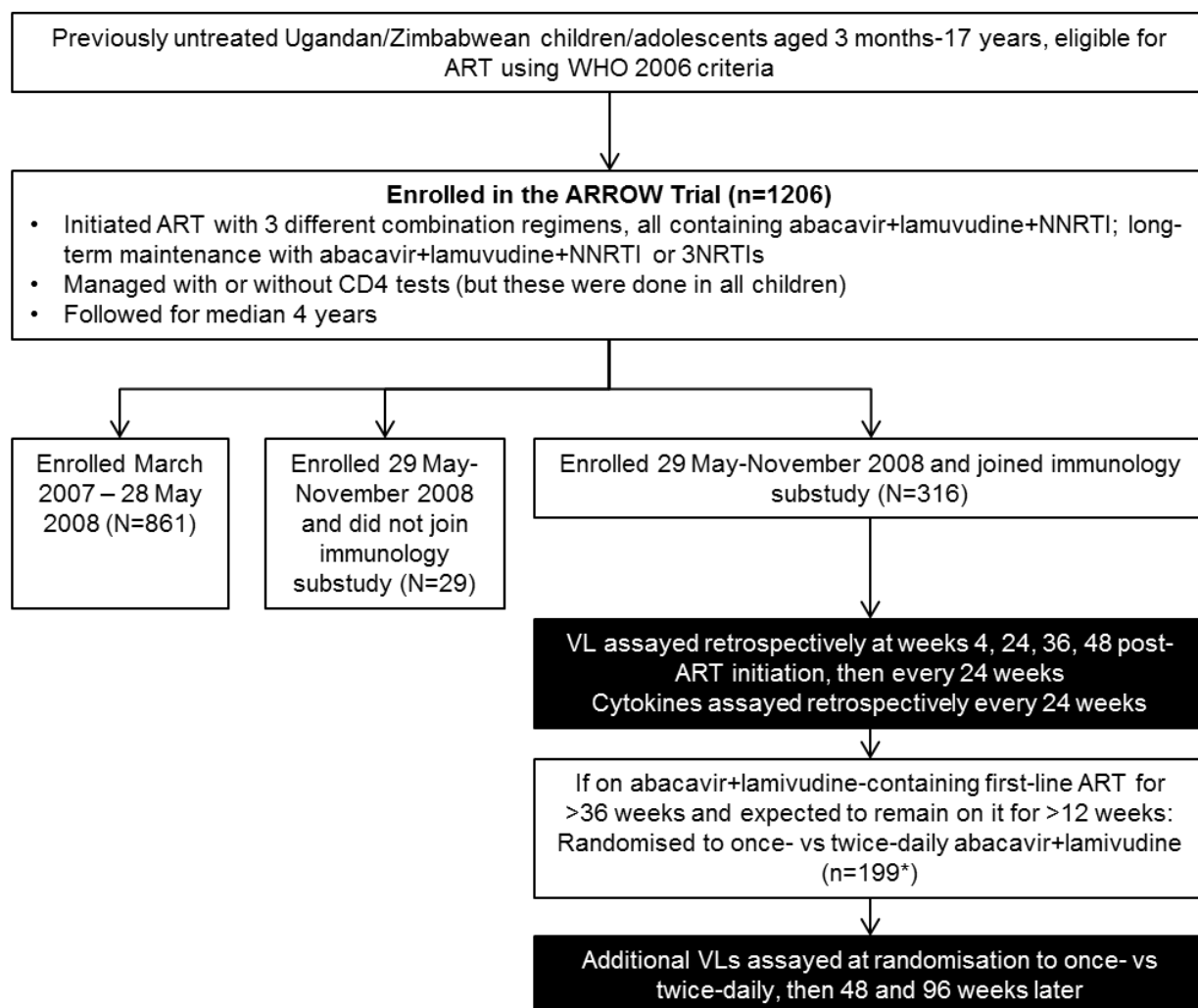


Study flow diagram



* Total 669 children randomised to once- vs twice-daily abacavir+lamivudine in the trial as a whole.

Note: NRTI=nucleoside reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor

SUPPLEMENTARY METHODS

Assay methods

VLs were assayed using Abbott m2000sp/rt or Roche COBAS Amplicor Monitor standard (baseline only) and ultrasensitive tests, v1.5. We used a lower limit of detection of 80 copies/ml because many samples were diluted 1:2 due to low volumes. Immunophenotyping used anti-CD4-PerCP (Becton Dickinson, BD), anti-CD45RA-APC (Caltag Medsystems), anti-CD31-PE (eBioscience) and either anti-Ki67-FITC (BD; after nuclear membrane permeabilization) or anti-HLA-DR-FITC (BD), with data acquired on a BD FACSCalibur flow cytometer. Analysis was undertaken using Cellquest. Plasma inflammatory biomarkers and IL-7 were measured in singlicate by ELISA (R&D Systems, Oxford, UK), according to manufacturer instructions.

Definitions of VL blips

Viral load (VL) measurements ≥ 80 copies/ml were classified as a blip if the child had a previous VL < 80 copies/ml and either (1) subsequently re-suppressed < 80 copies/ml (one or two VL ≥ 80 copies/ml allowed; $n=150$) or (2) their last measurement was a single value ≥ 80 copies/ml ($n=31$). The latter is because, firstly, blips were much more common than persistent low-level viraemia (pLLVL), and so, on the balance of probability, these observations would be more likely to be blips than pLLVL. Secondly, excluding them from analyses completely (effectively ignoring this last VL observation) would necessarily bias the estimate of the percentage of children experiencing blips downwards, because at least some of these children would indeed have blipped (and a smaller percentage would have pLLVL). Therefore by including them all as blips, the percentage of children who remain consistent VL responders is not over-estimated.

Reference values for weight-for-age and height-for-age

Height-for-age was calculated using WHO references¹. Weight-for-age was calculated using UK references², as the WHO weight-for-age reference is only available to 10 years (Spearman correlation between UK and WHO references=0.99 in 248 children < 10 years).

