	101	3.6	2	C	1	107	15	+	+	-	=	n.a.	T cell ≥50
	787.1a	18	20	3.1	1	C	1	20	28	+	-	+	=	n.a.	T cell ≥50
	634.1	96	117	5.4	2	C	1	121	36	+	-	+	↑	n.a.	T cell ≥50
	671.1	52	66	2.3	1	C	1	48	70	+	-	+	↑	n.a.	T cell ≥50

UPN indicates unique patient number; n.a., not applicable; ?, not analyzed; CDV, cidofovir.
Treatments are ordered based on T cell reconstitution and HAdV load change. The decimal denotes the first, second or third HSCT and the letter indicates the cidofovir treatment episode.

[*] Days after hematopoietic stem cell transplantation (HSCT).
[†] Plasma HAdV DNA levels in log copies per millilter.
[‡] Days from start cidofovir treatment.
[§] T cell numbers in peripheral blood ≥50 cells/μL within 14 days after treatment initiation.
[‖] ≥5-fold expansion of NK cell number in peripheral blood.
[¶] Prednisone ≥1 mg/kg body weight during cidofovir treatment.
[#] Reduction (↓)/Increase (↑): plasma HAdV DNA levels changed ≥1 log c/mL after initiation of cidofovir treatment. Stabilization (=): <1 log c/mL change of HAdV load. Clearance (c): plasma HAdV DNA levels below lower limit of detection at 2 consecutive time points.
[**] Follow-up was stopped at 1 week after the last dose of cidofovir or 1 week before T cell numbers reached 50 cells/μL.

(Supplemental Figure S1), resulting in 42 evaluable cidofovir treatments. Cidofovir treatment was initiated at a median of 31 (range, 7 to 214) days after HSCT. At treatment initiation, the HAdV load was median 4.1 (range, 1.7 to 6.5) log c/mL and median treatment duration was 16 (range, 1 to 99) days. Forty of 42 cases received ≥ 1 week (3 doses) of cidofovir (Tables 1, 2).

HAdV Load Dynamics in Relation to T Cell Reconstitution

T cells have been demonstrated to play a crucial role in viral control and could form a major confounder in the analysis of the antiviral effect of cidofovir. To test this hypothesis, we first divided the 42 cidofovir treatments in cases without (n = 20) and with (n = 22) T cell reconstitution in the first 2 weeks after treatment initiation (Table 2). The groups did not differ with respect to HSCT-related parameters and HAdV viremia characteristics (Supplemental Table S3).

In 11 of 20 cidofovir treatments (55%) with T cell numbers below 50 cells/μL, plasma HAdV DNA levels were stable in the first 2 weeks after treatment initiation. The HAdV load increased ≥ 1 log c/mL in 5 of 20 treatments (25%) and HAdV load reduction—but no clearance—was measured in 4 of 20 treatments (20%) without T cell reconstitution (Figure 1A).

In contrast, in 14 of 22 cidofovir treatments (64%) with concomitant T cell reconstitution, HAdV load reduction or HAdV clearance was observed within 2 weeks after treatment initiation. The HAdV load was stable in 6 of 22 treatments (27%) with T cell reconstitution and increased ≥1 log in only 2 of 22 cases (9%), who both received high-dose (>1 mg/kg methylprednisolone) steroid treatment (Figure 1B,C). Hence, it can be concluded that the evaluation of the antiviral effect of cidofovir treatment is strongly influenced by T cell reconstitution ($P = .008$, Figure 1A).

HAdV Load Dynamics in the Absence of T Cell Reconstitution

To exclude the confounding effect of T cells, HAdV load dynamics was further analyzed in the 20 cidofovir treatments with < 50 T cells/μL of peripheral blood (Table 2, Figure 2). In these cases, the evaluation period was extended from 14 days used in the previous section to the time frame between treatment initiation and 1 week after the last dose of cidofovir (n = 14) or 1 week before T cell numbers reached 50 cells/μL (n = 6).

A ≥1 log HAdV load reduction was observed in 7 of 20 treatments (35%) during a median 28-day evaluation period in the absence of T cells (Figure 2A). In 8 of 20 cidofovir treatments (40%), the HAdV load did not change significantly between the start and end of the evaluation period (median 18 days, Figure 2B). In the 5 remaining cases (25%), the HAdV load increased ≥1 log despite cidofovir treatment during median 21 days of follow-up in the absence of T cells (Figure 2C).

