

Cidofovir in addition to antiretroviral treatment is not effective for AIDS-associated progressive multifocal leukoencephalopathy: a multicohort analysis

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Objective: To establish the effectiveness of cidofovir for AIDS-related progressive multifocal leukoencephalopathy (PML) in patients concomitantly receiving combination antiretroviral therapy.

Design: Analysis of raw data pooled from one prospective and five cohort studies.

Setting: Tertiary care centers for the treatment of HIV-associated complications.

Patients: Three hundred seventy HIV-infected PML patients diagnosed from 1996 treated with combination antiretroviral therapy with or without cidofovir. All studies had already published their results but for four of them, additional patients and follow-up data are included in this report. Follow-up was started from the date of first abnormal neuroimaging; those treated with cidofovir were entered at risk at the date of cidofovir initiation.

Main study outcomes were time to PML-related death and odds of 12-month moderately severe to severe disability (Rankin score ≥ 4).

Results: Sixty-four percent of the PML cases were confirmed by histopathology or JC virus DNA detection in cerebrospinal fluid; 185 (50%) received cidofovir (median five cycles). During 463 person-years of follow-up, 167 PML-related deaths occurred (36.6 per 100 person-years of follow-up). Estimated 1 year survival was 0.56 (95% confidence interval, 0.50–0.61). In multivariate models stratified by cohort and adjusted for type of diagnosis and relevant prognostic confounders, cidofovir treatment was not associated with survival (hazard ratio for death 0.93, 0.66–1.32). Results were similar using time to death from any cause as the outcome. Furthermore, 12-month moderately severe to severe disability was not associated with the use of cidofovir.

Conclusion: In combination antiretroviral therapy-treated PML patients, cidofovir use did not influence PML-related mortality or residual disability. New treatments for AIDS-related PML are urgently needed.

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by the JC virus (JCV), and generally occurs in HIV-infected as well as other individuals as a result of severe immunodeficiency. In HIV-infected people, the disease is characterized by progressive neurological deficits leading to death after a median of 4–6 months and represents an important cause of morbidity and mortality [1,2]. The introduction of potent combination antiretroviral therapy (cART) significantly prolonged survival of HIV-infected patients in general [3] and, according to some small studies, improved prognosis of AIDS-associated PML [4–10]. In fact, poorer survival has consistently shown to be associated with lower CD4 cell count at PML diagnosis [7,11,12] and the favorable effect of cART might be mediated by restoration of JCV-specific CD4 and CD8 T-cell responses [13,14]. Nonetheless, prognosis of cART-treated patients with AIDS-related PML remains poor, with 50% case fatality rates and severe chronic disability in survivors [15]. As no specific treatment with proven efficacy is available, the treatment guidelines for HIV-related opportunistic infections recommend only cART for patients with HIV infection and PML [16,17].

Cidofovir is an antiviral compound inhibiting replication of polyomaviruses *in vitro* [18]. Despite lack of *in-vitro* efficacy against JCV [19], some authors suggested that this drug might be of clinical benefit in patients with PML [20–23]. A retrospective study [12] suggested that addition of cidofovir to cART in patients with AIDS-associated PML was more effective in reducing cerebrospinal fluid (CSF) JCV DNA levels, improving neurological outcome and prolonging survival than antiretroviral therapy alone. Other retrospective studies reported either better survival in cidofovir-treated PML patients only after adjusting for other prognostic variables [24] or no beneficial effect of cidofovir compared with cART alone [11,25,26]. More recently, a prospective non-comparative study [27] conducted on 24 individuals observed high death rates in patients treated with cidofovir. Therefore, at present, the few published studies report partially contradictory results. The small number of patients in most studies may limit the possibility to generalize the results to the entire diseased population. Moreover, no randomized study to test the efficacy of cidofovir in AIDS-related PML has been conducted or is currently planned.

The aim of this study was to investigate the effectiveness of cidofovir for the treatment of AIDS-associated PML in patients already receiving cART pooled from six international cohorts or studies, with a focus on death or severe disability.