Analysis of rates of increase or decrease, overall and in each VL state

To estimate overall rates of increase or decrease in each outcome (CD4%; CD4-for-age; CD8-for-age; CD4/CD8 ratio; inflammatory biomarkers; CD4 subpopulations; weight-for-age; height-for-age), measurements were modelled using multi-level models with individual random intercepts and linear slopes. Where mean changes at each timepoint (as shown in **Figure 1**) suggested that a simple linear model did not adequately reflect the trajectory, piecewise linear models were used. For CRP, a time-dependent indicator variable was added to the final model to reflect possible impacts of being randomised to stop prophylaxis with cotrimoxazole vs. randomised to continue/not randomised³.

To estimate rates of increase or decrease in each outcome in each VL state (consistent response, blip/post-blip, pLLVL, rebound), measurements were modelled similarly using multi-level models with individual random intercepts and slopes based on measurements restricted to that state only. Inflammatory biomarkers, CD4-for-age and CD8-for-age were log₂ transformed for normality. For the consistent VL response state, these models included measurements only from when children were stable on ART (post-week 48; or for TNF- α /CRP/% CD4 Ki67+ post-week 72, see **Figure 1D**) and time was measured from this fixed timepoint post-ART initiation. For blip/post-blip, low-level VL and rebound, time was measured from the first measurement in that state unless this measurement was before week-48 (week-72 for TNF- α /CRP/% CD4 Ki67+), in which case time was measured from week-48 (week-72 for TNF- α /CRP/% CD4 Ki67+) to match consistent VL response and ensure that comparisons across VL states were unbiased by duration on ART.

Measurements in the four states were modelled simultaneously for each outcome to enable direct tests of whether changes in each outcome differed according to VL state. Wherever possible we allowed correlation between the initial measurement in each state and the subsequent slope in that state for each child, but not with the initial measurement or slope in other states due to lack of model convergence, and included heteroskedastic residuals in each

state. For some outcomes, there was too little variability in outcomes to incorporate these additional random effects.

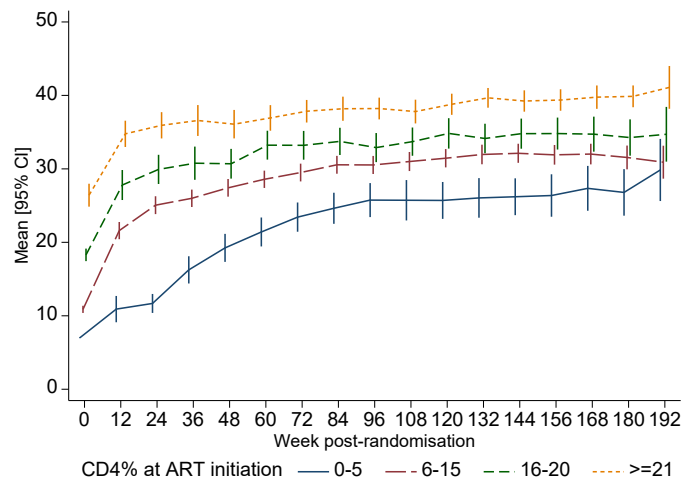
For each biomarker, we first conducted a global (df=3) test of whether the trajectory (slope of any increase or decrease) differed across all four VL states (**Supplementary Table 1A**). Where there was evidence of a difference between the VL states on this overall (global) test, we also considered pairwise tests comparing consistent VL suppression versus each other state, rebound vs pLLVL, and consistent suppression/blips versus pLLVL/rebound (sustained loss of control). We compared the first value in each state (intercept) across states similarly (**Supplementary Table 1B**).

Supplementary References

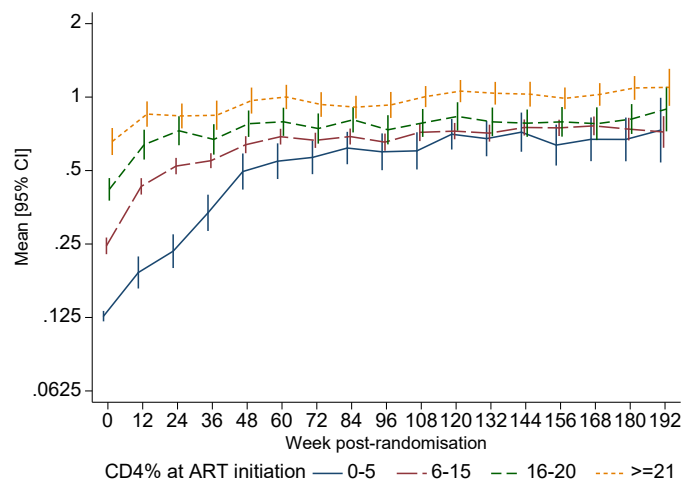
1. De Onis M, Onyango AW, Borghi E, Siyam A, Nishida C and Siekmanna J. Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization*. 2007; 85: 660-7.
2. Wade A and Ades A. Age-related reference ranges: significance tests for models and confidence intervals for centiles. *Statistics in Medicine*. 1994; 13: 2359-67.
3. Prendergast AJ, Bwakura Dangarembizi M, Musiime V, et al. Lower Inflammatory Biomarkers in Children Randomized to Prolonged Cotrimoxazole Prophylaxis. *18th Conference on Retroviruses and Opportunistic Infections*. Boston, MA: CROI, 2014.

