Persistence of HIV reservoir following successful haematopoietic stem cell transplant for juvenile myelomonocytic leukaemia in a child with perinatally acquired HIV

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Abstract

This report describes a case of juvenile myelomonocytic leukaemia (JMML) on a background of both perinatally acquired HIV infection and congenital cytomegalovirus, and management of antiretroviral therapy during haematopoietic stem cell transplant. Peripheral blood HIV viral load remained below the lower limit of detection throughout and following transplant and is currently <20 RNA copies/mL. The child is currently in remission from JMML, but HIV DNA remains detectable despite myeloablative conditioning and sustained plasma HIV viral suppression.

Introduction

HIV remission following haematopoietic stem cell transplant (HSCT) from a CCR5-negative donor has been demonstrated in one adult (reviewed in [1]). Children with perinatally acquired HIV who have achieved early and sustained virological suppression with combination antiretroviral therapy (cART), in part due to minimised HIV reservoir size, are currently considered to have significant potential for successful HIV remission or eradication using novel strategies [2].

This is a report of a child diagnosed with juvenile myelomonocytic leukaemia (JMML) on a background of both congenital cytomegalovirus (CMV) and perinatally acquired HIV infection. Complexities of antiretroviral management in the context of HSCT and novel aspects of JMML management are highlighted. Despite early suppressive ART, myeloablative conditioning and ongoing HIV suppression throughout HSCT, HIV reservoir is still detectable during the post-transplant period, albeit at the lower limit of detection of available assays.

Case report

Background

As previously reported [3], a male infant at 28 + 5 weeks' gestation was born by emergency caesarean section to a mother antenatally diagnosed with HIV. His mother had not received any antenatal care, having spent much of the pregnancy abroad, and presented to the hospital with pre-eclampsia at 28 weeks' gestation. Maternal raltegravir, nevirapine, zidovudine and lamivudine were initiated 5 days prior to delivery. Intravenous (IV) zidovudine was given intrapartum. The infant was born in fair condition, with Apgar scores of 4 at 1 minute and 9 at 5 minutes, weighing 1150 g.

Due to high risk of HIV transmission, a three-drug infant regimen for prevention of mother-to-child transmission (PMTCT) was commenced: nevirapine, zidovudine and lamivudine [4]. The infant's day 1 HIV blood RNA PCR result was positive, with a viral load (VL) of 3000 copies/mL, and baseline resistance testing demonstrated a subtype C wild-type virus. The PMTCT antiretroviral dosing was changed to full treatment dosing at 2 days of age. Abacavir was added when HLA-B*5701 was confirmed negative on day 8 of life. In view of the slow virological response on initial therapy and concerns about the potential for transmitted resistance, nevirapine was changed to ritonavir-boosted lopinavir at 2 months of age. At 5 months, zidovudine was discontinued in view of ongoing anaemia. An HIV VL of <50 copies/mL was achieved from 5 months of age.

Congenital CMV infection was diagnosed in the first week of life by urine DNA PCR and IV ganciclovir was commenced at 3 weeks, by which time he had a negative CMV DNA PCR on blood testing, and this was converted to oral valganciclovir, which was continued for 6 months of total treatment. Cranial ultrasounds, cranial magnetic resonance imaging and ophthalmological reviews were normal, and his CMV plasma DNA PCR was <150 copies/mL at 28 months of age.

Diagnosis of malignancy

At 3 months of age, the infant developed pancytopenia thought to be related to antiviral treatment and was treated with granulocyte colony-stimulating factor, erythropoietin and blood transfusions.

At 11 months of age, he presented with a rash and hepatosplenomegaly. Full blood count showed haemoglobin 95 g/L, platelets 47×10^9/L and white blood cells of 26.5×10^9 cells/L due to monocytosis and a leucoerythroblastic film, with dysplastic monocytes. The initial presentation and laboratory findings have previously been reported [3]. Bone marrow examination revealed hypercellular marrow, reduced erythropoiesis and an abnormal expansion of monocyte and promonocyte populations accounting for 30% of cells. These findings, coupled with a monosomy 7 on cytogenetics in 80% of cells and an NRAS codon Gln61Lys, confirmed a diagnosis of JMML. He did not have a raised fetal haemoglobin. At this time, blood CMV, HIV, Epstein–Barr virus,

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human herpesvirus 6 and eight blood PCRs were negative. 6-Mer-captopurine (6MP) 50 mg/m^2 was commenced. One month later, the child presented with neutropenic sepsis and hepatitis possibly secondary to 6MP and JMML. Adenoviraemia with peripheral blood VL of 41,000 copies/mL was detected and cidofovir was commenced with good effect. Due to hepatitis on 6MP and ongoing hepatosplenomegaly, the child was transferred to Great Ormond Street Hospital for a trial of two cycles of azacitidine with a partial response as assessed by a reduction in organomegaly and an improvement in peripheral counts. He was subsequently given a single course of acute myeloid leukaemia (AML) chemotherapy following an exacerbation of disease coincident with *Streptococcus viridans* sepsis. He responded well to AML chemotherapy with a reduction in monosomy 7 clone down to 7% and a substantial reduction in organomegaly.

