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Clinical Trial Report

Potent anti-retroviral therapy with or without cidofovir for AIDS-associated progressive multifocal leukoencephalopathy: Extended follow-up of an observational study

Andrea De Luca,[1] Maria Letizia Giancola,[2] Adriana Ammassari,[1] Susanna Grisetti,[2] Antonella Cingolani,[1] Dora Larussa,[1] Lucia Alba,[2] Rita Murri,[1] Giuseppe Ippolito,[2] Roberto Cauda,[1] Antonella d'Arminio Monforte,[3] and Andrea Antinori[2]

[1]Department of Clinical Infectious Diseases, Università Cattolica del S Cuore, Rome, Italy; [2]National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy; and [3]Institute of Infectious and Tropical Diseases, University of Milan, L Sacco Hospital, Milan, Italy

To analyze the clinical efficacy of cidofovir combined with highly active anti-retroviral therapy (HAART) in AIDS-related progressive multifocal leukoencepalopathy (PML), a multicenter observational study was performed. Consecutive HIV-positive patients with histologically or virologically proven PML and at least 4 weeks of treatment after diagnosis were examined: 27 patients were treated with HAART, whereas 16 patients were treated with HAART plus cidofovir 5 mg/kg intravenously per week for the first 2 weeks and every other week thereafter. JC virus DNA was quantified in cerebrospinal fluid (CSF) by PCR. Baseline virologic, immunologic, and clinical characteristics as well as HIV RNA and CD4 responses to HAART were homogeneous between the groups. The median follow-up was 132 weeks. In one case (6%), cidofovir was permanently discontinued because of severe proteinuria. One-year cumulative probability of survival was 0.61 with cidofovir and 0.29 without (log rank test $P = 0.02$). After adjusting for baseline CD4 counts, JC viral load in CSF, Karnofsky, and use of HAART prior to the onset of PML, the use of cidofovir was independently associated with a reduced risk of death (hazard ratio, 0.21, 95% confidence interval, 0.07–0.65; $P = 0.005$). A randomized study will definitively establish whether cidofovir confers significant advantage over HAART alone in AIDS-related PML. *Journal of NeuroVirology* (2001) **7,** 364–368.

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Progressive multifocal leukoencephalopathy (PML) is still a relevant cause of morbidity and mortality in patients with AIDS. In the era of highly active anti-retroviral therapy (HAART), its reduction seems less evident than that of other opportunistic disorders of the central nervous system (Moore and Chaisson 1999; Ammassari *et al*, 2000). With potent anti-retroviral therapies, a significantly longer survival of patients affected by PML has also been shown (Miralles *et al*, 1998; Dworkin *et al*, 1999; Tassie *et al*, 1999). Nevertheless, many HIV-infected patients with this neurological disorder have a poor prognosis despite potent anti-retroviral therapies (De Luca *et al*, 1998). The anti-viral compound cidofovir inhibits the replication of simian polyomaviruses *in vitro* (Andrei *et al*, 1997). Several anecdotal reports and a previous retrospective study by our group have shown a clinical benefit of adding cidofovir to HAART in AIDS-related PML (Blick *et al*, 1998; Brambilla *et al*, 1999; De Luca *et al*, 1999; Meylan

Address correspondence to Andrea De Luca, M.D., Istituto di Clinica delle Malattie Infettive, Università Cattolica del S. Cuore, Largo F. Vito, 1–00168 Roma, Italy. E-mail: andeluc@tin.it
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et al, 1999; De Luca *et al*, 2000b). We report the extended follow-up of our initial study with the aim to analyze whether the observed benefit is maintained over time.

Results

Baseline characteristics of patients

From September 1996 to December 2000, 57 HIV-infected patients with PML were observed in the 3 centers. Six were excluded for insufficient diagnostic criteria and 8 (3 from group A, 5 from group B) because of death earlier than 4 weeks after diagnosis. Therefore, 43 patients were analyzed: 27 from group A and 16 from group B. Diagnosis relied on the clinical and MRI picture in all cases, a detectable JC virus DNA in CSF in 41 cases and was histologically confirmed in 4 cases (postmortem). Baseline characteristics are summarized in Table 1. There was a higher proportion of intravenous drug users and a lower proportion of heterosexual HIV transmission in group A. The other variables were homogeneous, including factors previously demonstrated to be associated with prolonged survival in AIDS-associated PML (Berger *et al*, 1998; Taoufik *et al*, 1998;Yiannoutsos *et al*, 1999; De Luca *et al*, 2000a).

