Title:

Immediate antiretroviral therapy reduces risk of infection-related cancer during early HIV infection

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Summary: In START, immediate cART initiation reduced risk of infection-related cancer by 74% and infection-unrelated cancer by 51%. Adjustment for potential mediators, such as HIV RNA, appeared to attenuate HRs of immediate/deferred cART for infection-unrelated cancer more than infection-related cancer. cART benefit in reducing cancer risk may be mediated by mechanisms beyond HIV suppression.

Abstract

Background: In the START study, immediate combination antiretroviral therapy (cART) reduced cancer risk by 64%. We hypothesized that risk reduction was higher for infection-related cancer and determined by differences in CD4 counts and HIV RNA between the study arms.

Methods: Incident malignancies in START were categorized into infection-related and infection-unrelated cancer. We used Cox models to assess factors associated with both cancer categories. We used sequential adjustment for baseline covariates, cancer risk factors and HIV-specific variables to investigate potential mediators of cancer risk reduction with immediate cART.

Results: There were 14 cancers among persons randomized to immediate cART (6 infection-related and 8 infection-unrelated) and 39 cancers in the deferred arm (23 infection-related and 16 infection-unrelated) (Hazard Ratios; 95% CI; of immediate vs deferred cART initiation were 0.26; 0.11-0.64; for infection-related and 0.49; 0.21-1.15; for infection-unrelated cancer). Independent predictors of infection-related cancer were older age, higher BMI, low-middle income region, HIV RNA and baseline CD8 count. Older age and baseline CD8 count were independent predictors of infection-unrelated cancer. Adjustment for latest HIV RNA level had little impact on the protective effect of immediate cART on infection-related cancer. Adjustment for latest HIV RNA level, but not for CD4 count or cancer risk factors, attenuated the effect of immediate cART on infection-unrelated cancer.

Conclusions: Immediate cART initiation significantly reduces risk of cancer. Though limited by small sample size, this benefit doesn't appear to be solely attributable to HIV RNA suppression and may be also mediated by other mechanisms.

Introduction

HIV+ persons have an increased risk of cancer when compared to the general population. Many reasons have been postulated for this including immunodeficiency [1-4], higher prevalence of traditional cancer risk factors [5] and co-infection by oncogenic viruses, direct pro-oncogenic effects of HIV [6], long term combination antiretroviral therapy (cART) toxicity [7,8] and activated inflammation and coagulation [9,10]. Because of this inherent increased risk and given the fact that HIV+ persons are now living much longer [11], cancer has emerged as a major cause of morbidity and death in the cART era [12].

HIV infection [13] and other immunodeficiency disorders [14,15] are associated with a higher risk of infection-related cancer. According to recent estimates, up to 40% of cancer cases among HIV+ persons in the USA are infection-related [16]; an attributable fraction ten times as high as in the general population. HIV+ persons also have an increased risk of infection-unrelated cancer [17]. Reduced control of oncogenic viruses and impaired immune surveillance of malignant cells may facilitate carcinogenesis during HIV infection [18].

The Strategic Timing of Antiretroviral Treatment (START) trial randomized HIV+ adults with a CD4 count over 500 cells/mm^3 to immediate cART initiation or cART deferral until CD4 counts dropped below 350 cells/mm^3. Immediate cART reduced of risk of cancer by 64% [19]. This was a surprising finding because START recruited young participants with early HIV infection and a median CD4 count of 651 cell/mm^3 at study entry. Here, we set out to determine factors associated with cancer development among START participants and assess mediators of the benefit of immediate cART in reducing cancer risk. Our *a priori* hypothesis was that the reduction in cancer risk was higher for infection-related versus infection-unrelated cancer and mainly determined by differences in CD4 counts and HIV RNA levels between the study arms.

Methods

The methods and results of the START study have been described in detail elsewhere [19]. In short, START randomised 4,685 HIV+ participants from 35 countries to start cART immediately or to defer cART initiation until CD4 counts dropped below 350 cells/mm^3 or AIDS-defining illnesses developed. After a mean follow up of 3.0 years, the START data and safety monitoring board determined that pre-defined stopping rules were met for the primary endpoint of a composite of serious AIDS-related and non-AIDS-related events and recommended that all participants be offered cART. In the immediate cART arm, average CD4 counts during follow up were 194 cells/mm^3 higher than in the deferred cART arm [19].

