Consequences of prior use of full-dose ritonavir as single protease inhibitor as part of combination antiretroviral regimens on the future therapy choices in HIV-1 infected children

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ABSTRACT

Background: South African HIV-infected infants below age 6 months and children younger than 3 years on concomitant antimycobacterial treatment received full-dose ritonavir single PI (RTV-sPI), together with two nucleoside reverse transcriptase inhibitors (NRTIs), from 2004 until 2008. Use of RTV-sPI has been described as a risk factor for PI drug resistance, but the extent of this resistance is unknown.

Aim: This research assesses clinical and virological outcome of a pediatric RTV-sPI cohort at a large South African antiretroviral therapy (ART) site in a high-burden tuberculosis setting, including resistance mutations in those failing ART.

Methods: All children initiated at Kalafong hospital before December 2008, who ever received RTV-sPI-based regimens, were assessed for patient outcome, virological failure and drug resistance. HIV viral loads were done 6-monthly and HIV genotyping since 2009.

Results: There were 178 children who ever received RTV-sPI, with a mean age at ART initiation of 1.4 years. Of the 135 children (76%) with >6 months follow-up, 17 children (13%) never had viral suppression, while another 25 (18%) developed virological failure later. Nineteen of 26 children (73%) with genotypic resistance results had major PI mutations.

Conclusions: Treatment failure is not a universal feature in children with prior exposure to RTV-sPI-regimens, but the significant proportion (31%) with virological failure are of concern due to high prevalence of major PI- and multi-class mutations. These children currently have no treatment options in the South African public sector, highlighting the urgent need for access to alternative ART regimens to ensure improved outcomes.

The large-scale antiretroviral therapy (ART) roll-out in South Africa started in 2004.[1] This occurred against the backdrop of widespread use of single-dose nevirapine (sdNVP) for prevention of mother-to-child transmission (PMTCT), posing a risk for transmission of drug-resistant HIV.[1,2] Furthermore, there were limited options available for drug substitution and no salvage therapy after failed second-line ART.[1,2]

Fortunately, South African children started on triple-ART regimens according to a standardized protocol.[1,3] Children under 3 years of age received two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI), due to lack of safety data for the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) in young children and widespread use of sdNVP for PMTCT. The PI was boosted lopinavir (LPV/r), except for the use of ritonavir (RTV) as full-dose single PI (RTV-sPI) between 2004 and 2008 in children aged <6 months, before LPV/r safety data became available in 2007.[4] RTV-sPI regimens were also used in children co-treated for tuberculosis, as data on additional RTV-boosting of LPV/r, to overcome the increased hepatic LPV-metabolism due to rifampicin co-administration, only became available in 2008.[5]

Prolonged non-suppressive ART increases the risk of acquiring additional viral mutations, with subsequent increased cross-resistance and drug failure over time.[6] Mutations conferring PI resistance are uncommon, but higher rates of PI mutations have been observed in children, compared with adults.[7] Known factors that increase the risk of HIV drug resistance in children include higher baseline HIV-1 viral loads (VL), complex adherence issues, inadequate pharmacokinetic information and unavailability of suitable pediatric ART formulations.[8] The high incidence of *Mycobacterium tuberculosis* co-infection in developing countries, resulting in concomitant use of rifampicin, is a further risk factor for HIV treatment

failure and subsequent drug resistance.[5,9] Several naturally occurring polymorphisms have also been observed in the protease region in cohorts with subtype C in South Africa, with possible decreased susceptibility to PIs.[7]

The use of RTV-sPI in children has been identified as a risk factor for PI drug resistance, but there is inadequate data available to determine the extent of PI drug resistance and virological failure in a high tuberculosis-burden setting such as South Africa, where children previously received RTV-sPI.[10] Kalafong hospital, a public sector hospital in Gauteng, was one of the country's first ART sites and, in accordance with the initial centralized model of care, became a large ART clinic for adults and children. This research assesses the proportion of children initiated on PI-based regimens who ever received RTV-sPI ART as per prior protocol (2004)[1] and describes the clinical and virological outcome of the entire RTV-sPI cohort, including resistance mutations in those failing ART.