Treatments and toxicity

Group A patients were treated with 2 nucleoside reverse transcriptase inhibitors (NRTI) + 1 protease inhibitor (PI) (in 24 cases) and 1 or 2 NRTI + 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) + 1 PI (in 3 cases). Group B individuals were treated as follows: 2 NRTI + 1 PI (in 13 cases), 2 NRTI + 1 NNRTI + 1 PI (in 2 cases), and 2 NRTI + 2 PI (in 1 case) and they were concomitantly treated with intravenous cidofovir (5 mg/kg per week the first 2 weeks and every other week thereafter) in association with probenecid. The median number of cidofovir cycles was 8 (range 4–30). There was only one (6%) cidofovir-related World Health Organization grade-3 toxicity (proteinuria) that led to permanent discontinuation of the drug and reverted after interruption. In 4 other cases (25%), there were delays in the administration of the drug due to mild proteinuria (WHO grade 1). There was no clinically relevant episode of ocular toxicity.

Neurological response

After 2 months of therapy, in an intention to treat analysis (using a noncompleter = nonresponder principle) 7 of 27 (26%) group A and 9 of 16 (56%) group B patients showed clinical response (chi-square $P = 0.047$). After 6 months, by intention to treat analysis, responders were 7 of 24 (29%) in group A and 6 of 12 (50%) in group B (P = ns).

Virological and immunological responses

Two months after therapy (range 45–75 days), JCV-DNA in the CSF became undetectable in 5 of 12 (42%) tested patients from group A and in 7 of 8 (87%) from group B ($P = 0.04$). The proportion of patients ever reaching less than 500 HIV-RNA copies/ml of plasma during follow-up was 75% in both groups. The mean (±SD) changes of HIV-RNA in CSF after 2 (±0.5) months of therapy were −0.45 (±1.36) $\log_{10}$ copies/ml in group A and −0.50 (±1.41) $\log_{10}$ copies/ml in group B (P = ns). Peripheral blood CD4+ T lymphocyte changes 3 months after therapy for PML were +26 (±SD 88) cells/mm^3 in group A and +58 (±85) cells/mm^3 in group B ($P = 0.15$).

Survival analysis

Patients were followed throughout January 31, 2001, when the median follow-up was 922 days: 1143 days

Table 1 Baseline characteristics of patients according to treatment group

	Group A (n = *27*)	*Group B* (n = *16*)	P *value*
Median age (range), years	36 (28–54)	37 (27–55)	0.16
Sex (M/F)	18/9	11/5	0.97
Transmission category—MSM (%)	7	19	0.26
—IDU (%)	81	50	0.03
—Heterosexual (%)	11	31	0.10
Median Karnofsky (range)	50 (20–100)	60 (20–80)	0.58
% PML as first AIDS-defining illness	56	44	0.45
% Enhancement on MRI	15	12	0.83
% HAART prior to PML	37	50	0.40
CD4+ median (IQR) × 10^6 cells/L	51 (26–98)	36 (15–81)	0.39
Plasma HIV-RNA median (IQR), $\log_{10}$ copies/ml	4.00 (2.70–5.77)	4.94 (3.46–5.30)	0.30
CSF HIV-RNA median (IQR), $\log_{10}$ copies/ml	3.11 (2.37–3.60)	2.90 (1.90–3.79)	0.63
CSF JCV-DNA median (range), $\log_{10}$ copies/ml	3.30 (3.20–6.30)	3.30 (3.20–6.30)	0.95

Group A = HAART; group B = HAART + cidofovir; IQR = interquartile range; MSM = men who have sex with men; IDU = intravenous drug users; MRI = brain magnetic resonance imaging; CSF = cerebrospinal fluid.