Methods

Study design, patients and treatments

For the purpose of the present study, the raw datasets of six international clinical cohorts or studies of HIV-positive individuals with PML were pooled. Presumptive diagnosis of PML was based on characteristic clinical symptoms and signs together with neuroradiological abnormalities highly suggestive of this disorder. Confirmed PML diagnosis included criteria for presumptive diagnosis and either characteristic histopathological findings in brain biopsy or autopsy specimens or detection of JCV DNA in CSF tested by PCR [28]. Patients were selected from the AIDS Clinical Trials Group (ACTG) 363 study, a prospective, multicenter single arm study of cidofovir treatment in confirmed cases of PML [27]; the Gesida 9/99 study, a Spanish multicenter cohort study of patients with presumptive or confirmed PML [11]; the cohort from the Bicêtre hospital, a reference treatment center for neurology and neuro-rehabilitation that enrolled patients with presumptive or confirmed diagnosis from the Paris region [24]; the Università Cattolica and the Hospital San Raffaele cohort, two Italian single reference center cohorts located in Rome and Milan, respectively that recruited and followed PML patients with presumptive and confirmed diagnosis [10,29]; and the IRINA cohort, an Italian multicenter cohort of AIDS patients with neurological disorders, that enrolled patients with presumptive or confirmed PML [30]. Study results from ACTG 363 and Gesida have been published [11,27]. Data from the other cohorts have been published [10,24,29,30], but in the analysis reported herein, additional patients have been enrolled and follow-up was extended until last available visit or April 30, 2004. The only criterion to allow inclusion of patients in this analysis was a PML diagnosis in or after calendar year 1996 and receipt of cART [defined as a treatment with at least three antiretroviral drugs including at least one non-nucleosidic reverse transcriptase inhibitor (NNRTI) or one protease inhibitor) after PML diagnosis. Karnofsky performance status, the presence of brainstem involvement (defined as the presence of at least one of the following neurological signs: oculomotor palsies, central vestibular syndrome or difficulties with swallowing related to bulbar or pseudobulbar syndrome, all with an magnetic resonance imaging confirmation [31]), CD4 cell counts and plasma HIV RNA levels at baseline and during follow-up, prior AIDS, prior use of antiretroviral drugs and type of cART regimen after PML diagnosis (cART containing a protease inhibitor, cART containing a NNRTI, cART containing a NNRTI and protease inhibitor as well as the number of neuroactive drugs [32]), qualitative CSF JCV DNA test results and

levels (quantified by different real-time PCR techniques by individual studies [10,24,27,33,34]) were included in the analyses.

Prescription of cART and cidofovir were not dictated by a common study protocol but prescribed by the caring physician, with the exception of ACTG 363 study patients, who received cidofovir according to an established protocol [27]. In all cases, cidofovir was administered by intravenous infusion at baseline, after 1 week and every 2 weeks thereafter. The standard dose of 5 mg/kg was used when renal function was normal and cidofovir treatment was deferred or discontinued in case of impaired renal function, neutropenia or thrombocytopenia. Probenecid at standard dose was given orally before and after cidofovir administration and adequate hydration was given.

Study outcomes

The primary outcome was time to PML-related death. In a secondary analysis, death from any cause was also considered. The modified Rankin disability scale [35] at 12 months of follow-up was used to classify patients centrally into the following groups: 0, no symptoms at all; 1, no significant disability despite symptoms (able to carry out all usual duties and activities); 2, slight disability (unable to carry out all previous activities but able to look after own affairs without assistance); 3, moderate disability (requiring some help but able to walk without assistance); 4 moderately severe disability (unable to walk without assistance and unable to attend to own bodily needs without assistance); 5, severe disability (bedridden, incontinent, and requiring constant nursing care and attention).

Statistical analysis

Patients treated with cidofovir and those without were compared according to general HIV-related and PML-related characteristics. Differences in proportions were analyzed by the Chi-square test and between continuous variables by the Mann-Whitney test, as appropriate. Two-sided *P* values less than 0.05 were considered statistically significant.

