Supplementary Text 1 Laboratory methodologies

Genotyping was performed at two laboratories. The details of methodologies used for each laboratory is given below.

Virco (Beerse, Belgium):

Dideoxy sequencing reactions were performed on the purified amplicon (ABI Prism Big Dye Terminator Cycle Sequencing Kit, Version 3.1, Applied Biosystems) with a set of eight sequence-specific primers distributed over the PR-RT sequence for both strands: F1, 5'-GAGAGCTTCAGGTTTGGGG-3' ; F2, 5'-AATTGGGCCTGAAAATCC-3' ; F3, 5 -CCTCCATTCCTTTGGATGGG-3' ; F5, 5'-CACTCTTTGGCAACGACCC-3'; R1, 5'-CTCCCACTCAGGAATCC-3'; R3, 5'-CTTCCCAGAAGTCTTGAGTTC-3'; R5, 5'-GGGTCATAATACACTCCATG-3'; R6, 5'-GGAATATTGCTGGTGATCC-3'. Reactions were purified using a DyeEx Purification Protocol (Qiagen), and analyzed using the ABI 3730xl DNA Analyzer (Applied Biosystems).

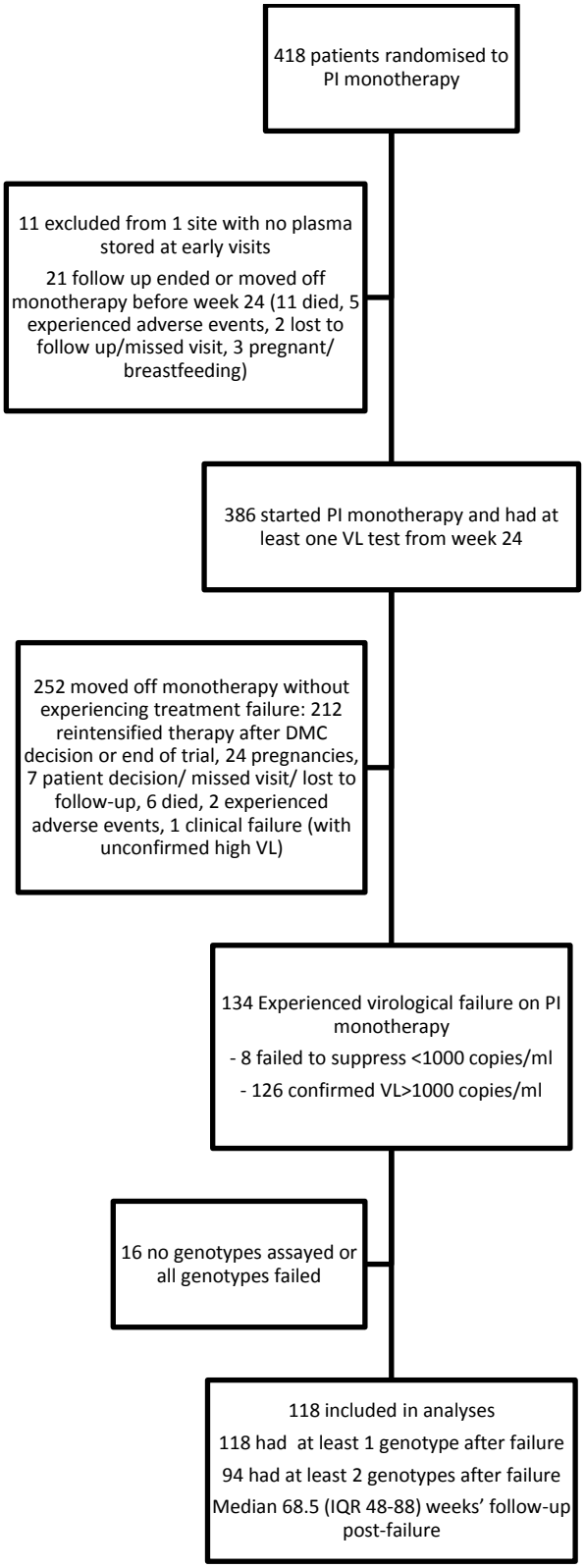
Sequence data files were grouped per sample identifier (ID) and aligned against the reference HXB2 reference sequence by means of the Sequencher TM Program V 4.1.4 (Gene Codes Corp.). Using the reference sequence as an aid, the data were trimmed to size and points of ambiguity were checked by visual observation of the associated chromatograms to obtain a consensus sequence. A 25% scoring rule was used to define the mixtures observed and this mixture scoring procedure was compared head to head with the 454 deep sequencing to evaluate the frequency of mixture scoring between the 2 sequencing methodologies [6] . The validated sequence from the reviewed data were exported for resistance analysis using virco TYPE HIV-1.