Changes in CD4, CD8, subpopulations and inflammatory biomarkers on first-line ART according to baseline CD4%

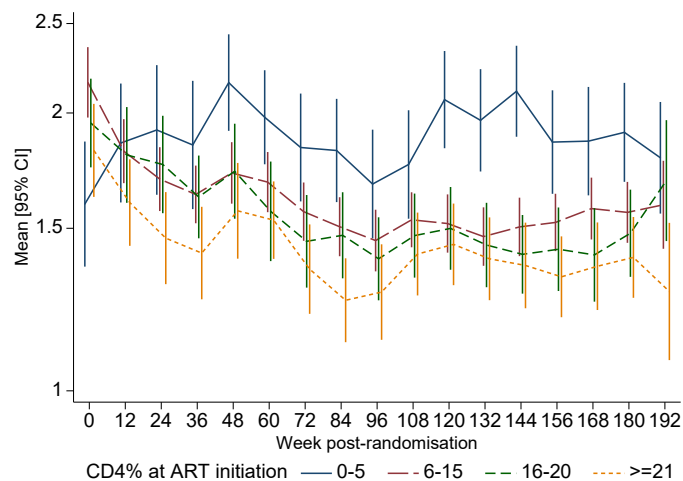
(a) CD4%



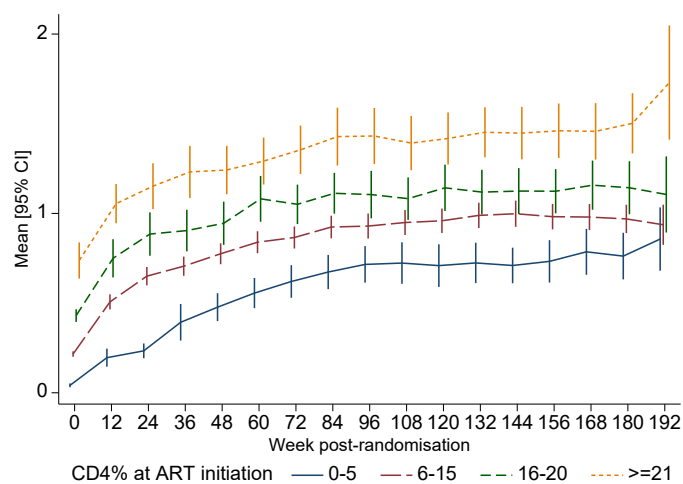
(b) CD4-for-age



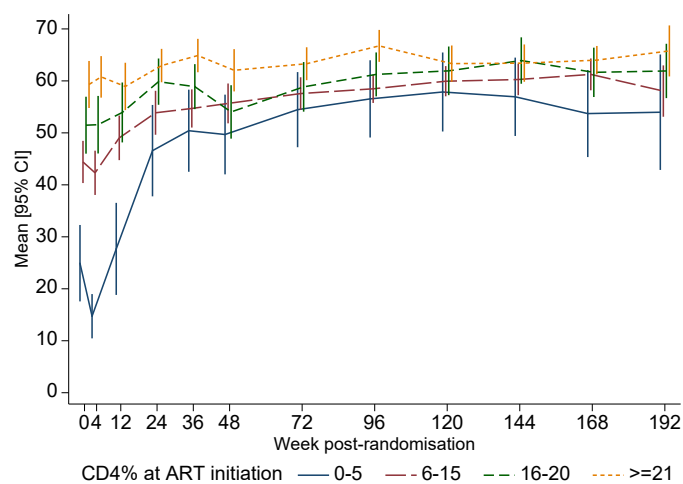
(c) CD8-for-age



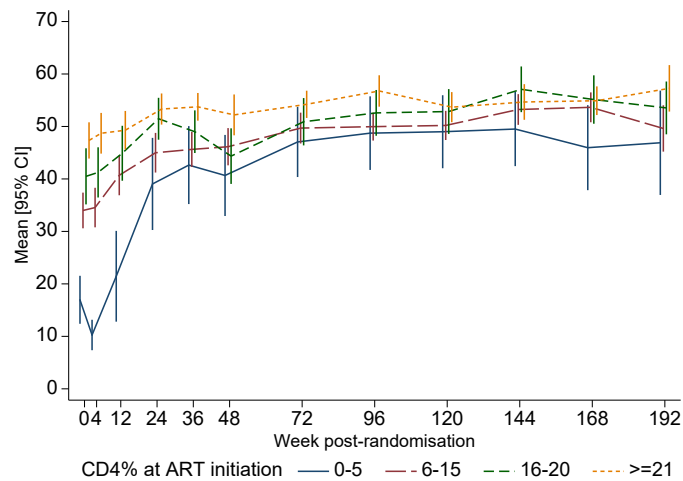
(d) CD4/CD8 ratio



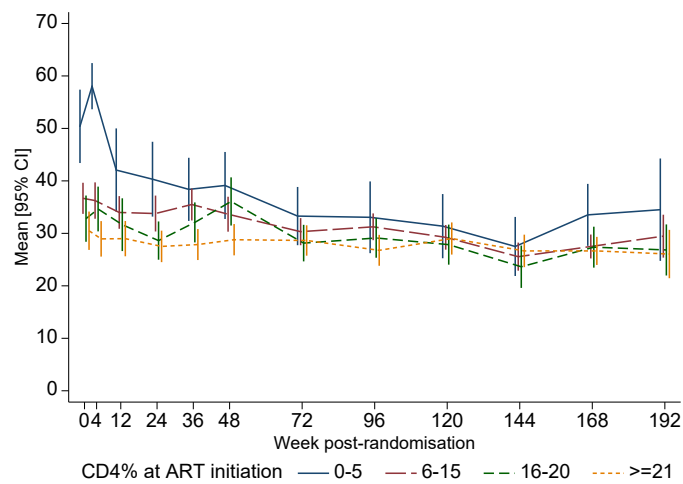
(e) Naïve (CD45RA+) (% of CD4)



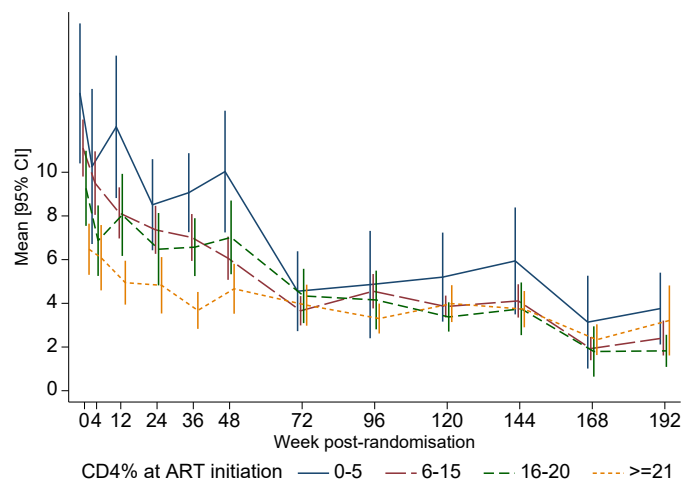
(f) CD45RA+CD31+ (% of CD4)