Study baseline was defined as the date of the first abnormal neuroimaging examination. Because cidofovir was usually given after the baseline, which could introduce potential bias associated with better survival, the effect of cidofovir was evaluated by entering the patients treated with cidofovir at risk in the analysis at the date of drug initiation [36]. Survival time was calculated from baseline to PML-related death, to death from all causes, or, if alive, to the last date of follow-up. Kaplan-Meier survival estimates were calculated for the entire study population, stratifying by study site, and stratified according to treatment with cART only or with cART

combined with cidofovir. Differences between the survival curves were tested by the log-rank test.

Cox proportional hazards regression models were used to detect associations with survival. Crude and adjusted hazards ratios of death and relative 95% confidence intervals (CI) are reported. Use of cidofovir, as a dichotomous variable was evaluated as an intention-to-continue-treatment (i.e., if cidofovir treatment was stopped for any reason, the variable remained coded as 'yes' for cidofovir use). Multivariable models stratified by cohort included variables for which the univariate *P* value of the hazard ratio for death was less than 0.20 or which were considered potentially relevant. CD4 cell counts were analyzed in separate models using the baseline and the time-updated values.

To identify variables associated with worse disability score at 12 months of follow-up, the modified Rankin scale was dichotomized as less than 4 (no, slight or moderate disability) versus at least 4 (moderately severe to severe disability). Factors for which the *P* values less than 0.05 in univariate logistic regression analysis were included in a multivariate regression model together with cidofovir use as a dichotomous variable.

Results

Patients characteristics

Three hundred seventy patients are included in the analysis. All patients were diagnosed between January 1996 and February 2004. PML was diagnosed during the calendar years 1996–1999 in 61% and during the years 2000–2004 in 39% of patients. Table 1 summarizes the principal characteristics of patients according to each cohort or study. Of all cases, 47% had been prescribed prior antiretroviral therapy: 12% had received only nucleoside reverse transcriptase inhibitor -based therapy, 35% had received cART. Median duration of symptoms before PML diagnosis was 20 days [interquartile range (IQR), 5–47]. After PML diagnosis, cART was initiated or continued in all patients. Information on the type of cART used after PML diagnosis was available for 189 patients (51%): 145 received cART containing a protease inhibitor, 20 received cART containing a NNRTI whereas 24 received cART containing a NNRTI and a protease inhibitor. The median number of neuroactive antiretroviral drugs in these regimens was 2 (IQR, 1.5–2.5). Cidofovir treatment was administered to 185 patients (50%). It was initiated before 8 weeks after PML diagnosis in 75% of patients and was administered for a median of five cycles (IQR, 3–11). The proportion of patients treated with cidofovir ranged from 38.1% in the Spanish Gesida cohort to 100% in the ACTG363 study. Patients enrolled in the different studies differed in HIV-related and PML-related

Table 1. Characteristics of patients shown by study cohort.