METHODS:

The study population consisted of children who received RTV-sPI at Kalafong hospital during the period October 2003 to November 2008, either due to very young age (<6 months) or concomitant antimycobacterial treatment (with age <3years or weight <10kg), as per South African HIV guidelines.[1] For the purpose of this study, virological failure was defined as two consecutive HIV-1 VLs of >1000 copies/ml.[11] Children who initiated therapy outside of Kalafong hospital were excluded.

Data collection included demographic and anthropometric measurements, World Health Organization (WHO) HIV clinical staging, CD4 T-cell counts/ percentages and HIV-1 VLs at ART initiation. We also recorded ART regimens and concomitant antimycobacterial

treatment, as well as clinical outcome (treatment success, death, loss-to-follow) and viral suppression.

The study endpoint for children, who had not exited the clinic, was either January 2012 (for active children with virological suppression) or time at genotyping. ART regimens were classified according to the PI-component as either 'RTV-sPI only' or both 'RTV-sPI and LPV/r consecutively'. HIV clinic physicians switched ART regimens using the national protocol as guideline.[1] Reasons for ART switches included concomitant antimycobacterial treatment (mostly between LPV/r and RTV); advancing age (PI changed to EFV when >3 years old); side effects (mostly stavudine (d4T) changed to abacavir (ABC)); or switch to second-line ART due to treatment failure. Clinical interventions in cases of virological failure also included strategies to improve treatment adherence, social interventions if appropriate, and treatment of opportunistic infections.

VLs were done routinely at 6-monthly intervals and virological response was classified as early VL suppression (<1000 copies/ml on first VL at 6 months of ART) or late VL suppression (<1000 copies/ml at 12 months of ART or later) or never suppressed. The estimated failing time on ART was calculated as the period from the midpoint between the last undetectable VL and VL >1000 copies/ml and the genotype date, or in those never suppressed, the period from midpoint between ART initiation and first VL >1000 copies/ml and genotype date.

Genotyping was requested in all children with virological failure still in follow-up, if adherence interventions had failed to result in virological suppression. The Department of Immunology, University of Pretoria, did population-based genotyping on samples with

confirmed VL >1000 copies/mL, using a drug resistance assay developed in-house.[12] RNA was extracted from 200µl of plasma using Nuclisens miniMAG extraction kit (bioMéRIEUX, SA). A PCR product containing 1315bp was generated from the HIV-1 subtype C *pol* region. Sequencing was performed on HIV-1 protease and the first 300 codons of the reverse transcriptase (RT) gene. RNA was reverse transcribed into cDNA with the Superscript III kit (Invitrogen Corporation, Carlsbad, CA) and a gene specific primer: RT21 as described by Mansa *et al*. Platinum taq polymerase (Invitrogen Corporation, Carlsbad, CA) and a nested PCR protocol were used to amplify the protease and RT gene regions from cDNA. The amplicon was sequenced using 4 primers.[13] Sequencing reactions were run on an automated 3500xl, 24 capillary Genetic Analyzer (Applied Bioscience).

Sequences were assembled and manually edited using CLC DNA Workbench 5.7.1 software and then submitted to Stanford HIVDB website (http://hivdb.stanford.edu) to identify HIV-1 drug resistance mutations, while the HIV-1 subtype was assigned on the Stanford database using the Rega HIV-1 subtyping tool. Frequencies of mutations were determined and resistance patterns analyzed with emphasis on clinical relevance.

Statistical analysis was done using SPSS version 20. Statistical significance of continuous variables was tested using two-sided t-tests. Categorical variables were examined using two-sided chi-square or Fisher's exact tests. p-values of ≤ 0.05 were considered significant.

The Ethics Review Committee, Faculty of Health Sciences, University of Pretoria, approved the study protocol.

RESULTS:

In this high burden tuberculosis-setting there were 178 (43%) of 416 children on PI-based regimens who were treated with RTV-sPI before December 2008, because of either concomitant antimycobacterial treatment (n=157; 88%) or ART initiation before age of 6 months (n=5; 3%) or both (n=16; 9%). The mean age at ART initiation (RTV-sPI cohort) was 1.4 years (range 27 days to 3.8 years), with a male-to-female ratio of 1.2:1. The mean duration of follow-up was 44 months (range 0 – 94 months), with a mean duration on RTV-sPI of 8 months (range 0 – 28 months), and an additional 23 months (range 0 – 91 months) on LPV/r-regimens. The clinical characteristics of the RTV-sPI cohort, as shown in table 1, reveal the poor nutritional state, advanced clinical disease and high VLs at ART initiation.

