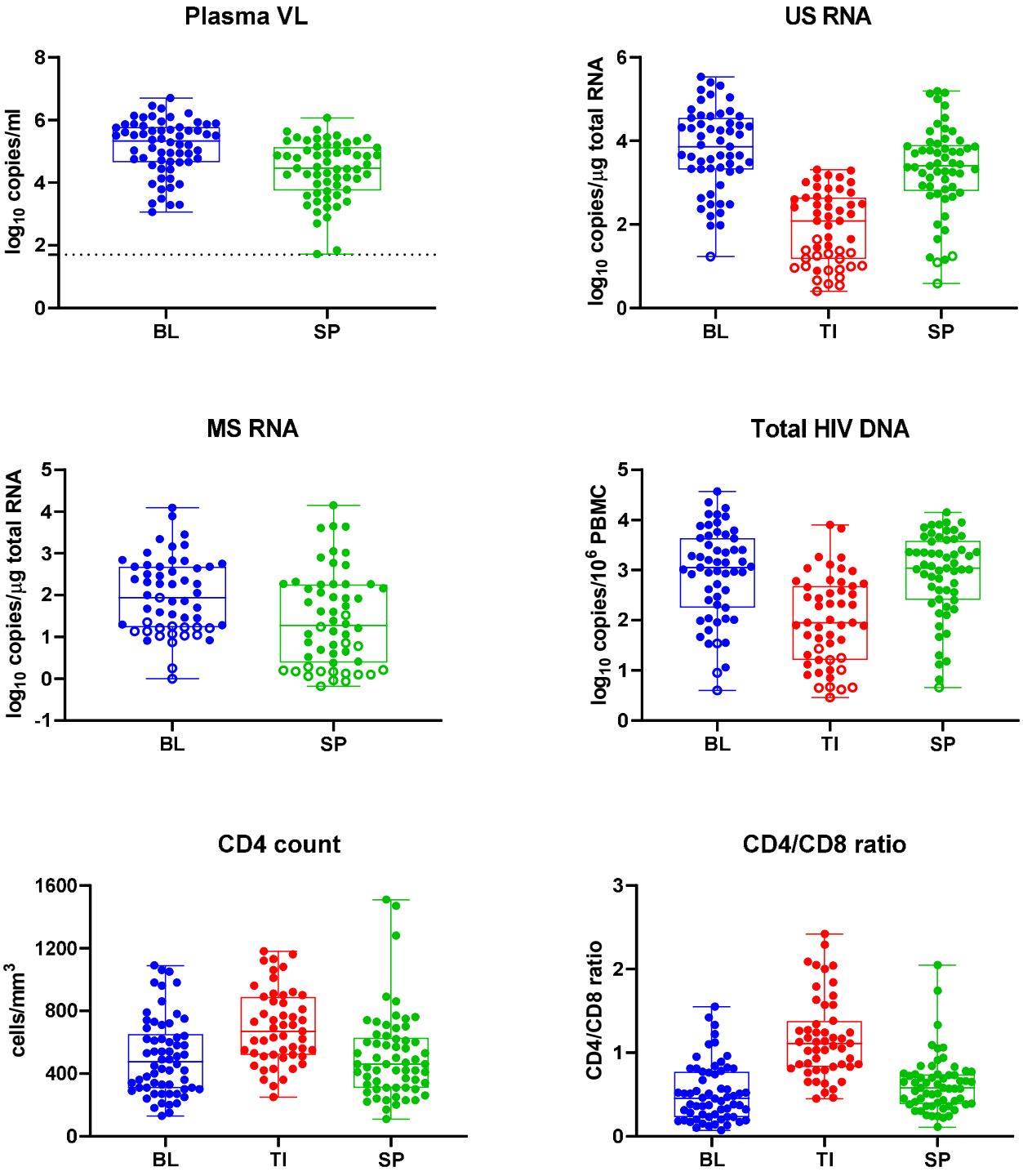
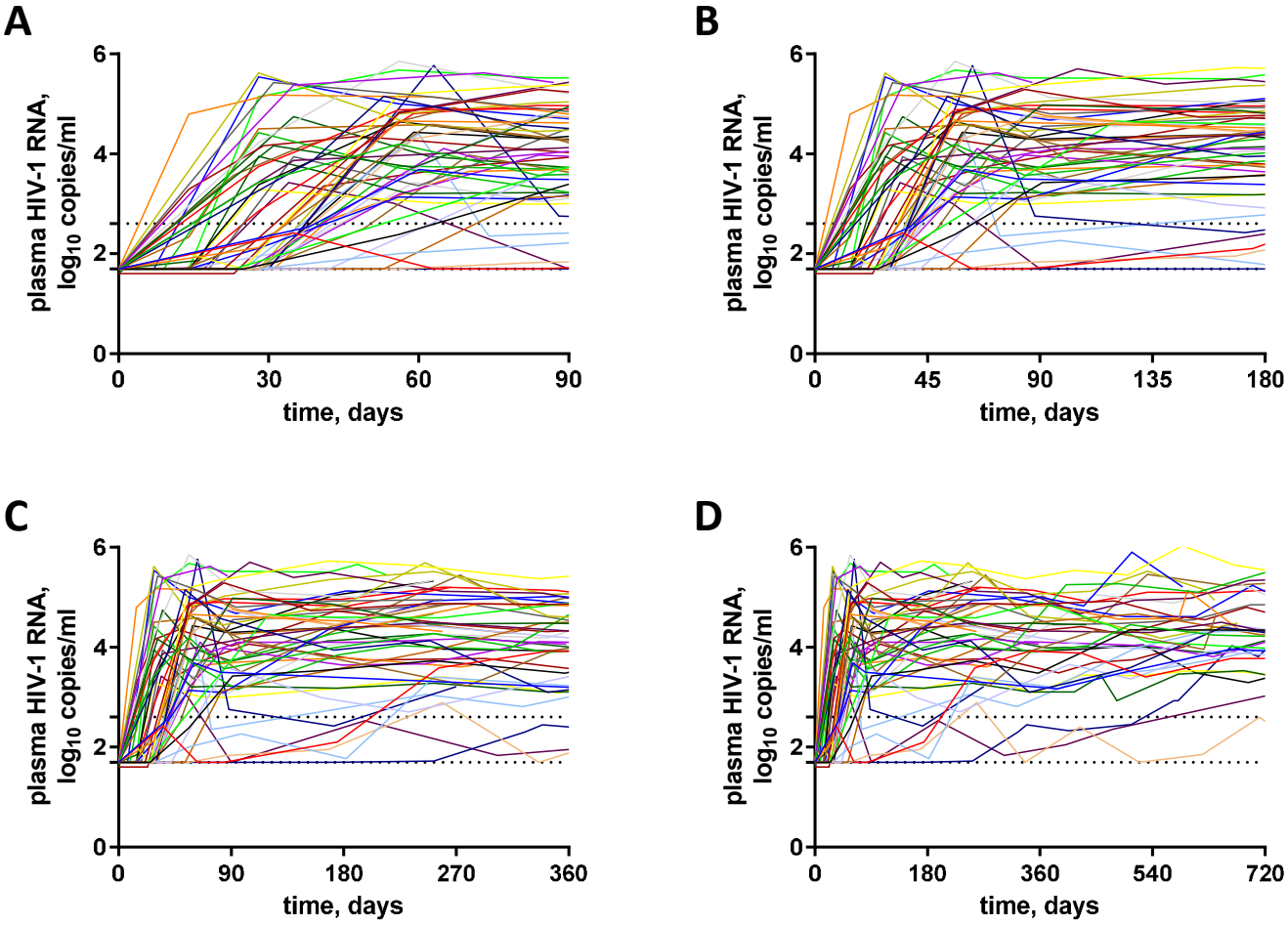