START was conducted in compliance with the Declaration of Helsinki Guidelines, was registered on clinical trial databases (ClinicalTrials.gov number: NCT00867048) and reviewed by an independent data and safety monitoring board. The institutional review board at each study site approved the original study protocol, and written informed consent was obtained from all participants.

An endpoint review committee independently validated all reported cancer events. All cancer cases were considered definitive events with the exception of one case of leukemia that was classified as probable. We categorized incident malignancies in START into infection-related and infection-unrelated cancer after individual review of case report forms and source documentation. Infection-related cancer was defined as cancer driven by the following infectious agents: human herpesvirus 8 (HHV-8) (Kaposi sarcoma), Epstein-Barr virus (EBV) (non-Hodgkin lymphoma, Hodgkin lymphoma), human papillomavirus (anal cancer, cervical cancer) [20]. All other malignancies were classified as infection-unrelated cancer, including one liver cancer in a participant with no evidence of hepatitis B or C co-infection and one gastric cancer in a participant without previous history of *Helicobacter pylori* infection. No cases of pharyngeal lymphoepithelioma or Merkel-cell carcinoma were observed.

Statistical analyses

Participants were followed from study entry until first cancer event, death, loss to follow-up, or May 25th 2015 (date of database closure), whichever occurred first. We calculated annual rates of any type cancer in each study arm and examined the trends within study group using unadjusted Poisson regression models. Kaplan-Meier (KM) curves giving the cumulative percentage of participants with infection-related and infection-unrelated cancer were constructed for each study arm. We tested for the difference in treatment group hazard ratios (HRs) for infection-related cancer versus infection-unrelated cancer by fitting a Cox regression model that simultaneously estimated both treatment group HRs and stratified by type of event.

Multivariable Cox models identified factors independently associated with risk of infection-related and infection-unrelated cancer. The statistical power was insufficient to investigate individual cancer types. Epidemiologically important and biologically plausible baseline variables were investigated: demographics (age, sex, race and region), medical history (current smoking, alcohol abuse, body mass index, hepatitis B or C co-infection and prior cancer) and HIV-specific variables (CD4 count, CD8 count, CD4:CD8 ratio and HIV RNA level). Variables associated with cancer risk in univariate analyses were subsequently mutually adjusted for.

To explore potential mediators of the reduced cancer risk from immediate cART, Cox proportional hazards models with an indicator for treatment group were adjusted sequentially for demographics, traditional cancer risk factors, and baseline and time-updated HIV-specific variables. The following models were used: A) univariable with a single treatment group indicator; B) adjusted for baseline covariates: age, gender, race, geographical region, smoking, BMI, hepatitis B/C, CD4 count and $\log_{10}$ HIV RNA; C) adjusted for latest CD4 count, modelled as a continuous variable; D) adjusted for latest HIV RNA, modelled as ≤200 copies/mL vs HIV RNA >200 copies/mL; E) adjusted for latest CD4 count and latest HIV RNA (≤200 vs >200 copies/mL); F) as in B) with further adjustment for latest CD4 count and latest HIV RNA (≤200 vs >200 copies/mL); G) adjusted for latest $\log_{10}$ HIV RNA and H) adjusted for latest CD4:CD8 ratio

Secondary analyses

We repeated analyses excluding premalignant or low-grade lesions not requiring chemotherapy (one participant had micro-invasive squamous cell carcinoma and anal intraepithelial neoplasia grade III which was classified as anal cancer in the main analyses), localised skin squamous carcinoma (n=2) and Kaposi sarcoma restricted to the skin without internal organ involvement (n=7). This was done because these lesions have low morbidity or can be reversed or cured by cART initiation alone [21,22].

Results

There were 14 malignancies among persons randomized to the immediate cART arm (6 infection-related and 8 infection-unrelated) and 39 malignancies in the deferred arm (23 infection-related and 16 infection-unrelated). Types of infection-related and infection-unrelated cancer stratified by treatment group are listed in Table 1. Baseline characteristics of participants with no cancer and with infection-related and infection-unrelated cancer are shown in Table 2.

