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No Clinically Significant Drug Resistance Mutations In HIV-1 subtype C Infected Women After Discontinuation Of NRTI-Based Or PI-Based HAART For PMTCT In Botswana

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Abstract

Risk of developing drug resistance after stopping antiretroviral regimens to prevent mother-to-child HIV-1 transmission (PMTCT) is unknown. Mma Bana Study randomized treatment-naïve pregnant women with CD4 ≥200 cells/mm^3 to receive either abacavir/zidovudine/lamivudine (triple NRTI arm) or lopinavir/ritonavir/zidovudine/lamivudine (PI arm). Drugs were discontinued after 6 months breastfeeding. One month post-discontinuation, 29 NRTI arm samples and 25 PI arm samples were successfully genotyped. No clinically significant antiretroviral resistance mutations were detected. Eight minor resistance mutations were found among 11(20%) women (3 from NRTI arm, 8 from PI arm), occurring at similar frequencies to those reported in HIV-1 subtype C treatment naive cohorts.

Keywords

Mother-to-Child HIV transmission; Highly Active Antiretroviral Therapy; Minor drug resistance mutations; Botswana

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Background

World Health Organization (WHO) guidelines for prevention of mother-to-child HIV-1 transmission (PMTCT) include options to use highly active antiretroviral therapy (HAART) for all HIV infected pregnant women from pregnancy through the end of the breastfeeding period[1]. However, no resistance data has been reported for the period immediately after stopping HAART used for PMTCT during breastfeeding.

Treatment interruption studies in non-pregnant adults indicate that stopping treatment may lead to negative long-term health outcomes[2–4], including development of resistance mutations[5–9]. In PMTCT settings, lower risk of resistance after short-course HAART compared with ZDV/single dose Nevirapine (sd-NVP) has been reported shortly after delivery in non-breastfeeding studies[10–13]. No report has evaluated emergence of drug resistance mutations after discontinuing triple NRTI or PI-based HAART regimens following breastfeeding. We therefore evaluated development of drug resistance at 7 months postpartum among HIV-1 subtype C infected pregnant women who discontinued HAART for PMTCT after 6 months breastfeeding.

Materials and methods

Mma Bana Study[14] enrolled 560 HIV-1 subtype C infected treatment-naïve pregnant women in Botswana with CD4 counts ≥200 cells/mm^3 who elected to breastfeed. Women were randomized, starting between 26–34 weeks gestational age, to receive either abacavir (ABC)/zidovudine (ZDV)/lamivudine (3TC) co-formulated as Trizivir (TZV) in the NRTI arm or lopinavir/ritonavir (LPV/r)/ZDV/3TC co-formulated as Kaletra (KAL)/Combivir (CBV) in the PI arm. PMTCT regimens were discontinued at 6 months postpartum visit after confirmation of weaning just prior to this visit unless HAART was required for maternal health according to the Botswana guidelines.

The Botswana Health Research Development Committee and the Harvard School of Public Health Human Subjects Committee approved the study protocol and amendments.

For the resistance sub-study, we extracted RNA from 85 samples collected from women (42 from the NRTI arm, 43 from the PI arm) at 7 months postpartum, including all women from each randomization arm who had a sample available and had been at highest risk for virologic failure (on HAART that concluded at 6 months postpartum with detectable HIV RNA >400 copies/mL at 1, 3, or 6 months postpartum). Twenty-three (27%) of these 85 women, 11 (26%) in the NRTI arm and 12 (28%) in the PI arm, were in the highest risk category because they had detectable HIV RNA >400 copies/mL at postpartum. PCR amplification and sequencing were attempted for all 85 samples.