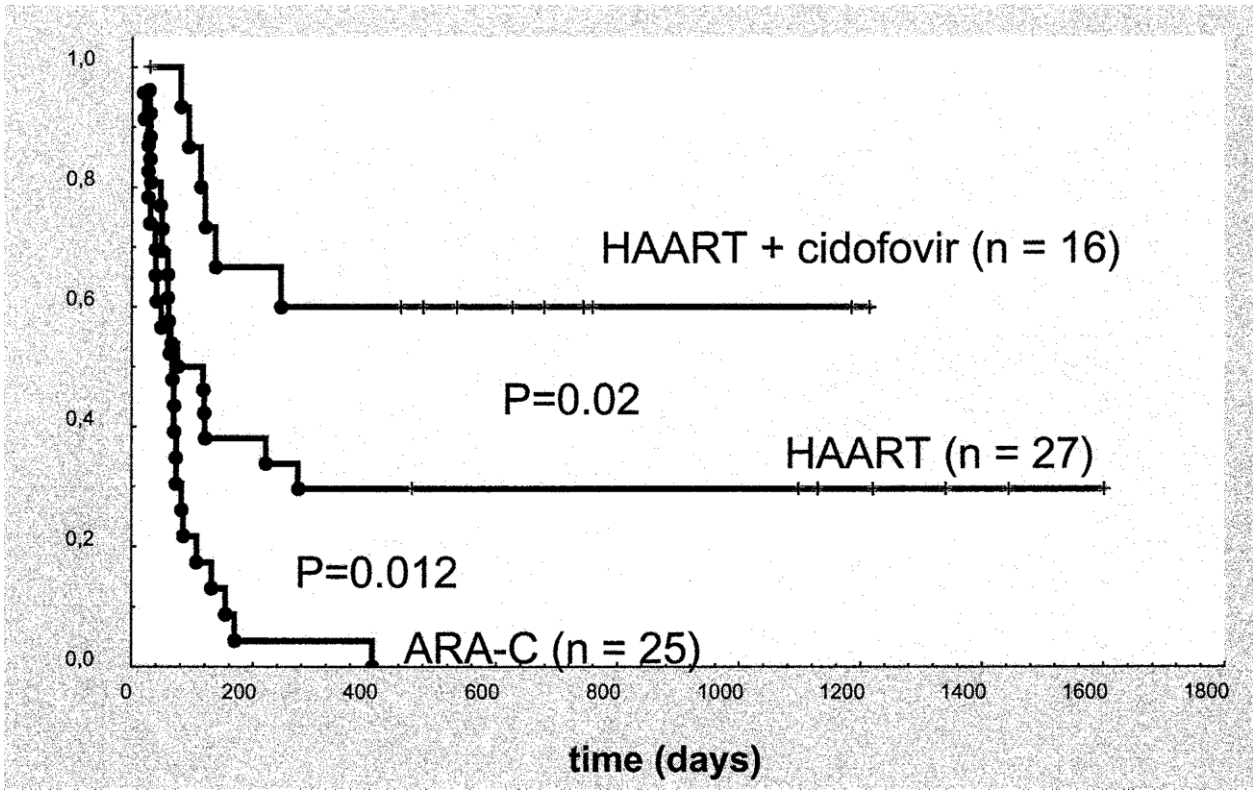

Figure 1 Kaplan–Meier curves indicating cumulative proportion of patients survival according to treatment group. An historical pre-HAART group of patients treated with cytarabine was added for comparison (De Luca, 2000a).

in group A and 590 days in group B. There were 24 deaths, all related to PML: 18 in group A and 6 in group B. Kaplan–Meier analysis showed that the group of patients treated with cidofovir in addition to HAART had a longer survival that the group treated with HAART alone (see Figure 1). The cumulative proportion of patients surviving after 1 year was 0.29 in the HAART group and 0.61 in the HAART plus cidofovir group (log-rank $P = 0.02$). For the purpose of comparison, survival in both treatment groups was plotted together with survival of an historical control group of 25 patients from the pre-HAART era (De Luca *et al*, 2000a): a significant survival advantage was already evident with HAART alone and a further increased survival with cidofovir was observed (see Figure 1). Baseline variables significantly associated with longer survival in univariate analysis were a cerebrospinal fluid JC viral load lower than 50,000 (4.7 $\log_{10}$) DNA copies/ml ($P = 0.0014$), a Karnofsky performance status of 60 or more ($P = 0.019$), beginning HAART before the onset of PML ($P = 0.042$), and a baseline CD4+ cell counts higher than 100/mm^3 ($P = 0.046$).