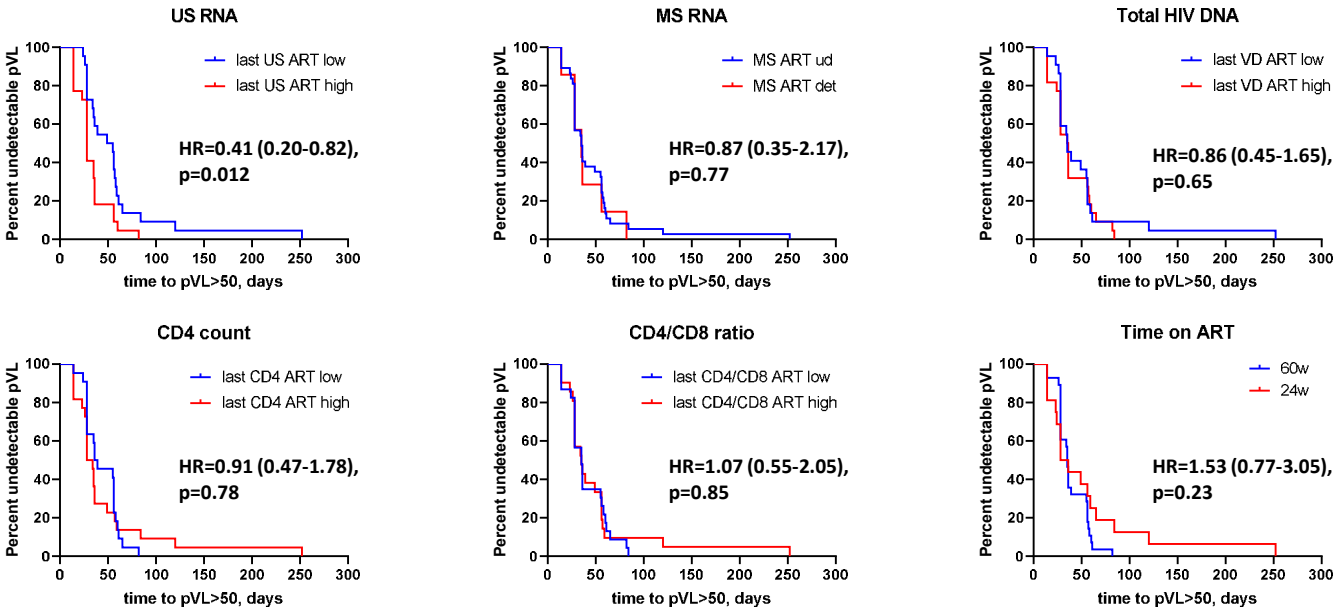