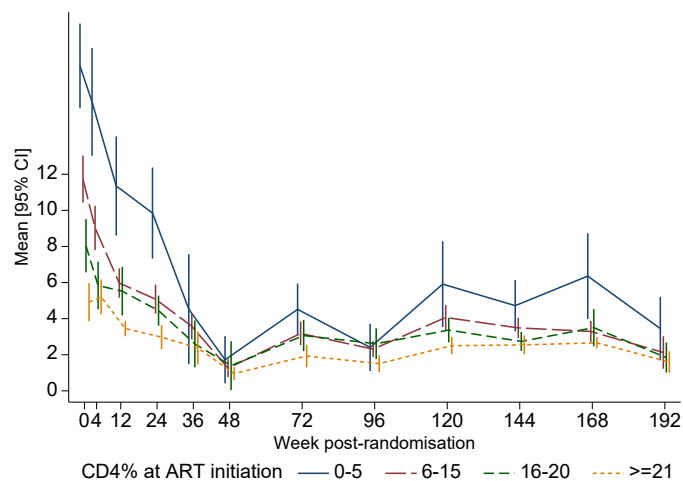
(g) CD45RA-CD31- (% of CD4)



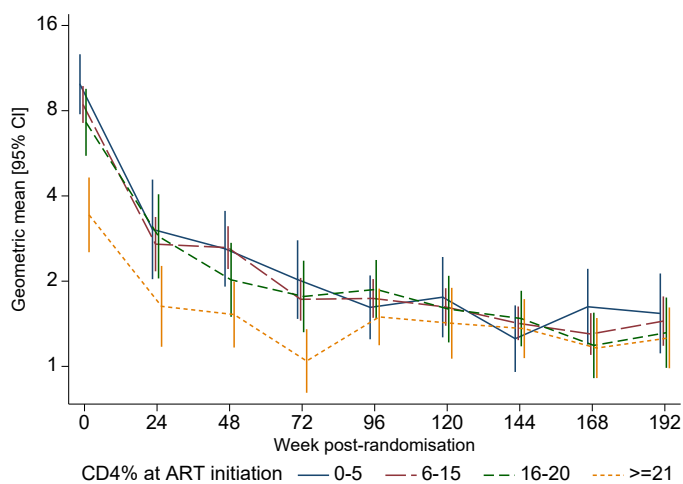
(h) Activated CD4 (HLA-DR+) (% of CD4)



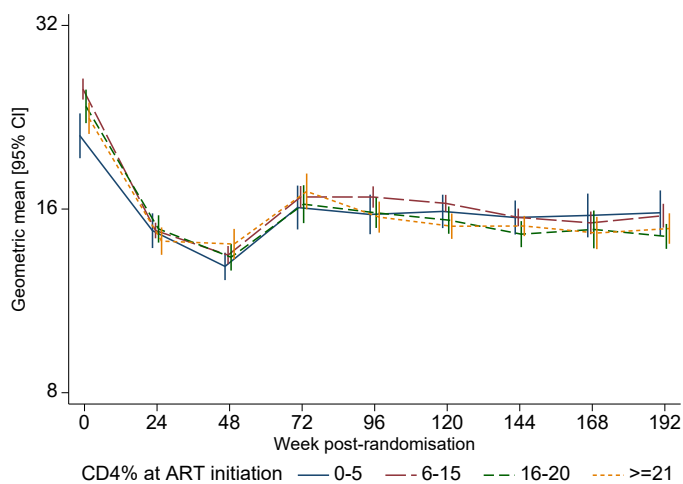
(i) Proliferating CD4 (Ki67+) (% of CD4)



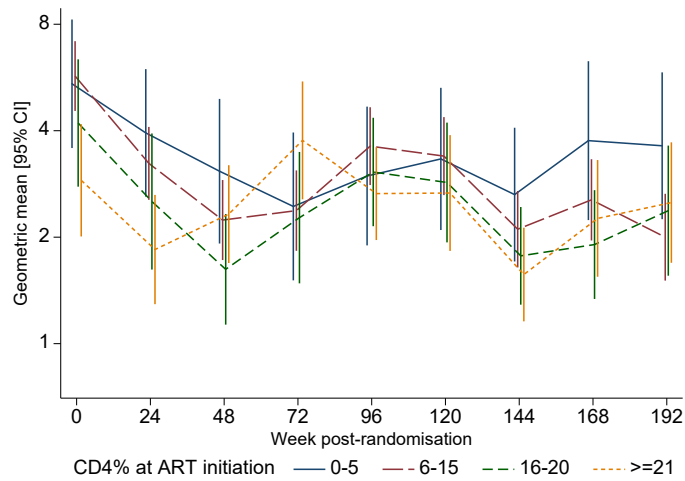
(j) IL-7 (pg/mL)



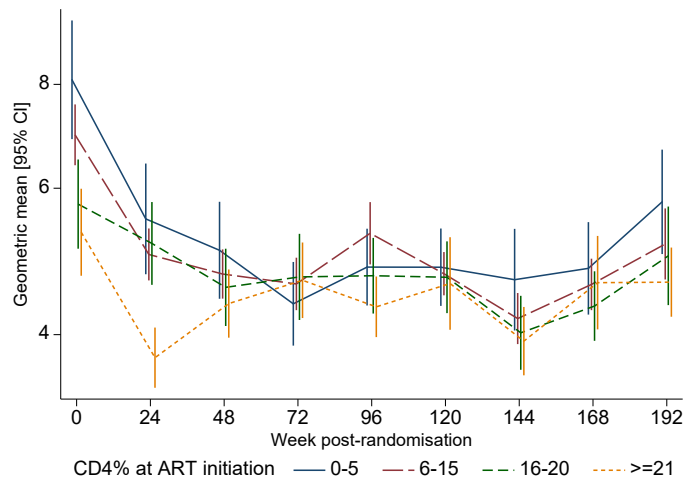
(k) TNF- α (pg/mL)