Population sequencing of a segment of HIV-1 *pol* was conducted using an in-house HIV drug resistance genotyping assay as previously detailed elsewhere[15]. Briefly, viral RNA was extracted using either QIAamp viral RNA extraction Kit (Qiagen) or ViroSeq kit (Celera). The RNA was reverse transcribed into cDNA and the resulting cDNA was PCR amplified using primers CWF1 (5′-GAAGGACACCAAATGAAAGAYTG-3′) and CWR1 (5′-GCATACTTYCCTGTTTTCAG-3′). The PCR product was purified sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and then sequenced in the ABI Prism 3100 genetic analyzer. ViroSeq HIV-1 Genotyping Systems Software Version 2.7 (Celera) was used to generate consensus sequences. The consensus sequences were analyzed for known ART resistance mutations using the Stanford HIV Drug Resistance Database.

Statistical analysis was performed using nonparametric methods in the Python SciPy package. Statistical significance was assessed with a family-wise type I error rate of 0.05 using the Bonferroni approach.

Results

Maternal Characteristics

Among the 85 samples, 61 had known HIV RNA levels (range <400 – 750,000 copies/ml) and 24 had unknown RNA levels 1 month after discontinuing HAART. Fifty-four (64%) samples were successfully amplified and sequenced, 29 (69%) from NRTI arm and 25 (58%) from PI arm. Of these women, 7 (24%) mothers from the NRTI arm and 8 (32%) from the PI arm had HIV RNA>400 copies/mL in the postpartum treatment period.

Among women with genotyped samples, the median age was 27 years at enrollment and median duration of HAART was 266 days (Table 1). Median baseline (pre-treatment) HIV RNA for women with genotyped samples was 41,600 copies/mL in the NRTI and 29,500 copies/mL in the PI arm. At delivery, 47 (87%) women had HIV RNA <400 copies/mL, while the remaining 7 (13%) had HIV RNA ranging from 448 to 9,460 copies/mL. At the time of discontinuation of HAART, after 6 months breastfeeding, 47 (87%) women had HIV RNA < 400 copies/mL. Among the 7 (13%) women not suppressed, 3 were from the NRTI arm (range of 1,750–63,000 copies/mL) and 4 from the PI arm (range 606–42,200 copies/mL). At 7 months postpartum, one month after HAART discontinuation, median HIV RNA among genotyped women with known viral loads was 56,900 copies/mL (IQR 15,300–217,750 copies/mL). These values did not differ significantly by arm, or exceed the corresponding HIV RNA values at baseline.

For women with genotyped samples, median CD4 cell count at baseline was 389 cells/mm^3 for the NRTI arm and 403 cells/mm^3 for the PI arm (Table 1). At the time of HAART discontinuation at 6 months postpartum, median CD4 count was 595 cells/mm^3 (IQR 519–734 cells/mm^3) in NRTI arm and 610 cells/mm^3 (IQR 495–846 cells/mm^3) in the PI arm. Self-reported adherence of mothers revealed that 8 (15%) mothers had missed HAART for 2 or more days: 3 (10%) in NRTI arm and 5 (20%) in PI arm. Nine (17%) women reported missing at least one day of HAART.

All sequences were HIV-1 subtype C by phylogenetic analysis.

HIV Drug Resistance Detected One Month After Discontinuation of HAART

One month after discontinuing HAART only minor resistance mutations were detected in 11 (20%) of the 54 samples, 3 (10%) in the NRTI arm and 8 (32%) in the PI arm (Table 2a). Nine (17%) women (2 from the NRTI arm who did not receive any PI drugs and 7 from the PI arm) had one or more of the protease mutations L10V/I, Q58E, A71V/T, and T74S (P = 0.07 for difference between arms). Notably, of the 9 women carrying a PI mutation, 7 (78%) harbored only one isolated PI mutation and 2 (22%) samples showed two PI mutations. The minor NRTI mutation, V118I, was detected in 2 (7%) women from the NRTI arm only. One of these women in the NRTI arm also had A71T, a PI mutation.

Four (7%) of the 54 women had E138A mutation (2 in NRTI, 2 in PI arm), which is associated with NNRTI resistance (none received NNRTIs during the study). Both of the women in the NRTI arm with E138A mutation also exhibited V118I. One of these women harbored all three classes of minor ARV mutations: A71T (PI), V118I (NRTI), and E138A (NNRTI). In the PI arm, one E138A mutation was observed along with PI mutations (L10V, T74S). Nine (82%) of the 11 women with minor mutations reported 100% adherence to HAART from antenatal enrollment to 6 months postpartum.