HIV-RNA levels in plasma and CSF both at baseline and during follow-up, gender, age, HIV-transmission categories, and a history of previous AIDS-defining events did not influence survival. Follow-up variables significantly associated with a longer survival were a clinical response at 2 months ($P = 0.002$) and reaching undetectable levels (<1600 copies/ml) of JCV-DNA in the CSF ($P = 0.01$). The treatment group and all baseline variables significantly associated with survival were included in a multivariate model. Stepwise logistic regression analysis showed that the variables independently predictive of a lower hazard of death were the use of cidofovir (hazard ratio, HR, 0.21, 95% confidence interval, CI, 0.07–0.65; $P = 0.005$) and HAART before the onset of PML (HR 0.31, 95% CI 0.11–0.92; $P = 0.035$). Baseline JCV-DNA $\geq$4.7 log10 copies/ml (HR 3.48, 95% CI 1.38–8.82; $P = 0.008$) and Karnofsky $\leq$50 (HR 2.55, 95% CI 1.00–6.50; $P = 0.049$) were independently associated with increased risk of death.

Discussion

In a previous analysis, we had already shown a significant advantage of cidofovir when added to HAART in the treatment of AIDS-associated PML. There was a more rapid decline of JC virus DNA in the CSF, a significantly higher percentage of patients on cidofovir showing clinical response after 2 months of therapy, and a significantly longer survival in patients on HAART plus cidofovir as compared to those treated with HAART alone. That observation, added to several case reports, suggested that cidofovir might

be clinically useful in AIDS-associated PML (Blick *et al*, 1998; Brambilla *et al*, 1999; De Luca *et al*, 1999; Meylan *et al*, 1999; De Luca *et al*, 2000b).

We have now extended the follow-up of our observational analysis in the 3 clinical centers by 10 more months and recruited new patients observed during this time frame following the previously established selection criteria. The extended analysis confirmed that the use of cidofovir was independently associated with a significantly reduced risk of death. An obvious limitation of our study is its observational nature, which raises the question of possible bias in favor of one treatment group. Nevertheless, baseline characteristics, in particular variables previously proved to be relevant in terms of disease prognosis (Berger *et al*, 1998; Taoufik *et al*, 1998; Yiannoutsos *et al*, 1999; De Luca et al, 2000a), were similar in both treatment groups. Also, HIV RNA and CD4+ cell responses to treatment did not differ between groups, showing that efficacy of HAART was not a bias in favor of one group. Cidofovir with HAART was not always effective. Patients with a high JC viral load in the CSF and a low Karnofsky performance status had a poor prognosis.

Findings of this study are in apparent contrast with the negative results of cidofovir in some patients with PML unrelated to AIDS (Houston *et al*, 2001) and with the poor neurological response obtained in a prospective uncontrolled study of cidofovir (Marra *et al*, 2001). Different patients selection criteria and an excess of toxicity-related treatment discontinuations in the prospective study may account for the prognostic difference between studies. On the other hand, another two large observational studies involving a total of more than 170 patients (more than 60 treated with cidofovir) showed that, in patients treated with HAART, the use of cidofovir was independently associated with a reduced risk of death (Berenguer *et al*, 2001; Gasnault *et al*, 2001).

Given the possible beneficial effect of cidofovir reported by 3 independent, large, retrospective studies and other apparently negative results, there is now clearly the need to test this agent in a randomized, controlled clinical trial. Such a study will require a multicenter and multinational effort to reach the sufficient statistical power to definitively establish whether there is a significant benefit of adding cidofovir over optimal antiretroviral therapy alone for the treatment of PML in the context of HIV infection.

