

Case report

Successful rescue therapy with a darunavir/ritonavir and etravirine antiretroviral regimen in a child with vertically acquired multidrug-resistant HIV-1

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An increasing prevalence of antiretroviral therapy (ART) resistance in ART-experienced and ART-naïve pregnant women has been reported. Some studies suggest that antiretroviral drug-resistant viruses might have decreased replication capacity and transmissibility. However, cases of perinatal transmission of multidrug-resistant HIV type-1 (HIV-1) have been described. Here, we report the case of one child with vertically-acquired multidrug-resistant HIV-1 and the outcome of a rescue therapy with a darunavir/ritonavir- and etravirine-containing

antiretroviral regimen. During the 15 months of therapy, the child showed clinical improvement, including no side effects, persistent suppression of viral replication and a great increase in CD4⁺ T-cell count. Paediatric HIV specialists should be prepared to manage a small, but increasing, number of babies with a 'nightmare' multidrug-resistant virus with no available treatment options. The use of experimental agents might become a compelling issue in vertically HIV-infected children born in the era of highly active ART.

An increasing prevalence of antiretroviral therapy (ART) resistance in ART-experienced and ART-naïve pregnant women has been reported [1,2]. Some studies suggest that antiretroviral drug-resistant viruses might have decreased replication capacity and transmissibility [3]. However, vertical transmission of multidrug-resistant (MDR) virus has been recently reported [4–6]. Therefore, developing effective combination therapy for HIV-infected children harbouring MDR virus could become one of the greatest challenges in the clinical management of paediatric HIV infection. Recent data showed that a darunavir/ritonavir and etravirine combination is a successful salvage regimen in HIV-infected adults lacking effective treatment options [7,8]. Here, we report the clinical, virological and immunological outcome of a vertically infected child with an MDR HIV type-1 (HIV-1) treated with a darunavir/ritonavir and etravirine combination over the course of 15 months.

The patient was male, born in November 2004 by an emergency caesarean section for premature rupture of membranes at 33 weeks of gestation to a mother who had

failed several lines of ART. The mother's viral load and CD4⁺ T-cell count at delivery were 4.44 log₁₀ copies/ml and 76 cells/mm³, respectively, and genotypic analysis detected 11 point mutations at the reverse transcriptase site (D67N, T69N, K70R, A98S, K103N, V118I, V179E, M184V, Y188L, T215F and K219E) as well as at the protease site (L10I, K20M, M36I, M46I, L63P, A71V, G73S, V77I, I84V, L90M and I93L).

The patient's plasma viraemia at birth was 5.92 log₁₀ copies/ml and genotypic sequences showed an identical pattern of viral mutations as detected in the mother. From delivery to 18 months of age, the patient's CD4⁺ T-cell counts (cells/mm³) and HIV RNA (log₁₀ copies/ml) were, respectively, 650 and 5.92 at birth, 450 and 5.70 at 6 months, 210 and 5.81 at 12 months, and 48 and 5.80 at 18 months. During the same period, his clinical condition rapidly deteriorated from moderately to severely symptomatic and he sequentially received the antiretroviral drugs lamivudine, lamivudine plus didanosine, stavudine plus lopinavir/ritonavir and zidovudine plus tenofovir plus lopinavir/ritonavir plus enfuvirtide. At 21 months of age,

after being monitored by two other paediatric clinics, the child was admitted to our department. His clinical and laboratory data included wasting syndrome, severe motor delay, recurrent episodes of fever, 8.0 mg/dl of haemoglobin, 190 neutrophils/mm³, alanine aminotransferase increased at 3× upper limit of normal, CD4⁺ T-cell count 10 cells/mm³, 5.80 log₁₀ copies/ml of HIV RNA and the same genotypic resistances detected at birth plus gp41-associated mutations (V38G, Q40H and L45M). In November 2006, darunavir/ritonavir and etravirine were provided for compassionate use by Tibotec (Cork, Ireland) and their use was approved by Luigi Sacco Hospital Ethical Committee (Milan, Italy). At 24 months of age, the patient began darunavir at a dose of 150 mg in combination with ritonavir 20 mg twice daily plus etravirine at a dose of 50 mg twice daily (increased to 100 mg twice daily 2 months later according to the pharmacokinetic results) plus lamivudine at a dose of 4 mg/kg twice daily. Pharmacokinetics assessment was performed on days 7 and 70 and showed a darunavir pre-dose plasma concentration (C_{0h}) of 3,770 and 5,270 ng/ml, respectively, and an etravirine C_{0h} of 26.3 and 650 ng/ml, respectively. The plasma concentrations measured at day 70 are consistent with those observed in recent paediatric and adult studies [9,10]. During the 15 months of follow-up, the new ART regimen was well tolerated and the patient's condition greatly improved showing absence of fever, no new AIDS event, weight (+3.8 kg) and height (+13 cm) increase, and normalization of haemoglobin, neutrophils and liver enzymes values. The adherence to ART was excellent throughout the entire period of follow-up. HIV RNA load decreased sharply and progressively decreased between 3 and 12 months of treatment (from 5.38 to 2.27 log₁₀ copies/ml) and it reached