Discussion

The use of HAART for PMTCT has been a resounding success. However, some HAART regimens have tradeoffs for maternal health, including the development of drug resistance mutations that may compromise future treatment. In this study, there was no major antiretroviral drug resistance mutation detected after stopping either NRTI-based or PI-based HAART at 6 months postpartum. Only minor or accessory antiretroviral drug resistance mutations were detected (L10I/V, Q58E, A71T/V, T74S, E138A, and V118I), nearly all polymorphisms that have been reported among antiretroviral naïve adults in Botswana[16] and elsewhere [17–21]. All 4 women with E138A had previous pregnancies, and thus possible exposure to ZDV and sd-NVP through the Botswana PMTCT program, although only one woman self-reported to have taken ZDV during a previous pregnancy.

Our results contrast with studies that reported high rates of drug resistance mutations after stopping NVP[5–8,22] or NFV-based HAART[9,23–26], but are in accord with the results of a study in Germany that demonstrated the absence of acquired drug resistance after stopping LPV/r-based HAART immediately after delivery[13]. Few studies have evaluated stopping HAART after breastfeeding cessation (rather than after delivery), but we believe that the higher percentage of virologically suppressed women at this time point may reduce the risk for resistance development during the drug washout period (if fewer women have detectable virus exposed to low-levels of drug).

The drug regimens used in the Mma Bana Study were chosen in part because of the safety of stopping these regimens in treatment settings[22,27–35]. Upon virologic failure[27,35] or discontinuation[13] of LPV/r-based HAART, the prevalence of resistance mutations is often low. This reflects the high genetic barrier to resistance for LPV/r, which typically requires the accumulation of several mutations in the active site of protease, in addition to multiple accessory mutations[17].

Similar half-lives of ARVs used in our study may have reduced unintended monotherapy following discontinuation of HAART, thus partially explaining the lack of major resistance mutations[36,37]. ZDV decays rapidly ($t_{1/2}$=2.8 hrs)[38] while 3TC maintains longer inhibitory levels ($t_{1/2}$=12.5 hrs)[39]. Compared with the half-lives of NFV and NVP (3.5 and 27.5 hrs)[40,41], those of ABC and LPV/r (6.4 and 6.2 hrs)[41–43] better match that of 3TC, narrowing the window of functional monotherapy after treatment termination and possibly avoiding selection of drug resistance.

This study focused on detecting significant resistant subpopulations. While it has been shown that minority resistant mutants can be detected in both drug-experienced and drug-naive patients, their impact on the success of antiretroviral therapy remains to be established[10,44–47]. Minority subsets of drug-resistant virus present at baseline can proliferate under drug pressure and lead to virologic failure in treatment-naive patients, most frequently among patients who receive NNRTI-based HAART regimens[44,48–53]. In contrast, other studies (with boosted PIs) showed no significant difference between the outcome of first-line therapy in acutely or recently HIV-1 infected patients who carried minority variants of drug-resistant viruses and those who did not[45,46,54,55].

Our study had some limitations. The viral load requirement of our genotyping assay may have caused us to miss mutations in samples with less-fit (thus slower rebounding) mutant virus. We note that structured treatment interruption studies[34,56] reported that no NRTI or PI resistance mutations were detected during delayed or absent viral rebounds after treatment interruptions. Furthermore, we highlight the fact that the baseline viral loads of genotyped mothers were significantly higher than those of the remainder of the Mma Bana cohort ($P < 0.001$). High baseline viral load was previously found to be significantly associated with

development of drug resistance during HAART[57]. Also, in a recent study, women who had HIV-1 RNA levels above 400 copies/mL during pregnancy-limited antiretroviral therapy were found to be 2.7 times more likely to have the M184V mutation detected postpartum than those remaining aviremic through delivery[26]. Thus, we can reasonably infer that the probability of drug resistance among the genotyped mothers represents an approximate upper bound for the entire Mma Bana cohort.