Patients and methods

Patients selection and clinical assessment

Patients from 3 large HIV tertiary-care centers with a confirmed diagnosis of PML, who were exposed to HAART with or without cidofovir for at least 4 weeks were included in the study. Required diagnostic criteria were the concomitant presence of a compatible clinical and neuroradiological picture, plus either the presence of JC virus DNA in the CSF (by PCR), or the presence of characteristic histopathological features in brain tissue (demyelination, oligodendrocytes with enlarged, hyperchromatic nuclei, and enlarged bizarre astrocytes). Cytomegalovirus, herpes simplex virus, varicella zoster virus, and Epstein–Barr virus infections were excluded by PCR assays of the CSF. Patients were classified as those treated with HAART (3 or more antiretroviral drugs, group A), and those treated with HAART and cidofovir (group B). Neurological assessment was standardized, as described (De Luca *et al*, 2000a). Findings from baseline, month 2 and month 6 neurological examinations were analyzed. Patients with improvement or stability of the neurological picture were defined as clinical responders; those with neurological progression were defined as clinical nonresponders.

Virological assays

JC virus DNA was quantified in all CSF samples by a central laboratory by a semiquantitative technique, as described (De Luca *et al*, 2000a). The detection limit of the assay was 1600 copies per ml of CSF and the quantitation range was 2×10^3–2×10^7 copies/ml.

Plasma HIV-RNA was measured using a branched DNA assay (Quantiplex 2.0, Chiron, Emeryville, CA) with a detection limit of 500 copies/ml. HIV-RNA levels in CSF were determined either by an ultrasensitive RT-PCR (Roche, Branchburg, NJ), with a detection limit of 20–50 copies/ml, or by NASBA (Organon Teknika, Baxtel, The Netherlands) with a detection limit of 80 copies/ml.

Statistical analysis

Viral concentrations in body fluids were transformed in log units/ml before calculations. Differences between proportions were analysed by the chi-square test, differences between continuous variables by the Student's *t*-test, or the Mann–Whitney *U*-test, as appropriate. Survival analysis was performed using Kaplan–Meier curves. Stepwise logistic regression was employed to detect the independent association of variables with the risk of death. Two-sided *P* values $\leq$0.05 were considered statistically significant. All analyses were performed using the Statistica 5.0 software package (Statsoft, Padova, Italy).

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References

Ammassari A, Cingolani A, Pezzotti P, De Luca A, Giancola ML, Larocca LM, Antinori A (2000). AIDS-related focal brain lesions in the era of HAART. *Neurology* **55:** 1194–1200.

Andrei G, Snoeck R, Vandeputte M, De Clercq E (1997). Activities of various compounds against murine and primate polyomaviruses. *Antimicrob Agents Chemother* **41:** 587–593.

Berenguer J, Miralles P, Arrizabalaga J, Ribera E, Dronda F, Baraia J, Domingo P, Marquez M, Rodriguez-Arrondo FJ, Laguna F, Rubio R, Lopez-Aldeguer J, De Miguel V, and the Gesida 11/99 Study Group (2001). Clinical course and prognostic factors of AIDS-associated progressive multifocal leukoencephalopathy (PML) in patients treated with HAART (GESIDA 11/99). *The 8th conference on retroviruses and opportunistic infections*, Chicago, February 4–8, 2001, Foundation for Retrovirology and Human Health, p 45, Abstract 10.

Berger JR, Levy RM, Flomenhoft D, Dobbs M (1998). Predictive factors for prolonged survival in acquired immunodeficiency syndrome-associated progressive multifocal leukoencephalopathy. *Neurology* **44:** 341–349.

Blick G, Whiteside M, Griegor P, Hopkins U, Garton T, LaGravinese L (1998). Successful resolution of progressive multifocal leukoencephalopathy after combination therapy with cidofovir and cytosine arabinoside. *Clin Infect Dis* **26:** 191–192.

Brambilla AM, Castagna A, Novati R, Cinque P, Terreni MR, Moioli MC, Lazzarin A (1999). Remission of AIDS-associated progressive multifocal leukoencephalopathy after cidofovir therapy. *J Neurol* **246:** 723–725.