Supplemental Figure 1. Levels of virological and clinical biomarkers. Plasma HIV viral load (VL), cell-associated HIV unspliced (US) and multiply spliced (MS) RNA, total HIV DNA, CD4+ count, and CD4/CD8 ratio were measured at the baseline (BL), early therapy interruption (TI), and virological setpoint (SP). Plasma VL at TI was undetectable (<50 copies/ml) for all except two participants and therefore not shown. MS RNA at TI was undetectable for almost all participants and not shown. Median values, interquartile ranges, and total ranges are shown by box plots. Open circles depict undetectable values, censored to 50% of assay detection limits. The latter depended on the amount of input cellular RNA or DNA in the assay and therefore differed between samples. The dashed line shows the limit of detection for plasma VL (50 copies/ml).

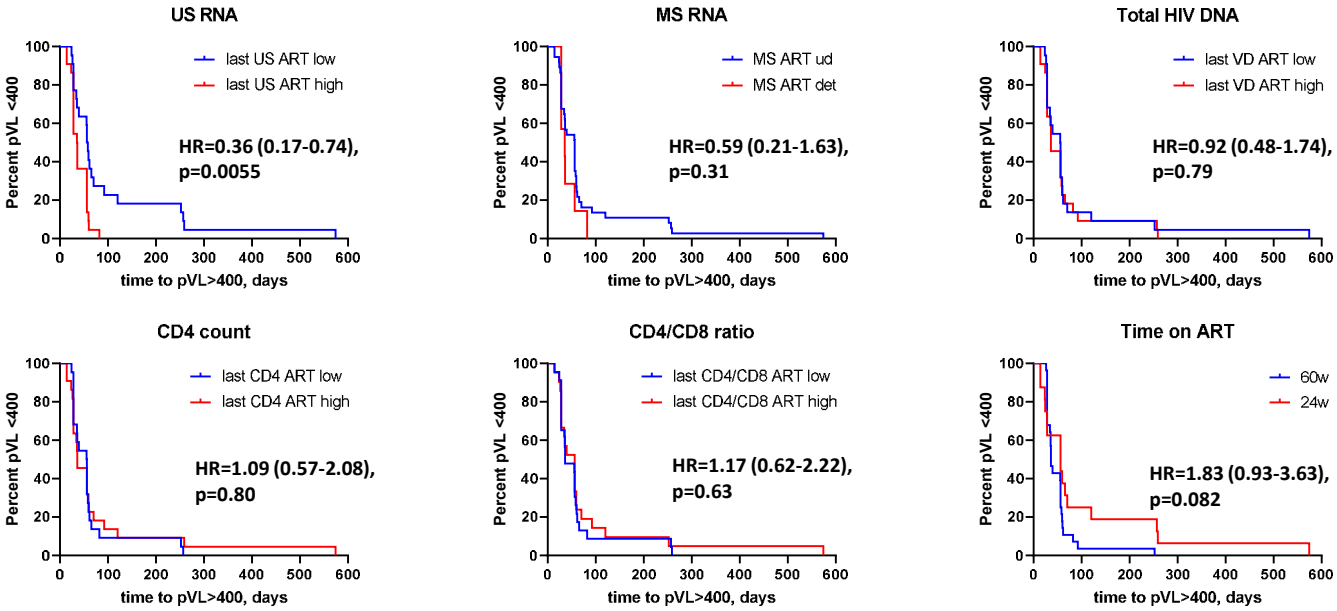


Supplemental Figure 2. Longitudinal dynamics of viral rebound. Shown is dynamics of plasma HIV-1 load during the first (A) 90, (B) 180, (C) 360, and (D) 720 days post-TI. Thresholds of 50 and 400 copies/ml are shown by dashed lines. Individuals are color coded.

Time to pVL >50



Time to pVL >400



Supplemental Figure 3. On-ART predictors of time to viral rebound after interruption of early ART. Restricted to participants with plasma VL <50 copies/mL at the measurement time points (n=44). Kaplan-Meier survival analysis for cell-associated HIV unspliced (US) RNA, total HIV DNA (VD), CD4+ count, CD4/CD8 ratio, measured at the latest time point available before interruption of early ART, as well as detectability of cell-associated HIV multiply spliced (MS) RNA at any time point on early ART (det=detectable, ud=undetectable), and time on early ART (60 or 24 weeks) (n=51). The endpoint of the analysis was viral rebound to >50 or >400 copies/ml. Levels of US RNA, total HIV DNA, CD4+ count, and CD4/CD8 ratio were stratified into “high” and “low” strata based on median values. Hazard ratios (HR) and 95% confidence intervals were calculated using Mantel-Haenszel method. P values were calculated using log-rank tests. TI, treatment interruption.