Haematopoietic stem cell transplant

The initial search for a closely HLA-matched CCR5 coreceptor negative donor was unsuccessful. On finding a suitable stem cell donor, CCR5 depletion by gene editing using zinc finger nucleases was planned. However, the child's JMML disease progression with further episodes of sepsis meant this strategy was abandoned to prioritise HSCT.

Prior to HSCT, the HIV VL remained <50 copies/mL, with a CD4 cell count of 1380 cells/µL (64%) and a CD4:CD8 ratio of 2.3. Weight was 8.45 kg (ninth centile). Prophylactic liposomal amphotericin B and cotrimoxazole were commenced. Azacitidine therapy was ongoing. In view of planned conditioning agents and antifungal, antimicrobial and antiviral prophylaxis and given the potential interactions with boosted protease inhibitors, special consideration was required in managing HIV through HSCT. A regimen was planned in the paediatric HIV virtual clinic [5] with these considerations in mind: raltegravir, lamivudine, abacavir with potential addition of IV zidovudine and enfuvirtide during periods of decreased absorption of oral medication.

Conditioning included treosulphan, cyclophosphamide, melphalan and antithymocyte globulin (Genzyme, USA). The child received haematopoetic stem cells from a 10/10-matched unrelated CMV-positive female donor.

Raltegravir therapeutic drug monitoring (TDM) was employed to optimise dosing. On developing mucositis during transplant, IV zidovudine and IV enfuvirtide (2 mg/kg bd) were added and oral ART was continued as tolerated (days 3–20). Given the nature of the intermittent oral intake with variable mucositis, it was considered preferable to have periods with five antiretroviral agents rather than stopping oral medication completely. The child remained on oral zidovudine after stopping IV zidovudine until cerebrospinal fluid HIV PCR was confirmed <50 copies/mL and raltegravir TDM was in therapeutic range (1 month post transplant).

Although chest X-rays were unremarkable, CT of the chest showed left lower lobe consolidation. It was unclear if this was secondary to fungal infection or aspiration; therefore, he continued liposomal amphotericin B. At discharge, a repeat CT of the chest showed an almost full resolution of the consolidation and his medication was converted to oral posaconazole prophylaxis.

There was a brief period of CMV viraemia from 30 to 43 days post transplant, with a maximum VL of 4130 copies/mL in peripheral blood, which did not require treatment. There were no signs of CMV disease and subsequent CMV screening was negative.

Nasogastric tube was the preferred route of medication administration through transplant, allowing continued enteral ART administration.

He was discharged 7 weeks after HSCT with 100% donor engraftment in all cell lineages, in full haematological remission and without any signs of graft-versus-host disease (GVHD).

Follow up

Nineteen months post transplant, he has good immune reconstitution, continues 100% donor engraftment and remains clear of GVHD. He remains on raltegravir (chewable tablets), lamivudine and abacavir (liquids) twice daily with a CD4 cell count of 867 cells/µL (30%), a CD4:CD8 ratio of 0.7 and an undetectable HIV plasma VL (<20 copies/mL). HIV-1/2 fourth-generation Ag/Ab testing is presently negative.

HIV peripheral blood DNA reservoir measurement

Measurement of proviral DNA was as previously described [6]. Briefly, viral DNA was extracted from peripheral blood mononuclear cells, and HIV DNA was measured with a duplexed PCR using patient template DNA with forward and reverse primer pairs for PDH, PDH probe, LTR and LTR probe plus Qiagen Multiplex Mastermix (Qiagen, Hilden, Germany). The total HIV DNA was 1.3 and 10.5 copies per million cells at 5 and 15 months after the transplant. For a graphical overview of the case, see Figure 1.

Discussion

This case is a first report of successful HIV suppression throughout and following HSCT for JMML in a child with perinatally acquired HIV. HIV VL was <50 copies/mL from 5 months of age and remained below the lower limit of detection of conventional assays during and after HSCT on an ART regimen designed to minimise chances of drug interactions and toxicity.