ART regimens and clinical outcome are summarized in figure 1. The RTV-sPI cohort (n=178) included 66 children who were never switched to a boosted PI, either because of death while still on RTV-sPI, loss to follow/ referral or switch to a NNRTI regimen, most often because they attained the age of 3 years. The remaining 112 children were either started on RTV-sPI and switched to LPV/r or the reverse, depending on the need for concomitant antimycobacterial therapy.

Subjects lost from the cohort included 31 children who were referred to other ART sites, 25 children who were lost-to-follow and 27 who died (20 deaths occurred within six months of starting ART). Nearly all (90%) deceased children had WHO stage 4 disease. A comparison of baseline characteristics of children who were alive and those who died at 6 months of ART showed that the children who died were significantly more underweight-for-age (p=0.002), had a lower body mass index (BMI) (p=0.011), and were more likely to have baseline VLs greater than 6 $\log_{10}$ (p=0.04) and lower CD4 counts (p=0.04).

Virological suppression was assessed for those 135 children (76%) who were on ART for longer than 6 months (figure 2). Fifty-seven children (42%) had early VL suppression, while another 57 (42%) had late VL suppression. The remaining 21 children (16%) were never virologically suppressed, although this included four children with follow-up of <12 months. Twenty-five (18%) children with initial VL suppression had virological failure later. In 28 children (21%), viral suppression was lost intermittently, but they were re-suppressed after adherence interventions. ART interruptions had occurred in 6 children (4%), but they re-suppressed after treatment restart.

Therefore 86 of the 135 children (64%) with follow-up of longer than 6 months, had viral suppression – either sustained from ART initiation or re-suppressed after greater attention was paid to adherence. Children with early VL suppression were more likely to have sustained viral control. Forty-two children (31%) had virological failure on follow-up, of whom 26 have genotyping results. Reasons for lack of genotyping included loss to follow-up (n=7), referrals (n=3), death (n=3), genotype testing difficulties (n=2) and virological failure due to frequent ART interruptions (n=1). Seven of the 135 children (5%) had insufficient time in follow-up to assess the sustainability of their medium-term virological response.

The study endpoint was reached by 95 children (53.5%), of whom 69 children were still on active treatment and virologically suppressed in January 2012, while the other 26 children had virological failure with genotyping (figure 1). Of these, 12 patients had been on PI regimens only and 14 patients had also received NNRTI regimens. The latter group included 4 children on mixed regimens, with two children previously switched to NNRTI regimens

and then changed back to PI regimens before genotyping, while the other two children were on both PI and NNRTI at genotyping.

Results of the genotype resistance tests revealed that all patients were infected with HIV-1 subtype C and all had HIV drug resistance mutations. Nineteen of 26 (73%) genotype results had major PI mutations. The major and minor PI mutations found in the cohort are shown in figure 3. The most common major PI mutations were I54V (54%), V82A (54%), M46I (31%) and L76V (19%).

All of the genotypes had NRTI mutations and, as expected, the M184V mutation (conferring resistance to 3TC), was the most common (81%). Thymidine analogue mutations (TAMs) were found in 38%, with seven out of these ten genotypes showing multiple (≥3) TAMs and the majority following the TAM 2 pathway. The K65R mutation was rare in this patient population (3%).

Furthermore, NNRTI mutations were found in 58% of resistance results. Most common (>10% of genotypes) were K103N (27%), V106M (23%) and Y188L (15%). Y181C (12%) was the most common mutation which also confers resistance to etravirine, with L100I (4%) and K101P (4%) occurring at low levels, and none of the combination mutations, known to compound etravirine resistance, were found.

The HIV mutations and resulting drug resistance are summarized in table 2. Patients are divided into three groups: 1) Those who were only on PI-regimens before genotyping, 2) those initially on PI regimens and then switched to NNRTI-regimens and 3) those on mixed regimens (both PI and NNRTI). The majority of subjects (73%) had developed major PI

mutations and all with NNRTI-exposure had developed NNRTI mutations. Furthermore all genotypes had at least one NRTI mutation. Thus the majority (88%) now had dual class mutations and 71% of those who had been exposed to an NNRTI had resistance mutations to all three classes.