	Total (n = 370)	ACTG363 [25] (n = 20)	Gesida [11] (n = 105)	Bicêtre [24] (n = 76)	Irina [27] (n = 76)	UCSC [26] (n = 48)	HSR [10] (n = 45)	P value
General characteristics								
Age, years, median (IQR)	38 (34–42)	38 (34–44)	37 (33–40)	39 (35–44)	40 (37–43)	36 (33–41)	36 (34–39)	0.03
Male (%)	80	75	87	83	77	75	71	0.22
HIV transmission route								
Men who have sex with men (%)	16	60	12	29	6	13	4	<0.001
Injecting drug use (%)	54	5	68	38	62	65	49	
Heterosexual (%)	19	35	14	24	22	15	13	
HIV-related characteristics								
Previous AIDS-defining event (%)	33	10	38	32	29	33	38	0.21
CD4+ lymphocytes, cells/ μ l, median (IQR)	73 (29–157)	66 (21–199)	84 (38–160)	70 (26–135)	78 (32–171)	41 (15–119)	77 (34–168)	0.90
HIV-RNA, log ₁₀ copies/ml, (n = 303), median (IQR)	5.0 (4.0–5.4)	4.7 (3.2–5.1)	5.1 (4.3–5.0)	5.1 (4.6–5.4)	4.8 (3.6–5.3)	4.8 (3.3–5.3)	4.8 (3.3–5.3)	0.02
Prior NRTI-based treatment (%)	32	10	33	27	17	52	47	<0.001
Prior cART (%)	35	45	20	28	43	52	44	<0.001
PML-related characteristics								
Confirmed PML diagnosis (%)	64	100	40	82	53	77	82	<0.001
JCV DNA in CSF, log ₁₀ copies/ml, (n = 148), median (IQR)	3.7 (3.2–4.8)	3.1 (2.2–4.9)	NA	4.3 (3.3–5.2)	NA	3.5 (3.2–5.0)	3.6 (3.0–4.5)	0.047
JCV DNA in CSF positive/tested	238/302 (79%)	16/19 (84%)	33/61 (54%)	63/73 (86%)	40/62 (65%)	42/42 (100%)	44/45 (98%)	<0.001
Cidofovir therapy, (%)	50	100	38	58	46	46	56	<0.001
Days from baseline to start of cidofovir, median (IQR)	24 (14–54)	41 (30–72)	21 (21–22)	39 (18–62)	18 (5–41)	30 (17–75)	17 (7–33)	0.53
Brainstem involvement (%)	25	15	23	41	23	4	31	<0.001
Karnofsky score, median (IQR)	50 (40–70)	55 (40–70)	60 (60–70)	40 (30–50)	40 (40–60)	60 (50–70)	60 (40–80)	<0.01
PML-related death, (%)	45	45	33	45	52	60	47	0.04
Person-years of follow-up, median (IQR)	0.59 (0.20–2.07)	0.24 (0.14–0.60)	0.84 (0.25–2.40)	0.80 (0.29–3.04)	0.29 (0.09–1.26)	0.43 (0.21–1.35)	0.46 (0.20–2.54)	<0.001

ACTG, AIDS Clinical Trials Group; cART, combination antiretroviral therapy; CSF, cerebrospinal fluid; HSR, Hospital San Raffaele; IQR, interquartile range; JCV, JC virus; NA, not available; NRTI, nonnucleosidic reverse transcriptase inhibitor; PML, progressive multifocal leukoencephalopathy; UCSC, Università Cattolica.

characteristics and in duration of follow-up (see Table 1). This was not unexpected given the different designs of the studies and recruitment modalities. For this reason, survival analyses were stratified by study cohorts. JCV DNA quantification in CSF was available from four studies for a total of 148 patients. The virus was quantified a median of 1.6 weeks (IQR, 0–6.3) after baseline (the first positive neuroimaging) and 6.3 weeks (IQR, 3.7–11.9) after PML onset; 35% of these individuals were on prior cART, that had been administered for a median duration of 40 weeks (IQR, 23–79). Time from baseline or PML onset, prior cART exposure and its duration were not correlated to JCV DNA load (data not shown). There was a borderline significant difference of JCV DNA load among cohorts (see Table 1). A qualitative JCV DNA result was available for 302 patients, 78% had a detectable viral DNA in the CSF.

Comparison of baseline characteristics in cidofovir-treated and untreated patients showed that patients in the cidofovir group were diagnosed at later calendar years and that fewer acquired HIV as a consequence of injecting drug use (see Table 2). Other baseline characteristics did not significantly differ between the two groups of patients.

Patients survival and predictors

This analysis includes 463 person-years of follow-up. One hundred eighty-eight patients died and, in 167 (89%) of them, PML was considered the principal cause of death yielding a rate of 36.6 PML-related deaths per 100 person-years. Overall, the estimated proportion of patients surviving at 1 year was 0.56 (95% CI, 0.50–0.61). This proportion fell to 0.51 when patients treated with cidofovir were entered at risk at the time of cidofovir

initiation. The probability of survival was lower in PML patients treated with cidofovir, although this difference was not statistically significant after stratifying by study cohort (hazard ratio for PML-related death 1.14; 95% CI, 0.82–1.57; stratified log-rank test $P=0.44$) (Fig. 1a). The estimated 1 year probability of survival was 0.44 (95% CI, 0.34–0.53) in patients treated with cidofovir and 0.55 (95% CI 0.47–0.62) in patients treated with cART alone.