JCRC (Kampala, Uganda):

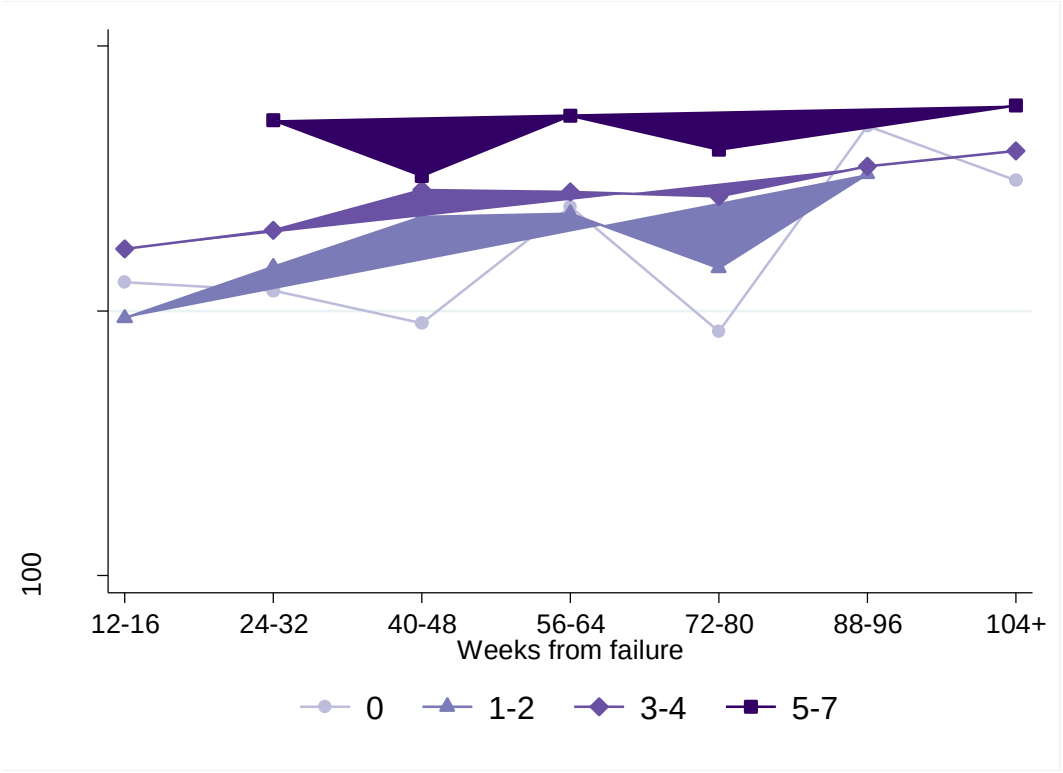
The primers spanning the full length HIV protease gene (1-99 amino acids) were used to sequence quantified and purified PCR product: RTS1 (5'- TAAACAATGGCCATTGACAGAAGA-3'), RTA4 (5'-CTGTATATCATTGACAGTCCAGCT -3'), PS2 (5'-TCCCTCAAATCACTCTTTGGCAAC-3') and RTS2R (5'-ATGGATTTTCAGGCCCAATTTTTGA -3').

PCR product was sequenced using the BigDye Terminator cycle sequencing kit (v3.1) (Thermo Fisher Scientific) according to manufacturer's instructions on ABI 3730xl sequencing platform (Life Technologies, Carlsbad, California, USA). Sequences were analyzed using SeqScape (version 2.7), and subsequently using RECall (beta v3.02) program as recommended by the WHO to generate a consensus sequence for the analysis.