On the one hand, in the immediate cART arm, annual incidences of any type cancer remained stable throughout the entirety of follow up (p value for trend= 0.790). On the other hand, in the deferred cART arm, annual incidences of any type cancer increased significantly during follow up (p= 0.026) (Figure 1). HR; 95% CI; of immediate versus deferred cART initiation was 0.28; 0.13-0.58; p<0.001; for any type cancer after the exclusion of cancer events occurring in the first year of follow up. Among participants with any type cancer, the latest median (interquartile range [IQR]) CD4 count at time of cancer diagnosis was 861 (680-1082) cells/mm^3 in the immediate cART arm and 586 (452-664) cells/mm^3 in the deferred cART arm. In the deferred arm, 13 malignancies (8 infection-

related and 5 infection-unrelated) were diagnosed after cART initiation. The median (IQR) time from cART initiation to cancer diagnosis was 134 (39-1,225) days in the deferred arm.

Immediate cART significantly reduced the risk of infection-related cancer by 74% (HR; 95% confidence interval [CI]; of immediate vs deferred cART initiation 0.26; 0.11-0.64; p=0.003). KM curves overlapped during the first year; afterwards, the lower risk of infection-related cancer in the immediate cART arm became evident and remained so for the rest of follow up (Figure 2). The lower risk of infection-related cancer in the immediate cART arm was driven mainly by a reduction of cases of Kaposi sarcoma and non-Hodgkin lymphoma (Table 1).

Immediate cART lowered the risk of infection-unrelated cancer by 51% (95% CI 21%-115%; p=0.103). This risk reduction did not reach statistical significance (Figure 3). This seemed to suggest that immediate cART reduced the risk for infection-related cancer more than infection-unrelated cancer. However, when we tested the difference in HRs for infection-related versus infection-unrelated cancer we found no statistical significance (p-value for comparing the treatment group HRs for infection-related vs infection- unrelated cancers: 0.27).

In adjusted analyses with both treatment groups combined, independent baseline predictors of infection-related cancer were older age (1.42; 0.99-2.02 per 10y older), higher BMI (1.47; 1.05-2.05 per 5Kg/m^2), low-middle income region (3.40, 1.44-8.02 versus high income region), baseline HIV RNA (2.01; 1.17-3.47 per 1log_{10} higher) and baseline CD8 count (1.13; 1.03-1.24 per 200 cells/mm^3 higher) (Figure 4). Older age (2.54; 1.72-3.73 per 10y older) and baseline CD8 count (1.12; 1.01-1.25 per 200 cells/mm^3 higher) were the only independent predictors of infection-unrelated cancer (Figure 4).

Figure 5 shows the impact of sequential adjustment for baseline and time-updated variables on the protective effect of immediate cART initiation on any type cancer risk. Adjustment for demographics, traditional cancer risk factors and CD4 counts (Models B-C) had no appreciable effect on the estimates of overall effect. Adjustment for latest HIV RNA level (Models D-G) and CD4:CD8 ratio (Model H) attenuated the protective effect of immediate cART on any type cancer by partially attenuating the overall effect and enlarging 95% CIs. The effect of immediate cART was no longer significant in models D-H suggesting that the effect of cART is partially mediated through effects on these parameters.

As for infection-related cancer, results were broadly consistent across the models with HRs varying from 0.26 to 0.48 (Figure 5). Adjustment for latest HIV RNA levels (Models D-G), in particular when modelled as continuous variable (Model G), and CD4:CD8 ratio (Model H) partially attenuated the effect of immediate cART on the risk of infection-related cancer.

With respect to infection-unrelated cancer, the measurable effect of sequential adjustments on the protective effect of immediate cART appears to be stronger (Models D-F) For instance, HRs (immediate cART/ deferred cART) for infection-unrelated cancer increased from 0.49 (Model A) to 1.07 (Model E) after adjustment. However, the overall number of cancer events was too small and no definitive statement can be made about the degree of attenuation of the protective effect of immediate cART after adjustment.

Secondary analyses excluding pre-malignant lesions and localized cancer did not change these conclusions.