NK Cell Expansion during HAdV Control in the Absence of T Cell Reconstitution

In the majority of the cases without T cell reconstitution, $CD3^{-}CD56^{+}$ NK cells were already present at the start of cidofovir treatment (Figure 2D-F). Reduction of the HAdV load during cidofovir treatment coincided with a dynamic increase in NK cell numbers in all 7 cases (8 to 29-fold NK cell expansion, Figure 2D). In comparison, NK cell expansion was observed in 4 of 8 cidofovir treatments with HAdV load stabilization (Figure 2E) and 2 of 5 treatments with HAdV load increase (Figure 2F, $P = .05$).

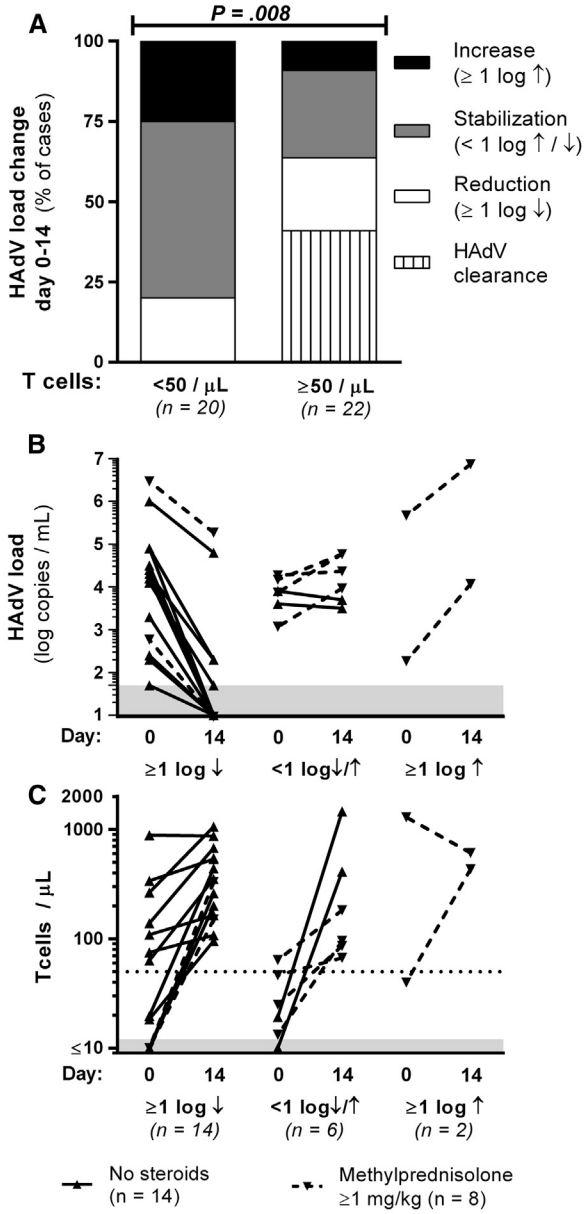

Figure 1. HAdV load dynamics in relation to T cell reconstitution. (A) Change of plasma human adenovirus DNA levels (HAdV load) between the start of treatment (day 0) and day 14 of cidofovir treatment in cases with T cell numbers <50 cells/μL (n = 20, left bar) and T cell numbers ≥50 cells/μL (n = 22, right bar) within this time period. HAdV load change: clearance: dashed, ≥1 log reduction: white, stabilization: gray, ≥1 log increase: black. *P* value: Pearson's chi-square test. (B) Change of HAdV load between day 0 and 14 in 22 cidofovir treatments with T cell numbers ≥50 cells/μL. Solid lines: cases without steroid treatment. Interrupted lines: methylprednisolone >1 mg/kg of body weight. Shaded area: HAdV load below limit of detection (1.7 log c/mL). (C) Change of T cell numbers between day 0 and 14 in 22 cidofovir treatments with T cell reconstitution. Solid lines: cases without steroid treatment. Interrupted lines: methylprednisolone >1 mg/kg of body weight. Shaded area: T cells below limit of detection (10 cells/μL).

HAdV Load Stabilization in the Absence of Both T and NK Cells

We finally analyzed the antiviral effect of cidofovir on the HAdV load in HSCT recipients lacking both T and NK cell reconstitution to exclude the possible contribution of NK cells to HAdV control as well. Two HSCT recipients fulfilled these criteria. In patient A (UPN 600.1a), no engraftment

