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Estimating the public health impact of the effect of HSV suppressive therapy on HIV-1 plasma viral load

Short title: Impact of HSV therapy on HIV transmission

Rebecca F. Baggaley[1], Jamie T. Griffin[1], Ruth Chapman[2], T. Déirdre Hollingsworth[1], Nicolas Nagot[3], Sinead Delany[4], Philippe Mayaud[5], Frank de Wolf[6], Christophe Fraser[1], Azra C. Ghani[1], Helen A. Weiss[2].

Objective: Trials of herpes simplex virus (HSV) suppressive therapy among HSV-2/HIV-1-infected individuals have reported an impact on plasma HIV-1 viral loads (PVL). Our aim was to estimate the population-level impact of suppressive therapy on female-to-male HIV-1 sexual transmission.

Design and methods: By comparing pre- and post-randomisation individual-level PVL data from the first two HSV suppressive therapy randomised controlled trials in sub-Saharan Africa, we estimated the effect of treatment on duration of asymptomatic infection and number of HIV-1 transmission events for each trial.

Results: Assuming that a reduction in PVL is accompanied by an increased duration of HIV-1 asymptomatic infection, 4-6 years of HSV suppressive therapy produce a one year increase in the duration of this stage. To avert one HIV-1 transmission requires 8.8 (95%CI 5.9-14.9) and 11.4 (95%CI 7.8-27.5) women to be treated from half-way through their HIV-1 asymptomatic period, using results from Burkina Faso and South Africa trials respectively. Regardless of the timing of treatment initiation, 51.6 (95%CI 30.4-137.0) and 66.5 (36.7-222.6) treatment-years are required to avert one HIV-1 infection. Distributions of set-point PVL values from sub-Saharan African populations suggest that unintended adverse consequences of therapy at the population level (i.e. increased HIV-1 transmission due to increased duration of infection) are unlikely to occur in these settings.

Conclusions: HSV suppressive therapy may avert relatively few HIV-1 transmission events per person-year of treatment. Its use as a prevention intervention may be limited; however further research into its effect on rate of CD4 decline and the impact of higher dosing schedules is warranted.

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Abstract

Objective: Trials of herpes simplex virus (HSV) suppressive therapy among HSV-2/HIV-1-infected individuals have reported an impact on plasma HIV-1 viral loads (PVL). Our aim was to estimate the population-level impact of suppressive therapy on female-to-male HIV-1 sexual transmission.

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Conclusions: HSV suppressive therapy may avert relatively few HIV-1 transmission events per person-year of treatment. Its use as a prevention intervention may be limited; however further research into its effect on rate of CD4 decline and the impact of higher dosing schedules is warranted.

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Introduction

Epidemiological and biological evidence show that herpes simplex virus type 2 (HSV-2) infection enhances acquisition of HIV-1 and may also increase levels of genital and plasma HIV-1 RNA within co-infected individuals and hence increase transmission of HIV-1 in populations [1-6]. It is therefore reasonable to assume that HSV suppressive therapy may reduce transmission of HIV-1 in populations with high prevalence of both viruses [7].

Recent randomised placebo-controlled trials (RCTs) have quantified the effect of HSV suppressive therapy on the HIV-1 infectiousness of dually HSV-2/HIV-1 positive individuals [8-15]. The first two trials from sub-Saharan Africa reporting the effect of three-month HSV suppressive therapy on plasma HIV-1 viral loads (PVL) were parallel-arm RCTs among dually HIV-1/HSV-2 seropositive women ineligible for highly active antiretroviral therapy (HAART) in Burkina Faso (n=140 [9]) and South Africa (n=300 [8]). Both reported significant reductions in mean PVL (0.53 $\log_{10}$ copies/ml, 95%CI 0.35-0.72 using valacylovir 500mg b.i.d. [9] and 0.34 $\log_{10}$ copies/ml, 95%CI 0.15-0.54 using acyclovir 400mg b.i.d. [8]). These trials also reported significant reductions in genital HIV-1 and HSV-2 shedding frequency and viral loads [8, 9]. Three cross-over trials involving intense follow-up of male and female participants in Peru and Thailand and using valacyclovir or high dose acyclovir (800mg b.i.d.) also reported significant reductions in PVL (0.26 $\log_{10}$ copies/ml, 95%CI 0.19-0.33 [14]; 0.43 $\log_{10}$ copies/ml, 95%CI 0.29-0.56 [15]; and 0.33 $\log_{10}$ copies/ml, 95%CI 0.23-0.42 [13]) as well as significant reductions in rectal and cervicovaginal HIV-1 RNA concentrations [13-15].

By reducing both genital and plasma HIV-1 viral loads, HSV suppressive therapy is likely to reduce infectiousness of HIV-1, as PVL is highly correlated with risk of HIV-1 transmission [16, 17]. In addition, reduced PVL may increase life expectancy by decreasing the rate of CD4 decline during the asymptomatic period of HIV-1 infection [18, 19], thus delaying the point at which HAART should be initiated. However, this reduced infectiousness may be offset by an increased duration of infection, providing more opportunity for transmission, albeit at a lower rate. In this paper, we translate results of the first two African HSV therapy trials [8, 9] into number of potential HIV infections averted by HSV suppressive therapy.

Methods

We assessed the population-level impact of HSV suppressive therapy on HIV transmission by estimating the transmission potential of each HIV-1 infected individual as described by Fraser et al [20]. For the asymptomatic period of HIV-1 infection, the transmission potential is defined as the product of infectiousness and the duration of asymptomatic infection i.e. the mean number of persons that one index case can infect over their whole asymptomatic period, estimated as a function of set-point PVL (defined as the PVL steady state reached after the peak PVL in early infection and before progression to AIDS).

The concepts we explore are illustrated in Figure 1 which shows duration of asymptomatic HIV infection, HIV transmission rate and HIV transmission potential against set-point PVL (using functions reported in Fraser et al [20]) and changes in these properties for two illustrative HIV-infected individuals when they start HSV suppressive therapy. At some point during asymptomatic infection, therapy may be initiated and, we hypothesise, be accompanied by a drop in set-point PVL (Figure 1a). We assume that this drop increases each individual's projected duration of infection (Figure 1b), and decreases their infectiousness (Figure 1c). We further assume that HSV-2 infection always precedes HIV-1 infection, as a simplifying assumption and to estimate maximum impact of HSV suppressive therapy. Figure 1d illustrates that the combined effect on transmission potential (i.e. number of onward HIV transmission events over each individual's asymptomatic period) depends on their original set-point PVL. The impact on individuals with high set-point PVL is to increase transmission potential, while the opposite is true for those with lower set-point PVL. Therefore the public health impact of interventions reducing PVL by a moderate amount depends on the distribution of set-point PVL within the population.

Plasma HIV-1 viral load data

There are limited data linking viral load measures to HIV-1 infectiousness. While a focus of the two African trials was the impact of therapy on genital HIV-1 RNA, to our knowledge, there are no data that quantify the risk of ongoing HIV-1 transmission by frequency or quantity of

genital HIV-1 RNA for female-to-male HIV-1 transmission. We therefore use PVL data rather than genital viral load to estimate infectiousness.

We used data on PVL pre- and post-intervention from the Burkina Faso and South African trials [8, 9] to estimate the potential change in infectiousness and change in duration of asymptomatic infection that each woman may experience if started on indefinite HSV therapy. This analysis is restricted to participants with a measurement available from the end of the study period (12 weeks). Details of the laboratory methods used for quantifying PVL for each study are provided in Supplementary Information.

Quantifying reduction in PVL with HSV suppressive therapy

We examined individual-level data from the two trials to investigate whether the reduction in PVL varied with pre-randomisation (baseline) PVL. Two and three pre-randomisation PVL measurements were taken from study participants in South Africa and Burkina Faso respectively. For purposes of comparison between studies, we used the baseline measurement taken closest to the time of randomisation. Those with undetectable PVL were assigned a value of half the detection threshold of the assay (detection thresholds of 300 and 50 HIV-1 RNA copies/ml for Burkina Faso and South Africa, respectively). Analysing changes in PVL in the two trials suggested a reduction in PVL among treated participants for all levels of baseline PVL recorded (except very low levels, <3.0 $\log_{10}$ copies/ml, where any further reduction in PVL is unlikely to be detected) but no such decrease among controls (see Supplementary Information). Therefore we estimate the effect of suppressive therapy on HIV-1 transmission using data from the treatment arms only.

Translating PVL into estimates of infectiousness and duration of asymptomatic infection

We followed the approach taken by Fraser et al [20]. Briefly, the risk of HIV-1 transmission per year, stratified by PVL of the index case, is quantified using data from a Zambian cohort [16]. To translate PVL data of each RCT participant, V, into an estimate of their infectiousness, defined as the transmission rate (number of HIV-1 transmission events per HIV-1 infected individual per year) βV, the following logistic function is used:

$$\beta\left(V\right) = \beta_{\max} V^{\beta_k} \Big/ \left[V^{\beta_k} + \beta_{50}{}^{\beta_k} \right] \tag{1}$$

where $\beta_{\max}$, the maximum infection rate per annum, is 0.317 per year; β_{50}, the PVL at which infectiousness is half its maximum, is 13,938 copies/mL and β_k, the steepness of the increase in infectiousness as a function of PVL is 1.02.

A logistic function is also used to estimate the mean duration of the asymptomatic period of HIV-1 infection as a function of PVL using data from the Amsterdam Seroconverters Cohort, and allows for variability in the duration, given set-point PVL [20]. (The Amsterdam Seroconverters Cohort prospectively recruited homosexual men from 1982 onwards; the cohort has been described elsewhere [19].) The PVL of each RCT participant, V, is translated into an estimate of their duration of asymptomatic infection, $D\left(V\right)$, using:

$$D\left(V\right) = D_{\max} \, D_{50}{}^{D_k} \Big/ \left[V^{D_k} + D_{50}{}^{D_k} \right] \tag{2}$$

where $D_{\max}$, the maximum duration of asymptomatic infection, is 25.4 years; D_{50}, the PVL at which the duration is half its maximum, is 3,058 copies/mL and D_k, the steepness of the decrease in duration as a function of PVL, is 0.41.

Calculating HIV-1 infections averted

Each intervention arm participant's transmission potential (number of transmission events during their entire duration of asymptomatic infection) was calculated as the product of their transmission rate and duration (from equations (1) and (2)) for their baseline PVL and similarly for their PVL at the end of the trial. The number of infections averted is the difference between these. This assumes that suppressive therapy starts at the beginning of asymptomatic infection; therefore alternative scenarios where treatment starts at different points during the incubation period were also explored. A bootstrap sampling method was used to derive 95% confidence bounds for each outcome [21].

Results

The number of study participants randomised to the intervention and control arms was 68 and 68 for Burkina Faso and 152 and 148 for South Africa, respectively. For the Burkina Faso (South Africa) trial, 62 (132) from the treatment and 63 (135) from the control arm had PVL measurements recorded at the end of the three-months follow-up and were included in our analysis. For Burkina Faso, 61 intervention and 60 control arm participants had a baseline PVL measurement at the time point closest to randomisation. The remaining four participants in the trial (one in treatment, three in control arms) had measurements recorded at three weeks pre-randomisation. For South Africa, one control arm participant with a PVL measurement at study end had no measurement at the time point closest to randomisation; her measurement recorded at her previous baseline visit (one week before) was used.

Figure 2 shows distribution of PVL among females in the Zambian study which links PVL to infectiousness (Figure 2a, [16]), transmission potential by set-point PVL (Figure 2b) and the distribution of PVL for study participants in the two African trials (Figures 2c-2f, [8, 9]). The Zambian data shows females only to make it comparable with the trials because there may be differences in PVL distributions between women and men [22, 23]. This distribution is skewed because of the selection of non-transmitting partners for this discordant couple study. Burkina Faso study participants at baseline had a similar PVL distribution to those from Zambia (mean PVL 4.4 and 4.5 $\log_{10}$ copies/ml respectively), while those from South Africa were slightly lower (mean 3.9 $\log_{10}$ copies/ml). However, both trials recorded more frequent undetectable PVL measurements than the Zambian study (Figures 2a,c,e). This may partly be due to greater immunosuppression among the Zambian cohort (>80% of participants had CD4 <400 cells/mm^3 compared to a median 446 (range 334-628) cells/mm^3 for Burkina Faso and 475 (251-1382) cells/mm^3 for South Africa). Figures 2d and 2f illustrate the shift in distribution of PVL for the treatment arms after three months, with no such shift apparent for the control arms (Figures 2c and 2e). The highest frequency PVL group for the controls before and at study end is close to the level at which transmission potential is predicted to peak.