Baseline genotyping was not done to determine if the observed accessory mutations were present before starting HAART or emerged during or after HAART. However, the lack of major resistance mutations is of paramount importance, and clinical decisions are unlikely to be affected by the minor variants detected. We used population sequencing to detect resistance-associated mutations present in at least 20% to 25% of the viral population. While this is the most common method to detect mutations, it is less sensitive than allele-specific real-time PCR, which allows detection of minority variants with a sensitivity as low as 0.01% [46,53,54]. We believe the strength of our design was sequencing 1 month after HAART discontinuation, at a period early in viral rebound when wild-type virus was unlikely to have out-competed virus harboring resistance mutations. Finally, due to limited finance, the sample size was small, and larger studies to confirm the safety of stopping these HAART regimens after use for PMTCT through breastfeeding are warranted.

In conclusion, the NRTI-based and boosted PI-based regimens used for PMTCT in this study yielded no detectable major drug resistance mutations after discontinuation at 6 months postpartum, and may preserve future treatment options for women receiving these PMTCT regimens through the breastfeeding period. Factors associated with safe discontinuation of these regimens may have included a high rate of adherence and virologic suppression through 6 months breastfeeding in our study, the high genetic barrier to resistance of LPV/r-based HAART, and the similar half-lives of at least 2 drugs used in each regimen.

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References

1. WHO. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010 revision. Geneva, Switzerland: World Health Organization; 2010.
2. El-Sadr W, Lundgren JD, Neaton J, et al. CD4+ count-guided interruption of antiretroviral treatment. New Engl J Med. 2006; 355(22):2283–2296. [PubMed: 17135583]
3. Danel C, Moh R, Minga A, et al. CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial. The Lancet. 2006; 367(9527):1981–1989.
4. Pogány K, Vanvalkengoed IG, Prins JM, et al. Effects of active treatment discontinuation in patients with a CD4+ T-cell nadir greater than 350 cells/mm3: 48-week Treatment Interruption in Early Starters Netherlands Study (TRIESTAN). JAIDS Journal of Acquired Immune Deficiency Syndromes. 2007; 44(4):395.
5. Oyugi JH, Byakika-Tusiime J, Ragland K, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. Aids. 2007; 21(8):965. [PubMed: 17457090]
6. Salmon, D.; Daniel, N.; Krivine, A. Viral rebound and risk of emergence of resistant strains after HAART interruption in HIV chronically infected patients (pts) with undectectable viremia. Paper presented at: The XIV International AIDS Conference: AIDS 20022002; Barcelona.