Supplemental Table 1. Variables associated with time to virological suppression (plasma VL <50 copies/ml) on early ART (n=44; univariable Kaplan-Meier analyses).

Variable	**HR (95% CI)**[a]	***P***[b]
Age (younger vs. older)[c]	1.25 (0.67-2.33)	0.48
HIV subtype (B vs. non-B)	0.47 (0.19-1.19)	0.11
Fiebig stage of infection at diagnosis (I-II vs. III-IV vs. V-VI)	-	0.24
NRTI backbone at start of early ART (AZT+3TC vs. FTC+TDF)	1.20 (0.64-2.24)	0.57
ART class at start of early ART (double-class vs. triple-class)	0.99 (0.38-2.56)	0.99
Gender (female vs. male)	1.91 (0.43-8.5)	0.40
Transmission route (MSM vs. other)	0.82 (0.37-1.82)	0.63

[a] Hazard ratios (HR) and 95% confidence intervals were calculated using Mantel-Haenszel method.
[b] P values were calculated using log-rank tests.
[c] Stratified into "younger" and "older" strata based on median values.

Supplemental Table 2. Variables associated with virological suppression (plasma VL <50 copies/ml) at 12 weeks of early ART (n=43; univariable Mann-Whitney or Fisher's exact tests).

Variable	**OR (95% CI)[a]**	***P*[b]**
Age	-	0.85
HIV subtype (B vs. non-B)	0.31 (0.08-1.32)	0.15
Fiebig stage of infection at diagnosis (I-II vs. III-IV vs. V-VI)	-	0.11
NRTI backbone at start of early ART (AZT+3TC vs. FTC+TDF)	2.37 (0.69-8.30)	0.22
ART class at start of early ART (double-class vs. triple-class)	0.82 (0.13-4.43)	>0.99
Gender (female vs. male)	2.71 (0.29-40.67)	0.58
Transmission route (MSM vs. other)	0.56 (0.15-2.48)	0.48

[a] Odds ratios (HR) and 95% confidence intervals were calculated using Fisher's exact tests.
[b] P values were calculated using Mann-Whitney tests (for age) or Fisher's exact tests (for other variables).

Supplemental Table 3. Variables associated with CD4/CD8 ratio >1 at 48 weeks of early ART (n=24; univariable Mann-Whitney or Fisher's exact tests).