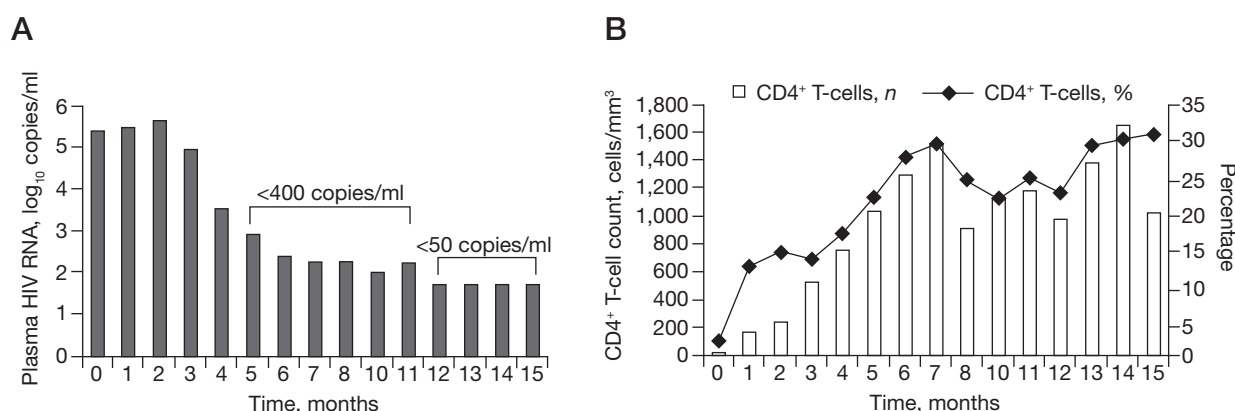
and maintained undetectable values (<50 copies/ml) between 12 and 15 months (Figure 1A).

CD4⁺ T-cell counts and percentage (Figure 1B) showed a marked increase in the first 6 months of the new ART regimen (from 14 to 1,294 cells/mm³ and from 1.9 to 26.9%) and this was maintained at 15 months of follow-up. Results of flow cytometric analysis are shown in Figure 2. From baseline to 12 months follow-up, activated CD4⁺ T-cell counts decreased sharply (from 46.6 to 1.8%), whereas activated CD8⁺ T-cell counts halved (from 84.1 to 43.5%; Figure 2A); naive CD4⁺ T-cell counts greatly increased (from 22 to 69.6%), whereas naive CD8⁺ T-cell counts remained unchanged (from 54.3 to 46%; Figure 2B). The expression of interleukin-7 (IL-7) receptor on CD4⁺ T-cell surface increased from baseline to 12 months follow-up (67% and 99.3%, respectively), but remained unchanged on CD8⁺ T-cells (from 52.6% to 68%; Figure 2C). The percentage of apoptotic non-necrotic cells (annexin V) sharply decreased in the CD4⁺ T-cell subset during the observation period (from 80 to 2.7%), but remained unchanged in the CD8⁺ T-cell subset (from 13 to 10.8%; Figure 2D).

De novo T-lymphocyte production, as measured by T-cell receptor excision circles (TRECs) analysis and expressed as TRECs copies/μl of peripheral blood, increased both in CD4⁺ T-cell and in CD8⁺ T-cell subsets during the 12 months period of treatment (from 0.05 to 1,485 copies/μl and from 0.48 to 7,363 copies/ml, respectively; Figure 2E). Finally, soluble plasma IL-7 levels decreased from 6 pg/ml at baseline to 2.3 pg/ml at 12 months follow-up (Figure 2F).

Thymus evaluation, before starting and 6 months after the new ART regimen, was performed using magnetic resonance imaging as previously described [11]. During this period the thymic volume increased by

Figure 1. Virological and immunological outcome under rescue therapy with a darunavir/ritonavir and etravirine combination in an HIV-1-infected child with vertically acquired multidrug-resistant strains