This case highlights the essential discussion between the multidisciplinary teams, including infectious diseases, pharmacy, transplant, virology and haematology services to plan ART during HSCT. In the ART era, the outcome of HSCT for haematological malignancy in adult individuals living with HIV is nearly comparable with that of individuals not living with HIV [1]. In this child's case, HSCT was the only curative option for JMML and aside from episodes of neutropenic sepsis and brief recurrence of CMV viral detection without disease, he completed HSCT with minimal complications.

This case has shown that in the absence of resistance, raltegravir, abacavir and lamivudine with IV zidovudine and enfuvirtide are effective in maintaining HIV control during HSCT. Further experience is needed to better guide ART in HSCT in children; however, this case shows that sustained virological suppression is possible despite the multiple challenges faced during HSCT.

In light of the report of HIV cure following CCR5 homozygous donor HSCT for AML in an adult [1], modification of donor cells for this individual to remove the gene encoding CCR5 was planned. Unfortunately, due to JMML rapid progression, this could not be undertaken in time. Cases to date have shown that interruption of ART post HSCT even in the context of CRR5 heterozygous or homozygous donors, results in HIV virological rebound [7,8].

In contrast to one recent case series in adults living with HIV requiring HSCT demonstrating absent reservoir with full donor engraftment, we have found evidence of low-level HIV reservoir post transplant [9]. The finding of a persistent HIV reservoir despite aggressive myeloablative conditioning and early and sustained HIV suppressions in this case indicates that alternative strategies are likely to be needed to induce long-term ART-free HIV remission following HSCT in children.
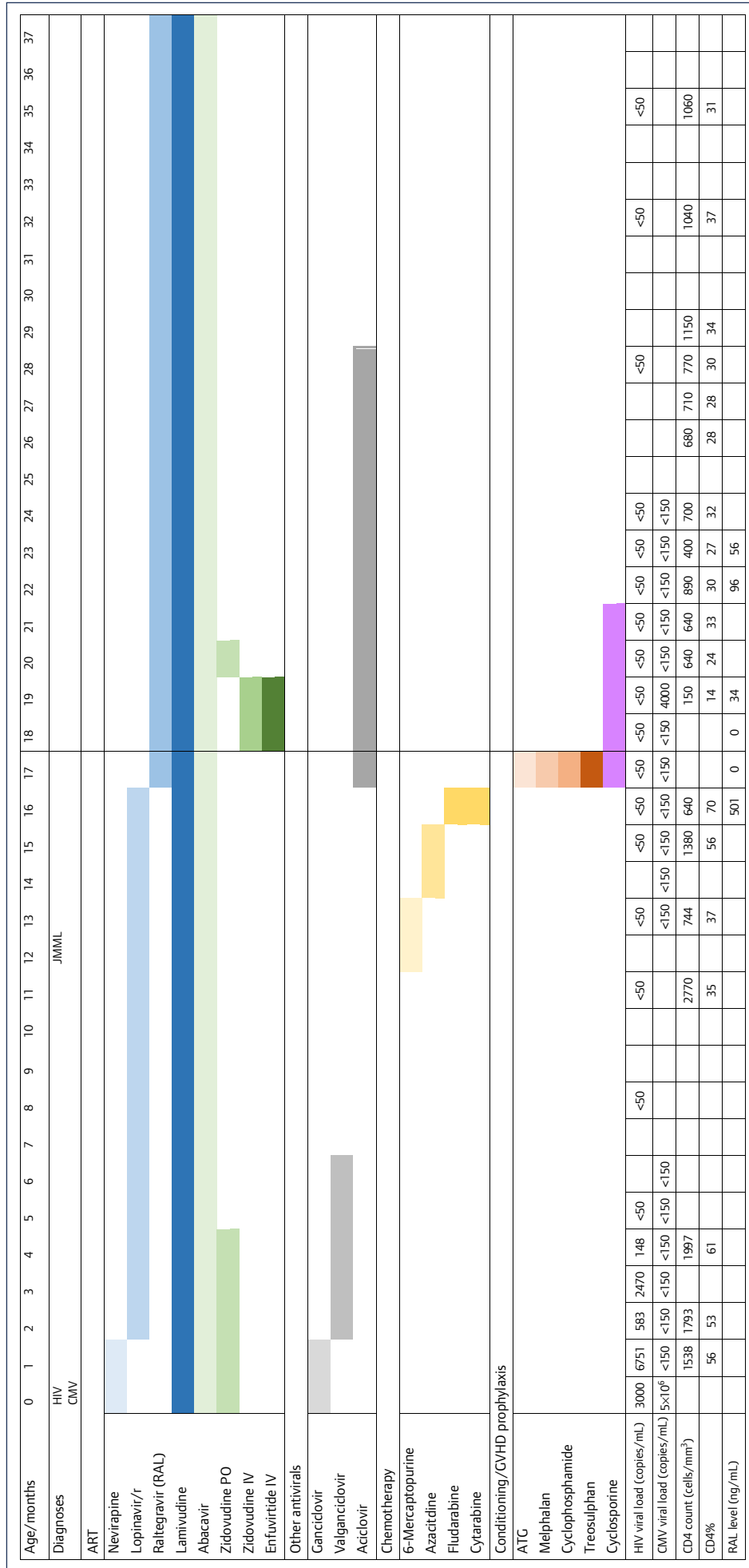
Figure 1. Overview of therapy and laboratory results from birth. ART: antiretroviral therapy; ATG: antithymocyte globulin; CMV: cytomegalovirus; GVHD: graft-versus-host disease; IV: intravenous; JMML: juvenile myelomonocytic leukaemia; PO: per os.