The drug resistance mutations translate into high-level resistance to LPV/r in the PI-regimen group, while in the NNRTI-group, high-level resistance was found to EFV and NVP, with additional accumulation of TAMs. For the entire patient group, darunavir (DRV/r) was the PI with the most favorable sensitivity profile at 23% resistance level (mainly low-level resistance), compared to tipranavir at 54% and atazanavir at 73%. Genotypic etravirine resistance was found in 42% of cases, although this includes potentially low-level resistance and genotypic resistance may not correspond to phenotypic resistance pattern.

We further compared the two groups with regards to baseline characteristics and drug resistance patterns (those who had remained on PI regimens versus those who had been switched to NNRTI regimens) and found no statistically significant differences regarding age (p=0.076), anthropometry (W/A: p=0.685, H/A: p=0.843, BMI/A: p=0.681), HIV VL (p=0.206), CD4 count (p=0.537) or CD4% (p=0.792) at ART initiation. The time on PI-regimens differed significantly (p=0.000), but not the time on RTV-sPI (p=0.062) or the estimated failure time (p=0.837).

There were significant differences in drug resistance patterns between the two groups (table 3), with those on PI-regimens having accumulation of PI mutations, but no TAMs, while those switched to NNRTI-regimens showing NNRTI-mutations in addition to accumulation of TAMs.

DISCUSSION:

Great progress has been made in ensuring survival of HIV-infected children in resource-poor countries by the rapid expansion of large-scale ART programmes.[3] Unfortunately this advancement is potentially threatened by development of HIV drug resistance, and this may, in hindsight, be partly due to suboptimal ART-regimen choices. Current knowledge of HIV drug resistance is mostly from developed countries with predominantly HIV-1 subtype B virus, showing mainly the consequences of sequential, non-suppressive regimens. [6,14] In contrast, much less information is available from sub-Saharan Africa regarding drug resistance in predominantly HIV-1 subtype C infection.[3] The high burden of concomitant HIV-disease and tuberculosis in African children complicates patient management and contributes to adverse patient outcomes.[9] It is therefore important to review the effect of the previous use of RTV-sPI regimens, especially since HIV drug resistance may complicate future management.

In the context of South Africa's rapid ART expansion and high tubercuolosis-burden,[1,11] a large percentage of children who initiated ART between 2004 and 2008 were at one stage exposed to RTV-sPI regimens, as shown by our study (43%). The study population mostly had advanced HIV-disease, as shown by the poor nutritional state, advanced WHO staging, common mycobacterial co-infection and high mortality (15%). This is indicative of the back-log of ill patients presenting for HIV-care in the first few years of the ART programme.

In a previous American study of children aged below 2 years, who received two NRTIs and RTV-sPI, sustained viral suppression of <400 copies/ml was observed in just over one-third of infants. [15] One important observation of our study is the reassurance that 64% of children, who were treated with RTV-sPI regimens and followed for at least 6 months, had virological

suppression. Reasons for the better virological outcome could include that RTV-sPI was only used for finite periods and that treatment changes occurred according to the National guidelines when suboptimal treatment response was identified. The two studies however used different viral suppression cut-offs, which limit comparison. Long term viral suppression in our study was observed for those children in whom early VL suppression had occurred. In another South African study of children below age 2 years who were started on PI-regimens, the mortality was 14% and the viral suppression (<400 copies/ml) was 71% at 26 weeks and 84% at 39 weeks, but only 39% of this cohort was co-treated for tuberculosis, and viral suppression rates were significantly lower in this latter group.[16]

In our study, all children with previous RTV-sPI exposure and virological failure had resistance mutations on genotyping, greatly limiting future ART options. Complex resistance mutations were common, as well as triple-class resistance (42%). NRTI mutations were universal – mostly the M184V mutation, while TAMs were also common. We found PI mutations in 73% of genotypes, substantially higher than the 17% to 44% reported in previous South African studies of children failing PI regimens, although these earlier studies included children on LPV/r-regimens in addition to RTV-sPI-regimens.[7,17,18] In Brazil, almost half of the children on either RTV-sPI or nelfinavir-based ART had PI mutations,[19] while in another South African study, major PI mutations were found in 71% of children with treatment failure and prior RTV-sPI exposure, a result that is very similar to the present findings.[10]