Supplementary Figure 1 CONSORT patient flow

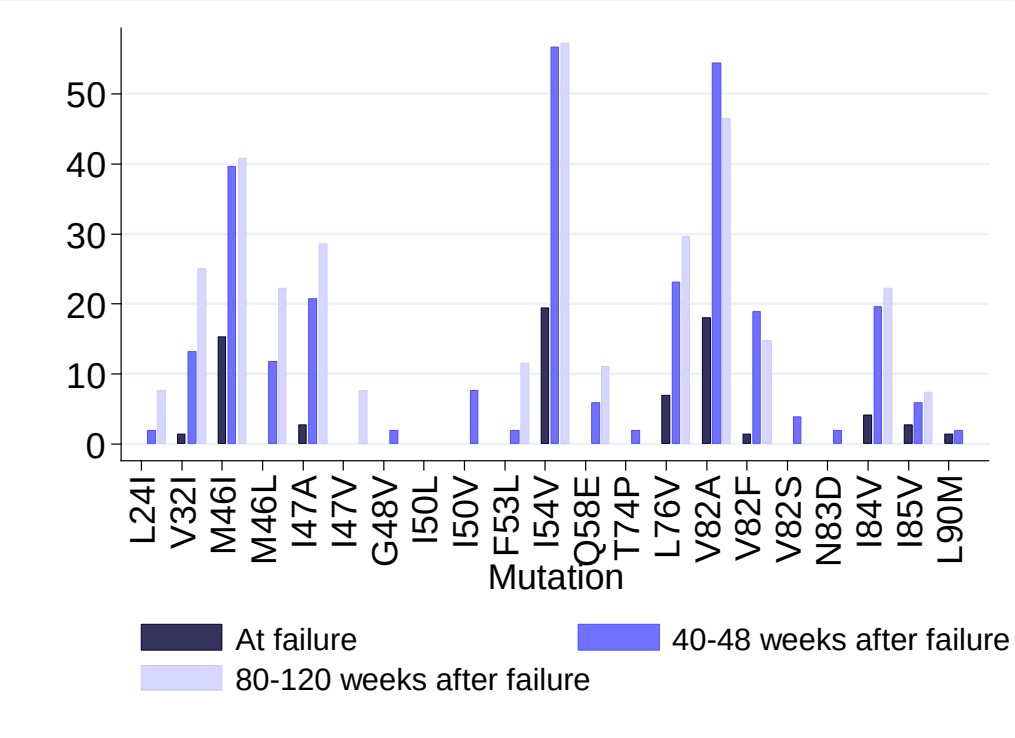


Supplementary Figure 2: Mean log_{10} viral load after virological failure by number of mutations in the previous sample

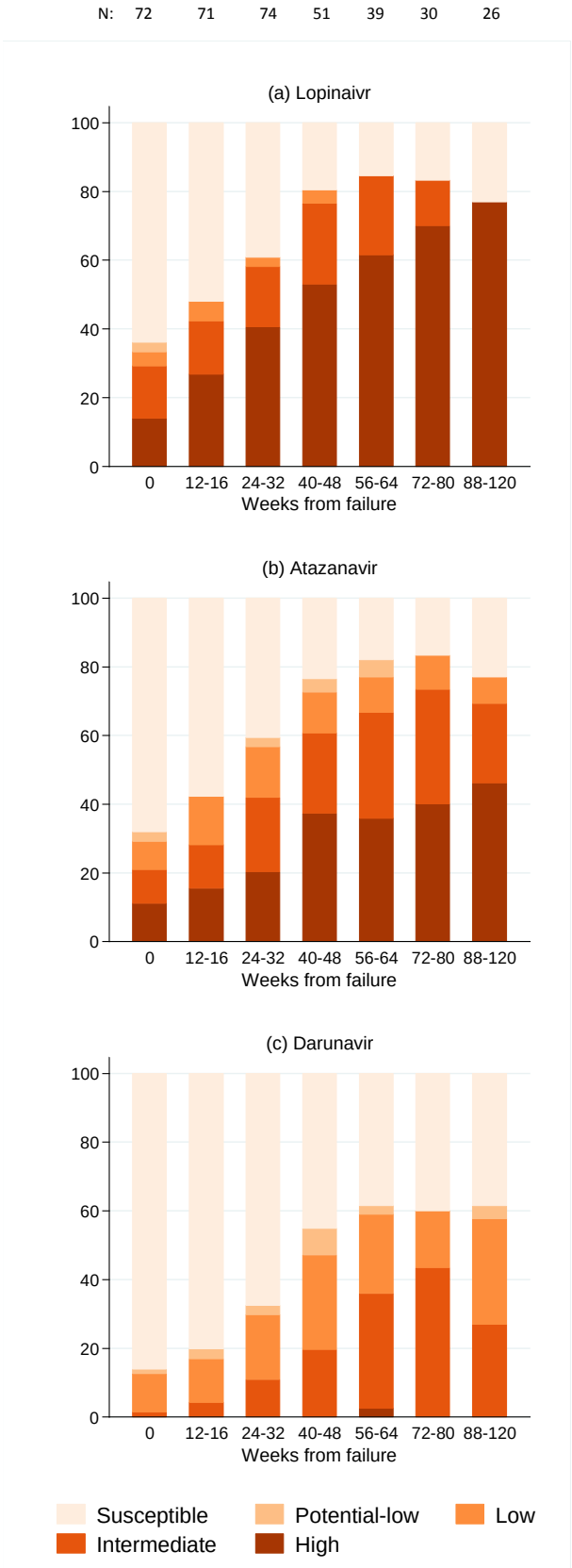


Supplementary Figure 3 Cumulative prevalence of individual mutations, without carrying absence of mutations backwards, at failure (darkest), 40-48 weeks (med), and 80-120 weeks after failure (lightest).