Figure 1b shows crude and adjusted hazard ratios for PML-related death and relative 95% CI for treatment with cidofovir versus cART alone stratified by study. Overall, no risk reduction was observed in patients receiving cidofovir and cART when compared with those

Table 2. Patients baseline characteristics according to the use of cidofovir.

Variable	cART alone (n = 185)	cART with cidofovir (n = 185)	P value*
Confirmed PML diagnosis (%)	62	66	0.40
Calendar year of diagnosis	1998	1999	<0.001
Male sex (%)	83	76	0.10
Risk group			0.006
Homosexual males (%)	12	20	
Injecting drug users (%)	63	45	
Heterosexual contacts (%)	16	22	
Age (years)	38	37	0.20
Prior AIDS (%)	67	67	0.91
Baseline CD4 cell count (cells/ μ l)	77	70	0.43
Plasma HIV RNA (log ₁₀ copies/ml)	4.99	4.94	0.39
CSF JCV DNA (log ₁₀ copies/ml)	3.75	3.68	0.74
Karnofsky score	60	50	0.48
Brainstem involvement (%)	27	23	0.40
Neuroactive antiretroviral drugs	2	2	0.20

Indicated values represent medians unless otherwise specified. cART, combination antiretroviral therapy; CSF, cerebrospinal fluid; JCV, John Cunningham virus; PML, progressive multifocal leukoencephalopathy.

*Using chi-square for categorical variables and Wilcoxon rank sum test for continuous variables.

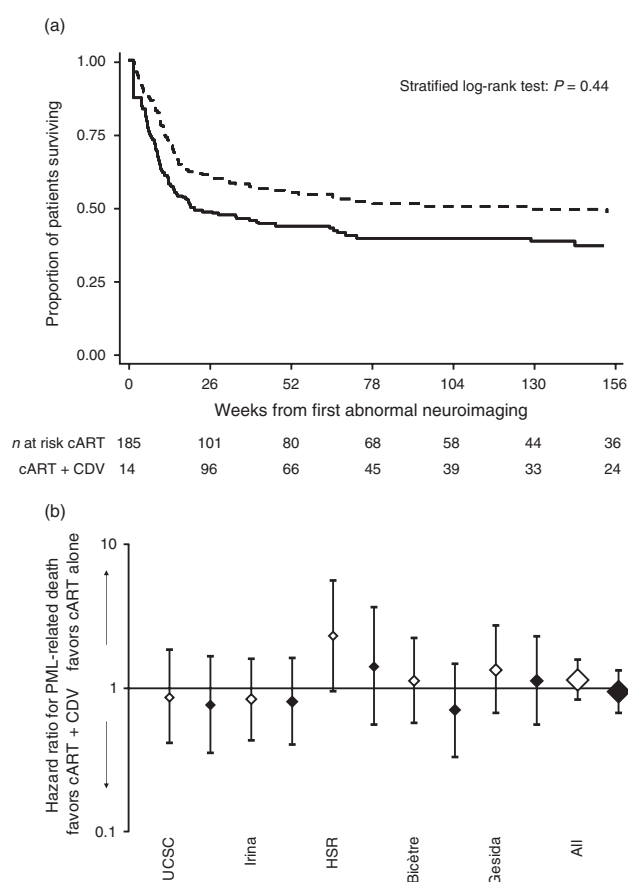


Fig. 1. Effect of cidofovir use on time to PML-related death. (a) Kaplan–Meier plots of the estimated proportion of patients surviving according to use of cidofovir (CDV) and cART (solid line) or cART only (dashed line): follow-up truncated at 156 weeks. (b). Hazard ratios for CDV + cART treatment versus cART alone shown by individual studies and overall. White diamonds indicate crude hazard ratios, black diamonds indicate hazard ratios adjusted for baseline CD4 cell counts and Karnofsky, whiskers indicate 95% CI. Size of the squares reflects square root of patient numbers. Data from ACTG 363 [27] are not included because there was no cART only arm.

Table 3. Crude and adjusted hazard ratios for the association of different variables with PML-related death.