7. Pilotto JH, Grinsztejn B, Veloso V, et al. High emergence of drug resistance after HAART interruption at delivery in a cohort of HIV+ pregnant women submitted to antiretroviral treatment to prevent mother-to-child transmission in Rio de Janeiro, Brazil. Retrovirology. 2009; 6(Suppl 1):O14.
8. Hauser A, Mugenyi K, Kabasinguzi R, Kuecherer C, Harms G, Kunz A. Emergence and Persistence of Minor Drug-Resistant HIV-1 Variants in Ugandan Women after Nevirapine Single-Dose Prophylaxis. PLoS ONE. 2011; 6(5):e20357. [PubMed: 21655245]
9. Soto-Ramirez LE, Rodriguez-Diaz R, Durán AS, et al. Antiretroviral resistance among HIV type 1-infected women first exposed to antiretrovirals during pregnancy: plasma versus PBMCs. AIDS research and human retroviruses. 2008; 24(6):797–804. [PubMed: 18507526]
10. Lehman DA, Chung MH, Mabuka JM, et al. Lower risk of resistance after shortcourse HAART compared with zidovudine/single-dose nevirapine used for prevention of HIV-1 mother-to-child transmission. Journal of acquired immune deficiency syndromes (1999). 2009; 51(5):522. [PubMed: 19502990]
11. Pérez H, Vignoles M, Laufer N, et al. Short communication Low rate of emergence of nevirapine and lamivudine resistance after post-partum interruption of a triple-drug regimen. Antiviral therapy. 2008; 13:135–139. [PubMed: 18389908]
12. Chokephaibulkit K, Chaisilwattana P, Vanprapar N, Phongsamart W, Sutthent R. Lack of resistant mutation development after receiving short-course zidovudine plus lamivudine to prevent mother-to-child transmission. Aids. 2005; 19(11):1231. [PubMed: 15990579]
13. Gingelmaier A, Eberle J, Kost BP, et al. Protease Inhibitor–Based Antiretroviral Prophylaxis during Pregnancy and the Development of Drug Resistance. Clinical Infectious Diseases. 2010; 50(6):890. [PubMed: 20166821]
14. Shapiro RL, Hughes MD, Ogwu A, et al. Antiretroviral regimens in pregnancy and breast-feeding in Botswana. N Engl J Med. 2010; 2010/06/18:2282–2294. [Multicenter Study Randomized Controlled Trial Research Support, N.I.H., Extramural]. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20554983. Accessed 24, 362. [PubMed: 20554983]
15. Wallis CL, Papathanasopoulos MA, Lakhi S, et al. Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1. Journal of virological methods. 2010; 163(2):505–508. [PubMed: 19917318]
16. Hermann Bussmann VN. William Wester1, HIV-1 subtype C drug-resistance background among ARV-naive adults in Botswana. Antiviral chemistry & chemotherapy. 2005; 16:103–115. [PubMed: 15889533]
17. Stanford University. HIV Drug Resistance Database.
18. Shafer R, Rhee SY, Pillay D, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. Aids. 2007; 21(2):215. [PubMed: 17197813]
19. Hamers RL, Wallis CL, Kityo C, et al. HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. The Lancet infectious diseases. 2011
20. Kandathil A, Kannangai R, Abraham O, Sudarsanam T, Pulimood S, Sridharan G. Genotypic resistance profile of HIV-1 protease gene: A preliminary report from Vellore, south India. Indian Journal of Medical Microbiology. 2008; 26(2):151. [PubMed: 18445952]
21. Bakhouch K, Oulad-Lahcen A, Bensghir R, et al. The prevalence of resistance-associated mutations to protease and reverse transcriptase inhibitors in treatment-naive (HIV1)-infected individuals in Casablanca, Morocco. The Journal of Infection in Developing Countries. 2009; 3(05):380–391.
22. Lockman S, Shapiro RL, Smeaton LM, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. New England Journal of Medicine. 2007; 356(2):135–147. [PubMed: 17215531]
23. Lyons FE, Coughlan S, Byrne CM, Hopkins SM, Hall WW, Mulcahy FM. Emergence of antiretroviral resistance in HIV-positive women receiving combination antiretroviral therapy in pregnancy. Aids. 2005; 19(1):63. [PubMed: 15627034]
24. Duran AS, Losso MH, Salomón H, et al. Drug resistance among HIV-infected pregnant women receiving antiretrovirals for prophylaxis. Aids. 2007; 21(2):199. [PubMed: 17197811]