The intriguing finding that use of PI-regimens is protective against development of resistance against co-administered antiretroviral drugs has been described in adults in relation to the M184V and K65R mutations.[20] Another adult study, comparing EFV-based regimens to

LPV/r-based regimens, found that the risk of dual class resistance was significantly higher in the EFV-group.[21] In our cohort, distinct mutation patterns were also observed according to ART use. Those children who remained on PI regimens had accumulation of multiple major PI mutations, but with no TAMs developing, despite the unsuppressed viral load. For those switched, NNRTI mutations and TAMs accumulated, and although they showed fewer PI mutations, their results need to be interpreted with care due to possible archiving of PI mutations. Extensive triple-class drug resistance developed in those on both PI and NNRTI regimens, although this group was small.

The interacting roles played by ART regimen potency, pharmacokinetics, treatment adherence and transmitted drug resistance are important, as all may negatively influence ART effectiveness.[6,18] ART drug classes and drugs themselves have different genetic barriers to resistance. Lamivudine and the NNRTIs, for example, have a low barrier to resistance, with a single mutation enough to confer complete resistance. When RTV is used as a single full-dose PI, it also has a low resistance barrier, while in contrast, boosted PIs like LPV/r, require a stepwise accumulation of mutations to reduce susceptibility.[22]

Due to high risk of disease progression if ART is discontinued, children will likely remain on failing regimens for extended periods, leading to accumulation of additional resistance mutations and resulting in high-level and multi-class resistance, as in this cohort. South African protocol currently only has first- and second-line ART options, with no or very limited access to third-line therapy.[11] Within the context of widespread use of NVP for PMTCT, infection with NNRTI-resistant HIV can be anticipated in many children, which, in reality, leaves only the first-line PI-regimen to achieve virological suppression.

For children with prior RTV-sPI exposure and virological failure, need for third-line therapy is thus a matter of urgency to ensure survival. Although currently not available at most South African ART sites, genotyping is recommended in this situation due to the multi-class resistance, to facilitate regimen choice. Care should be taken not to add a single new drug to a failing ART regimen. For those children who have remained on PI-regimens, switching them to NNRTI-regimens will likely not be sufficient to achieve viral suppression, as shown in this cohort. Required third-line drugs include boosted PIs darunavir (DRV/r) and tipranavir (TPV/r), which have high resistance barriers.[17] Choice of salvage PI depends on patient characteristics and genotype result,[17] but in our study, DRV/r had more favorable drug sensitivity results. Furthermore, access to the new generation NNRTI etravirine is needed, and although selected etravirine resistance mutations were identified in this cohort, phenotype remains the reference method to define etravirine susceptibility.[23] The complex resistance mutations already documented in this cohort also means that future treatment needs of children have to be anticipated in terms of licensing and development of suitable pediatric formulations of new ART drug classes like entry inhibitors and integrase inhibitors.

Study limitations include that this is a cohort from a single institution, albeit a large pediatric ART site. Moreover there were significant losses in patient follow-up, as this is a description of children receiving routine clinical care. Even though of great importance, treatment adherence and PMTCT history were not extensively documented in this clinical cohort and could not be used for analysis. The description of viral mutations is limited to those children with virological failure in whom a genotype was done, although the study does include genotypes on all children with virological failure who were still in follow-up.

This study, in conclusion, shows that not all children who previously used RTV-sPI-regimens are at risk of virological failure, but in those failing therapy, complex resistance patterns, with accumulation of major PI mutations, are common. Urgent access to third-line therapy is therefore necessary, to ensure that the prior policy which included RTV-sPI regimens does not contribute to suboptimal survival.

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Table 1: Clinical and laboratory findings at ART initiation of the entire RTV-sPI cohort