Discussion

In a large randomised HIV trial from across the globe, we report that immediate cART initiation significantly reduced the risk of infection-related cancer. This effect was mainly driven by a reduction in cases of Kaposi sarcoma and non-Hodgkin lymphoma. These were unexpected results because START enrolled participants with early HIV infection and follow up time was shorter than initially planned. Contrary to what we first hypothesised, adjustment for CD4 counts had no impact on the protective effect of immediate cART on any type and infection-related cancer and adjustment for HIV RNA levels only partially attenuated this association. Taken together, these findings suggest that the benefit of immediate cART in reducing cancer goes beyond HIV RNA suppression and immune status as assessed by CD4 counts and seems likely to be also mediated by other mechanisms impacting co-infections with pro-oncogenic virus and immune surveillance.

With the changing epidemiology of cancer during HIV infection, the traditional classification into AIDS-defining and non-AIDS-defining cancer, implemented in the early 90s for surveillance purposes [23], became outdated [18]. The classification of cancer into infection-related and infection-unrelated seems to us more appropriate because it considers a wider range of cancer types whose incidence is increased during immunosuppression and establishes a framework to better understand the interplay between viral co-infections, HIV and carcinogenesis. However, as pointed out by others [24], this classification is not perfect because, even when considering a given cancer type such as non-Hodgkin lymphoma, an infectious origin can only be ascertained in a subset of cases. This classification can only be definitive after a thorough search for pathogens in tissue samples using *in situ* hybridization.

Consistent with previous reports [25], we observed that infection-related and infection-unrelated cancer may have different predictors. Low-middle income region and baseline HIV RNA were associated with risk of infection-related cancer, which reflects a higher burden of these malignancies in resource constrained settings and suggests a stronger link between HIV RNA viremia and malignancies caused by pro-oncogenic viruses [26,27]. Older age and higher CD8 cells were independent predictors of both infection-related and infection-

unrelated cancer. Indeed, a recent report linked elevations in CD8 cells after cART initiation to viral co-infections and non-AIDS death [28].

Because cART improves immune function, lowers HIV viral load, and reduces inflammation [29], earlier cART initiation had previously been proposed as an approach to reduce cancer risk among HIV+ persons [2-4]. Furthermore, cohort studies had long identified a relationship between immunodeficiency and uncontrolled HIV RNA replication with risk of non-Hodgkin lymphoma and Kaposi sarcoma [1,2]. However, this benefit was estimated in previous studies when cART was initiated at much lower CD4 counts. In START, cART was initiated at higher CD4 counts before the development of overt immunosuppression; still, the benefit of immediate cART in reducing cancer risk seems to be larger than previously estimated by observational studies [30].

The benefit of immediate cART initiation in reducing infection-related cancer was determined by a marked decrease in cases of non-Hodgkin lymphoma and Kaposi sarcoma. The mechanisms by which EBV and HHV-8 may foster carcinogenesis are complex and not fully understood [31,32]. In both cases, common viral infections cause rare malignancies; this highlights the potential contribution of multifactorial non-viral factors to the development of infection-related cancer.

EBV establishes long-term latent infections in B cells and may act just as an initial promoter of cellular transformation from which subsequent progression to cancer is independent [31,32]. The dominant EBV latency patterns observed in non-Hodgkin lymphoma tissue differs between pre- [33] and post-cART studies [34]. It was hypothesised that cART increases immune surveillance of proteins expressed by cells latently infected by EBV resulting in a shift to a less oncogenic latency pattern [34].

As for HHV-8 infection, immunosuppression is clearly a major contributor to progression to Kaposi sarcoma, whereas recent evidence points out the importance of complex immune modulation mechanisms [35]. Questions remain as to how cART interferes with such mechanisms. In a randomised trial involving participants with tuberculous pericarditis predominantly co-infected with HIV, glucocorticoids and immunotherapy with non-pathogenic mycobacteria acted synergistically to increase risk of Kaposi sarcoma [35].

CD8 T-cell dysfunction in the setting of untreated HIV infection may also be an important contributor. Depleted response from EBV- and HHV-8-specific CD8 cytotoxic cells may facilitate carcinogenesis among immunocompromised hosts [36]. The extent to which the normalization of the CD4:CD8 ratio following cART initiation reflects a recovery of CD8 cell response is unclear [37], but this may explain why adjustment by CD4:CD8 partially attenuated the benefit of immediate cART in reducing cancer risk.