25. Ellis GM, Huang S, Hitti J, Frenkel LM. Selection of HIV Resistance Associated With Antiretroviral Therapy Initiated Due to Pregnancy and Suspended Postpartum. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2011; 58(3):241.
26. Paredes R, Cheng I, Kuritzkes DR, Tuomala RE. Postpartum antiretroviral drug resistance in HIV-1-infected women receiving pregnancy-limited antiretroviral therapy. AIDS (London, England). 2010; 24(1):45.
27. Walmsley S, Bernstein B, King M, et al. Lopinavir–ritonavir versus nelfinavir for the initial treatment of HIV infection. New England Journal of Medicine. 2002; 346(26):2039–2046. [PubMed: 12087139]
28. Ait-Khaled MRA, Griffin P, Cutrell A, Fischl MA, Clumeck N, Greenberg SB, Rubio R, Peters BS, Pulido F, Gould J, Pearce G, Spreen W, Tisdale M, Lafon S. Mutations in HIV-1 reverse transcriptase during therapy with abacavir, lamivudine and zidovudine in HIV-1-infected adults with no prior antiretroviral therapy. Antivir Ther Mar. 2002; 7(1):43–51.
29. Nolan M, Fowler MG, Mofenson LM. Antiretroviral prophylaxis of perinatal HIV-1 transmission and the potential impact of antiretroviral resistance. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2002; 30(2):216.
30. Gulick RM, Ribaudo HJ, Shikuma CM, et al. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. New England Journal of Medicine. 2004; 350(18):1850–1861. [PubMed: 15115831]
31. Eron JJ, Feinberg J, Kessler HA, et al. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naive HIV-positive patients: a 48-week randomized clinical trial. Journal of Infectious Diseases. 2004; 189(2):265–272. [PubMed: 14722892]
32. Melby, TTS.; Thorborn, D. Time to Appearance of NRTI-Associated Mutations and Response to Subsequent Therapy for Patients on Failing ABC/COM. Paper presented at: 8th Conference on Retroviruses and Opportunistic Infections; Feb 4–8, 2001; Chicago.
33. Lange, J.; Hill, A.; Ait-Khaled, M.; De Wolf, F.; Foudraine, N.; Maguire, M. Does the M184V mutation affect the efficacy of HAART? Evidence from sequential addition of protease inhibitors or abacavir to zidovudine/lamivudine or stavudine/lamivudine. Paper presented at: Poster presented at: 7th European Conference on Clinical Aspects and Treatment of HIV Infection; October 23–27, 1999; Lisbon, Portugal. p. Poster 384
34. García F, Plana M, Ortiz GM, et al. The virological and immunological consequences of structured treatment interruptions in chronic HIV-1 infection. Aids. 2001; 15(9):F29. [PubMed: 11416735]
35. Kempf DJ, King MS, Bernstein B, et al. Incidence of resistance in a double-blind study comparing lopinavir/ritonavir plus stavudine and lamivudine to nelfinavir plus stavudine and lamivudine. Journal of Infectious Diseases. 2004; 189(1):51–60. [PubMed: 14702153]
36. Taylor S, Boffito M, Khoo S, Smit E, Back D. Stopping antiretroviral therapy. Aids. 2007; 21(13): 1673. [PubMed: 17690564]
37. Lange J, Goudsmit J, Weverling G. Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomised, double-blind, placebo-controlled trial. Lancet. 2002; 359:1178–1186. [PubMed: 11955535]
38. Stretcher BN, Pesce AJ, Frame PT, Stein DS. Pharmacokinetics of zidovudine phosphorylation in peripheral blood mononuclear cells from patients infected with human immunodeficiency virus. Antimicrobial agents and chemotherapy. 1994; 38(7):1541–1547. [PubMed: 7979286]
39. Yuen GJ, Lou Y, Bumgarner NF, et al. Equivalent steady-state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily. Antimicrobial agents and chemotherapy. 2004; 48(1):176–182. [PubMed: 14693537]
40. Ford J, Cornforth D, Hoggard PG, et al. Intracellular and plasma pharmacokinetics of nelfinavir and M8 in HIV-infected patients: relationship with P-glycoprotein expression. Antiviral therapy. 2004; 9(1):77–84. [PubMed: 15040539]
41. Summary of product characteristics. Boehringer Ingelheim Pharmaceuticals, Inc; Ridgefield, CT, USA: 2005. Viramune® (nevirapine).