Diagnoses
- HIV (month 0)
- CMV (month 0)
- JMML (≈ month 11)

ART / antiviral / chemotherapy timeline (approximate age in months)

Row	Approximate duration (months)
Neviripine	~1
Lopinavir/r	~1–16
Raltegravir (RAL)	~17–37
Lamivudine	~1–37
Abacavir	~17–37
Zidovudine PO	~4–5; ~20
Zidovudine IV	~19–20
Enfuvirtide IV	~18–19
Other antivirals	
Ganciclovir	~0–1
Valganciclovir	~5–7
Aciclovir	~17–28
Chemotherapy	
6-Mercaptopurine	~12–13
Azacitidine	~13–15
Fludarabine	~15–16
Cytarabine	~16–17
Conditioning/GVHD prophylaxis	
ATG	~17
Melphalan	~17–18
Cyclophosphamide	~17–18
Treosulphan	~17–18
Cyclosporine	~18–21

Laboratory results (by age in months)

Age/months	HIV viral load (copies/mL)	CMV viral load (copies/mL)	CD4 count (cells/mm³)	CD4%	RAL level (ng/mL)
0	3000	5×10⁶	1538	56	
1	6751	<150	1793	53	
2	583	<150			
3	2470	<150	1997	61	
4	148	<150			
5	<50	<150			
6		<150			
8	<50				
11	<50		2770	35	
13	<50	<150	744	37	
14		<150			
15	<50	<150	1380	56	
16	<50	<150	640	70	501
17	<50	<150			0
18	<50	<150			0
19	<50	4000	150	14	34
20	<50	<150	640	24	
21	<50	<150	640	33	
22	<50	<150	890	30	96
23	<50	<150	400	27	56
24	<50	<150	700	32	
26			680	28	
27			710	28	
28	<50		770	30	
29			1150	34	
32	<50		1040	37	
35	<50		1060	31	

Acknowledgements

References

1. Kuritzkes DR. Hematopoietic stem cell transplantation for HIV cure. *J Clin Invest* 2016; **126**: 432–437.
2. Klein N, Palma P, Luzuriaga K *et al.* Early antiretroviral therapy in children perinatally infected with HIV: a unique opportunity to implement immunotherapeutic approaches to prolong viral remission. *Lancet Infect Dis* 2015; **15**: 1108–1114.
3. Bazin J, Karnik L, Tudor-Williams G *et al.* Juvenile myelomonocytic leukemia in an infant with congenital human immunodeficiency virus and cytomegalovirus infection. *Am J Hematol* 2017; **92**: 1391–1392.
4. Taylor GP, Dhar J, Kennedy MJ *et al.* British HIV Association guidelines for the management of HIV infection in pregnant women 2012 (2014 interim review). *HIV Med* 2014;**15 (suppl 4)**: 1–77.
5. Le Doare K, Mackie N, Kaye S *et al.* Virtual support for paediatric HIV treatment decision-making. *Arch Dis Child* 2015; **100**: 527–531.
6. Busby E, Whale AS, Bridget Ferns R *et al.* Instability of 8E5 calibration standard revealed by digital PCR risks inaccurate quantification of HIV DNA in clinical samples by qPCR. *Sci Rep* 2017; **7**: 1–7.
7. Sugarman J, Lewin SR, Henrich TJ *et al.* Ethics of ART interruption after stem-cell transplantation. *Lancet HIV* 2016; **3**: e8–e10.
8. Cummins NW, Rizza S, Litzow MR *et al.* Extensive virologic and immunologic characterization in an HIV-infected individual following allogeneic stem cell transplant and analytic cessation of antiretroviral therapy: a case study. *PLoS Med* 2017; **14**: 1–23.
9. Salgado M, Kwon M, Gálvez C *et al.* Mechanisms that contribute to a profound reduction of the HIV-1 reservoir after allogeneic stem cell transplant. *Ann Intern Med* 2018; **169**: 674–683.