Impact of treatment on infection duration and transmission – results for study participants

Table 1 shows estimated benefits of HSV suppressive therapy based on the duration of asymptomatic infection in the two trial populations. Greater benefits are produced the earlier in HIV-1 infection that therapy is initiated. A maximum increase in duration of asymptomatic infection of 2.8 years using Burkina Faso data and 1.9 years using South Africa data was estimated if therapy was initiated at the beginning of asymptomatic infection. The duration of therapy required to gain one HAART-free year extension to the asymptomatic period was independent of time at which therapy was initiated and was estimated as 4.2 years (95%CI 2.5-8.6) using Burkina Faso data and 6.2 years (95%CI 3.4-14.6) using South Africa data.

Table 1 also shows the impact of HSV suppressive therapy on number of infections averted. Unless therapy was initiated very early in infection and continued throughout the asymptomatic period, i.e. for more than a decade, relatively few HIV-1 infections could be averted per woman treated. To avert one HIV-1 transmission to a sexual partner of a HSV-treated woman requires 8.8 (95%CI 5.9-14.9) and 11.4 (95%CI 7.8-27.5) women to be treated from half-way through their HIV-1 asymptomatic period, predicted using Burkina Faso and South Africa data, respectively. If therapy can be initiated at the beginning of asymptomatic infection, these figures reduce to 4.4 (95%CI 3.0-7.5) and 5.7 (95%CI 3.9-13.8) respectively. There was a linear relationship between time on treatment and benefits achieved; therefore regardless of the timing of treatment initiation, 51.6 (95%CI 30.4-137.0) and 66.5 (36.7-222.6) treatment-years are required to avert one HIV-1 infection using Burkina Faso and South Africa data, respectively.

Impact of HSV suppressive therapy on HIV-1 transmission – generalised results
Figure 3 illustrates the effect on number of onward HIV-1 transmission events if therapy was administered to a hypothetical population of 1000 HSV-2/HIV-1 dually seropositive individuals, by mean set-point PVL (assumed normal distribution, standard deviation=1) if the reduction in PVL through suppressive therapy was as predicted for Burkina Faso, or South Africa, trial data respectively. All populations with a mean set-point PVL less than about 4.75 $\log_{10}$ copies/ml are likely to experience a net beneficial effect of an intervention such as this, which produces a modest reduction in PVL. Mean PVL for the trials' data and Zambian

dataset, as well as data from Uganda for HSV-2 seropositive individuals [24], illustrate that HSV-2 infected populations in Africa are likely to have distributions in PVL which would result in a positive impact of HSV suppressive therapy. The largest relative benefit of HSV suppression would occur among populations with modestly low (around median 3.5 $\log_{10}$ copies/ml, Figure 3) mean PVLs because it is at this point that changes in PVL have the greatest impact on transmission potential (shown by the steepest gradient of the curve in Figure 2b).

Discussion

Our analysis suggests that if HSV suppressive therapy reduces PVL of HIV-1/HSV-2 positive individuals as demonstrated by the first two African trials, it would reduce HIV-1 transmissions and increase the duration of asymptomatic HIV-1 infection, albeit by modest amounts. We estimate that treating between 9 and 11 HSV-2/HIV-1-infected individuals, starting therapy half-way through asymptomatic HIV-1 infection, would avert one HIV-1 infection. This compares favourably with the impact of male circumcision, where an estimated 5-15 surgeries would avert one HIV-1 infection over 10 years (Hankins et al, submitted). This is partly due to the use of suppressive therapy to prevent transmission rather than acquisition: there is no "wastage" of the intervention on those who will never be exposed to HIV-1 (the effect of HSV suppressive therapy on reducing acquisition of HIV-1 among HSV-2-positive/HIV-1-negative women has recently been investigated by two RCTs but no effect was observed [25, 26]). Results of the Partners in Prevention (PiP) study, following over 3400 HIV-1-discordant couples, will provide the first empirical data examining whether HSV suppressive therapy reduces HIV-1 transmission (www.clinicaltrials.gov *NCT00194519*).

While time from HIV-1 acquisition to symptomatic infection is longer for individuals with lower PVL [27], an intervention decreasing PVL in order to increase the duration of asymptomatic infection has not been previously assessed. Historical studies using high-dose acyclovir suggested moderate improvements in survival, although these typically treated patients with late stage rather than asymptomatic infection [28]. The potential of HSV therapy to reduce the rate of CD4 decline and thus increase the time before starting HAART has important

implications for HIV-1 patient management. PiP may have sufficient power to detect such an effect, which is also currently being investigated in Rakai, Uganda (www.clinicaltrials.gov *NCT00405821*), although many study participants started therapy while already at relatively late stage infection. To investigate impact for early HIV-1 infections, an ancillary study to the recent HPTN039 HSV suppressive therapy HIV-1 acquisition RCT [25] is maintaining HIV-1-seroconverters on their trial regimens (acyclovir or placebo) to investigate whether treatment alters HIV-1 set-point up to 6 months.

In contrast to the positive results from the two trials used in this analysis, a subsequent RCT among women in Tanzania reported no effect of acyclovir 400mg b.i.d. on genital HIV-1 RNA and HSV-2 DNA [10] at 6 or 12 months, while an RCT in Zimbabwe found no effect on genital HIV-1 RNA but an impact on genital HSV-2 DNA (OR 0.24, 95%CI 0.12-0.48) at 3 months [11]. Possible explanations for the varied findings include different durations of follow-up, drug regimens and levels of adherence achieved within these high-risk study populations, with lower adherence reported in the Tanzanian and Zimbabwean trials. Our estimate of averting one infection by treating 9 to 11 individuals, starting therapy half-way through asymptomatic infection, requires individuals to be treated for approximately six years, with adherence continuing at the high levels maintained in the trials, which could be challenging. However, suppressive therapy offers the additional benefits of reducing HSV-2 symptoms and would keep HIV-1-infected individuals within the healthcare system, allowing appropriate timing of HAART initiation, while loss of contact and delayed start of HAART would result in poorer prognoses.

Our analysis has a number of limitations. We assume that all partners of the participants are HIV uninfected, so this will overestimate the impact of therapy. Transmission potential was derived from data from discordant couples, which may underestimate infectiousness relating to more casual partnerships. Transmission potential is estimated for a scenario assuming random mixing between partners, which corresponds to a very high partner change rate. Mixing is seldom random, yet random mixing may be more appropriate for estimating transmission potential for these high risk women than the other extreme of serial

monogamous partnerships. A sensitivity analysis exploring the impact of these assumptions shows that modelling serially monogamous relationships would avert fewer HIV-1 infections with suppressive therapy because the transmission potential of each woman is reduced (see Supplementary Information).

We have made the simplifying assumption that by the end of each three-month trial the maximum impact of suppressive therapy has been reached, but there was a slight incremental benefit over time in Burkina Faso, which would increase the number of infections averted. However, such an effect may be mitigated if accompanied by waning adherence or if the effectiveness of HSV therapy actually decreases over time (there has been concern over the selection of resistant HIV mutants under the selective pressure of acyclovir in vitro [29]).

A recent review of serodiscordant couple studies estimated that the probability of transmitting HIV would increase by 20% and 40% for 0.3 and 0.5 $\log_{10}$ copies/ml increases in PVL respectively, with a 25% and 44% increased risk of progression to AIDS or death [30]. We believe the estimated changes in infectiousness and duration of asymptomatic infection used in our analysis are more reliable because x units change in either of these parameters does not change linearly with y units of $\log_{10}$ PVL, as assumed in the review. Our estimates depend not only on the change in PVL but also the original set-point because a small change in PVL when at high or low levels has less impact than at intermediate PVL (Figure 1). However, for comparison, using a "typical" viral set-point value of 4.5 $\log_{10}$ copies/ml (as used by others [31]) and formulas (1) and (2), infectiousness would increase by 18% and 26% and duration of infection before AIDS would decrease by 24% and 44% for 0.3 and 0.5 $\log_{10}$ copies/ml increases in PVL respectively using our methods, yet these values change substantially for different baseline viral set-points.

Our analysis suggests that while in theory, interventions to reduce PVL could risk the unintended consequence of increasing rather than decreasing overall HIV-1 transmission, this is unlikely to occur among sub-Saharan Africa target populations. This is because the distribution of set-point PVL is sufficiently low that any reduction in PVL likely moves the

population to a lower mean transmission potential. While the benefits of HSV suppressive therapy as a public health measure are predicted as modest using standard regimens, investigating the effect of higher dosing schedules may be important. Delaying the need to initiate HAART would be of particular value in resource-limited settings, where demand for HAART will likely continue to outstrip supply.

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Author contributions

R.F.B. conceived and co-wrote the article with substantial inputs from T.D.H., R.C., C.F., A.C.G. and H.A.W.. R.F.B. and J.T.G. conducted the statistical analysis. J.T.G., A.C.G., T.D.H., C.F. and H.A.W. advised on statistical analyses. A.C.G., T.D.H., R.F.B. and R.C. conceptualised and designed the schematic figures. All authors provided and contributed towards the analysis and interpretation of the data and to the development and critical revision of the manuscript, including Principle Investigators for each of the trial datasets used (N.N., S.D., P.M., F.d.W.) providing input based on their expertise and field experience. N.N. and P.M. were principal investigators of the ANRS1285 study in Burkina Faso, and H.A.W.

was the study senior statistician; S.D. and P.M. were principal investigators of the Wellcome Trust trial in South Africa. All authors have seen and approved the submitted version of the manuscript.

Conflicts of interest

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Appendix

Investigators in the ANRS 1285 study group (Burkina Faso trial) were as follows: *Centre Muraz, Bobo-Dioulasso, Burkina Faso:* E. Bahembera, A. Berthé, M. Coulibaly, M.-C. Defer, R. Diallo, D. Djagbaré, I. Konaté, F. Ky-Dama, G.T. M'Boutiki, N. Méda, I. Millogo, N. Nagot, A. Ouédraogo, D. Ouedraogo, F. Rouet, A. Sanon, H. Sawadogo, R. Vallo, L. Vergne [deceased January 2007]; *London School of Hygiene and Tropical Medicine, London:* P. Mayaud, N. Nagot, H.A. Weiss; *Montpellier University Hospital and Research Unit 145, Institute for Research and Development and University of Montpellier 1, Montpellier, France:* P. Becquart, V. Foulongne, M. Segondy, P. Van de Perre; *University Hospital of Bobo-Dioulasso, Burkina Faso:* J.-B. Andonaba, A. Sawadogo. Investigators for the South Africa trial were as follows: *University of Witwatersrand, Johannesburg, Republic of South Africa:* S. Delany, N. Mlaba, G. Akpomiemie, J. Dhookie, H. Rees, A. Capovilla, W. Stevens; *London School of Hygiene and Tropical Medicine, London:* T. Clayton, P. Mayaud; *Laboratoire de Microbiologie, Hôpital Saint-Louis, Paris, France:* J. Legoff; *Université Paris V, Equipe « Immunité et Biothérapie Muqueuse », Unité INSERM Internationale U743 (« Immunologie Humaine »), Centres de Recherches Biomédicales des Cordeliers, & Laboratoire de Virologie, Hôpital Européen Georges Pompidou, Paris, France:* L. Belec.

Table and Figure Legends

Table 1 Impact of HSV suppressive therapy based on results of the two treatment trials in terms of extending the duration of asymptomatic infection and number of HIV-1 infections averted.