	Univariate analysis hazard ratios (95% CI)	Multivariable analysis hazard ratios (95% CI)	
		Model 1	Model 2
Cidofovir treatment (yes vs no)	1.14 (0.82–1.57)	0.93 (0.65–1.32)	1.09 (0.77–1.54)
Baseline CD4 cell count (per ln cells/ μ l higher)	0.76 (0.67–0.85)*	0.75 (0.66–0.86)*	ND
CD4 cell count, time-updated (per ln cells/ μ l higher)	0.70 (0.62–0.80)*	ND	0.69 (0.60–0.78)*
Baseline HIV RNA \geq 500 vs < 500 cps/ml	1.43 (0.82–2.51)	1.27 (0.67–2.40)	1.23 (0.65–2.33)
Quantitative baseline JCV DNA (per log ₁₀ cps/ml higher) (<i>n</i> = 148)	1.26 (1.12–1.43)*	ND	ND
Baseline JCV DNA			
Negative	Reference	Reference	Reference
Positive	1.38 (0.86–2.21)	1.42 (0.85–2.39)	1.36 (0.81–2.28)
Not available	1.10 (0.63–1.94)	1.12 (0.62–2.04)	1.13 (0.62–2.06)
Karnofsky performance status, (per 10 points higher)	0.73 (0.65–0.81)*	0.74 (0.66–0.82)*	0.73 (0.65–0.82)*
Brainstem involvement, (yes vs no)	1.30 (0.90–1.87)	1.34 (0.92–1.97)	1.32 (0.90–1.94)
Confirmed vs presumptive PML diagnosis	0.78 (0.55–1.12)	0.76 (0.53–1.10)	0.83 (0.57–1.20)
Diagnosis during calendar year 1996 vs later calendar years	2.36 (1.05–5.33)**	2.08 (0.93–4.66)	2.17 (0.97–4.86)

The results of two separate multivariate Cox regression models are shown: in model 1, CD4 cell count was used as baseline value; in model 2, CD4 cell count was used as time-dependent variable. All analyses were stratified by cohort. CI, confidence interval; JCV, JC virus; ND, not done; PML, progressive multifocal leukoencephalopathy.

* $P < 0.001$.

** $P < 0.05$.

who received only cART. Table 3 summarizes the results of the univariate and multivariable analyses of hazard of PML-related death. In both proposed multivariable models, the use of cidofovir was not associated with survival time; survival was significantly longer in patients with higher CD4 cell count and better Karnofsky performance status. Of note, similar results were obtained when considering the CD4 cell count at baseline or when CD4 cell counts were time-updated. Calendar year 1996 was associated with shorter survival in univariate analysis, but was not an independent predictor in the multivariable models. Higher JC viral load at diagnosis was also strongly associated with shorter survival, but as this variable was available only in a subset of 148 (40%) patients, it was not included in the multivariable models. In the subgroup of 302 patients with a qualitative JCV DNA test result on CSF, the 238 patients with a positive test result did not show a significant difference in time to PML-related death as compared with those having a negative test result ($P = 0.18$). Baseline HIV RNA analyzed as a continuous variable or dichotomized as below and equal to or higher than 500 copies/ml, brainstem involvement and criteria for diagnosis were not associated with PML-related death. Age, sex, HIV transmission risk group, prior AIDS diagnosis, prior antiretroviral use and type of cART at or after PML diagnosis were not associated with survival (not shown).

Results were confirmed when death from any cause was used as the outcome (see Table 4). Of note, apart from Karnofsky and baseline or time-updated CD4, which protected against death, brainstem involvement was independently associated with an increased hazard ratio of death from any cause. As in the analysis of PML-related

death, cidofovir use was not associated with the hazard of death from any cause.

In a sensitivity analysis, we tested the effect of cidofovir use on PML-related death and on overall death in the subgroup ($n = 238$) with confirmed PML diagnosis, using the same multivariable models described above. Again, cidofovir use was neither associated with PML-related death nor with overall death in any of the models used ($P \geq 0.19$).