Our study had important limitations. First, this is a post hoc analysis of a trial whose sample size was not calculated to assess reductions in cancer risk. Second, START had a

follow up shorter than initially planned. As a result, we cannot exclude the possibility that immediate cART could have significantly reduced the risk of infection-unrelated cancer if a longer follow up with larger number of events had accrued. It is unclear whether the non-significant reduction in infection-unrelated cancer just reflected limited power to detect differences or was instead a true observation. The first possibility is favoured by the lack of significant difference in the HRs for infection-related versus infection-unrelated cancer (p=0.27). From our studies, activated inflammation is associated with an increased risk of both infection-related and infection-unrelated cancer [9]. Therefore, reduction of inflammation could have been a common mechanism by which cART may have reduced the risk of both types of cancer [38]. Third, we did not perform *in situ* hybridization analyses in cancer tissues; therefore, a definitive infectious origin could not be ascertained in cancer events classified as infection-related. Fourth, the number of cancer events was relatively small; this forced us to group heterogeneous malignancies into two categories and hampered our ability to quantify variation in risk of individual cancer types. We also had a hampered ability to fully explore potential mediators of the benefit of immediate cART and take our results as preliminary rather than definitive. A longer follow-up is needed in START to better understand the treatment differences and determine with accuracy which are the predictors for infection-related and infection-unrelated cancer. Nevertheless, few other randomised experiments are available to investigate the impact of cART on cancer risk. The Strategies for Management of Antiretroviral Therapy (SMART) study compared continuous cART use to CD4-guided structured cART interruptions [39]. As SMART participants who interrupted cART also had higher risk of cancer, in particular Kaposi sarcoma and non-Hodgkin lymphoma [40], an individual participant data meta-analysis combining START and SMART may be useful for a better estimation of the impact of cART on the risk of specific cancer types.

To conclude, immediate cART initiation significantly reduces risk of cancer during HIV infection. Adjustment for latest HIV RNA level appeared to attenuate HRs of immediate versus deferred cART for infection-unrelated cancers more than infection-related cancers. The benefit of immediate cART doesn't appear to be solely attributable to HIV RNA suppression and may also be mediated by other mechanisms, such as a curb on oncogenic virus coinfection and reduction of inflammation. Further research is needed to identify mediators of the benefit of immediate cART initiation in reducing cancer risk.

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Conflicts of interest

KH conducts research sponsored by GKS/ViV, Merck, Janssen, and Gilead. MKJ has received research grant support from Gilead Sciences, AbbVie, Janssen, GSK/ViiV Healthcare, Merck, Bristol-Myers Squibb. TW has received grant funding (paid to Weill Cornell Medicine) from Gilead Sciences, Glaxo Smith Kline/ViiV Health Care, and Bristol Myers Squibb. TW has also served as an ad hoc consult to Glaxo Smith Kline/ViiV Healthcare. The other authors have no conflicts of interest to declare.

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Author contributions

AHB, JN, AGB, TW and RM conceived the study. JN performed the statistical analyses. AHB drafted the manuscript. All authors contributed to data interpretation, critically revised the manuscript and approved the final version.

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Tables

Tables1: START Types of malignancies by treatment group

Table 2: START Baseline characteristics by cancer event status

Figures

Figure 1: Annual rates (95% CI) of any type cancer by year of follow up stratified by START arm. p-value for trend was 0.026 in the deferred cART arm and p for trend was 0.790 in the immediate cART arm.

Figure 2: Kaplan-Meier curves giving the cumulative procentage of participants who developed infection-related cancer in each START arm.

Figure 3: Kaplan-Meier curves giving the cumulative procentage of participants who developed infection-unrelated cancer in each START arm.

Figure 4: Factors independently associated with infection-related and infection-unrelated cancer. Hepatitis B/C could not be estimated for infection-related cancer.