Variable	**OR (95% CI)[a]**	***P*[b]**
Age	-	0.32
HIV subtype (B vs. non-B)	0.43 (0.03-4.02)	0.63
Fiebig stage of infection at diagnosis (I-II vs. III-IV vs. V-VI)	-	0.17
NRTI backbone at start of early ART (AZT+3TC vs. FTC+TDF)	0.33 (0.06-2.41)	0.39
ART class at start of early ART (double-class vs. triple-class)	1.00 (0.10-16.36)	>0.99
Gender (female vs. male)	-	>0.99
Transmission route (MSM vs. other)	1.44 (0.21-8.56)	>0.99

[a] Odds ratios (HR) and 95% confidence intervals were calculated using Fisher's exact tests.
[b] P values were calculated using Mann-Whitney tests (for age) or Fisher's exact tests (for other variables).

Supplemental Table 4. Baseline variables associated with time to viral rebound after interruption of early ART (n=43; Cox proportional hazards analysis).

Variable	Category	Rebound to >50 copies/ml				Rebound to >400 copies/ml			
		Univariable analyses		Multivariable model[a]		Univariable analyses		Multivariable model	
		HR (95% CI)	*P*	AHR (95% CI)	*P*	HR (95% CI)	*P*	AHR (95% CI)	*P*
Plasma viral load, per log_{10} copies/ml		1.30 (0.86-1.98)	0.22			1.51 (0.99-2.29)	0.056[b]	-[c]	-[c]
CD4+ count, per 100 cells/mm^3	-	0.90 (0.80-1.02)	0.091[b]	-[c]	-	0.85 (0.74-0.97)	0.017[b]	0.87 (0.76-1.00)	0.050
CD4/CD8 ratio, per 1	-	0.46 (0.17-1.29)	0.14			0.30 (0.10-0.89)	0.030[b]	-[c]	-[c]
Cell-associated HIV unspliced RNA, per log_{10} copies/µg total RNA	-	1.23 (0.89-1.70)	0.22			1.31 (0.92-1.87)	0.13		
Cell-associated HIV multiply spliced RNA, per log_{10} copies/µg total RNA	-	1.33 (0.93-1.90)	0.11			1.49 (1.02-2.17)	0.038[b]	-[c]	-[c]
Total HIV DNA, per log_{10} copies/10^6 PBMC	-	1.12 (0.82-1.52)	0.50			1.13 (0.82-1.56)	0.44		
Age, per 10 years	-	1.17 (0.87-1.58)	0.30			1.06 (0.78-1.43)	0.73		
HIV subtype	B (n=34)	2.00 (0.92-4.37)	0.080[b]	2.00 (0.92-4.37)	0.080	2.21 (0.97-5.04)	0.060[b]	1.82 (0.78-4.24)	0.17
	Non-B (n=9)	1	-			1	-		
Fiebig stage of infection at diagnosis	I-II (n=3)	0.41 (0.08-2.15)	0.29			1.32 (0.26-6.58)	0.74		
	III-IV (n=37)	0.71 (0.22-2.35)	0.58			1.65 (0.50-5.40)	0.41		
	V-VI (n=3)	1	-			1	-		
Gender	Female (n=3)	1.85 (0.56-6.14)	0.32			1.41 (0.43-4.63)	0.57		
	Male (n=40)	1	-			1	-		
Transmission route	MSM (n=34)	0.95 (0.43-2.08)	0.89			1.00 (0.46-2.18)	1.00		
	Other (n=9)	1	-			1	-		

[a] Fitted using backward stepwise regression.
[b] Included in the multivariable analysis.
[c] Not included in the final optimized model.

Supplemental Table 5. Variables associated with time to viral rebound after interruption of early ART (n=51; univariable Kaplan-Meier analyses).

Variable	Rebound to >50 copies/ml		Rebound to >400 copies/ml	
	HR (95% CI)[a]	*P*[b]	HR (95% CI)	*P*
Age (younger vs. older)[c]	0.48 (0.26-0.89)	0.020	0.68 (0.38-1.23)	0.21
HIV subtype (B vs. non-B)	1.85 (0.97-3.54)	0.062	1.90 (0.98-3.69)	0.059
Fiebig stage of infection at diagnosis (I-II vs. III-IV vs. V-VI)	-	0.64	-	0.94
NRTI backbone at interruption of early ART (3TC+TDF vs. AZT+3TC vs. FTC+TDF)	-	0.30	-	0.46
ART class at interruption of early ART (NNRTI- vs. PI- vs. NNRTI+PI-based)	-	0.78	-	0.89
Gender (female vs. male)	2.51 (0.49-12.98)	0.27	1.55 (0.37-6.50)	0.55
Transmission route (MSM vs. other)	0.87 (0.39-1.94)	0.73	1.00 (0.47-2.14)	>0.99

[a] Hazard ratios (HR) and 95% confidence intervals were calculated using Mantel-Haenszel method.
[b] P values were calculated using log-rank tests.
[c] Stratified into "younger" and "older" strata based on median values.