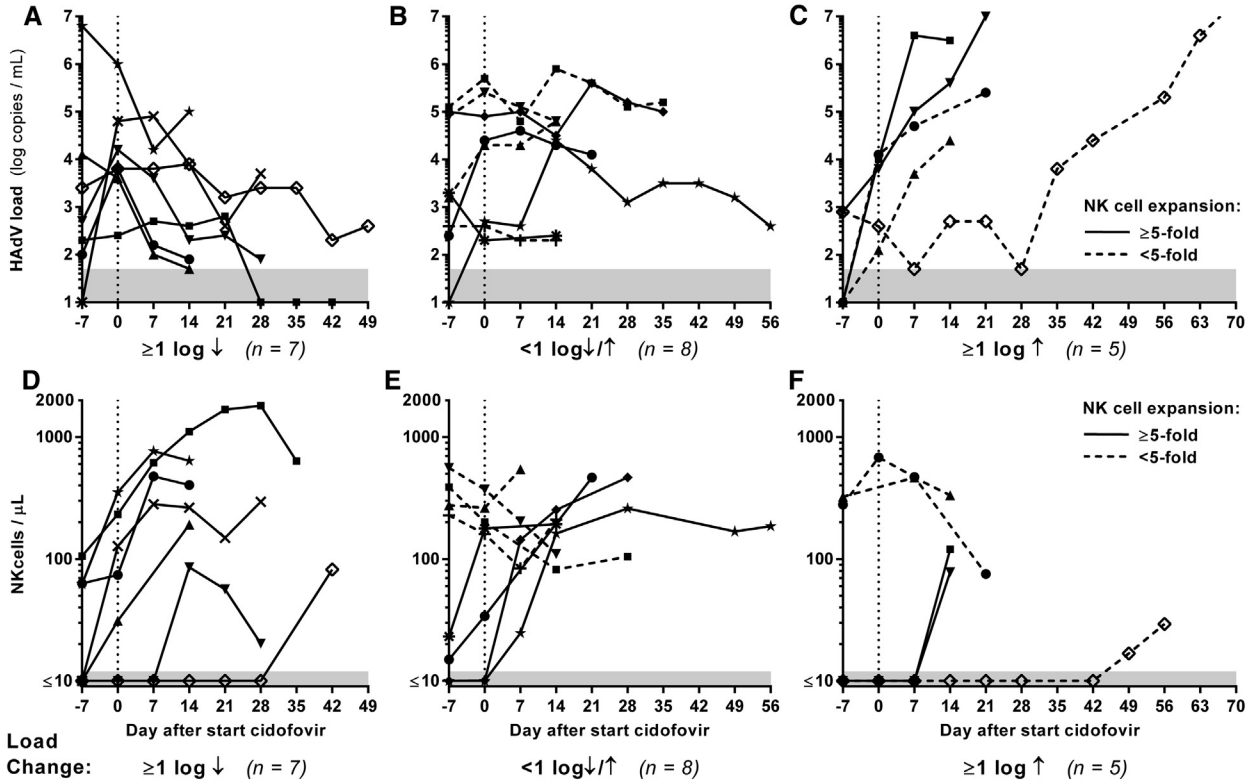

Figure 2. HAdV control in the absence of T cells. (A-C) Plasma human HAdV DNA levels in 20 cidofovir treatments with T cell numbers <50 cells/µL. Follow-up was stopped at 1 week after the last dose of cidofovir or 1 week before T cell numbers reached 50 cells/µL. HAdV load change: ≥1 log reduction (A, n = 7), stabilization (B, n = 8) or ≥1 log increase (C, n = 5). Solid lines: cases with ≥5 fold NK cell expansion, interrupted lines: cases without NK cell expansion. Vertical dotted line: start of treatment. Shaded area: HAdV load below limit of detection (1.7 log c/mL). (D-F) Absolute numbers of NK cells in peripheral blood in cases with HAdV load reduction (D), stabilization (E) and increase (F). Solid lines: cases with ≥5 fold NK cell expansion, interrupted lines: cases without NK cell expansion. Identical symbols in corresponding figures represent individual cases. Vertical dotted line: start of treatment. Shaded area: NK cells below limit of detection (10 cells/µL).

occurred and the HAdV viremia was stable during 3 weeks of cidofovir treatment in the absence of any lymphocytes (Figure 2A,D [open diamonds]; Supplemental Figure 1D). The viremia was ultimately cleared after lymphocyte reconstitution following a retransplantation. In patient B (UPN 545.1b), a graft rejection was treated with the lymphocyte depleting antibody alemtuzumab. The HAdV load was stable in the absence of lymphocytes over a 4-week period, after which HAdV dissemination occurred under cidofovir treatment (Figure 2C,F [open diamonds]; Supplemental Figure 1B).

DISCUSSION

Here, we report a systematic analysis of the effect of cidofovir treatment on HAdV viremia after HSCT, using plasma viral DNA levels as an objective parameter while taking concomitant lymphocyte reconstitution into account. In line with our hypothesis, rapid HAdV load reduction and HAdV clearance during cidofovir treatment were associated with concurrent T cell reconstitution. In the absence of T cell reconstitution, the HAdV viremia was controlled in 75% of cidofovir treatments, which can be attributed to cidofovir although a role of NK cell reconstitution cannot be excluded. Of note, in 2 cases, HAdV load stabilization was observed during a >3-week cidofovir treatment in the absence of both NK and T cells.