Figure 5: Immediate versus deferred cART initiation and the risk of any type, infection-related and infection-unrelated cancer in the START study.
1 infection-related cancer: Human Herpesvirus 8 (Kaposi sarcoma), Epstein–Barr virus (non-Hodgkin lymphoma, Hodgkin lymphoma), Human Papilloma virus (anal cancer, cervical cancer)
2 infection-unrelated cancer: prostate cancer, lung cancer, testis cancer, plasma cell myeloma, fibrosarcoma, breast cancer, bladder cancer, ureteric cancer, malignant melanoma, myeloid leukemia, thyroid cancer, leiomyosarcoma, squamous cell carcinoma of the head and neck, squamous cell cancer, gastric adenocarcinoma, liver cancer
A: univariable, estimated in a Cox proportional hazards model with a single treatment indicator

B: adjusted for baseline covariates: age, gender, race, geographical region, smoking, BMI, hepatitis B/C, baseline CD4 cell count and baseline log10 HIV RNA
C: adjusted for latest CD4 cell count, modelled as a continuous variable
D: adjusted for latest HIV RNA, modelled as $\leq$200 copies/mL vs HIV RNA >200 copies/mL
E: adjusted for latest CD4 cell count and latest HIV RNA
F: as in (B) with further adjustment for latest CD4 cell count and latest HIV RNA
G: adjusted for latest $\log_{10}$ HIV RNA
H: adjusted for latest CD4:CD8 ratio

Table 1: START types of malignancies by treatment group		
	Immediate cART	**Deferred cART**
Infection-related cancer		
Human herpes virus 8 Kaposi sarcoma	1	11
Epstein-Barr virus Non-Hodgkin lymphomas	2	9
Hodgkin lymphomas	1	1
Human papilloma virus Anal cancer	1	2
Cervical cancer	1	0
Total	**6**	**23**
Infection-unrelated cancer		
Prostate	2	3
Lung	2	2
Testicular	0	2
Multiple myeloma	1	0
Fibrosarcoma	1	0
Breast	0	1
Bladder	1	0
Ureteral	0	1
Malignant melanoma	0	1
Myeloid leukemia	0	1
Thyroid	0	1
Leiomyosarcoma	0	1
Squamous cell carcinoma of the skin	1	0
Squamous cell carcinoma of the head & neck	0	1
Gastric adenocarcinoma	0	1
Liver	0	1
Total	**8**	**16**

Table 2: START baseline characteristics by cancer event status			
	No Cancer	**Infection-related cancer**	**Infection-unrelated cancer**
No participants	4632	29	24
Demographics			
Age (years); median (IQR)	36 (29, 44)	44 (31, 48)	51 (36, 59)
Gender (% female)	26.9	10.3	25.0
Race (%)			
Asian	8.4	0.0	4.2
Black	30.3	13.8	16.7
Latino/Hispanic	13.7	10.3	4.2
White	44.2	75.9	75.0
Other	3.5	0.0	0.0
Region (%)			
High Income	45.8	51.7	79.2
Low-Middle Income	54.2	48.3	20.8
HIV History			
Likely mode of HIV infection (%)			
Sexual contact with person of same sex	55.1	79.3	54.2
Sexual contact with person of opposite sex	38.3	20.7	37.5
Injection drug use	1.4	0.0	4.2
Blood products/other/unknown	5.3	0.0	4.2
Time participant known to be HIV+ (years); median (IQR)	1.0 (0.4, 3.0)	0.7 (0.3, 3.4)	2.3 (0.6, 4.4)
Laboratory Results			
CD4[a] (cells/mm^3); median (IQR)	651 (584, 764)	671 (587, 770)	638 (562, 759)
CD4:CD8 ratio: median (IQR)	0.66 (0.48, 0.90)	0.57 (0.42, 0.73)	0.56 (0.38, 0.75)
CD8 (cells/mm^3); median (IQR)	1040 (774, 1400)	1152 (986, 1892)	1150 (821, 1499)
HIV RNA (log_{10}copies/mL); median (IQR)	4.1 (3.5, 4.6)	4.6 (4.0, 5.0)	4.2 (3.9, 4.5)
Highest HIV RNA[b] (log_{10}copies/mL); median (IQR)	4.3 (3.7, 4.8)	4.8 (4.1, 5.3)	4.5 (4.2, 5.0)
Medical History			
Hepatitis B (%)	2.8	0.0	0.0
Hepatitis C (%)	3.7	0.0	12.5
Prior non-AIDS cancer	0.4	0.0	4.2

(%)			
Current smoker (%)	31.8	44.8	41.7
Alcoholism or other substance dependence (%)	3.3	0.0	4.2
Body mass index (kg/m^2); median (IQR)	24.5 (22.1, 27.9)	25.9 (22.8, 29.6)	25.4 (22.3, 27.2)

[a] Average of screening and baseline values

[b] Documented in participant record