		Entire RTV-sPI cohort (n=178)
Age at ART initiation: (in years)	Mean (median) (SD)	1.4 (1.3) (0.8)
Anthropometry: • Weight-for-age Z-score • Height-for-age Z-score • BMI-for-age Z-score	Mean (median) (SD)	 -3.4 (-3.3) (1.8) -3.2 (-3.0) (1.6) -1.2 (-1.0) (2.0)
HIV staging:	WHO Stage 1: WHO Stage 2: WHO Stage 3: WHO Stage 4:	0 2 (1.1%) 49 (27.5%) 127 (71.4%)
CD4: Absolute count: **CD4 percentage:**	Mean (median) (SD) <u>Categories:</u> CD4% <15% CD4% 15% – 25% CD4% >25% Not recorded	680 (573) (544) 15.9% (14.0%) (9.3) 97 (54.5%) 59 (33.1%) 21 (11.8%) 1 (0.6%)
HIV Viral Load (VL) (log_{10} copies/ml):	Mean (median) (SD) <u>Categories:</u> VL < $log_{10}5$ VL ≥$log_{10}5$; <$log_{10}6$ VL ≥$log_{10}6$ Not recorded	5.7 (5.9) (0.8) 28 (15.7%) 62 (34.8%) 74 (41.6%) 14 (7.9%)

Abbreviations: ART= antiretroviral therapy; RTV-sPI= ritonavir as full-dose single protease inhibitor; SD= Standard deviation; BMI= Body mass index; WHO stage= HIV disease staging according to World Health Organization

Table 2: Summary of HIV-1 mutations and ART drug resistance for all patients with genotype results

	PI regimens only (n=12)	Switched to NNRTI (n=10)	Mixed regimens (PI & NNRTI)* (n=4)	Total group (n=26)
Time periods: (Median (SD) in months)				
• Time on PI	51.5 (13.8)	12.5 (9.6)	35 (24.6)	35 (22.9)
• Time on RTV-sPI	6.5 (4.1)	11 (6.6)	17 (2.4)	8 (5.9)
• Time off PI	Not applicable	50.5 (5.0)		
• Estimated failure time	41.5 (14.3)	43 (18.4)	52 (13.1)	45.5 (16.2)
Mutations: (Nr (%))				
1) **Genotypes with mutations**				
• Major PI mutations	9 (75%)	6 (60%)	4 (100%)	19 (73%)
• NNRTI mutations	1 (8%)	10 (100%)	4 (100%)	15 (58%)
• NRTI mutations				
○ Any	12 (100%)	10 (100%)	4 (100%)	26 (100%)
○ M184V	12 (100%)	8 (80%)	1 (25%)	21 (81%)
○ ≥1 TAM	0 (0%)	6 (60%)	4 (100%)	10 (38%)
○ ≥3 TAMs	0 (0%)	4 (40%)	3 (75%)	7 (27%)
• Dual class mutations	9 (75%)	10 (100%)	4 (100%)	23 (88%)
• Triple class mutations	1 (8%)	6 (60%)	4 (100%)	11 (42%)
2) **Mutations per genotype** (Median (range))				
• Any^	3.8 (1-6)	5.6 (2-8)	10.5 (10-11)	5.5 (1-11)
• Major PI mutations	2.5 (0-5)	0.6 (0-1) #	2.8 (2-4)	1.8 (0-5)
• NNRTI mutations	0.1 (0-1)	2.3 (1-3)	2 (1-3)	1.2 (0-3)
• NRTI mutations	1.2 (1-2)	2.8 (1-5)	5.8 (5-6)	2.5 (1-6)
ART drug resistance: (Nr (%))				
1) PI resistance:				
• Lopinavir	9 (75%)	6 (60%) #	4 (100%)	19 (73%)
• Darunavir	4 (33%)	1 (10%) #	1 (25%)	6 (23%)
• Atazanavir	9 (75%)	6 (60%) #	4 (100%)	19 (73%)
• Tipranavir	9 (75%) †	2 (20%) #	3 (75%)	14 (54%) †
2) NNRTI resistance:				
• Efavirenz	1 (8%)	10 (100%)	4 (100%)	15 (58%)
• Nevirapine	1 (8%)	10 (100%)	4 (100%)	15 (58%)
• Etravirine	1 (8%)	6 (60%) †	4 (100%) †	11 (42%)

*Mixed regimens-group includes 2 children, who had previously been switched to NNRTI regimens, but were switched back to PI regimens before genotyping, and another 2 children who were on both PI and NNRTI at genotyping