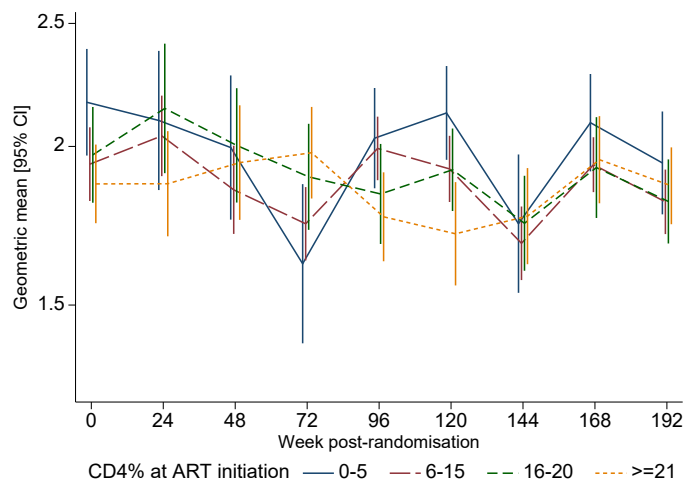
(l) CRP (mg/L)



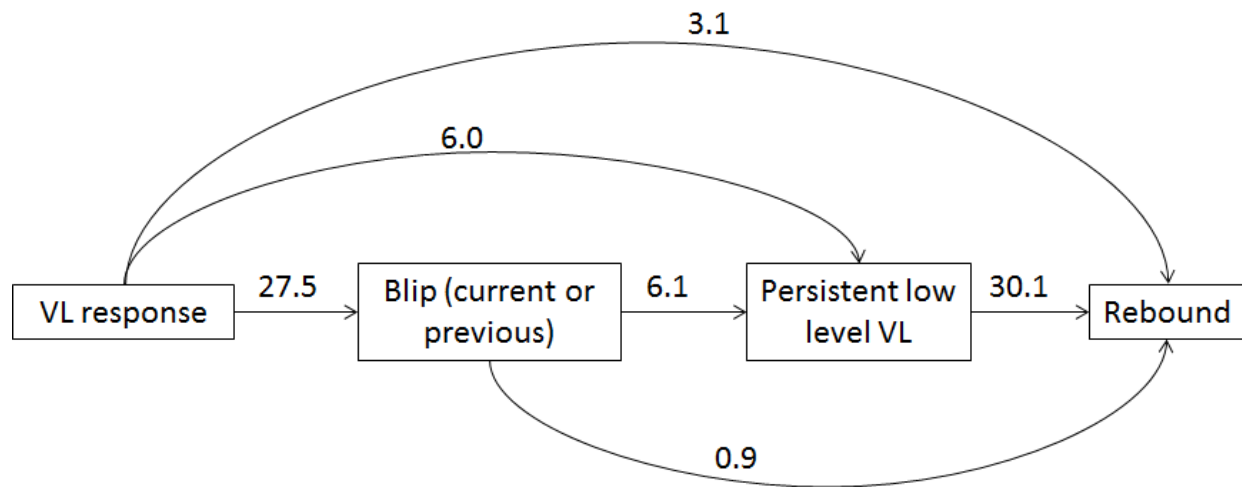
(m) IL-6 (pg/mL)



(n) sCD14 (mg/L)



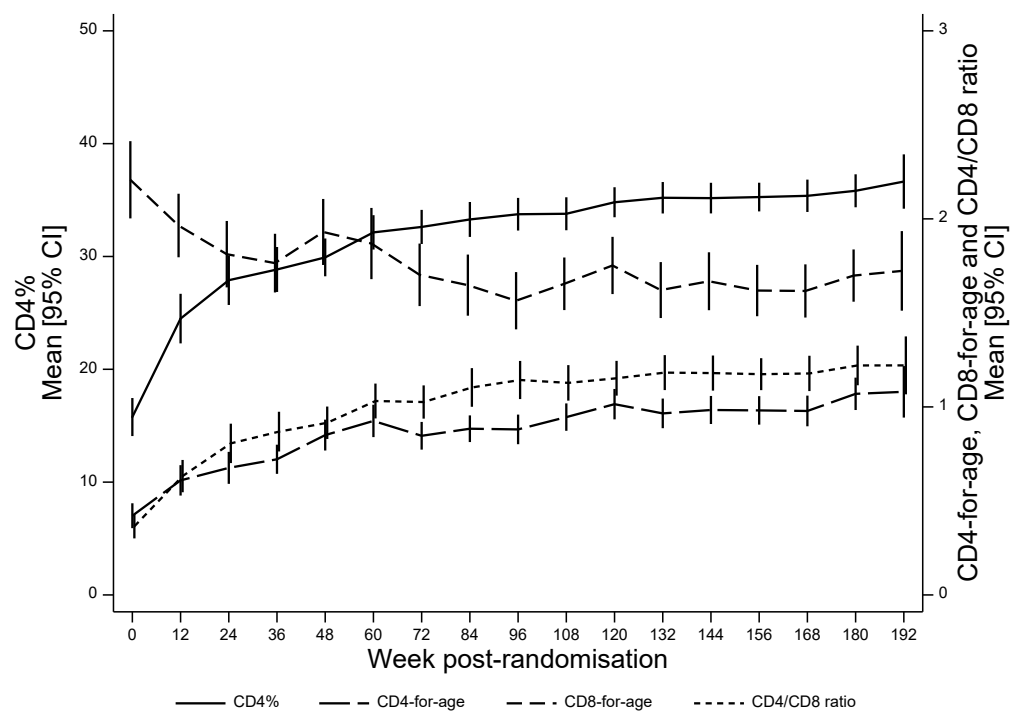
Post-week-48 transition rates (per 100 child-years at risk) between states*