Because of the strong correlation between T cell reconstitution and HAdV load reduction, as reported earlier [2-4] and supported by our data, we focused our analysis on the 20 cidofovir treatment episodes in HSCT recipients lacking T cell reconstitution. In view of the often fatal outcome of a progressing HAdV viremia [9], HAdV load reduction or stabilization can be regarded as a beneficial result. In line with the in vitro virostatic capacity of cidofovir [12-15], control of the HAdV viremia was observed in 15 of 20 cidofovir treatments in the absence of T cell reconstitution. However, reduction of the HAdV load (n = 7) always coincided with a ≥ 5-fold increase of NK cell numbers in the peripheral blood. Consequently, the contribution of NK cell reconstitution to HAdV control in HSCT recipients without T cell reconstitution cannot be excluded. Usually, NK cells are the first lymphocytes to reach normal levels after HSCT [26,27]. Although the NK cell response to HAdV is not as well described as the NK cell–mediated control of other viruses, such as influenza, cytomegalovirus, and hepatitis C virus [28-30], a limited number of studies reported NK cell activity against HAdV infected cells [31-33]. Our data further support a role for NK cells in the initial HAdV control in patients with a delayed T cell reconstitution. At the same time, NK cell expansion did not always coincide with HAdV load reduction, and the presence of NK cells was no guarantee for HAdV load stabilization.

In many studies, final outcome of HAdV viremia is used as a primary endpoint of cidofovir effectiveness, and concomitant lymphocyte reconstitution is often ignored. This bears the risk to overestimate the in vivo antiviral effect of cidofovir. Indeed, Walls et al. reported the clearance of HAdV viremia in 8 of 9 pediatric HSCT recipients with HAdV loads

>3 log c/mL in the absence of antiviral therapy [34]. For this reason, an unbiased evaluation of the antiviral capacity of cidofovir—and other pharmacological interventions—for human adenoviral infections after HSCT is only possible by monitoring HAdV load dynamics in patients who lack concomitant lymphocyte reconstitution. Only 2 patients in our cohort met this condition. In both cases, the HAdV viremia remained stable over a 3 to 4-week period. Consequently, cidofovir may also have contributed to the HAdV load stabilization observed in HSCT recipients lacking T cell reconstitution or with suppressed antiviral T cell responses due to high-dose systemic steroids [35-37].

Of note, cidofovir treatment could not prevent the progression of HAdV viremia in 7 of 42 cidofovir treatments (17%), all in the absence of T cell reconstitution or during high-dose steroid treatment. With respect to the cause of these treatment failures, no firm conclusions can be drawn. In vitro studies reported comparable cidofovir susceptibility between different HAdV species [12-15]. In our cohort, cidofovir treatment failures were observed both in patients with HAdV species A as well as those with species C. Whereas cidofovir resistant HAdV strains have been generated in vitro [38], no resistance was reported in clinical HAdV isolates from cidofovir-treated HSCT recipients [12-15]. Possibly, interpatient variations in cidofovir pharmacokinetics may have contributed to failure of cidofovir treatment as well. In view of the nephrotoxicity of cidofovir [21-24], the observed treatment failures emphasize the need for new and less toxic pharmacological interventions, such as brincidofovir (CMX001), the orally bioavailable lipid conjugate of cidofovir [39], as well as adoptive immunotherapy-based interventions [3,10] for patients with a delayed T cell reconstitution.

The sensitive PCR methods used for the virological monitoring after HSCT carry the risk of overtreatment, which might lead to avoidable toxicity [34,40]. Longitudinal monitoring of lymphocyte reconstitution can identify patients with better odds on a favorable outcome of HAdV viremia. Indeed, the presence of even a low number of T cells (≥50 cells/μL) in patients without steroid treatment was associated with a rapid HAdV load reduction. The frequent monitoring of T cell reconstitution can be a valuable tool to prevent the unnecessary installment or continuation of cidofovir treatment. A comparable approach has already been applied successfully in the management of cytomegalovirus and Epstein-Barr virus infections after HSCT [41,42].

Altogether, a subgroup of patients with HAdV viremia after HSCT might benefit from cidofovir treatment through a stabilization of the HAdV load pending lymphocyte reconstitution. Nevertheless, T cell reconstitution remains essential for viral clearance. For clinical decision-making, the combined monitoring of plasma HAdV DNA levels and lymphocyte reconstitution provides an objective tool for the guidance of personalized antiviral treatment and prevention of unnecessary exposure to cidofovir.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.10.012.

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