42. Piliero, P.; Shachoy-Clark, A.; Para, M.; Preston, S.; Lou, I.; Drusano, G. A study examining the pharmacokinetics of abacavir and the intracellular carbovir triphosphate (GSK protocol CNA10905). 2003.
43. Summary of product characteristics. Abbott Laboratories; 2004. Kaletra® (lopinavir/ritonavir). www.ema.europa.eu/docs/en_GB/document_library/EPAR
44. Metzner KJ, Giulieri SG, Knoepfel SA, et al. Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and-adherent patients. Clinical Infectious Diseases. 2009; 48(2):239–247. [PubMed: 19086910]
45. Metzner K, Rauch P, Von Wyl V, et al. Prevalence of minority quasispecies of drug-resistant HIV-1 in patients with primary HIV-1 infection in Zurich in the years 2002–2006. ANTIVIRAL THERAPY. 2007; 12(5):47. [PubMed: 17503747]
46. Peuchant O, Thiébaut R, Capdepont S, et al. Transmission of HIV-1 minority-resistant variants and response to first-line antiretroviral therapy. Aids. 2008; 22(12):1417. [PubMed: 18614864]
47. Cane PA. New developments in HIV drug resistance. Journal of Antimicrobial Chemotherapy. 2009; 64(suppl 1):i37–i40. [PubMed: 19675018]
48. MacLeod IJ, Rowley CF, Thior I, et al. Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART. Journal of Clinical Virology. 2010; 48(3): 162–167. [PubMed: 20427228]
49. Giuliano M, Palmisano L, Galluzzo CM, et al. Selection of resistance mutations in pregnant women receiving zidovudine and lamivudine to prevent HIV perinatal transmission. Aids. 2003; 17(10):1570. [PubMed: 12824800]
50. Church JD, Hudelson SE, Guay LA, et al. Short Communication: HIV Type 1 Variants with Nevirapine Resistance Mutations Are Rarely Detected in Antiretroviral Drug-Naive African Women with Subtypes A, C, and D. AIDS research and human retroviruses. 2007; 23(6):764–768. [PubMed: 17604538]
51. Coovadia A, Hunt G, Abrams EJ, et al. Persistent Minority K103N Mutations among Women Exposed to Single-Dose Nevirapine and Virologic Response to Nonnucleoside Reverse-Transcriptase Inhibitor–Based Therapy. Clinical Infectious Diseases. 2009; 48(4):462–472. [PubMed: 19133804]
52. Johnson JA, Li JF, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment–naive populations and associate with reduced treatment efficacy. PLoS Medicine. 2008; 5(7):e158. [PubMed: 18666824]
53. Li JZ, Paredes R, Ribaudo HJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure. JAMA: The Journal of the American Medical Association. 2011; 305(13):1327. [PubMed: 21467286]
54. Charpentier C, Dwyer DE, Mammano F, Lecossier D, Clavel F, Hance AJ. Role of minority populations of human immunodeficiency virus type 1 in the evolution of viral resistance to protease inhibitors. Journal of virology. 2004; 78(8):4234–4247. [PubMed: 15047838]
55. Metzner KJ, Rauch P, von Wyl V, et al. Efficient suppression of minority drug-resistant HIV type 1 (HIV-1) variants present at primary HIV-1 infection by ritonavir-boosted protease inhibitor-containing antiretroviral therapy. Journal of Infectious Diseases. 2010; 201(7):1063–1071. [PubMed: 20196655]
56. Ruiz, Lidia; Martinez-Picado, Javier; Romeu, Joan, et al. Structured treatment interruption in chronically HIV-1 infected patients after long-term viral suppression. AIDS. 2000; 14:397–403. [PubMed: 10770542]
57. Harrigan PR, Hogg RS, Dong WWY, et al. Predictors of HIV Drug-Resistance Mutations in a Large Antiretroviral-Naïve Cohort Initiating Triple Antiretroviral Therapy. Journal of Infectious Diseases. 2005; 191(3):339–347. [PubMed: 15633092]

Table 1

Maternal Characteristics.