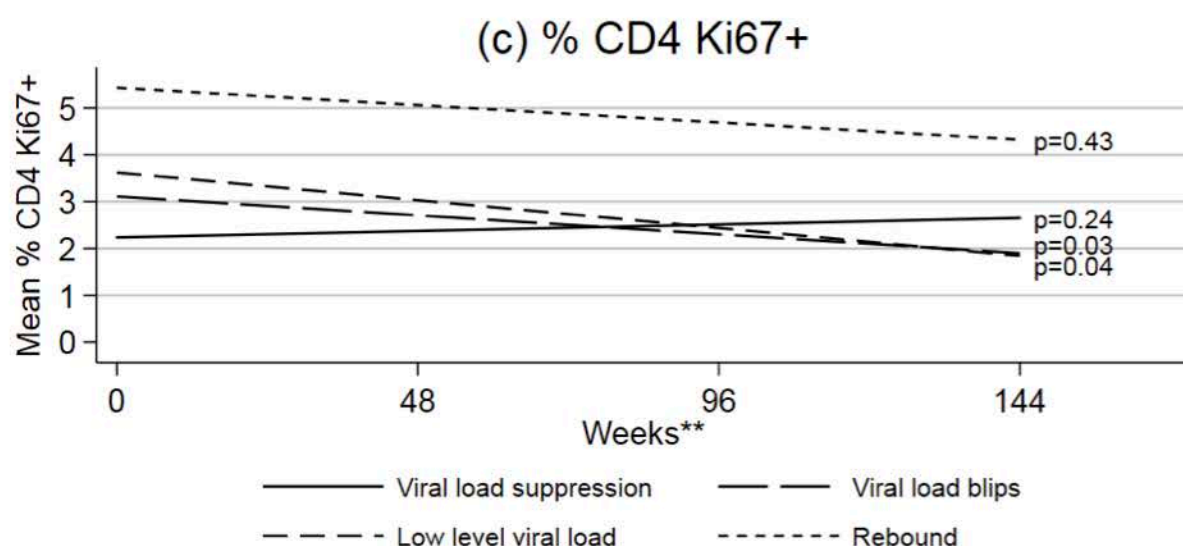
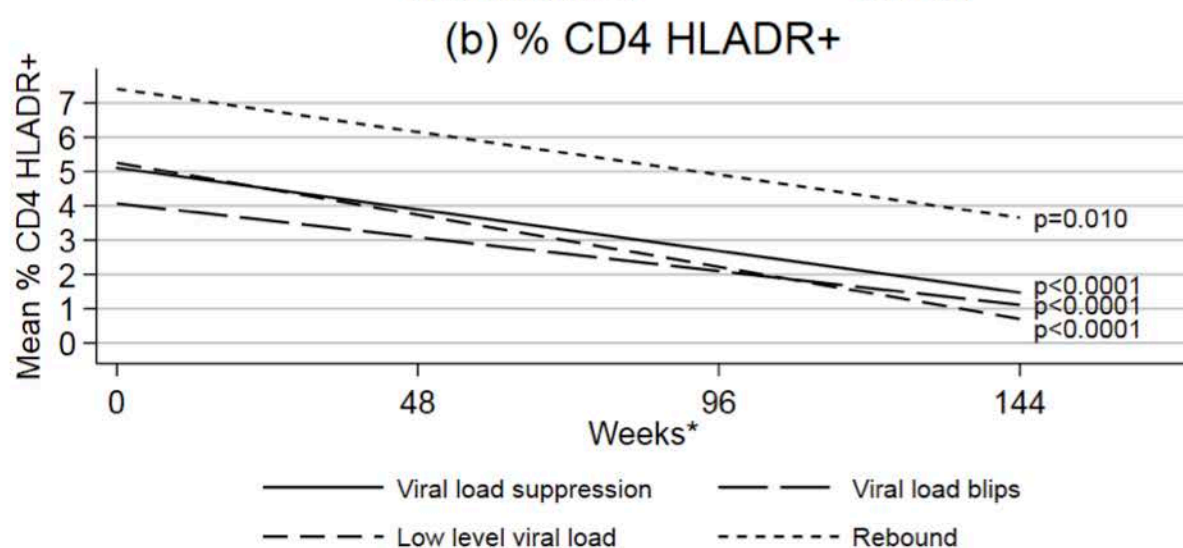
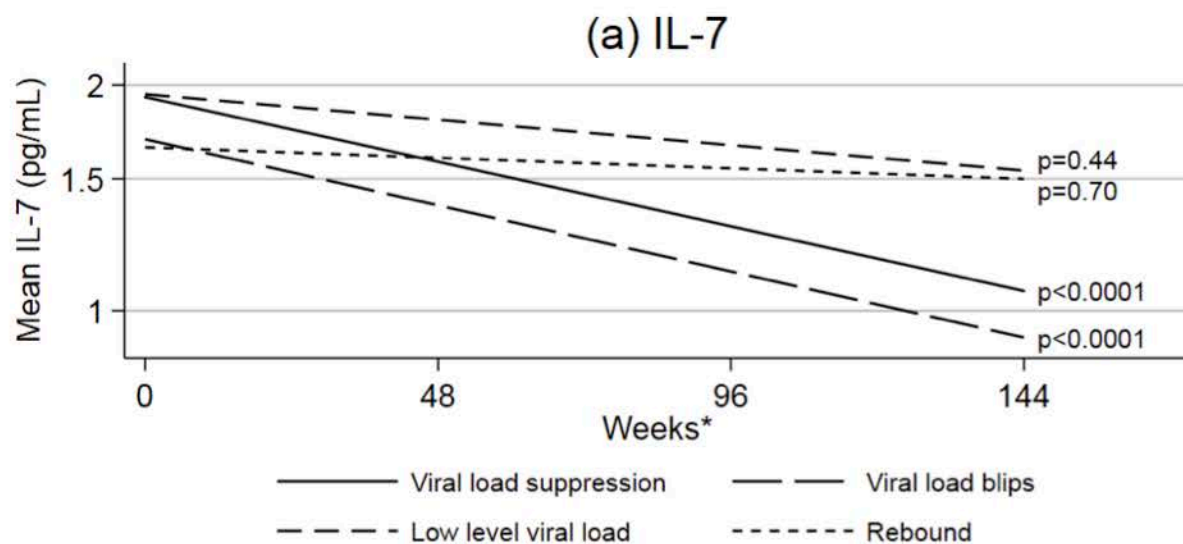


*308 children alive and in follow-up at 48 weeks

Note: for example, a rate of 27.5 per 100-child-years at risk means that for every 100 children spending a year in with consistent VL suppression (VL response), 27.5 would experience a blip.