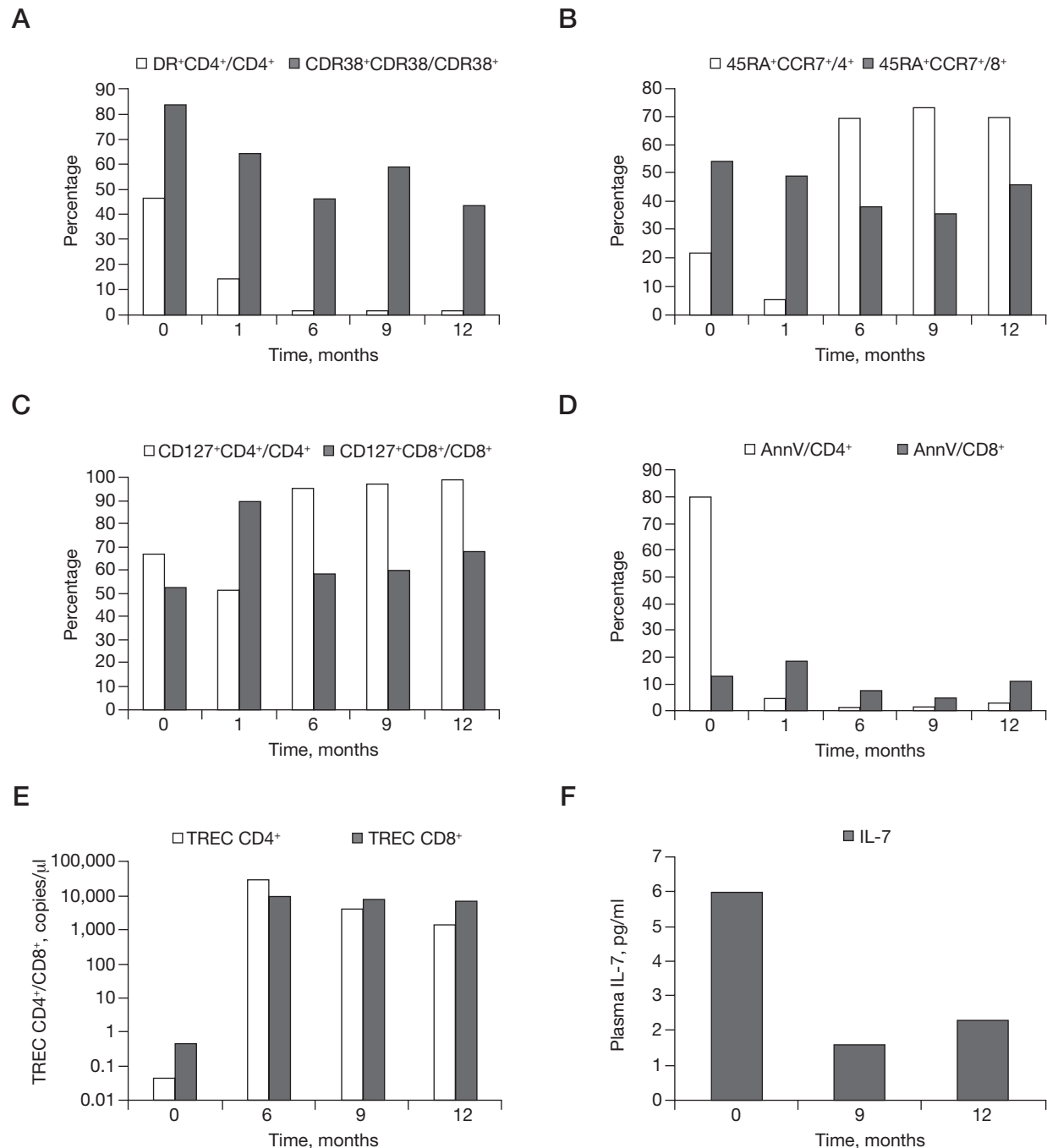
(A) Plasma HIV RNA load and (B) CD4⁺ T-cell count over 15 months. Data was not collected at 9 months of treatment. HIV-1, HIV type-1.

290%, thus increasing in size from 0.8 cm³ to 23.2 cm³ (Figure 3A & 3B).

Current estimates indicate that the prevalence of HIV-infected individuals with transmitted drug resistance is

highest in regions and populations with long-established use of ART [12]. The use of ART in pregnant HIV-infected women in resource-rich countries steadily increased in the highly active ART era from 5% in 1997 to 85%

Figure 2. Flow cytometric analyses



(A) Percentages of activated CD4⁺ T-cell counts (DR⁺CD4⁺/CD4⁺) and activated CD8⁺ T-cell counts (CD38⁺CD8⁺/CD8⁺). (B) Percentages of naive CD4⁺ T-cell counts (45RA⁺CCR7⁺/CD4⁺) and naive CD8⁺ T-cell counts (45RA⁺CCR7⁺/CD8⁺). (C) Percentages of interleukin-7 (IL-7) expression on CD4⁺ T-cell (CD127⁺CD4⁺/CD4⁺) and CD8⁺ T-cell surfaces (CD127⁺CD8⁺/CD8⁺). (D) Percentages of apoptotic non-necrotic cells in the CD4⁺ T-cell (AnnV/CD4⁺) and CD8⁺ T-cell (AnnV/CD8⁺) subsets. (E) T-cell receptor excision circles in the CD4⁺ T-cell (TREC CD4⁺) and CD8⁺ T-cell (TREC CD8⁺) subsets. (F) IL-7 plasma levels.