Maternal characteristics at enrolment (unless otherwise indicated)	Total sample N=54	NRTI arm (ABC/ZDV/ 3TC) N=29	PI arm (LPV/r/ZDV/3TC) N=25	P value
Study site, no. (%)				0.93†
City	13 (24)	7 (24)	6 (24)	
Town	8 (15)	5 (17)	3 (12)	
Village	33 (61)	17 (59)	16 (64)	
Median age, years (IQR)	27 (24–31)	29 (25–34)	26 (24–30)	0.09*
Marital Status, no. (%)				0.01†
Single	41 (76)	18 (62)	23 (92)	
Married/Cohabitating	13 (24)	11 (38)	2 (8)	
Gravida Including Current Pregnancy, no.(%)				0.06*
1	4 (7)	0 (0)	4 (16)	
2	22 (41)	10 (35)	12 (48)	
3	15 (28)	12 (41)	3 (12)	
4 or more	13 (24)	7 (24)	6 (24)	
Self-reported previous history of ARVs (ZDV) in pregnancy, no. (%)	6 (11)	4(14)	2 (8)	0.67†
Education, no. (%)				0.33*
None	3 (6)	3 (10)	0 (0)	
Primary	13 (24)	7 (24)	6 (24)	
Secondary	36 (66)	18 (63)	18 (72)	
University	2 (4)	1 (3)	1(4)	
Personal monthly income, in US dollars, (%)				0.24*
None	33 (61)	20 (69)	13 (52)	
< 100	11(20)	5 (17)	6 (24)	
101–200	5 (9)	1 (3)	4 (16)	
>200	5 (9)	3 (10)	2 (8)	
Median days on HAART (IQR)	266 (252–273)	261 (245–266)	268 (252–273)	0.18*
Self-reported adherence to HAART, total amount missed (%)				0.59*
>=2 days	8 (15)	3 (10)	5 (20)	
1 day	1 (2)	1 (3)	0 (0)	
1 dose	11 (20)	6 (21)	5 (20)	
None	34 (63)	19 (66)	15 (60)	
Median baseline HIV-1 RNA, copies/mL (IQR) Min–Max	32,900 (9,085–91,975) 2,070 – 750,000	41,600 (10,400 –95,700) 2,650 – 334,000	29,500 (9,080 –80,800) 2,070–750,000	0.74*

Maternal characteristics at enrolment (unless otherwise indicated)	Total sample N=54	NRTI arm (ABC/ZDV/ 3TC) N=29	PI arm (LPV/r/ZDV/3TC) N=25	P value
Median HIV-1 RNA at 1 month post-HAART, copies/mL (IQR) Min–Max	56,900 (15,450–217,750) 2,960–750,000 (N =36)	60,550 (9.945–121,250) 2,960–750,000 (N=20)	54,450 (35,325–319,500) 3,610–740,000 (N=16)	0.53 *
Median baseline CD4+ cells/mm3 (IQR) Min–Max	396 (330–510) 208–884	389 (343–516) 255–884	403 (282–473) 208–715	0.22 *

*Wilcoxon Rank Sum Test,

†Fisher's Exact Test.

Table 2a

Per-patient distribution of mutations detected one month after HAART cessation, categorized according to drug class association.

	Patient	PI	NRTI	NNRTI
NRTI Arm	4		V118I	E138A
	10	L10I		
	21	A71T	V118I	E138A
PI Arm	30	A71V		
	35	L10V, T74S		E138A
	38			E138A
	39	Q58E		
	44	A71T		
	47	T74S		
	52	A71T		
	54	L10I, A71T		

Table 2b

Summary of minor mutations detected with consensus sequencing of plasma RNA one month after HAART cessation.

Drug class association	Minor resistance mutations detected	Frequency of mutation (%)		Frequency in treatment naïve HIV-1 subtype C patients[17] (%)
		PI Arm ZDV/3TC/LPV/r	NRTI Arm ZDV/3TC/ABC	
PI	L10I	1/25 (4.0)	1/29 (3.4)	(2.1)
	L10V	1/25 (4.0)	0/29 (0.0)	(1.4)
	Q58E	1/25 (4.0)	0/29 (0.0)	(0.3)
	A71V	1/25 (4.0)	0/29 (0.0)	(0.2)
	A71T	3/25 (12.0)	1/29 (3.4)	(1.0)
	T74S	2/25 (8.0)	0/29 (0.0)	(9.7)
NRTI	V118I	0/25 (0.0)	2/29 (6.9)	(2.1)
NNRTI	E138A	2/25 (8.0)	2/29 (6.9)	(5.0)