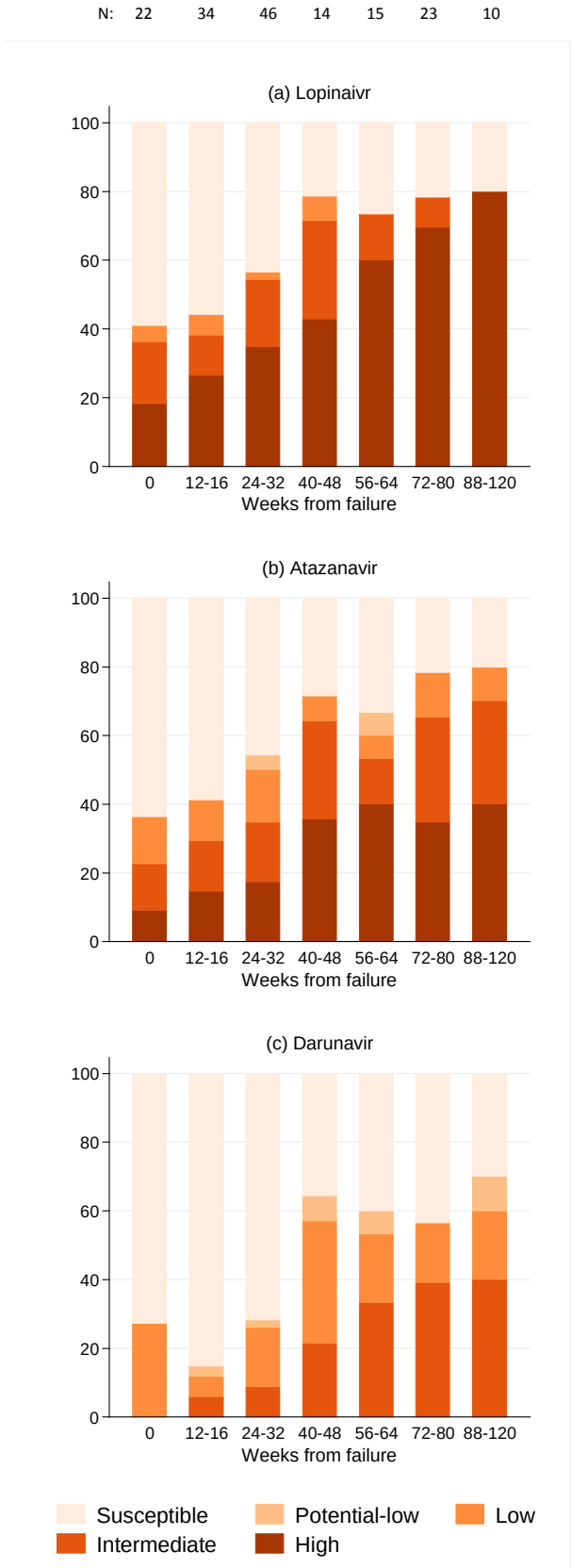
Note: Denominator number varies for each mutation because of carrying forward previously present mutations. At failure: N=72, 40-48 weeks N=51-60, 80-120 weeks after failure N=26-28.