Supplemental Table 6. Variables associated with disease progression (CD4+ count <350 cells/mm^3 or restart ART) after interruption of early ART (n=51; univariable Kaplan-Meier analyses).

Variable	**HR (95% CI)[a]**	***P*[b]**
Age (younger vs. older)[c]	0.90 (0.51-1.60)	0.73
HIV subtype (B vs. non-B)	2.47 (1.29-4.72)	0.0062
Fiebig stage of infection at diagnosis (I-II vs. III-IV vs. V-VI)	-	0.99
NRTI backbone at interruption of early ART (3TC+TDF vs. AZT+3TC vs. FTC+TDF)	-	0.14
ART class at interruption of early ART (NNRTI- vs. PI- vs. NNRTI+PI-based)	-	0.76
Gender (female vs. male)	0.50 (0.20-1.29)	0.15
Transmission route (MSM vs. other)	1.09 (0.53-2.21)	0.82

[a] Hazard ratios (HR) and 95% confidence intervals were calculated using Mantel-Haenszel method.
[b] P values were calculated using log-rank tests.
[c] Stratified into "younger" and "older" strata based on median values.

Supplemental Table 7. Variables associated with the rates of disease progression (time to CD4+ count <350 cells/mm^3 or restart ART) after virological setpoint (n=56; univariable Kaplan-Meier analyses).

Variable	**HR (95% CI)**[a]	***P***[b]
Age (younger vs. older)[c]	0.59 (0.33-1.05)	0.073
HIV subtype (B vs. non-B)	1.97 (0.99-3.89)	0.053
Gender (female vs. male)	0.85 (0.21-3.33)	0.81
Transmission route (MSM vs. other)	0.86 (0.39-1.89)	0.70

[a] Hazard ratios (HR) and 95% confidence intervals were calculated using Mantel-Haenszel method.
[b] P values were calculated using log-rank tests.
[c] Stratified into "younger" and "older" strata based on median values.

Supplemental Methods

Timing of the measurements. For the baseline analysis of treated individuals (n=44), plasma VL, CD4+ count, and CD4/CD8 ratio were measured at the day of ART initiation in 25 participants and a median of 4 days (IQR, 2-6 days; maximum, 25 days) before ART initiation in 19 participants. CA RNA and total HIV-1 DNA were measured at the day of ART initiation in 24 participants and at a median of 6 days (IQR, 4-10 days, maximum, 24 days) before ART initiation in 20 participants.

For the TI analysis (n=51), variables were measured at the latest visit before the TI from which samples were available. CD4+ count and CD4/CD8 ratio were measured immediately before TI (at the same day) in 47 participants, within a month prior to TI in 2 participants, and 2.5 months prior to TI in 2 participants. US RNA and total DNA were measured immediately before TI in 32 participants, 12 weeks prior to TI in 18 participants, and 24 weeks prior to TI in one participant. For 44 out of 51 participants, concurrent plasma VLs at the TI PBMC sampling dates were <50 copies/mL, irrespective of the exact time points. For six participants they were between 50 and 400 copies/mL, and one participant had a plasma VL of 534 copies/mL. In six out of these seven cases, these low but detectable plasma VLs were due to incomplete virological suppression 12 weeks prior to TI in participants who were treated for 24 weeks, and in one case, a participant had a detectable plasma VL (74 copies/mL) at TI after having been suppressed to <50 copies/mL in the preceding visits. To exclude any possible influence of incomplete virological suppression on the analysis, we performed a sensitivity analysis of predictors of time to viral rebound, excluding these seven participants with detectable plasma VLs at TI. This did not change the results, arguing that the exact plasma VL at sampling did not confound the predictive value of CA RNA for viral rebound (Supplemental Figure 3).

Virological setpoint was defined as the closest visit (median divergence, 7 days, IQR, 1-20 days) to 36 weeks from TI or randomization (for the untreated arm). Plasma VL, CD4+ count and CD4/CD8 ratio (n=56) were measured at the setpoint. CA RNA and total DNA were also measured at the setpoint in 38 participants and at a median of 83 days (IQR, 72-92 days, range 11-169 days) before or after the setpoint in 18 participants.