^Excludes minor PI mutations

#Needs to be interpreted with care due to possible archiving of mutations

†Includes potentially low resistance

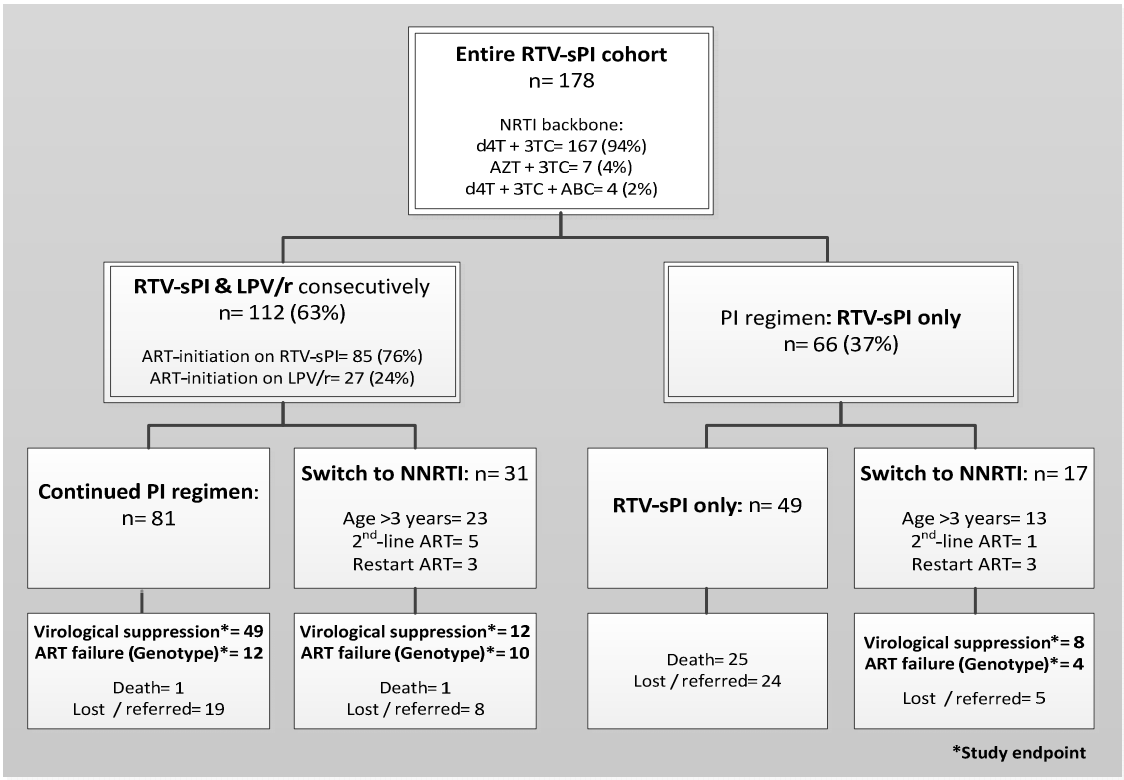
Abbreviations: ART= antiretroviral therapy; PI= protease inhibitors; NNRTI= non-nucleoside reverse transcriptase inhibitors; SD= standard deviation; RTV-sPI= ritonavir as full-dose single protease inhibitor; NRTI= nucleoside reverse transcriptase inhibitors; TAM= thymidine analogue mutations

Table 3: Comparison of genotype results of patients who remained on PI regimens versus those who were switched to NNRTI regimens

	PI regimens only (n=12)	Switched to NNRTI (n=10)	p-value
Mutations:			
1) Genotypes with mutations: (Nr (%))			
• Major PI mutations: ≥3	8 (67%)	0 (0%)	**p= 0.002**
• NNRTI mutations: ≥1	1 (8%)	10 (100%)	**p= 0.000**
• NRTI mutations: ≥1 TAM	0 (0%)	6 (60%)	**p= 0.003**
2) **Mutations per genotype:** (Mean (SD))			
• Any	3.8 (1.9)	5.6 (1.7)	**p= 0.026**
• Major PI mutations	2.5 (1.7)	0.6 (0.5)	**p= 0.002**
• NNRTI mutations	0.1 (0.3)	2.3 (0.7)	**p= 0.000**
• NRTI mutations: Nr of TAMs	0 (0)	1.6 (1.6)	**p= 0.011**