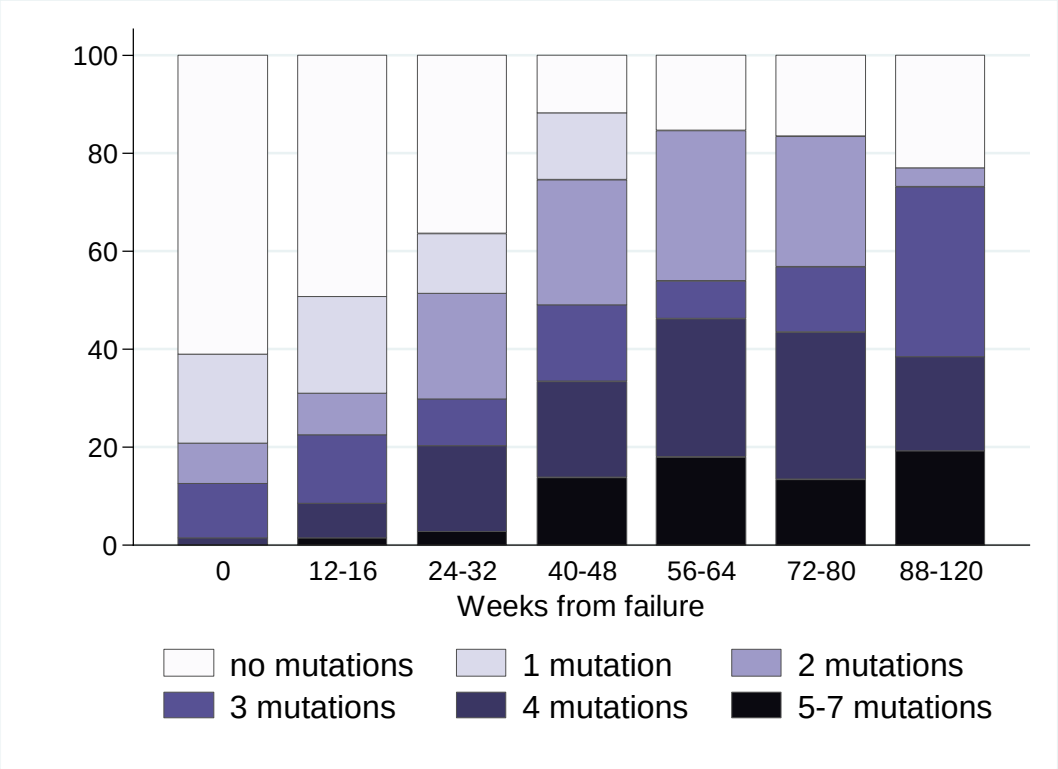
Supplementary Figure 4.1 Cumulative predicted drug susceptibility of (a) lopinavir (b) atazanavir and (c) darunavir by time since second-line virological failure without carrying absence of mutations backward.



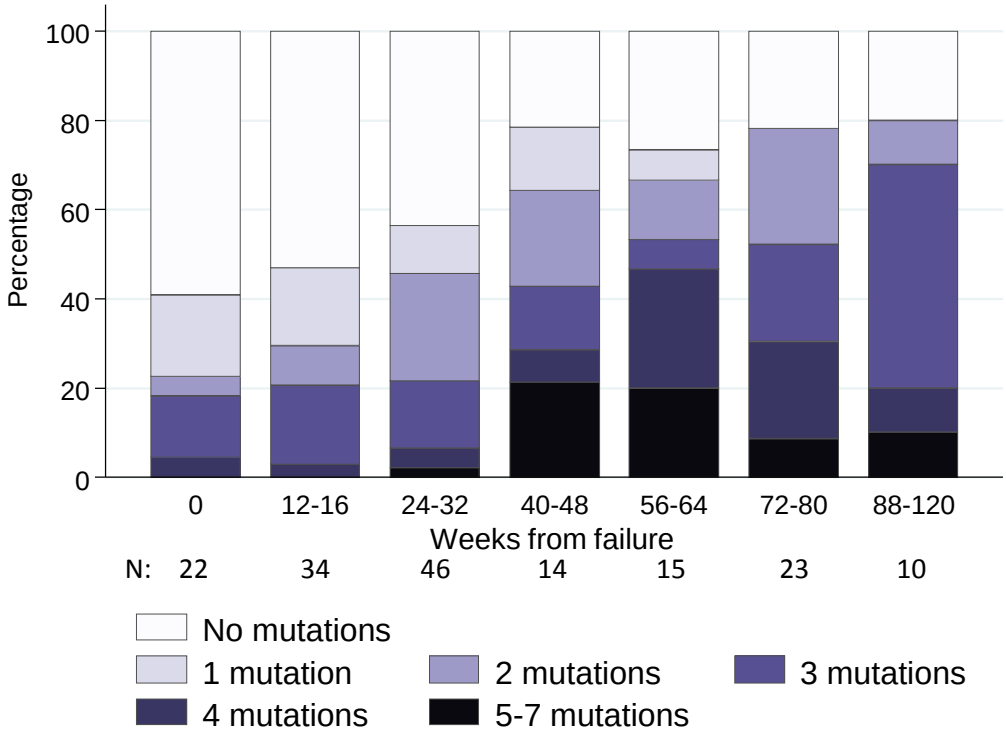
Supplementary Figure 4.2 Cumulative predicted drug susceptibility of (a) lopinavir (b) atazanavir and (c) darunavir by time since second-line virological failure without carrying absence of mutations backward restricted to 48, 96, 144 weeks from switch to second-line



Supplementary Figure 5.1: Cumulative number of PI-mutations present by time since second-line virological failure without carrying absence of mutations backward



Supplementary Figure 5.2: Number of PI-mutations present by time since second-line virological failure without carrying absence of mutations backward restricted to 48, 96, 144 weeks from switch to second-line



Supplementary Table 1 Completeness of VLs and genotypes

Viral loads for detecting failure:
Includes all patients who start monotherapy and have at least one VL from week 24 onwards whilst in follow-up [a]