Point of asymptomatic period at which treatment initiated	Mean duration on HSV therapy, years		Mean increase in duration of asymptomatic infection, years		Infections averted[1]		Number to treat to avert one HIV-1 transmission event	
Burkina Faso Trial (95%CI)								
Start	11.7	(7.9-23.4)	2.8	(1.0-9.5)	14.0	(8.3-20.9)	4.4	(3.0-7.5)
¼ way	8.8	(6.0-17.5)	2.1	(0.7-7.2)	10.5	(6.2-15.7)	5.9	(4.0-10.0)
1/3 way	7.8	(5.3-15.6)	1.9	(0.6-6.4)	9.3	(5.5-13.9)	6.6	(4.5-11.2)
½ way	5.8	(4.0-11.7)	1.4	(0.5-4.8)	7.0	(4.2-10.4)	8.8	(5.9-14.9)
2/3 way	3.9	(2.6-7.8)	0.9	(0.3-3.2)	4.7	(2.8-7.0)	13.3	(8.9-22.4)
¾ way	2.9	(2.0-5.8)	0.7	(0.2-2.4)	3.5	(2.1-5.2)	17.7	(11.9-29.9)
South Africa Trial (95%CI)								
Start	11.7	(7.9-25.9)	1.9	(0.6-7.9)	23.2	(9.6-34.0)	5.7	(3.9-13.8)
¼ way	8.8	(5.9-19.4)	1.4	(0.4-5.9)	17.4	(7.2-25.5)	7.6	(5.2-18.3)
1/3 way	7.8	(5.3-17.3)	1.3	(0.4-5.3)	15.5	(6.4-22.7)	8.5	(5.8-20.6)
½ way	5.9	(4.0-13.0)	0.9	(0.3-4.0)	11.6	(4.8-17.0)	11.4	(7.7-27.5)
2/3 way	3.9	(2.6-8.6)	0.6	(0.2-2.6)	7.7	(3.2-11.3)	17.0	(11.6-41.3)
¾ way	2.9	(2.0-6.5)	0.5	(0.1-2.0)	5.8	(2.4-8.5)	22.7	(15.5-55.0)

[1] Predicted number of infections averted by treating all 62 (132) women in the intervention arm for the Burkina Faso (South Africa) study.

Figure 1 Schematic illustrating the predicted impact of HSV suppressive therapy on duration of asymptomatic HIV-1 infection, HIV-1 transmission rate (hazard of transmission: probability of HIV-1 transmission per year) and HIV-1 transmission potential (product of hazard of transmission and duration of asymptomatic infection) for two hypothetical patients (red and blue). Time starts at the beginning of each individual's asymptomatic period (i.e. after high PVL accompanying primary infection). The figure shows that all patients experiencing a drop in PVL with therapy would experience increases in duration of asymptomatic infection and infectiousness, but direction of change to transmission potential depends on baseline set-point PVL and reduction in PVL with therapy.

Figure 2 Comparison of a) distribution of HIV-1 PVL for female study participants from Zambia participating in an HIV serodiscordant couple study from which transmission rate estimates were calculated (n=134) [16]; and b) transmission potential (onward HIV transmission events during asymptomatic infection) by PVL estimated by Fraser et al [20]; with distributions of PVL for the HSV suppressive therapy trials: c) Burkina Faso control arm (n=63); d) Burkina Faso treatment arm (n=62) [9]; e) South Africa control arm (n=135); and South Africa treatment arm (n=132) [8]. Mean PVL for females from the Zambian study was 4.51 $\log_{10}$ copies/ml [16]. Mean PVL was 4.22 and 4.61 $\log_{10}$ copies/ml at baseline and 3.70 and 4.65 $\log_{10}$ copies/ml at study end for the Burkina Faso treatment and control arms, respectively. For the South Africa study, these values were 4.01 and 3.87 $\log_{10}$ copies/ml at baseline and 3.66 and 3.92 $\log_{10}$ copies/ml at study end, respectively.

Figure 3 Change in total number of onward HIV-1 transmission events if HSV suppressive therapy were administered to hypothetical populations of 1000 HSV-2/HIV-1 dually seropositive individuals, by mean set-point PVL of the population (assumed normal distribution, standard deviation (SD) 1). Estimates for the impact if the reduction in PVL through suppressive therapy was as predicted for Burkina Faso (-0.53 $\log_{10}$ copies/ml) and South Africa (-0.34 $\log_{10}$ copies/ml) are shown, drawing from a normal distribution with the mean reduction in PVL and standard deviation calculated using reported 95%CIs. Plotted are mean HIV-1 PVL for the study participants of the Burkina Faso and South Africa trials (both