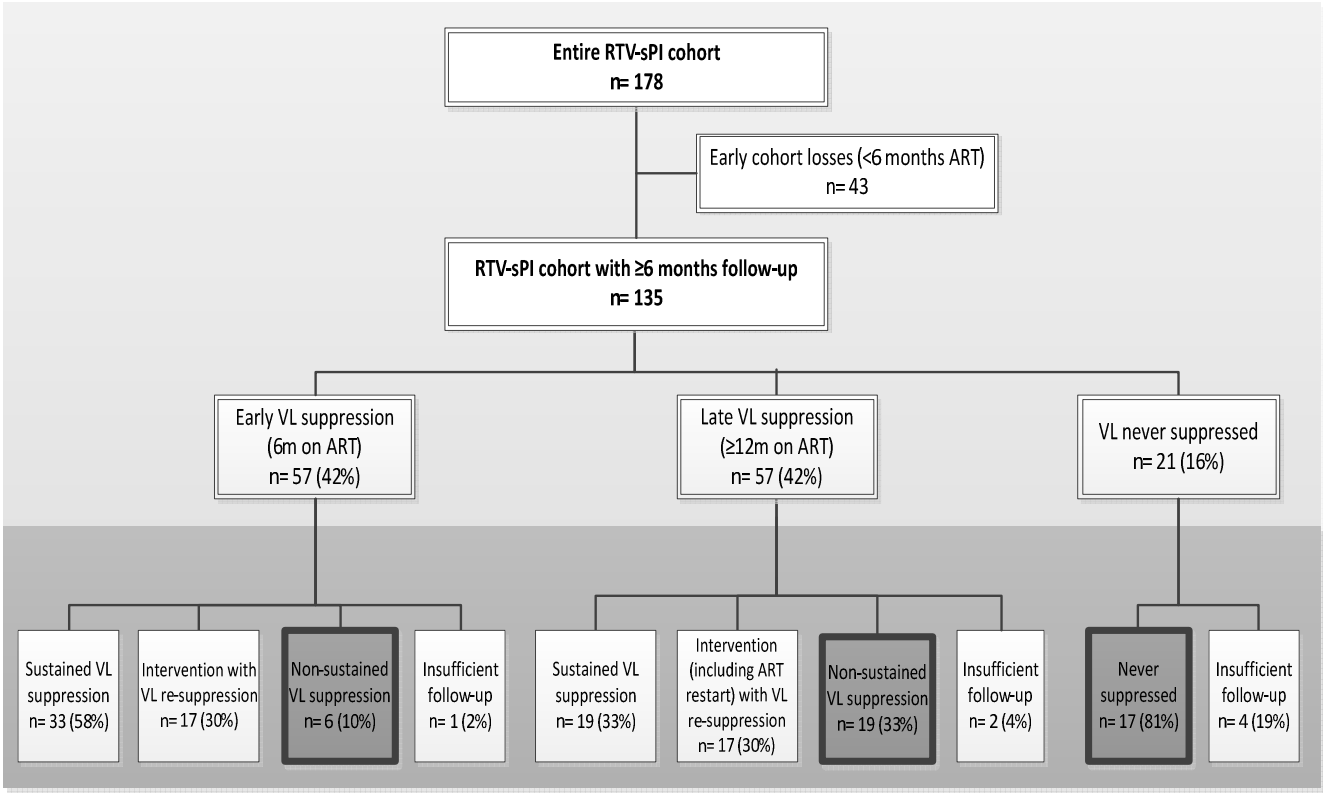
Abbreviations: PI= protease inhibitors; NNRTI= non-nucleoside reverse transcriptase inhibitors; NRTI= nucleoside reverse transcriptase inhibitors; TAM= thymidine analogue mutations; SD= standard deviation

Figure 1: Summary of the antiretroviral regimens and clinical outcome of the entire RTV-sPI cohort



Abbreviations: RTV-sPI= ritonavir as full-dose single protease inhibitor; NRTI= nucleoside reverse transcriptase inhibitors; d4T= stavudine; 3TC= lamivudine; AZT= zidovudine; ABC= abacavir; LPV/r= boosted lopinavir; ART= antiretroviral therapy; PI= protease inhibitors; NNRTI= non-nucleoside reverse transcriptase inhibitors

Figure 2: Virological suppression in RTV-sPI cohort with more than 6 months follow-up



Abbreviations: RTV-sPI= ritonavir as full-dose single protease inhibitor; ART= antiretroviral therapy; VL= viral load; m= months

Figure 3: Protease inhibitor (PI) drug resistance mutation patterns identified in the cohort