Weeks from start of second-line	VLs available/ patients in follow-up (%)
4 [b]	359/386 (93%)
12 [b]	374/386 (97%)
24	364/383 (95%)
36	361/378 (96%)
48	365/372 (98%)
64	215/362 (59%)
80	192/359 (53%)
96	343/351 (98%)
112	161/287 (56%)
128	104/161 (65%)
144	59/59 (100%)
Total from week 24 onwards	**2164/2712 (80%)**

Viral loads and genotypes in patients included in analysis (Experienced failure and has one or more genotype at or post-failure)

Weeks from start of second-line	VLs available/ patients in follow-up (%)	Genotypes available/ patients in follow-up (%) [c]
0	118/118 (100%)	72/118 (61%)
12-16	104/112 (93%)	71/112 (63%)
24-32	93/106 (88%)	74/106 (70%)
40-48	74/89 (83%)	51/89 (57%)
56-64	55/68 (81%)	39/68 (57%)
72-80	38/48 (79%)	30/48 (63%)
88-120	29/31 (94%)	26/31 (84%)
Total	**551/572 (89%)**	**363/572 (63%)**

[a] Weeks 4 and 12 includes those that start PI monotherapy and had at least one VL test from week 24. See supplementary figure 1 for reasons patients were excluded. From week 24 onwards, patients were in follow-up if they were on PI monotherapy at the time the sample was scheduled to be collected. At weeks 4-48, 96, and 144 all samples were tested for VL. Samples at weeks 64, 80, 112, and 128 were only tested if weeks 48 or 96 were detectable (VL>400 copies/ml).

[b] Weeks 4 and 12 were used to assess early suppression.

[c] Weeks 48, 96, and 144 were genotyped for all patients on monotherapy who had experienced VL failure with VL>1000. Intermediate time-points were sequenced if samples at any of weeks 48, 96, or 144 contained a PI mutations and VL>1000. In addition, the last sample before the patient returned to combination therapy was also tested if VL>1000. Selected samples were similar with a cut off of VL>400 as most samples with VL>400 had VL>1000. Samples not available include a combination of samples not run and failed sequences.

Supplementary Table 2: Univariable associations between mutations and mutations in previous sample