arms, all HSV-2 positive: mean 4.4 $\log_{10}$ copies/ml, SD 0.97 and mean 3.9 $\log_{10}$ copies/ml, SD 1.10, respectively); for women from the Zambia transmission study (HSV-2 prevalence not stated: mean 4.5 $\log_{10}$ copies/ml, SD 0.82); and for a study from Uganda comparing PVL for HSV-2 infected and uninfected individuals (HSV-2 positives only: mean 4.6 $\log_{10}$ copies/ml, SD 0.94) [24].
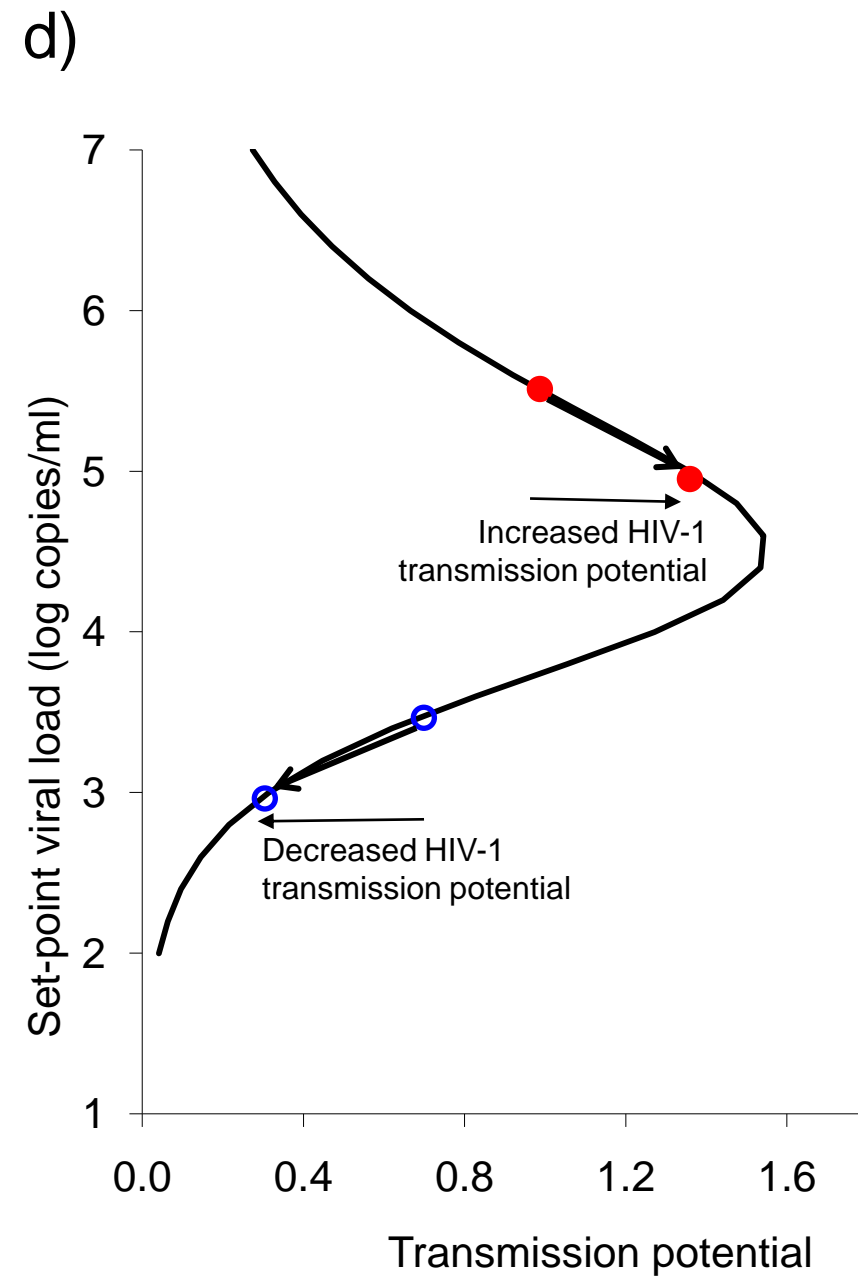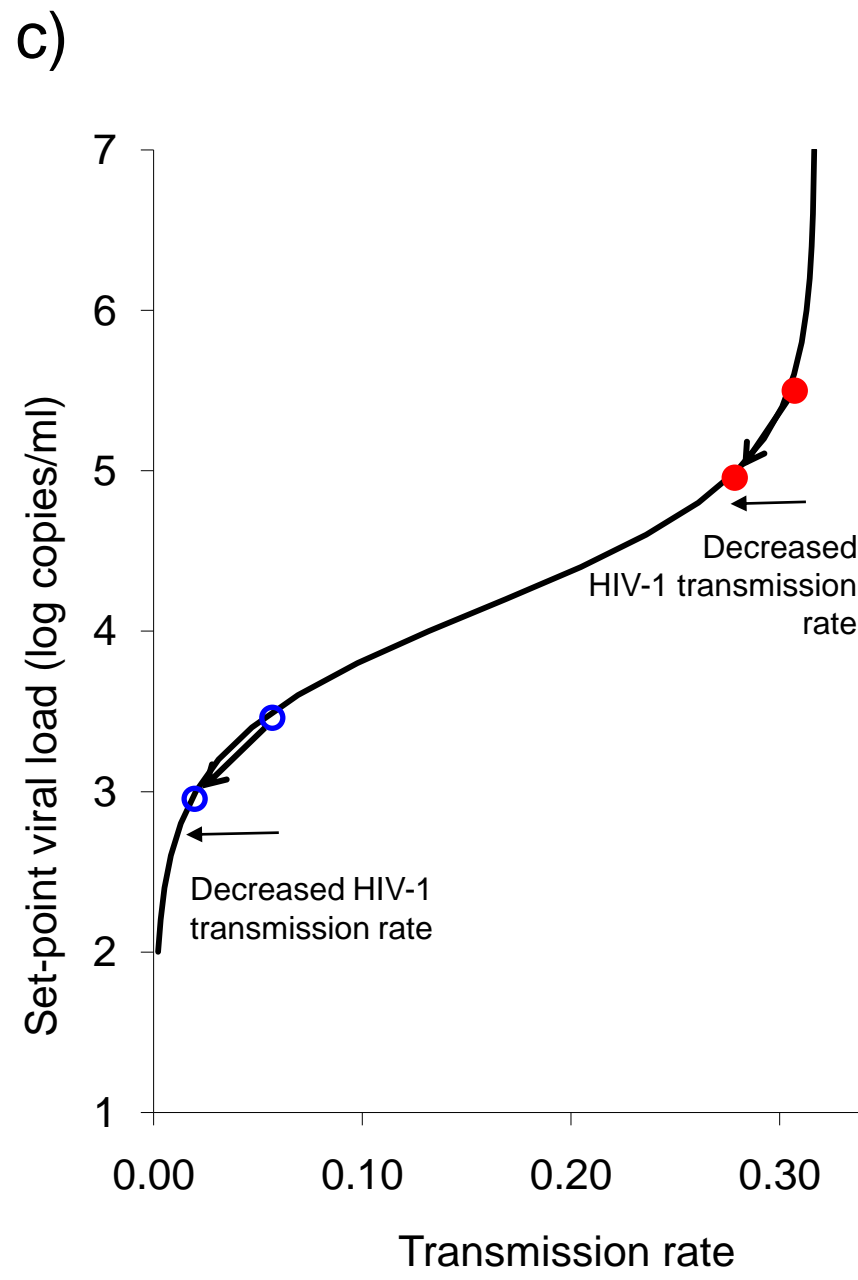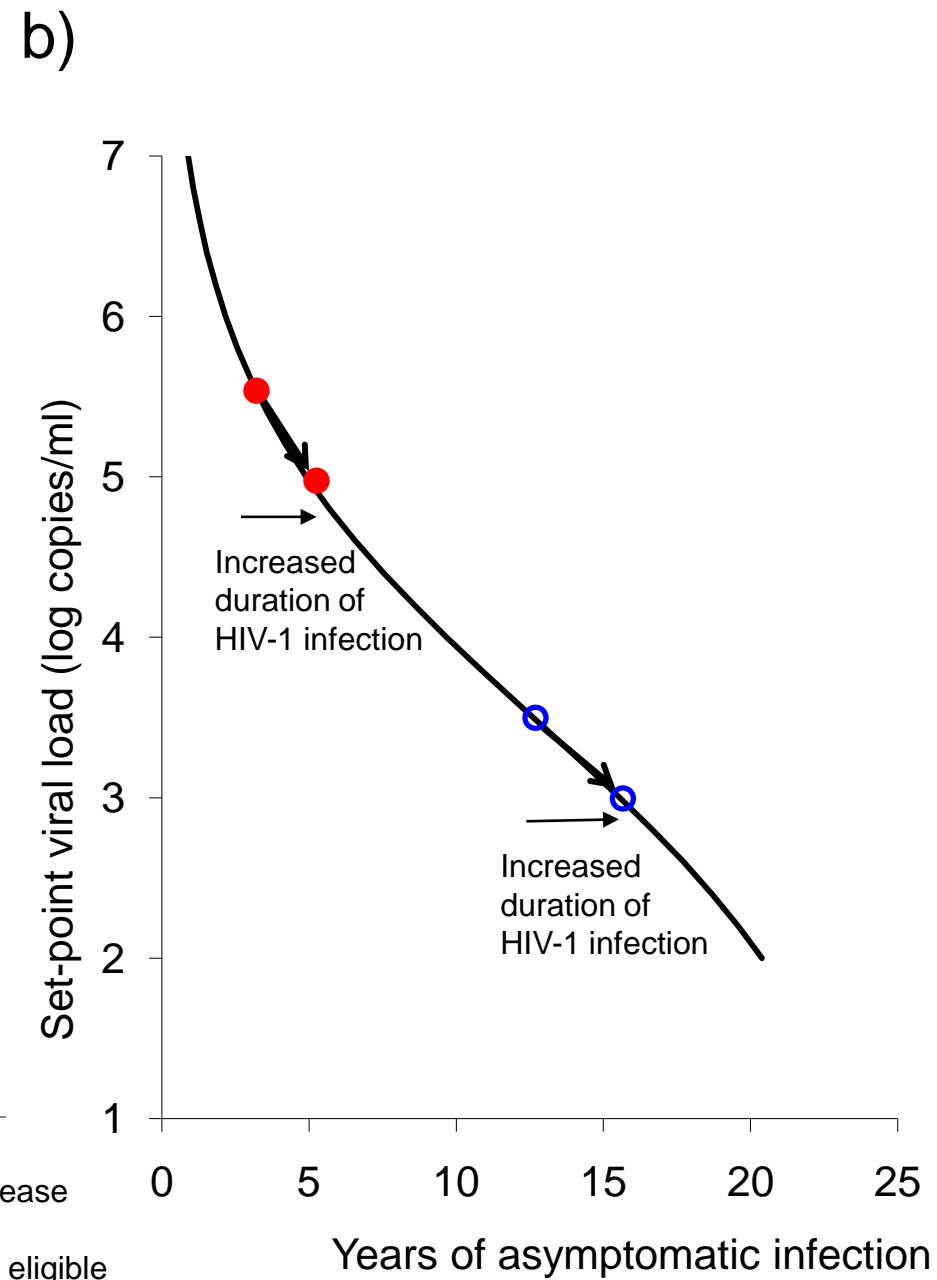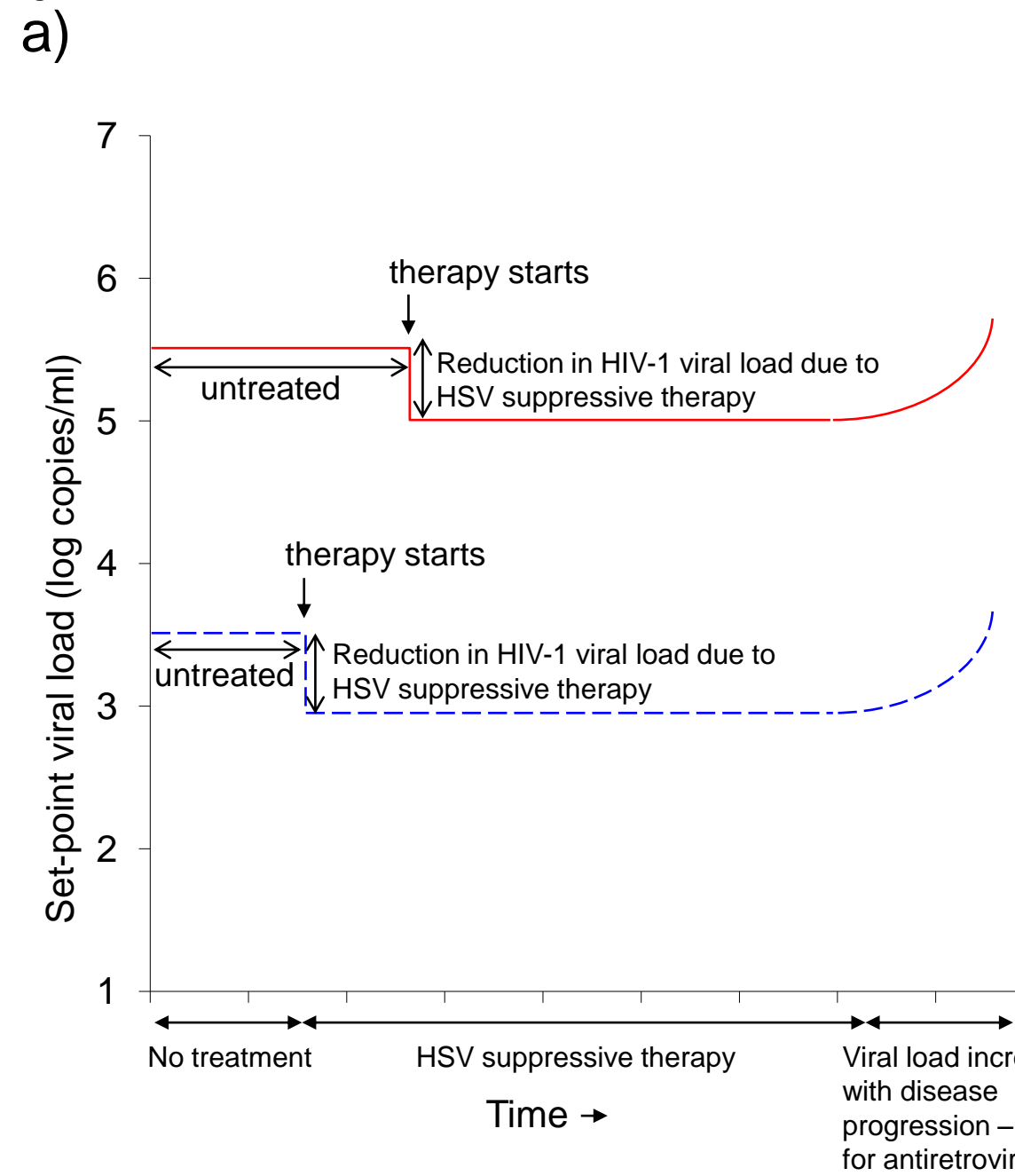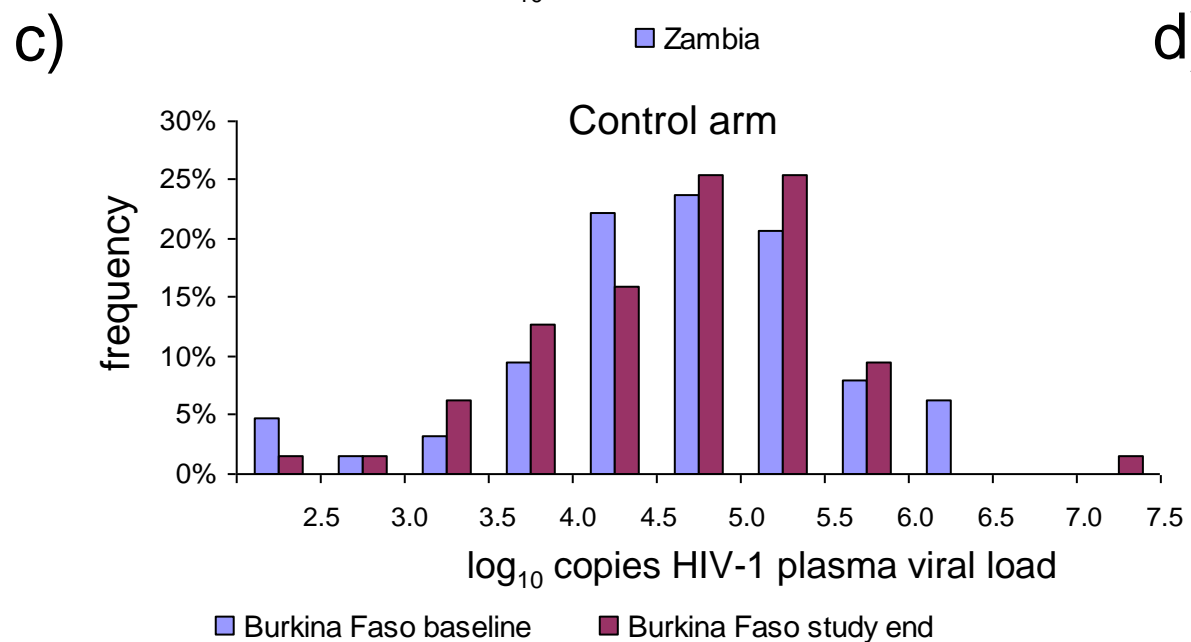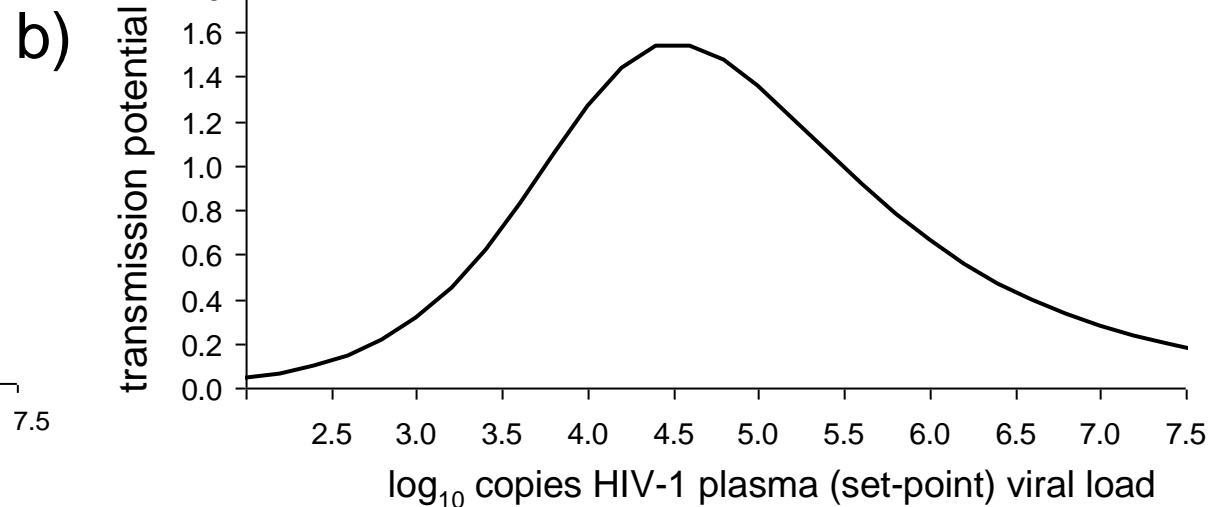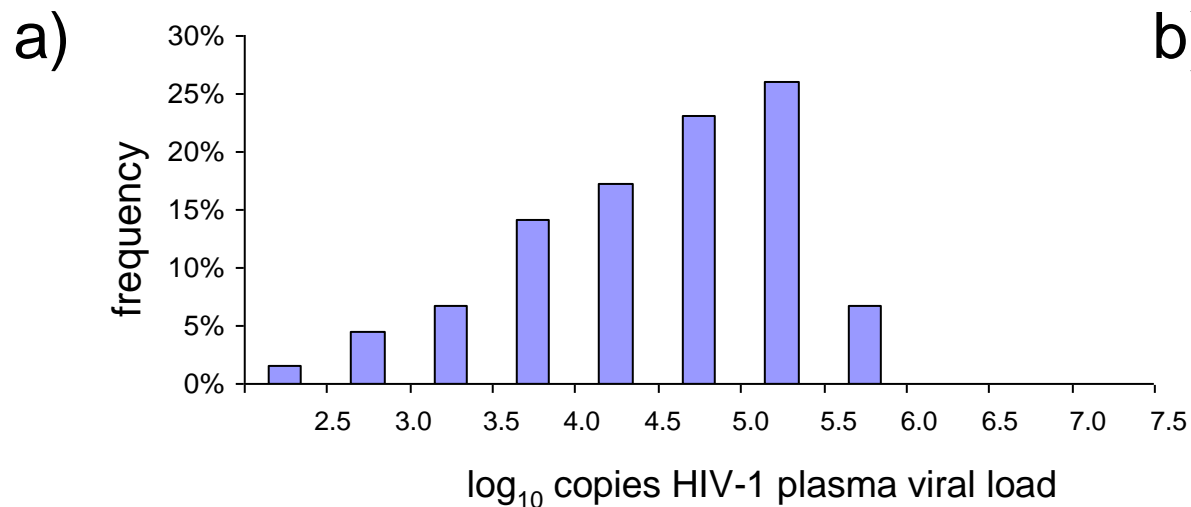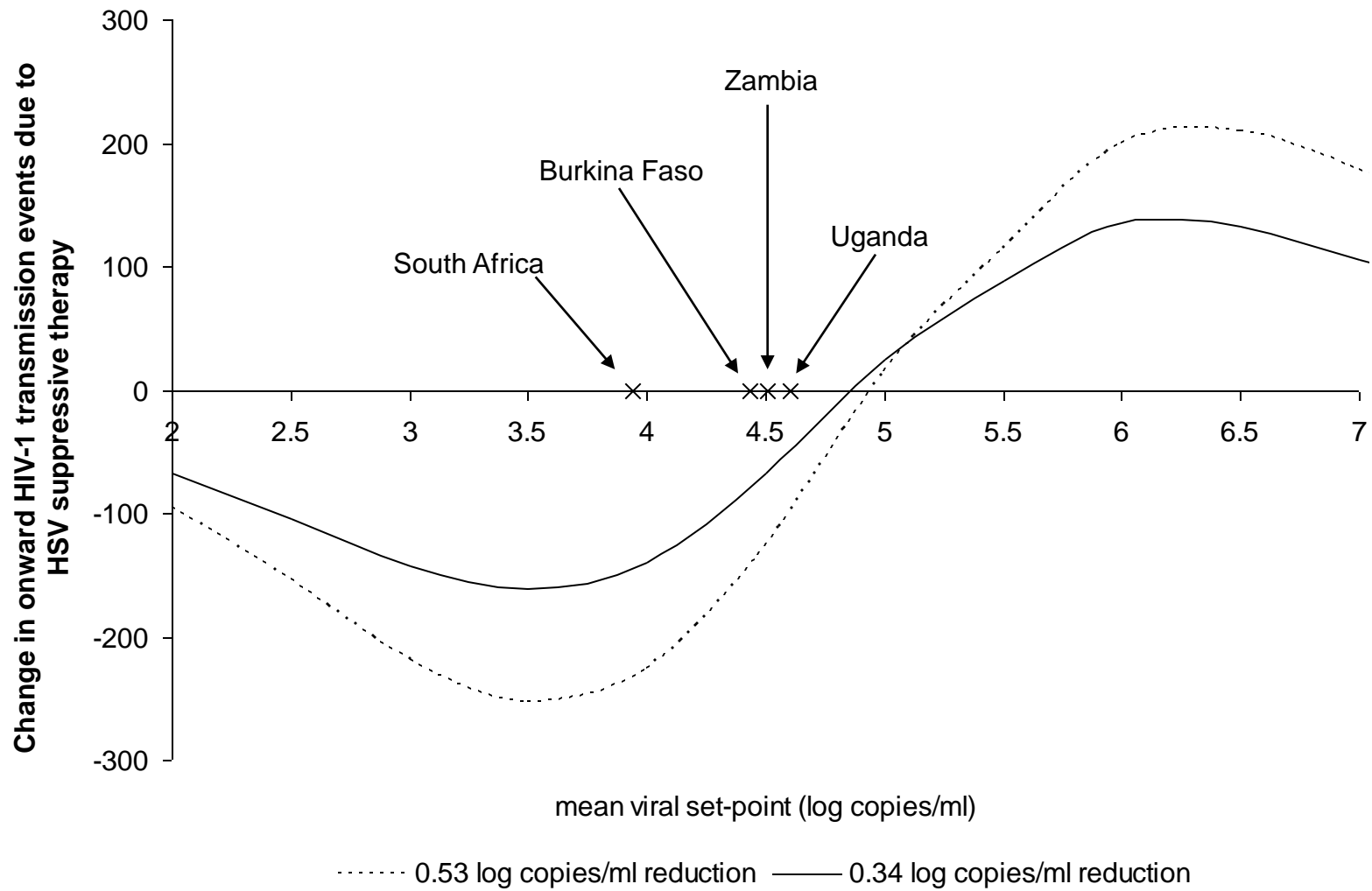
Figure 1