Changes in CD4 and CD8 in children with consistent VL suppression





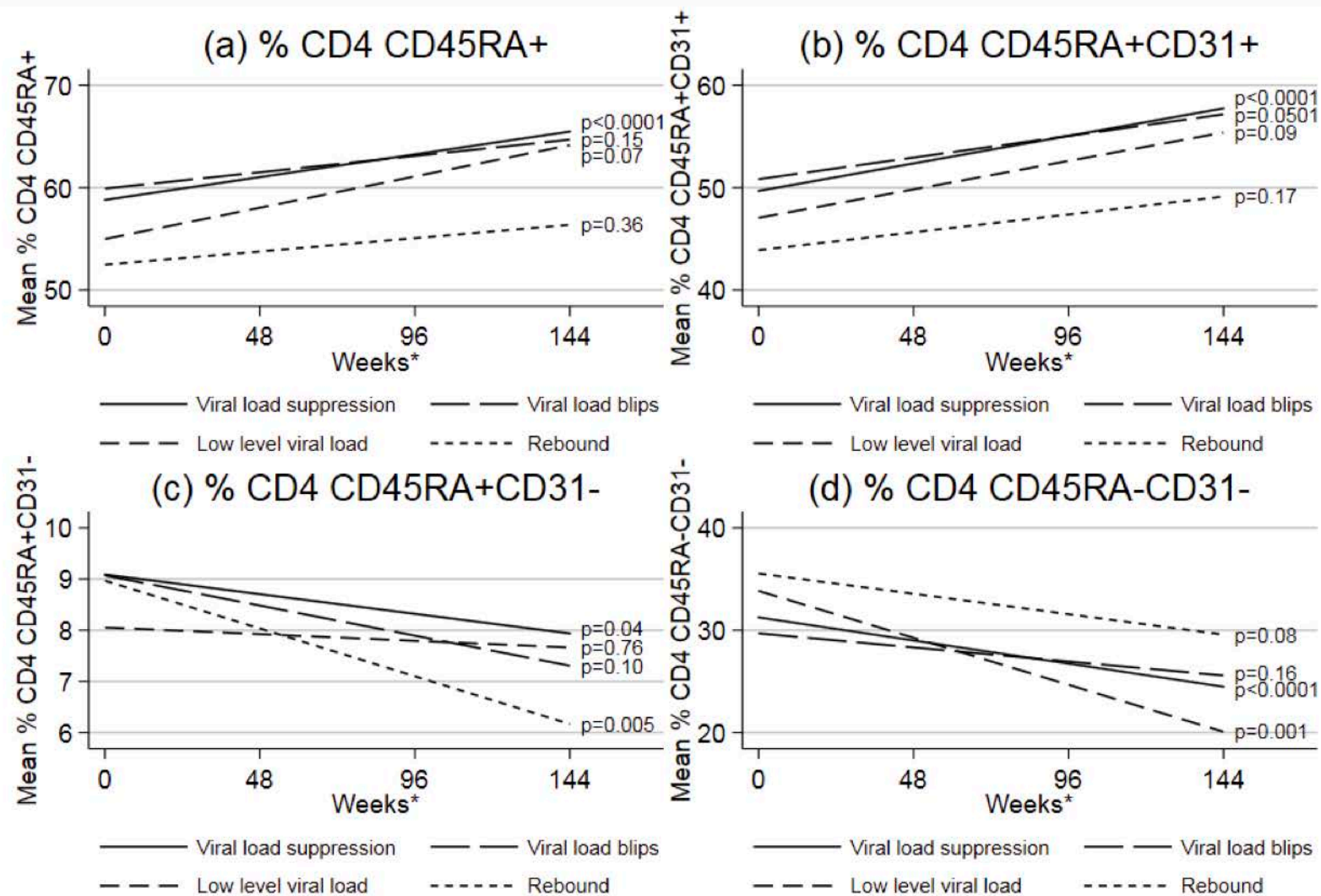
P-values test each slope against no change

See S1 Table for comparisons of suppression with blips, low level viral load and rebound; and low level viral load with rebound

*Viral load suppression: weeks post-week 48; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 48 (whichever latest)

**Viral load suppression: weeks post-week 72; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 72 (whichever latest)

1 **Figure 5 CD4 subpopulations over time with consistent VL suppression, previous VL blips, pLLVL and rebound**



P-values test each slope against no change
 See S1 Table for comparisons of suppression with blips, low level viral load and rebound; and low level viral load with rebound
 *Viral load suppression: weeks post-week 48; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 48 (whichever latest)

Comparisons of initial values of CD4 subpopulations, inflammatory biomarkers, weight-for-age and height-for-age at first observation in stable consistent VL suppression, blip, persistent low-level VL or rebound