	Mutation in current sample										
Mutation in previous sample	**L24I**	**V32I**	**M46I**	**M46L**	**I47A**	**I54V**	**Q58E**	**L76V**	**V82A**	**V82F**	**I84V**
I47A	1.59 (0.03, 18.41) p=1.00	26.91 (3.14, 292.28) p=0.002	0.52 (0.05, 3.48) p=0.73	4.37 (0.58, 26.98) p=0.16		0.05 (NE, 0.30) p=0.0003	2.37 (0.20, 16.27) p=0.58	0.20 (NE, 1.36) p=0.11	0.48 (0.07, 2.45) p=0.51	0.60 (0.01, 5.17) p=1.00	0.77 (0.02, 7.05) p=1.00
I54V	8.11 (0.75, 419.41) p=0.10	0.09 (NE, 0.58) p=0.007	1.47 (0.47, 4.60) p=0.62	0.42 (0.04, 2.37) p=0.49	0.06 (NE, 0.38) p=0.0008		5.74 (0.94, 62.31) p=0.06	3.85 (1.11, 14.21) p=0.03	0.39 (0.06, 1.76) p=0.29	0.72 (0.07, 4.46) p=1.00	0.42 (0.04, 2.28) p=0.47
I84V	0.92 (NE, 7.35) p=0.94	0.43 (NE, 3.05) p=0.45	0.46 (0.04, 2.84) p=0.60	0.68 (0.01, 5.96) p=1.00	0.43 (NE, 3.13) p=0.46	0.39 (0.04, 2.60) p=0.47	0.85 (0.02, 7.74) p=1.00	0.52 (0.01, 4.57) p=0.95	0.12 (NE, 0.85) p=0.03	0.76 (0.02, 6.88) p=1.00	
L24I		1.55 (NE, 15.17) p=1.00		1.53 (NE, 14.95) p=1.00	1.09 (NE, 10.56) p=1.00		4.02 (NE, 53.78) p=1.00	0.98 (NE, 9.47) p=0.99		1.39 (NE, 13.54) p=1.00	1.37 (NE, 13.34) p=1.00
L76V	0.74 (NE, 5.84) p=0.80	0.27 (NE, 1.84) p=0.21	0.77 (0.07, 5.79) p=1.00	0.27 (NE, 1.81) p=0.21	0.19 (NE, 1.25) p=0.09	2.09 (0.28, 24.46) p=0.68	2.82 (0.39, 16.71) p=0.36		0.60 (0.09, 3.38) p=0.77	0.34 (NE, 2.30) p=0.31	7.08 (1.52, 33.95) p=0.01
M46I	9.77 (0.90, 506.22) p=0.07	0.46 (0.05, 2.45) p=0.55		0.19 (0.00, 1.49) p=0.16	0.56 (0.09, 2.39) p=0.61	0.35 (0.07, 1.33) p=0.14	7.86 (1.27, 85.88) p=0.02	1.07 (0.22, 4.30) p=1.00	0.52 (0.12, 1.92) p=0.42	0.31 (0.01, 2.58) p=0.48	0.46 (0.05, 2.48) p=0.56
M46L	2.78 (0.05, 35.17) p=0.76	1.64 (NE, 16.17) p=1.00	0.91 (0.07, 8.49) p=1.00		1.95 (NE, 25.66) p=1.00	0.14 (NE, 1.09) p=0.06	1.61 (0.03, 16.77) p=1.00	0.35 (NE, 2.51) p=0.34	2.21 (0.11, 134.79) p=0.94	0.85 (0.02, 8.07) p=1.00	0.79 (NE, 6.28) p=0.85
Q58E	6.50 (NE, 89.29) p=1.00	2.49 (NE, 32.87) p=1.00		1.53 (NE, 14.95) p=1.00	1.75 (NE, 23.05) p=1.00	1.02 (NE, 39.98) p=1.00		0.98 (NE, 9.47) p=0.99	1.11 (NE, 43.11) p=1.00	1.39 (NE, 13.54) p=1.00	1.37 (NE, 13.34) p=1.00
V32I	2.78 (0.05, 35.17) p=0.76		0.91 (0.07, 8.49) p=1.00	4.74 (0.35, 47.69) p=0.27		0.08 (NE, 0.59) p=0.01	4.47 (0.35, 36.02) p=0.27	0.35 (NE, 2.51) p=0.34	0.49 (0.04, 3.67) p=0.70	0.85 (0.02, 8.07) p=1.00	0.73 (NE, 5.75) p=0.79
V82A	8.45 (0.78, 436.54) p=0.09	0.16 (0.00, 1.22) p=0.09	0.98 (0.28, 3.29) p=1.00	1.73 (0.37, 7.66) p=0.60	0.42 (0.07, 1.74) p=0.31	1.87 (0.50, 7.47) p=0.44	2.02 (0.35, 11.87) p=0.55	2.27 (0.65, 7.95) p=0.23		0.34 (0.03, 1.79) p=0.29	0.87 (0.18, 3.57) p=1.00
V82F	1.48 (NE, 12.48) p=1.00	0.55 (NE, 4.06) p=0.61	1.64 (0.11, 23.93) p=1.00	0.54 (NE, 4.00) p=0.60	0.50 (NE, 3.75) p=0.55	0.56 (0.01, 11.27) p=1.00	0.91 (NE, 6.97) p=0.94	3.28 (0.43, 22.09) p=0.30	0.11 (NE, 0.83) p=0.03		1.20 (0.02, 12.49) p=1.00

Supplementary Table 3: Univariable association with the presence of any PI mutations at failure

	No PI mutations N=79	PI mutations N=28	Crude Odds ratio (95% CI)	P value
Subtype				0.01
A	40 (51%)	9 (32%)	1	
C	20 (25%)	16 (57%)	3.56 (1.34, 9.45)	
D	11 (14%)	3 (11%)	1.21 (0.28, 5.26)	
Recombinant	8 (10%)	0		
Years to VL failure median (IQR)	0.7 (0.2-1)	1.3 (0.7-1.6)	3.98 (1.73, 9.17)	0.001
Percent adherence median (IQR)	86% (70%-100%)	93% (86%-100%)	0.07 (0.004, 1.16)	0.06

Supplementary Table 4: Independent predictors of mutation development after VL failure including previous resistance to lopinavir

Factor	Adjusted Rate Ratio (95% CI)	p value
Weeks since failure [a]		0.34
16	1	
32	1.13 (0.88, 1.45)	
48	1.18 (0.83, 1.64)	
64	1.20 (0.83, 1.74)	
Resistance to Lopinavir in previous sample		0.03
Susceptible	1	
Potential-low	0.73 (0.17, 3.11)	
Low	1.17 (0.57, 2.40)	
Intermediate	1.08 (0.73, 1.61)	
High	0.60 (0.42, 0.87)	
Adherence per 10% higher	1.01 (0.92, 1.10)	0.90
Viral subtype		0.28
A	1	
C	0.77 (0.54, 1.12)	
D	0.80 (0.52, 1.21)	
Other	0.58 (0.30, 1.10)	
CD4 in previous sample per 100 cell higher	1.11 (0.99, 1.25)	0.07
VL at switch to second-line per $\log_{10}$ higher	1.20 (0.95, 1.53)	0.13
Age [c]		0.01
20	1	
30	2.08 (1.27, 3.41)	
40	2.23 (1.23, 4.04)	
50	1.78 (0.94, 3.36)	