Figure 2
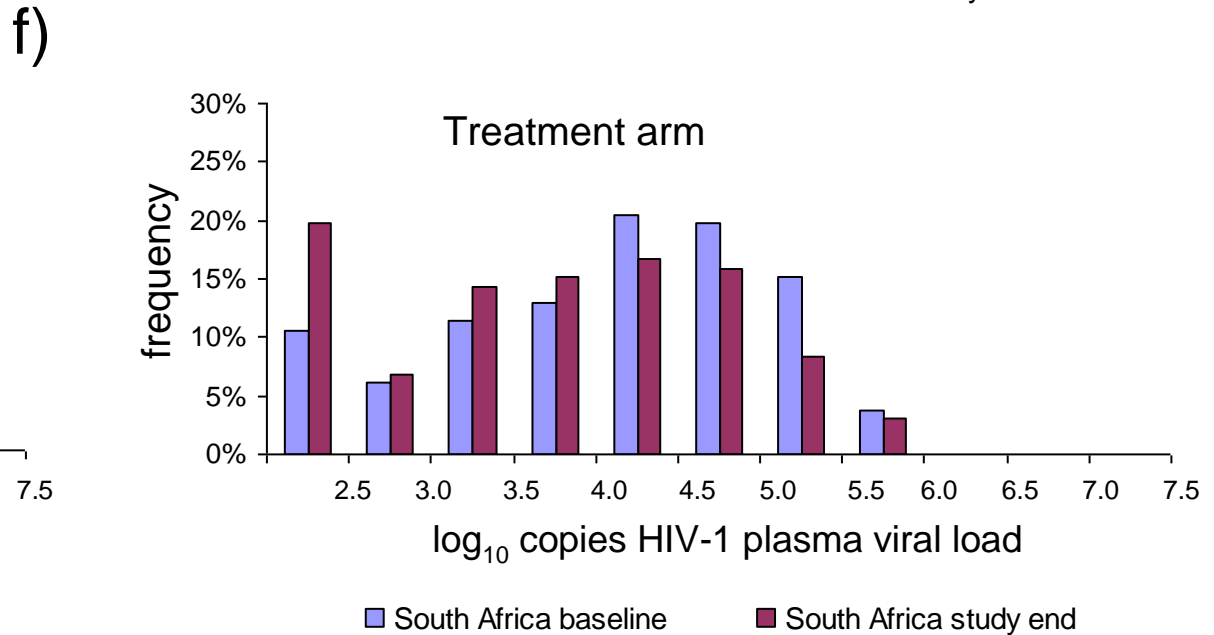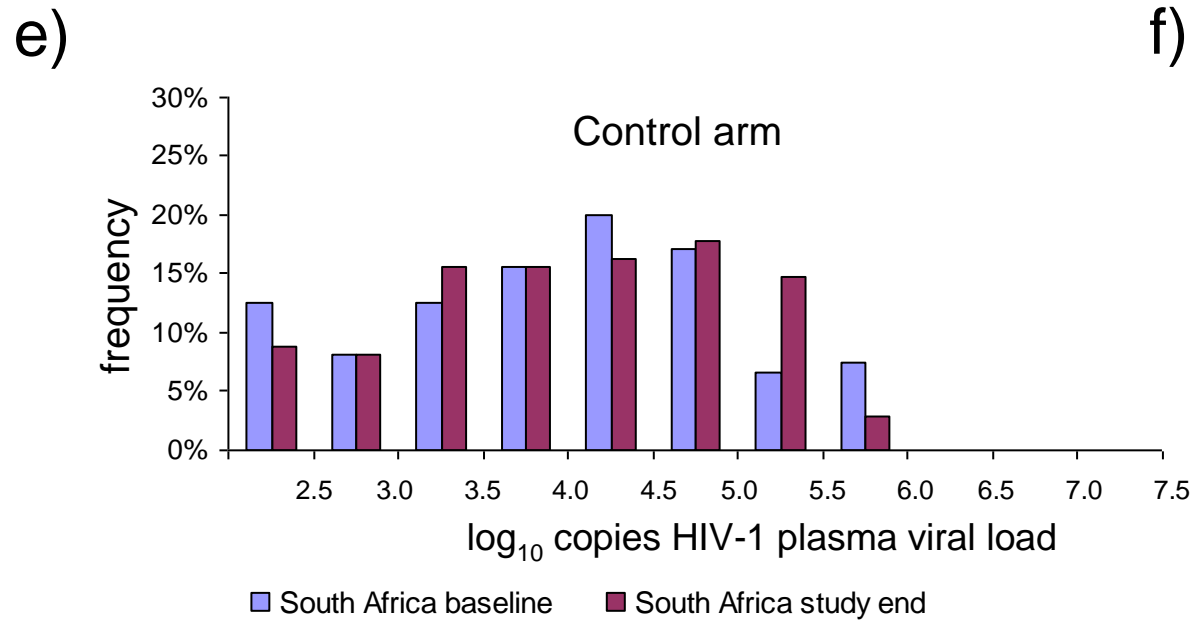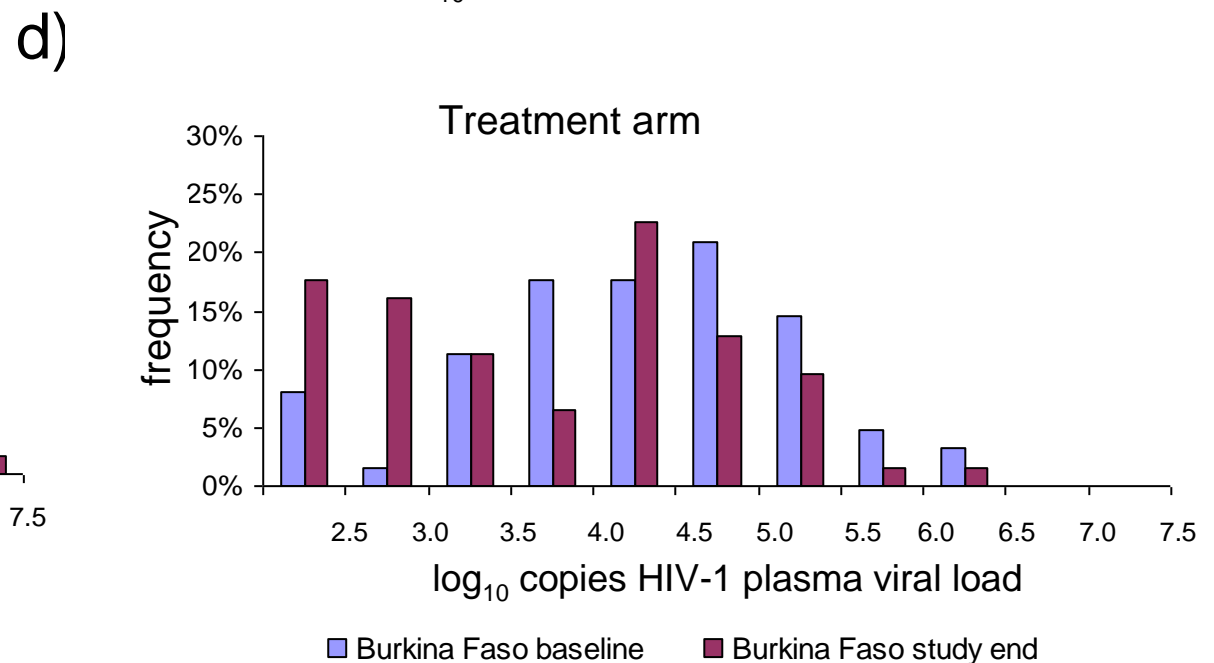
Figure 3

Supporting information for "Estimating the public health impact of the effect of HSV suppressive therapy on HIV-1 plasma viral load"

Baggaley et al.

1 Supplementary Methods

Quantification of plasma viral load (PVL) in each study: Nagot et al (Burkina Faso) quantified PVL using real-time polymerase-chain reaction (PCR) using the ABI 7000 system and manual nucleic acid extraction (Qiagen RNA kit) as previously described [1]. Delany et al (South Africa) used the ultrasensitive Roche Amplicor Monitor version 1.5 assay (Roche Molecular Systems, Branchburg, NJ, USA). Fideli et al (Zambian infectiousness data used to translate PVL measures into infectiousness) used the Roche Amplicor version 1.0 assay (threshold 400 $\log_{10}$ copies/ml). Several different assays were used for the Amsterdam Seroconverters Cohort (used to translate PVL into estimates of duration of asymptomatic HIV-1 infection), including reverse-transcriptase-PCR assays of several types and sensitivities and the Quantiplex branched DNA amplifier assay [2].