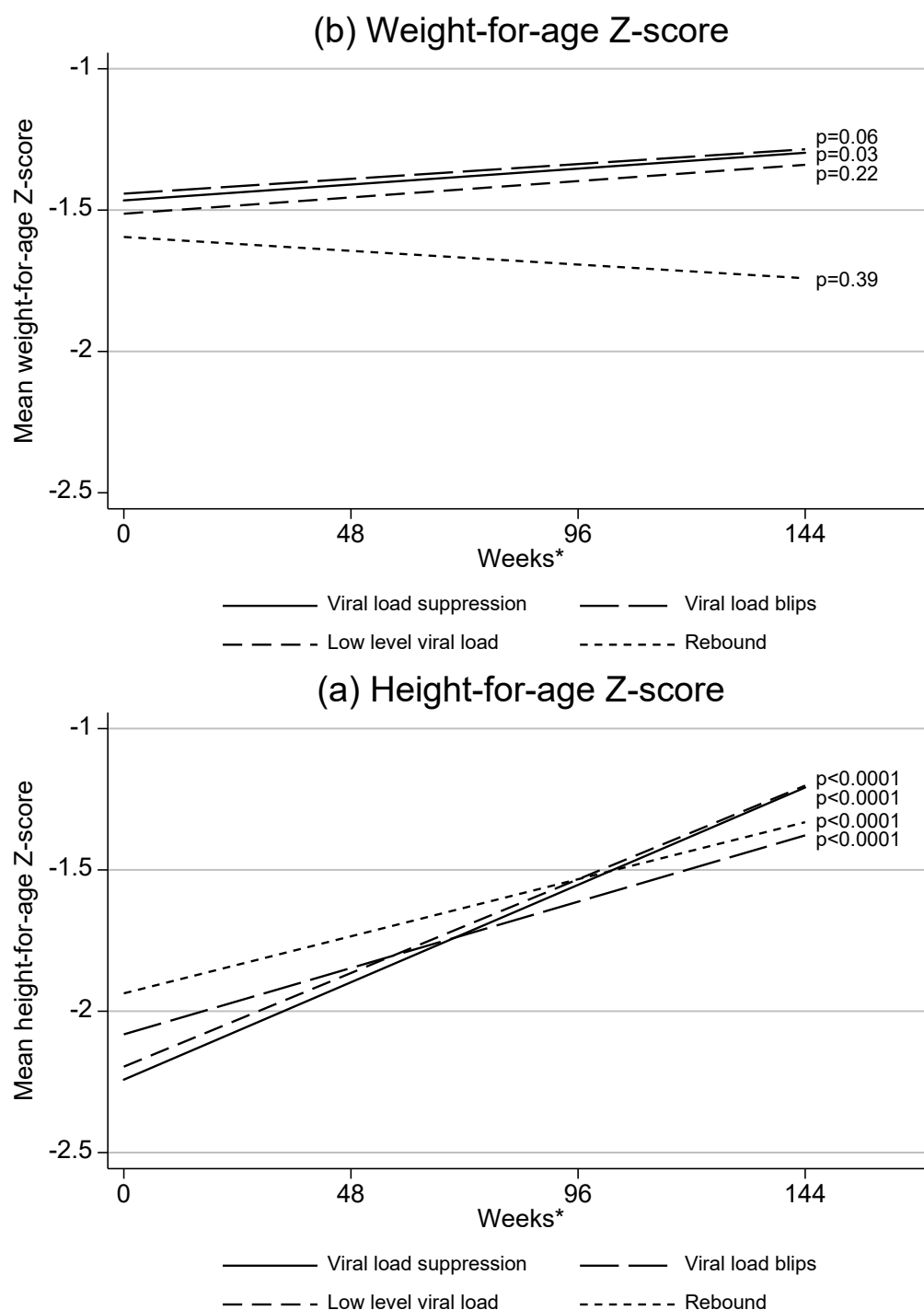
	Consistent suppression vs. blips vs. pLLVL vs. rebound	Consistent suppression vs. blips	Consistent suppression vs. pLLVL	Consistent suppression vs. rebound	Rebound vs. pLLVL	Consistent suppression+blips vs. pLLVL+rebound
CD4%	<0.0001	0.02	0.52	<0.0001	<0.0001	0.0001
CD4-for-age	<0.0001	0.39	0.27	<0.0001	0.0003	0.0002
CD8-for-age	0.0496	0.15	0.65	0.07	0.08	0.30
CD4/CD8 ratio	<0.0001	0.07	0.54	<0.0001	<0.0001	<0.0001
% CD4 CD45RA+	0.009	0.55	0.13	0.007	0.40	0.04
% CD4 CD45RA+CD31+	0.01	0.51	0.27	0.006	0.25	0.04
% CD4 CD45RA+CD31-	0.56	0.98	0.17	0.87	0.32	0.53
% CD4 CD45RA-CD31-	0.02	0.32	0.22	0.02	0.49	0.054
TNF- α **	0.002	0.30	0.11	0.0001	0.07	0.003
IL-6	0.046	0.23	0.01	0.097	0.48	0.07
CRP**	0.62	0.62	0.24	0.34	0.83	0.51
sCD14	0.54	0.93	0.18	0.78	0.37	0.30
IL-7	0.46	0.20	0.95	0.28	0.35	0.22
% CD4 Ki67+**	<0.0001	0.0009	0.0001	<0.0001	0.007	<0.0001
% CD4 HLADR+	<0.0001	0.005	0.78	0.002	0.008	0.11
Height-for-age Z-score	0.31	0.20	0.82	0.09	0.30	0.15
Weight-for-age Z-score	0.82	0.84	0.78	0.42	0.70	0.38

* Suppression: week 48 (children, "stable," on ART); blips/persistent low-level viral load/rebound: first measurement in state/week 48 (whichever latest; see Methods)

** Suppression: week 72; blips/persistent low-level viral load/rebound: first measurement in state/week 72 (whichever latest)

Note: for example e.g. CD4%: mean initial measurement in blips greater than in suppression (as per Figure 2A) (p=0.02), but subsequent rates of change similar during suppression and blips (p=0.94, Table 2). pLLVL-persistent low-level viral load

(a) Height- and (b) weight-for-age over time during viral load suppression, viral load blips, persistent low level viral load and rebound



P-values test each slope against no change

See Supplementary Table 1 for comparisons of suppression with blips, low level viral load and rebound; and low level viral load with rebound

*Viral load suppression: weeks post-week 48; viral load blips/low level viral load/rebound: weeks post-first measurement in state/week 48 (whichever latest)