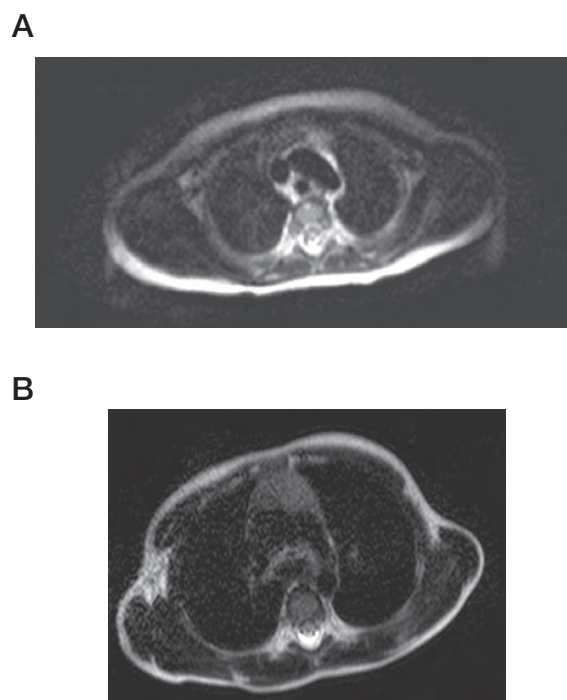
in 2003 [13]. As a consequence, ART drug resistance could continue to increase among HIV-positive pregnant women, which could favour perinatal transmission of MDR HIV-1. Therefore, the availability of new effective therapies could become one of the greatest tasks in the management of HIV-infected children harbouring MDR virus.

The only published data currently available on the use (as the rescue therapy) of etravirine combined with darunavir in multidrug-experienced HIV-infected patients, came from studies performed in adults [7,8]. Overall, the patients enrolled in both these studies showed a less severe immune impairment and displayed a lower number of resistance-associated mutations than our paediatric patient. Nevertheless, our patient achieved comparable results with those observed in the two randomized trials in terms of virological response, but with a greater immunological benefit. In humans there is strong evidence that thymic-dependent pathways are required for rapid CD4⁺ T-cell regeneration and that aging negatively affects the efficiency of immune reconstitution. Our results are consistent with the faster CD4⁺ T-cell regeneration observed in children compared with adults after intensive chemotherapy and with the observed relevant contribution of the thymus to the immune reconstitution in vertical HIV-infected children treated with successful ART [9,14].

The in-depth analysis of peripheral lymphocyte changes showed a well-known two-phase kinetic model of CD4⁺ T-cell recovery following the introduction of an effective ART regimen [15]. The first phase of CD4⁺ T-cell count increase was driven by two main events: the sharp reduction in the percentage of activated cells undergoing apoptotic processes and the quick release of CD4⁺ T-cells with a memory phenotype from lymphoid tissue. The second phase was mainly due to the proliferation and production of new CD4⁺ T-cells with naive phenotype (re)expressing IL7-receptor, which also corroborated with the sharp increase in total CD4⁺ T-cell TREC content maintained up to 12 months follow-up [16,17]. In line with the progressively reconstituting CD4⁺ T-cell pool, a reduction in IL-7 levels was shown in plasma. This finding, together with the overall rise in both TRECs and IL-7+CD4⁺ T-cell counts, indicate a T-cell pool that is overall receptive to the IL-7-mediated positive effects on peripheral T-cell homeostasis [18]. The changes in CD8⁺ T-cell subset appear more complex than those observed in CD4⁺ T-cells, possibly reflecting diverse recovery dynamics in the two subpopulations.

In conclusion the administration of the two investigational antiretroviral drugs was safe and effective in our patient with vertically-acquired MDR HIV-1 and drove a sustained quantitative and qualitative immune recovery. Strategies for the management of patients with MDR HIV infection are limited in

Figure 3. Thymus size assessed by magnetic resonance imaging



Magnetic resonance imaging was carried out (A) prior to and (B) 6 months after rescue therapy with darunavir/ritonavir and etravirine combination.

adults and even more in paediatric patients. Our observation is relevant, mainly if we consider that our paediatric patient harboured a virus with a broad extent of resistance-associated mutations, involving all the classes of antiretroviral drugs available in clinical practice. The awareness of risks coming from the use of experimental agents is mandatory, especially in paediatric patients, both in terms of unknown side effects and potential unfavourable pharmacokinetic interactions. Nevertheless, we believe that the use of experimental agents, with a careful follow-up, should be considered in paediatric patients with limited treatment options and that it will be a compelling issue in the vertically HIV-infected children born in the era of highly active ART.

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Disclosure statement

The authors declare no conflicting interests.

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