One year disability score and predictors of severe disability in survivors

In the 188 patients surviving at 12 months, the median modified Rankin scale was 3 (IQR, 2–4); 39% were classified as having a moderately severe to severe disability (score ≥ 4). Univariable analysis showed that the use of cidofovir [odds ratio (OR), 1.75; 95% CI, 1.11–2.73; $P = 0.016$], and JCV DNA load in CSF ($n = 64$; OR, 1.60 per log₁₀ copies/ml higher; 95% CI, 1.23–2.08, $P = 0.001$) conveyed increased risk of moderately severe or severe disability, whereas baseline CD4 cell counts (OR, 0.73 per ln cells/ μ l higher; 95% CI, 0.56–0.97; $P = 0.027$) and baseline Karnofsky (OR 0.73 per 10 points higher, 95% CI 0.65–0.83, $P < 0.001$) conveyed decreased odds of moderately severe to severe disability. Sex, age, HIV transmission risk group, baseline HIV RNA levels, prior use of antiretroviral therapy before diagnosis, baseline brainstem involvement, type of diagnosis, prior AIDS, calendar year and type of cART were not associated with disability. In a multivariable analysis, a higher baseline CD4 cell count (per ln increase OR 0.71; 95% CI 0.52–0.98, $P = 0.038$) and a better baseline Karnofsky performance status (per 10 points

Table 4. Crude and adjusted hazard ratios for the association of different variables with death from any cause.

	Univariate analysis hazard ratios (95% CI)	Multivariable analysis hazard ratio (95% CI)	
		Model 1	Model 2
Cidofovir treatment (yes versus no)	1.10 (0.81–1.49)	0.91 (0.65–1.27)	1.06 (0.76–1.47)
Baseline CD4 cell count (per ln cells/ μ l higher)	0.78 (0.70–0.88)*	0.78 (0.68–0.88)*	ND
CD4 cell count, time-updated (per ln cells/ μ l higher)	0.72 (0.64–0.81)*	ND	0.70 (0.61–0.79)*
Baseline HIV RNA \geq 500 versus <500 copies/ml	1.45 (0.84–2.49)	1.27 (0.69–2.33)	1.24 (0.68–2.28)
Quantitative baseline JCV DNA (per log ₁₀ copies/ml higher) (<i>n</i> = 148)	1.23 (1.10–1.38)*	ND	ND
Baseline JCV DNA			
Negative	Reference	Reference	Reference
Positive	1.45 (0.93–2.24)	1.41 (0.86–2.30)	1.36 (0.83–2.22)
Not available	1.05 (0.62–1.80)	1.11 (0.62–1.97)	1.11 (0.62–1.98)
Karnofsky performance status (per 10 points higher)	0.73 (0.66–0.80)*	0.74(0.66–0.82)*	0.73(0.66–0.81)*
Brainstem involvement, (yes versus no)	1.42 (1.01–1.98)**	1.45 (1.01–2.07)**	1.43 (1.00–2.04)**
Confirmed vs presumptive PML diagnosis	0.86 (0.61–1.21)	0.84 (0.59–1.19)	0.90 (0.63–1.28)
Diagnosis during 1996 versus later calendar years	1.75 (0.92–3.32)	1.73 (0.88–3.44)	1.81 (0.91–3.59)

The results of two separate multivariable Cox regression models are shown: in model 1, CD4 cell count was used as baseline value; in model 2, CD4 cell count was used as time-dependent variable. All analyses were stratified by cohort. CI, confidence interval; JCV, JC virus; ND, not done.

**P* < 0.001.

***P* < 0.05.

higher OR, 0.71; 95% CI, 0.62–0.82; *P* < 0.001) were independently associated with a lower odds of moderately severe to severe disability at 1 year, but cidofovir use was not (OR, 1.37; 95% CI 0.72–2.61).