2 Change in PVL over duration of study by baseline PVL

Figure S1 shows the changes in PVL in the two trials stratified by intervention arm. In the treatment arms the reduction in PVL was similar regardless of baseline PVL. However, among controls, there was a decreasing trend in the difference in PVL with baseline PVL. This reflects fluctuations in PVL during asymptomatic infection: those with low PVL recorded at one time point were likely to record a higher PVL when next measured, and vice versa (i.e. regression to the mean). Figure S1 suggests a reduction in PVL by study end observed among treated participants for all levels of baseline PVL recorded (except very low levels, <3.0 $\log_{10}$ copies/ml, where any further reduction in PVL is unlikely to be detected) but no such decrease among controls. Therefore it appears reasonable to estimate effect of suppressive therapy on HIV-1 transmission using data from the treatment arms only.

3 A Model with Partner Change

Our analysis has focused on the transmission potential as the potential number of people one woman could infect in a situation of random mixing between partners. This will clearly never hold, and different situations, partner networks and partner change rates will lead to the transmission potential being realised to different extents. We have conducted a similar sensitivity analysis to those conducted for previous publications [3, 4] in order to investigate the sensitivity of our conclusions to the random mixing assumption.

One possible extension to our model is to consider a situation of serial monogamous partnerships, formed and reformed as a random (Poisson) process with constant rate c. For an infection with sequential Markov disease stages of duration D_i and infectiousness β_i, (where i denotes the stage), the probability of transmission is approximately given by $\beta_i / \left(\beta_i + c + 1/D_i \right)$. We hypothesise that this formula remains approximately valid in our case where we use a realistic survival distribution for the asymptomatic stage of infection (i.e. non-Markov stages), and furthermore we continue to use a mass-action model, but with effective infection rate given by

$$\widetilde{\beta_i} = \beta_i c \big/ \left(\beta_i + c + 1/D_i \right).$$

If $\beta(V)$ is the transmission hazard within a partnership, then the overall transmission hazard will be well approximated by $\widetilde{\beta}(V) = \beta(V) c \big/ \left(\beta(V) + c + 1/D(V) \right)$. The effect of this on the re-scaled transmission potential denoted $\widetilde{TP}(V)$ and the estimated effectiveness of the intervention in terms of total HIV infections averted and infections averted per woman treated with HSV suppressive therapy is illustrated for a range of values of the mean partnership duration ($s = 1/c$) in the attached Figure S2.

Figure legends

Figure S1 Change in HIV-1 PVL over duration of study for control and treatment arm study participants, stratified by baseline PVL for Burkina Faso (a and b) and South Africa (c and d). Middle line of each box represents the median PVL; upper and lower ends of the box represent the 75th and 25th percentiles, respectively. Bars denote upper and lower adjacent values and dots denote outliers.

Figure S2: Effect of varying the partner change rate on a) transmission potential by viral set-point; total onward HIV infections averted by treating all intervention arm participants for b) the Burkina Faso and c) the South Africa trial, by point during asymptomatic infection that HSV suppressive therapy is initiated; and HIV infections averted per woman treated for d) the Burkina Faso and e) the South Africa trial. The predictions in the main manuscript are made for a model with random mixing i.e. corresponding to a very high partner change rate. The effect of varying the partner change rate in a simple model of serial-monogamous partnerships is illustrated against the random mixing model. The mean duration of partnerships is defined as s and is varied from 0.25 to 2 years. The transmission potential is reduced and shifts slightly to the left as partnerships are formed and reformed less frequently. The longer the partnership duration and
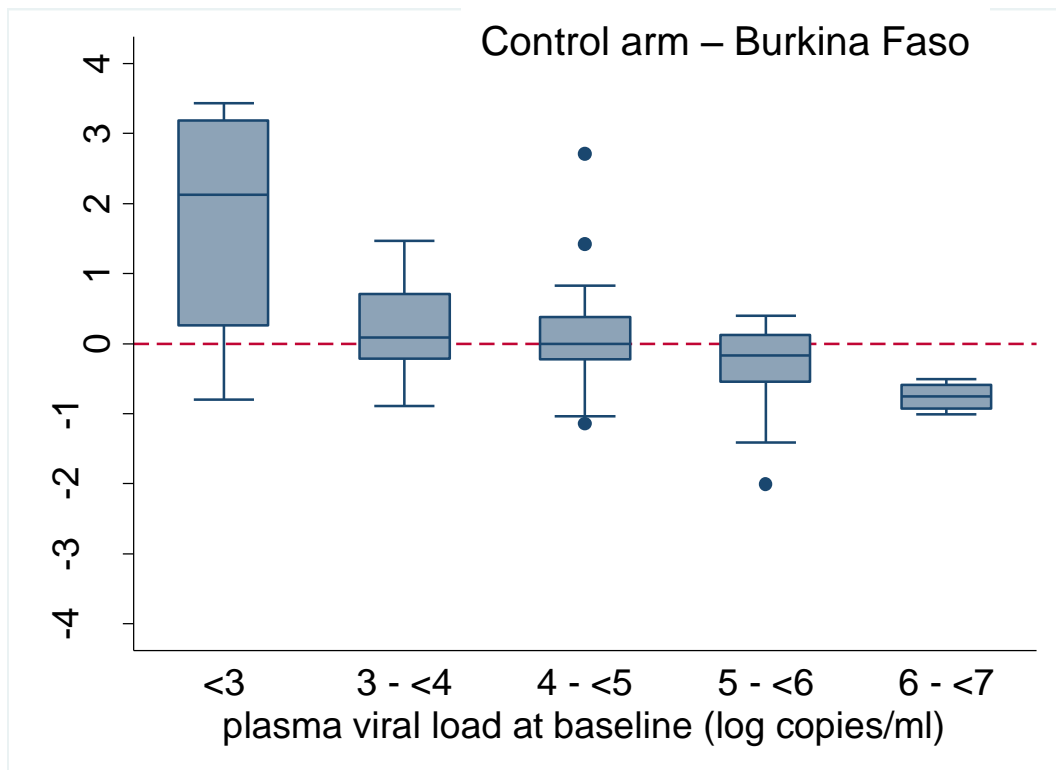
smaller the partner change rate, the lower the transmission potential and thus the fewer HIV infections averted by HSV suppressive therapy.
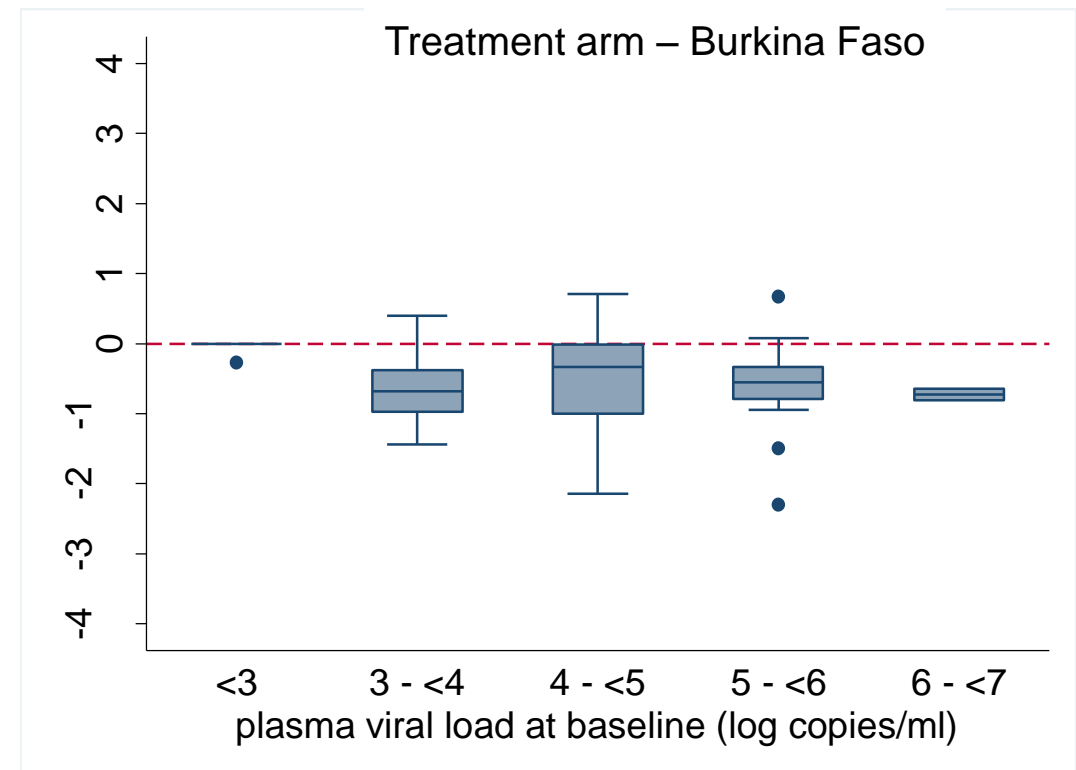

References

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2.   Donnelly CA, Bartley LM, Ghani AC, Le Fevre AM, Kwong GP, Cowling BJ*, et al.* **Gender difference in HIV-1 RNA viral loads**. *HIV Med* 2005,6:170-178.
3.   Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP. **Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis**. *Proc Natl Acad Sci U S A* 2007,104:17441-17446.
4.   Hollingsworth TD, Anderson RM, Fraser C. **HIV-1 transmission, by stage of infection**. *J Infect Dis* 2008,198:687-693.

a)