De Luca A, Ammassari A, Cingolani A, Giancola ML, Antinori A (1998). Disease progression and poor survival of AIDS-associated progressive multifocal leukoencephalopathy despite highly active antiretroviral therapy. *AIDS* **12:** 1937–1938.

De Luca A, Fantoni M, Tartaglione T, Antinori A (1999). Response to cidofovir after failure of highly active antiretroviral therapy alone in a patient with AIDS-associated progressive multifocal leukoencephalopathy. *Neurology* **52:** 891–892.

De Luca A, Giancola ML, Ammassari A, Grisetti S, Cingolani A, Paglia MG, Govoni A, Murri R, Testa L, d'Arminio Monforte A, Antinori A (2000a). Cidofovir added to HAART improves virological and clinical outcome in AIDS-associated progressive multifocal leukoencephalopathy. *AIDS* **14:** F117–F121.

De Luca A, Giancola ML, Ammassari A, Grisetti S, Paglia MG, Gentile M, Cingolani A, Murri R, Liuzzi G, d'Arminio Monforte A, Antinori A (2000b). The effect of potent antiretroviral therapy and JC virus load in cerebrospinal fluid on clinical outcome of patients with AIDS-associated progressive multifocal leukoencephalopathy. *J Infect Dis* **182:** 1077–1083.

Dworkin MS, Wan PC, Hanson DL, Jones JL (1999). Progressive multifocal leukoencephalopathy: improved survival of human immunodeficiency virus-infected patients in the protease inhibitor era. *J Infect Dis* **180:** 621–625.

Gasnault J, Kousignian P, Marchadier E, Karaterki A, DelFraissy JF, Taoufik Y (2001). Cidofovir in AIDS-associated progressive multifocal leukoencephalopathy: a monocenter observational study with clinical and JC virus load monitoring. *J NeuroVirol*, submitted.

Houston S, Roberts N, Mashinter L (2001). Failure of cidofovir therapy in progressive multifocal leukoencephalopathy unrelated to human immunodeficiency virus. *Clin Infect Dis* **32:** 150–152.

Marra CM, Rajicic N, Barker DE, Cohen B, Clifford D, and the ACTG 363 team (2001). Prospective pilot study of cidofovir for HIV-associated progressive multifocal leukoencephalopathy. *The 8th conference on retroviruses and opportunistic infections*, February 4–8, 2001, Foundation for Retrovirology and Human Health, p 223, Abstract 596.

Meylan PR, Vuadens P, Maeder P, Sahli R, Tagan MC (1999). Monitoring the response of AIDS-related progressive multifocal leukoencephalopathy to HAART and cidofovir by PCR for JC virus DNA in the CSF. *Eur Neurol* **41:** 172–174.

Miralles P, Berenguer J, Garcia de Viedma D, Padilla P, Cosin J, Lopez-Bernardo de Quiros JC, Munoz L, Moreno S, Bouza E (1998). Treatment of AIDS-associated progressive multifocal leukoencephalopathy with highly active antiretroviral therapy. *AIDS* **12:** 2467–2472.

Moore RD, Chaisson RE (1999). Natural history of HIV infection in the era of combination antiretroviral therapy. *AIDS* **13:** 1933–1942.

Taoufik Y, Gasnault J, Karaterki A, Pierre Ferey M, Marchadier E, Goujard C, Lannuzel A, Delfraissy JF, Dussaix E (1998). Prognostic value of JC virus load in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. *J Infect Dis* **178:** 1816–1820.

Tassie JM, Gasnault J, Bentata M, Deloumeaux J, Boue F, Billaud E, Costagliola D (1999). Survival improvement of AIDS-related progressive multifocal leukoencephalopathy in the era of protease inhibitors. Clinical Epidemiology Group. French Hospital Database on HIV. *AIDS* **13:** 1881–1887.

Yiannoutsos C, Major E, Curfman B, Jensen P, Gravell M, Hou J, Clifford D, Hall C (1999). Relation of JC virus DNA in the CSF to survival in AIDS patients with biopsy-proven progressive multifocal leukoencephalopathy. *Ann Neurol* **45:** 816–820.