Supplementary Table 5: Independent predictors of change in viral load after failure, including interactions with time since failure

Factor	Adjusted Effect on log_{10} VL (95% CI)	p value
Time since failure per year later		
Before I47A [a]	+0.29 (+0.12, +0.46)	0.001
After I47A	-0.42 (-0.99, +0.14)	0.14
Mutations in previous sample		
I47A	+1.36 (+0.63, +2.10)	0.0003
Other	+0.15 (+0.08, +0.23)	0.0001
Adherence per 10% higher	-0.08 (-0.13,-0.03)	0.003
Subtype		0.45
A	1	
C	+0.09 (-0.16, +0.34)	
D	+0.14 (-0.17, +0.45)	
Other	+0.30 (-0.08, +0.68)	
Time on second line before failure per year longer	-0.33 (-0.56, -0.11)	0.004
Age per 10 year older	-0.11 (-0.21, -0.01)	0.03
Time on first line ART per year longer	-0.06 (-0.11, -0.01)	0.02

[a] Interaction between time since failure and presence of I47A p=0.01. No evidence of other interactions with time since failure p>0.15

Supplementary Table 6: Rate of mutation development under different missing data assumptions

Assumption	Rate (95% CI)
Accumulation of mutations, not carrying back absence of mutations. Including all time points	2.0 (1.7, 2.3)
Accumulation of mutations, not carrying back absence of mutations. Restricting to weeks 48, 96, and 144 where all patients' genotypes were assayed	1.8 (1.4, 2.2)

Supplementary Table 7: Independent predictors of mutation development after failure, observed cumulative mutations (not carrying backwards absences of mutations)

Factor	Adjusted Rate Ratio (95% CI)	p value
Weeks since failure [a]		0.72
16	1	
32	0.96 (0.76, 1.21)	
48	0.94 (0.69, 1.29)	
64	0.93 (0.66, 1.34)	
Mutations in previous sample [b]		<0.0001
0	1	
1	1.50 (1.05, 2.12)	
2	1.02 (0.74, 1.39)	
3	0.70 (0.79, 0.99)	
4	0.50 (0.33, 0.78)	
5	0.38 (0.22, 0.65)	
Adherence per 10% higher	0.97 (0.89, 1.06)	0.54
Viral subtype		0.12
A	1	
C	0.74 (0.52, 1.06)	
D	0.70 (0.46, 1.06)	
Other	0.54 (0.28, 1.03)	
CD4 in previous sample per 100 cell higher	1.11 (0.99, 1.24)	0.09
VL at switch to second-line per $\log_{10}$ higher	1.16 (0.93, 1.46)	0.19
Age [c]		0.002
20	1	
30	2.37 (1.44, 3.92)	
40	2.57 (1.40, 4.69)	
50	1.95 (1.03, 3.68)	

Poisson model with time since failure, adherence and subtype forced into the model. Other factors selected using backward elimination (exit p>0.1). Factors not selected: age, sex, years on first-line, CD4 at switch to second-line, proportion of VL>50, viremia copy-years, or time on second-line before failure, VL in previous sample, VL or CD4 second-line failure.

[a] Included in model using fractional polynomials with 2 d.f as week $^{-1}$.

[b] Included in model using fractional polynomials with 4 d.f as previous-mutations $^{-2}$ + ln(previous-mutations

[c] Included in model using fractional polynomials with 4 d.f as age $^{-2}$ + age ^{3}.

Supplementary Table 8: Independent predictors of change in viral load after failure, observed cumulative mutations (not carrying backwards no mutations)

Factor	Adjusted Effect on log_{10}(VL) (95% CI)	p value
Time since failure per year longer	+0.34 (+0.16, +0.52)	0.0002
Mutations in previous sample		
I47A	+0.45 (+0.14, +0.77)	0.005
Other	+0.13 (+0.05, +0.20)	0.001
Adherence per 10% higher	-0.05 (-0.11, +0.01)	0.09
Subtype		0.59
A	1	
C	-0.01 (-0.28, +0.28)	
D	+0.13 (-0.22, +0.48)	
Other	+0.27 (-0.16, +0.71)	
Time on second-line before failure per year longer	-0.29 (-0.54, -0.04)	0.02
Age per 10 years older	-0.10 (-0.22, 0.01)	0.07
Time on first line ART per year longer	-0.06 (-0.12, -0.01)	0.03