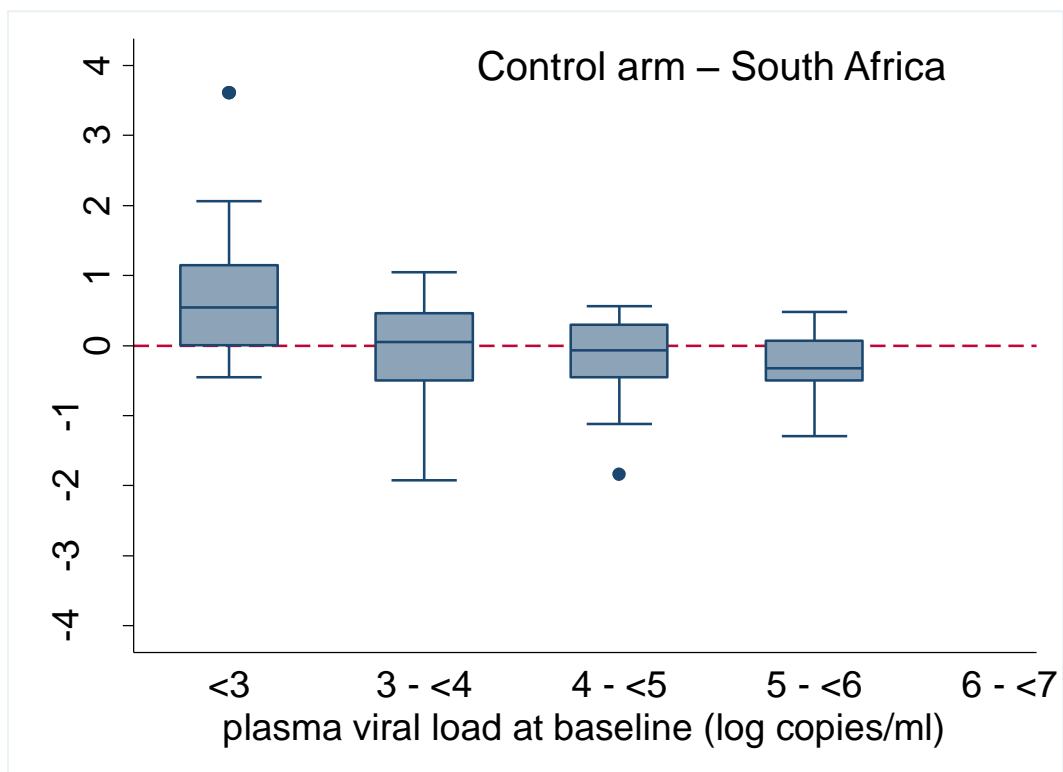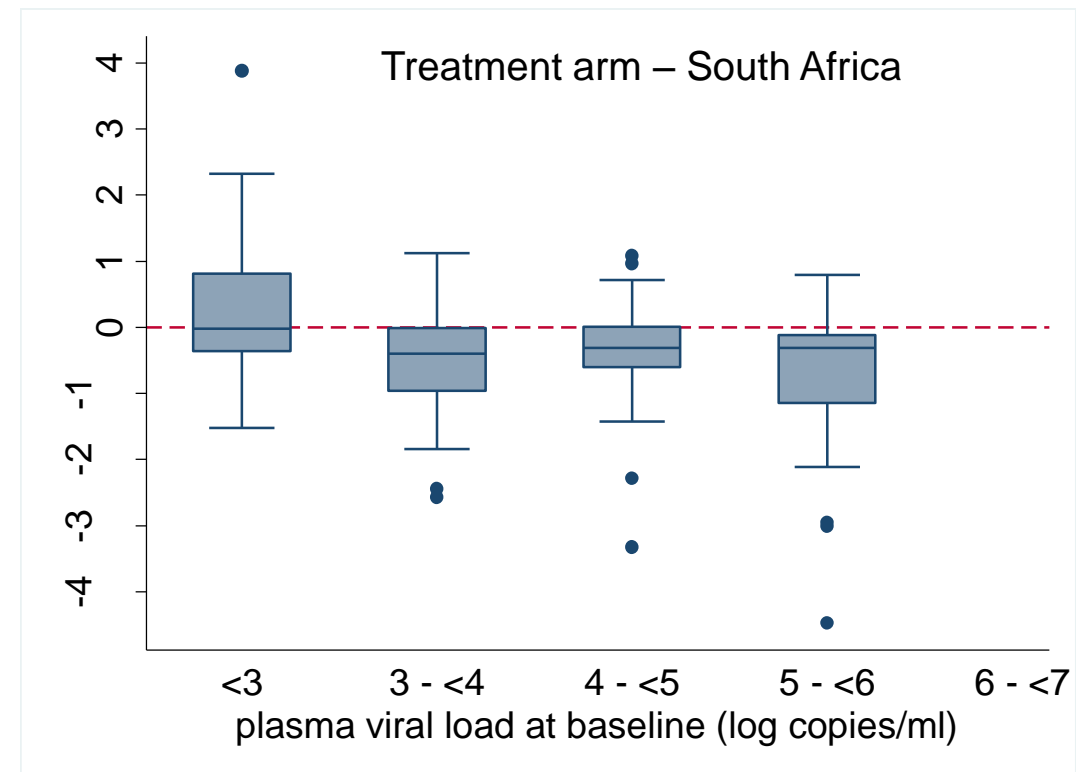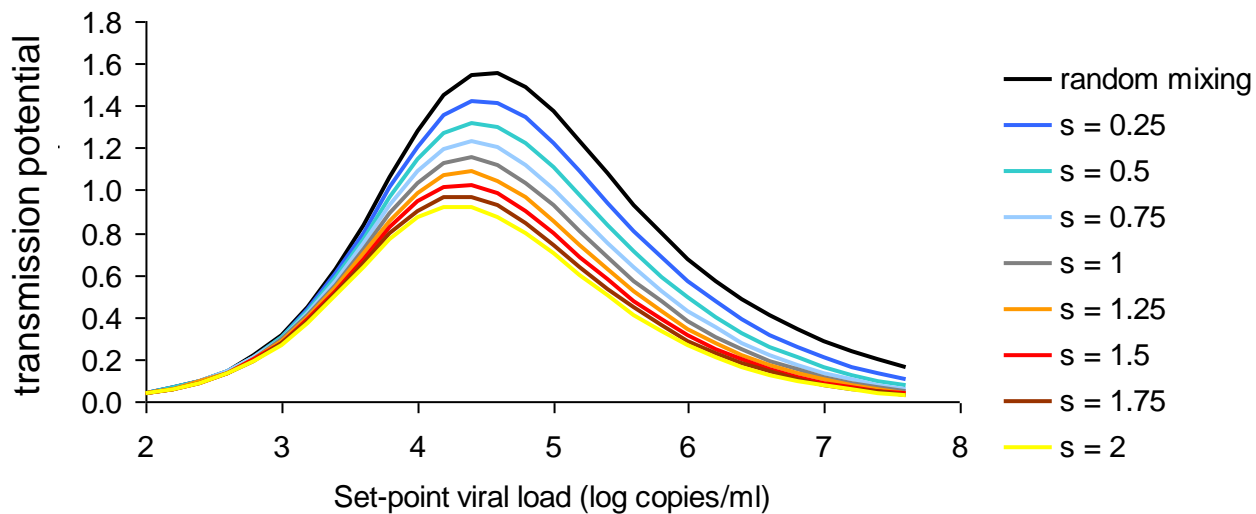b) Burkina Faso

c) South Africa

d) Burkina Faso

e) South Africa
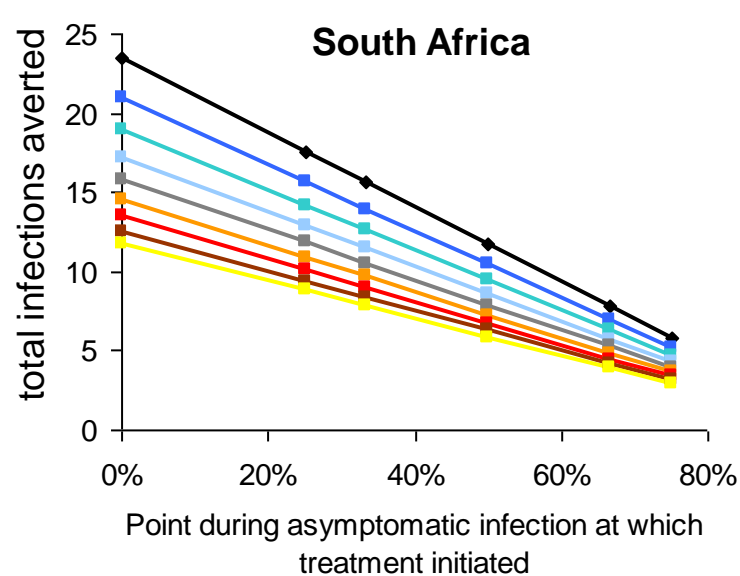
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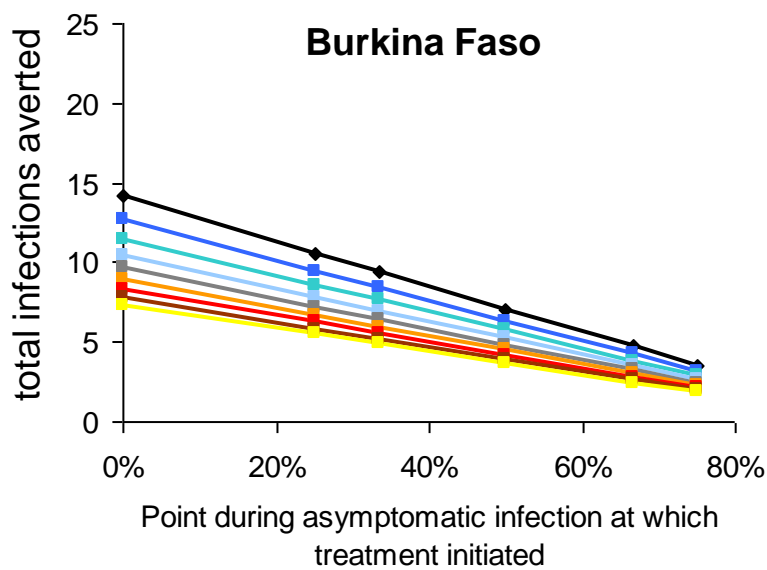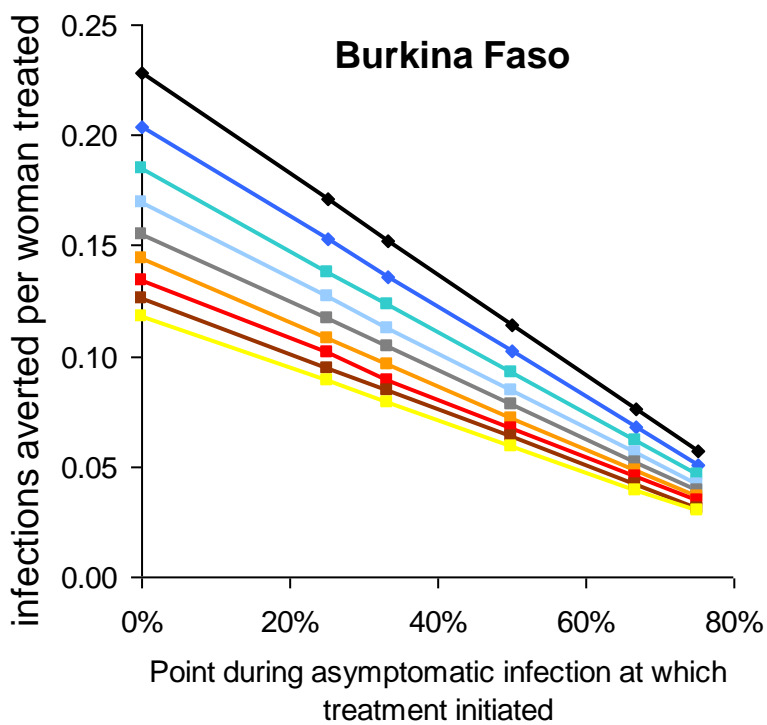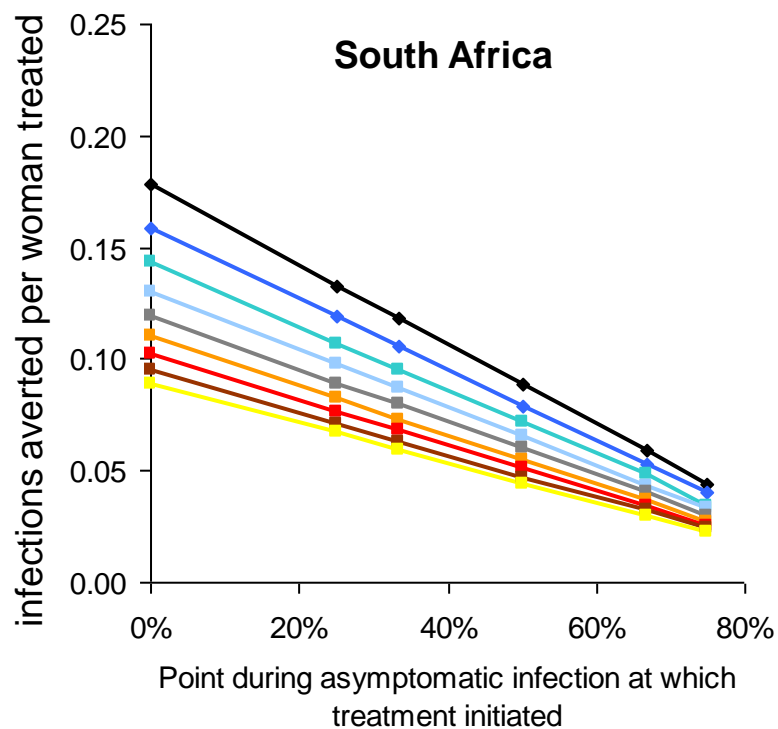
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3rd February 2009   **Rebecca Baggaley MA MSc PhD**

Chris Mowat
Editorial Coordinator
AIDS Editorial Office

Dear Dr Mowat,

AIDS submission AIDS-D-08-01379: Estimating the public health impact of the effect of HSV suppressive therapy on HIV-1 plasma viral load

Thank you for your email of 12th January with the reviewers' comments on our manuscript. Both reviewers provided helpful comments and suggestions and we have revised the paper in line with these, which has improved the paper. Please find attached a response sheet detailing all changes and justifying these in light of all the points raised.

We hope that you will be satisfied that we have responded appropriately to all the comments and suggestions and that our revised manuscript will be suitable for publication in AIDS.

I look forward to hearing from you.

Yours sincerely,

Rebecca Baggaley
Imperial College London

Response sheet

Response to reviewers' comments for manuscript AIDS-D-08-01379: *Estimating the public health*

impact of the effect of HSV suppressive therapy on HIV-1 plasma viral load.

Reviewer 1

The presentation of data/methods is at times challenging. On one hand, this is a statistical modeling analysis, so one might expect more summarization and less detailing of supporting data. On the other hand, at some points it seems like the authors try to present very specific primary data from the individual clinical trials. Any effort to harmonize these approaches might be useful.