Discussion

The present study of 370 cART-treated AIDS-related PML cases, 50% of whom were also treated with cidofovir, represents the largest observational study published in this field. Data were pooled from six distinct published studies or cohort analyses and were diagnosed from 1996 to 2004. To account for observed differences in mortality between the distinct cohorts, stratification by cohort was used for all survival analyses. In univariate and multivariate models, compared with treatment with cART alone, treatment with cART and cidofovir did not confer a survival benefit. The hazards of PML-related death and death from any cause were independently lower in patients with higher baseline and time-updated CD4 cell counts and better baseline Karnofsky performance status. Lower CSF JCV DNA load was also associated with lower hazard of PML death and overall death in the subset analyzed. The hazard ratios of death from any cause, but not from PML-related death, were lower in patients with brainstem involvement at baseline. The odds of moderately severe to severe disability at 12 months were lower in patients with higher baseline CD4 cell counts, higher baseline Karnofsky performance status and, in the tested subset, in those with lower CSF JCV DNA load but were not related to cidofovir use. Plasma HIV RNA levels, type of diagnosis (presumptive or confirmed), prior antiretroviral treatment and type of cART used at or after PML diagnosis were not associated

with disability. In the subset of 238 patients with confirmed PML diagnosis, cidofovir use was not associated with survival.

The present study has several limitations. Patients treated with cidofovir began this treatment after the time of diagnosis. Thus, this treatment group could have a better prognosis than those treated with cART alone because they survived long enough to receive treatment with cidofovir. However, we accounted for this potential bias in patients treated with cidofovir by entering patients at risk at the date of cidofovir initiation. On the contrary, confounding by indication cannot be ruled out. Indeed, patients may have had a higher likelihood of receiving cidofovir because their clinical condition was worsening. Thus, patients who received this drug may have been selected as those with a worse outcome. However, our analysis was adjusted for known prognostic factors, including CD4 cell counts and Karnofsky performance status. Although we were able to analyze CD4 cell counts as a time-updated covariate, we were not able to perform such analyses with the other variables because they were not systematically recorded at the time of cidofovir initiation. Therefore, a potential selection of patients with more negative prognosis in the cidofovir group could not be completely excluded or corrected by the analysis.

The study confirms results of previous observations showing that higher CD4 cell counts and better Karnofsky performance score are the main prognostic indicators in AIDS patients with PML [9]. Compared with subsequent years, PML diagnosis in calendar year 1996 showed a trend towards a shorter time to PML-related death, suggesting an improved efficacy of cART regimens over time. As in a previous study [15], patients with brainstem involvement were significantly more likely to die from any cause. The

lack of a clear association between brainstem involvement and PML-related death may have been owing to misclassification of the cause of death as not PML-related (e.g. in case of aspiration pneumonia) despite the fact that PML may have been the initial cause.

Although JCV DNA levels were quantified in a subset of patients only, ours is the largest analysis of the prognostic role of this marker in PML [10,12,33,34,37]. There is no standardized method for quantifying JCV DNA and different real-time PCR techniques have been used in the distinct studies. Despite this limitation and the fact that JCV DNA was quantified at different times after disease onset, higher JCV DNA levels conferred increased risk of death or disability. However, the association of CSF JCV load was not verified in multivariable models because of the limited subset of tested study patients. This finding requires to be verification by a standardized technique.

Despite limitations in this study due to its observational nature, the overall results point against a major effectiveness of cidofovir in the treatment of AIDS-related PML. The strength of this study is the size of the population analyzed as well as the fact that no randomized comparison, which would be the only way to determine the efficacy of cidofovir treatment in addition to cART compared with cART alone, has ever been performed or planned. Results of our study are in agreement with a small study of PML in patients not infected with HIV, where no beneficial effect of cidofovir was observed [38]. In light of the observed lack of effectiveness and of the potential renal and ocular toxicity [39], we conclude that cidofovir should be abandoned in patients with AIDS-related PML. The prognosis of AIDS-related PML remains poor despite cART. New agents and treatment strategies are urgently needed for the treatment of this uncommon but frightening opportunistic infection.

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A. De Luca and A. Antinori designed the study and interpreted results of the statistical analyses. A. De Luca coordinated data collection and merging and contributed

to manuscript completion. A. Ammassari contributed to study design and results interpretation and wrote the first draft of the manuscript. P. Pezzotti performed data merging, cleaning and statistical analyses. P. Cinque, J. Gasnault, J. Berenguer, S. Di Giambenedetto, A. Cingolani, Y. Taoufik, P. Miralles, C.M. Marra and A. Antinori contributed data, gave relevant scientific input, contributed to interpretation of the findings and reviewed the final manuscript.

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