We appreciate that our original manuscript included a fair amount of detail regarding the empirical data used, which may have made it harder for the reader to follow the concepts and methods relating to our modelling analysis. However presentation of some of this information is unavoidable as it is important for the reader to have some background knowledge of the data gathered from the four studies without having to refer back to each original publication, and because of the different parameters required for our analysis. Therefore we have retained some information for this purpose, but have transferred Figure 3 and the accompanying text and description of the laboratory measurements for PVL to the Supplementary Information, as suggested by Reviewer 1, which improves the balance of the paper substantially.

General comments:

1) *Is it truly necessary to present the laboratory methods for plasma viral load sequencing (Roche etc) for these studies as well as for the comparator studies for modeling? Particularly if methodology is not considered again later?*

   Details of the laboratory methods used, presented on p.6 2nd paragraph from "Nagot et al" onwards, have now been moved to the Supplementary Information document and replaced with the sentence, "Details of the laboratory methods used for quantifying PVL for each study are provided in Supplementary Information". We have retained this paragraph in Supplementary Information because it is still useful to have a summary of the methods used for all four studies (Burkina Faso, South Africa, Zambia and The Netherlands) for comparison.

2) *Figure 1 is useful. That said, panel 1a illustrates an important limitation of this model that the authors do not acknowledge sufficiently - the trials of HSV suppression to date have been very short (12 weeks maximum) and there are no data to know definitively whether the effect is persistent with long-term treatment. Indeed, recent in vitro work suggesting direct anti-HIV effects of acyclovir, with the potential for HIV to develop specific genotypic resistance (McMahon JBC 2008), may indicate the impact could fade over time. Acknowledgement of this as a potential limitation is important.*

   We acknowledged that effectiveness may decrease over time due to possible waning adherence (p.13 3rd paragraph of original manuscript: "Increased benefit over time on therapy would increase the number of infections averted; however, such an effect may be mitigated if accompanied by waning adherence") yet also discussed the possible increased effect over time that was suggested by the Burkina Faso study. However, we did not acknowledge that effectiveness may wane even with sustained adherence levels, and so the end of the 2nd paragraph, p12 of the new manuscript, reads: However, such an effect may be mitigated if accompanied by waning adherence or if the effectiveness of HSV therapy actually decreases over time (there has been concern over the selection of resistant HIV mutants under the selective pressure of acyclovir in vitro [McMahon et al JBC 2008])".

3) *Figure 2 is quite long and involved. If any way to make more approachable, would do so. May consider whether panels c and e are truly needed, or could just be explained in the text. And doesn't panel b reflect something similar to Figure 1 panel d?*

We would prefer to retain Figure 2 in its current form, especially as Figure 3 has now been removed. In Figure 2, five of the six graphs illustrate the same property – distribution of plasma viral loads (PVLs) – comparing the two trials with the cohort study (Fideli et al) which we used to estimate infectiousness. This enables the reader to compare the distributions between control and intervention groups for the two trials and then, even more importantly, compare these to the transmission potential graph (Fig 2b). From this, the reader can appreciate that the peak frequency of PVLs among the control arm subjects and intervention arms subjects at baseline is around the peak of transmission potential, while the intervention arm PVL distributions at study end suggest a shift to PVLs conferring a lower transmission potential. The figure thus reinforces the conceptual ideas introduced in Figure 1 but also provides a simple illustration of how our results and conclusions have been made.

4) *A small point, but if CD4 counts for the two trials are going to be presented (top of page 9), they should at least be presented in the same manner.*

This has been changed to, "compared to a median 446 (range 334-628) cells/mm^3 for Burkina Faso and 475 (251-1382) cells/mm^3 for South Africa."

5) *Figure 3 is interesting to see presented, yet it takes a fair amount of thought to get through. Ultimately, for the purposes of a modeling paper such as this, it may just be necessary to state that treatment effect was present for baseline viral loads >3 log10 copies/mL - that could be done solely in text, without the entire figure. This is an example of analysis of the primary trial data becoming more prominent than the modeling analysis.*

We appreciate that this manuscript includes a lot of information, both describing the original trials and describing our analysis. Information on the original trials is important because it summarises the results without the reader having to refer to several other papers to understand fully this current piece of work. Furthermore, Figure 3 shows whether the effect of suppressive therapy changed as a function of PVL, which is important since we are modelling how survival and infectiousness change as a function of PVL, and this relationship is not presented in the original publications. We would like to move Figure 3 and the accompanying text from the manuscript (p. 9 middle paragraph of original manuscript) to the Supplementary Information. We have added the following text to the Methods section, p. 6 end of *Quantifying reduction in PVL with HSV suppressive therapy* paragraph: "Analysing changes in PVL in the two trials stratified by intervention arm suggested a reduction in PVL by study end observed among treated participants for all levels of baseline PVL recorded (except very low levels, <3.0 $\log_{10}$ copies/ml, where any further reduction in PVL is unlikely to be detected) but no such decrease among controls (see Supplementary Information). Therefore it appears reasonable to estimate effect of suppressive therapy on HIV-1 transmission using data from the treatment arms only".

6) *Table 1 is useful. A point that is unclear is exactly what clinical expectations have gone into the statement in the text about a gain of one year in HAART-free period. Is HAART assumed to be initiated when HIV is symptomatic?*

The intention of HSV suppressive therapy would be to extend life expectancy (and reduce infectiousness) while patients CD4 counts are high enough for HAART not to be clinically warranted, after which, as CD4 counts eventually decline, HAART would be initiated (which would have been at 200 cells/mm^3 at the time these studies were undertaken). A point which we have not elaborated on in the text is the possible continuation of HSV treatment alongside HAART, as there is evidence that it can suppress PVL to lower levels than HAART alone (Ouedraogo et al AIDS 2006). However the trial data we use are from women not yet requiring HAART and given the word count restraint, we do not discuss this. In order to clarify the issue raised by Reviewer 1, we have amended the text: Introduction p.4 penultimate line, added "thus delaying the point at which HAART should be initiated" after references [15,16].

7) *It might be useful for the authors to discuss the nadir in Figure 4* [now Figure 3]. *The largest benefit of HSV suppression appears to occur in populations with modestly low (3.5 log) plasma viral loads.*
The following has been added at the end of the Results section: "The largest relative benefit of HSV suppression would occur among populations with modestly low (around median 3.5 $\log_{10}$ copies/ml, Figure 3) mean PVLs because it is at this point that changes in PVL have the greatest impact on transmission potential (shown by the steepest gradient of the curve in Figure 2b)." Similarly the worst possible impact would be among populations with median PVL around 6-6.5 $\log_{10}$ copies/ml because that is the point with the steepest gradient on the other side of the curve.

8) *In the Discussion, would separate out adherence into its own paragraph, since that would be a key issue if HSV suppression were truly to be used as an adjunctive treatment for HIV infection. Comparisons to Bactrim prophylaxis for individuals not yet needing HAART might be useful.*
The third paragraph of the Discussion now concentrates on the issue of adherence.

9) *The comparisons against the studies from Tanzania and Zimbabwe are important. Both had poor adherence to study drug and it might be useful to explicitly cite those studies when the relevant comment about adherence is made.*
We have amended the now-third paragraph of the Discussion section to reflect this: "There are many possible reasons for the varied findings of these trials: different durations of follow-up, drug regimens and levels of adherence achieved within these high-risk study populations, with lower adherence notable in the Tanzanian and Zimbabwean trials.

10) *The paragraph addressing the recent paper by Modjarrad et al. (ref 30) is both specific and vague. I am not sure how to compare the two analyses, other than the authors of the present study think their approach was better. Although the basic findings of the Modjarrad analysis are presented, they are not given a parallel comparison of the present findings. Is there any way to state some direct comparison or at least a more direct statement about the contribution of each study?*
The following has been added to this paragraph: "However, for comparison, using a "typical" viral set-point value of 4.5 $\log_{10}$ copies/ml (as used by others [Wilson et al Lancet 2008]) and formulas (1) and (2), infectiousness would increase by 18% and 26% and duration of infection before AIDS would decrease by 24% and 44% for 0.3 and 0.5 $\log_{10}$ copies/ml increases in PVL respectively using our methods, yet these values change substantially for different baseline viral set-points". While the sets of estimates are reasonably comparable when we assume 4.5 $\log_{10}$ copies/ml PVL, they would differ substantially for other set-point values because infectiousness and risk of/time to AIDS are not linearly related to log PVL values.

11) *I am surprised that the authors do not mention the ongoing clinical trials that are actually measuring the effect of HSV suppression on the endpoints of this modeling exercise: the large discordant couples study that will look at both heterosexual transmission and HIV disease progression (clinicaltrials.gov NCT00194519), the follow-up analysis of seroconverting participants from HPTN 039 (NCT00076232) who were maintained on acyclovir during early infection, and the trial of suppression in chronic infection in Rakai that will examine time to symptomatic disease and ART initiation (NCT00405821).These may be the true measures of whether HSV suppression has a role to play for the treatment of HIV.*
P.11 end of first paragraph of the Discussion section, we have added: "Results of the Partners in Prevention (PiP) study, following over 3400 HIV-1-discordant couples, will provide the first empirical data examining whether HSV suppressive therapy reduces HIV-1 transmission (www.clinicaltrials.gov *NCT00194519)".*
P.12 end of first paragraph: We have added: "The potential of HSV therapy to reduce the rate of CD4 decline and thus increase the time before starting HAART has important implications

for HIV-1 patient management. PiP may have sufficient power to detect such an effect, which is also currently being investigated in Rakai, Uganda (www.clinicaltrials.gov *NCT00405821*), although many study participants started therapy while already at relatively late stage infection. To investigate impact for early HIV-1 infections, an ancillary study to the recent HPTN039 HSV suppressive therapy HIV-1 acquisition RCT [25] is maintaining HIV-1-seroconverters on their trial regimens (acyclovir or placebo) to investigate whether treatment alters HIV-1 set-point up to 6 months."

Reviewer 2

1) *The model utilized looks primarily at the potential reduction in transmission from the dual infected transmitter in the context of discordant couples but does not look at the potential reduction in transmission by reduced susceptibility of the non-HIV infected and now non-HSV infected HIV-1 exposed person taken outside of the discordant couple scenario. This should be expanded in the discussion.*
We have added the following sentence after "never be exposed to HIV-1" in the first paragraph of the Discussion, p.11: "(The effect of HSV suppressive therapy on reducing acquisition of HIV-1 among HSV-2 positive HIV-1 negative women has recently been investigated by two RCTs but no effect was observed [Watson-Jones et al N Eng J Med 2008 and Celum et al Lancet 2008].)". Our analysis is not restricted to looking at discordant couples; in fact it assumes random mixing with a high partner change rate, but we also investigate the scenario of serial monogamy (see analysis in Supplementary Information).

2) *The potential further impact on viral load and CD4 and durability of the ART free time (as long as viral load stays low and transmission potential remains low) would be value added in resource limited settings where ART need will continue to potentially outstrip ART availability. This component does deserve additional study as noted by the authors.*
To emphasise this point we have added the following to the end of the Discussion section: "Delaying initiating HAART would be of particular value in resource-limited settings, where HAART need will likely continue to outstrip supply."

Editor's comments

1) *Authors need to shorten discussion by about 250 words.*
The original length of the Discussion was 1207 words. It is now 1032 words, with the total paper being 3190 words and one less than the maximum number of figures/tables allowed. We have worked hard to cut the Discussion down as much as possible, but in order to incorporate all the suggestions made by the reviewers, we have been able to cut 200 rather than 250 words.

2) *Please ensure that limits to length of structured abstracts (250 words) and titles (120 characters) are not exceeded, and that authorship is limited to those who have made a substantial contribution to the paper - justification of more than 10 names should be submitted to the Editors. More than 12 authors is not acceptable.*
The author list exceeds 10 because this analysis involved use of multiple datasets and it was vital to utilise the experience of the Principle Investigators for the trials as well as those individuals who were involved in creating the methodology we used, which translates plasma viral load data into transmission potential.

3) *Ensure that the references are not saved as endnotes or footnotes, and inform us in advance if figures are to be reproduced in colour - The cost for any colour used in publication is $1250.*
We do not need our figures to be reproduced in colour. We have amended Figure 1 slightly so the blue circles used in graphs b to d are now outlines rather than solid colour, to distinguish them from the red circles. The only figure which requires colour is Figure S2 of the Supplementary Information, but as this will remain electronic, we assume it is exempt.