



OPEN Associations between weight gain, integrase inhibitors antiretroviral agents, and gut microbiome in people living with HIV: a cross-sectional study

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Dolutegravir and bictegravir are second-generation HIV integrase strand transfer inhibitors (INSTIs) that were previously associated with abnormal weight gain. This monocentric cross-sectional study investigates associations between weight gain during the first year after initiation of dolutegravir, bictegravir or other anchor drugs and gut microbiome diversity as well as taxa composition. The study enrolled 79 participants receiving dolutegravir, 32 receiving bictegravir and 10 receiving non-INSTI based regimens. Most of them were treatment experienced at initiation of those anchor drugs agents. Although weight gain was not linked to overall bacterial diversity, strong associations with specific taxa were demonstrated (FDR $q < 0.01$). Using multiple linear regression, we identified 4 distinct groups of bacteria associated with either dolutegravir, bictegravir, weight loss or weight gain under treatment, allowing a machine learning model to predict 15.9% of the weight gain variability regardless of sex, age and body mass index (RMSE: 0.0126). *Dysosmobacter* sp. and *Haemophilus* sp., two bacteria previously associated with host metabolism, were among the strongest predictors. Our findings link INSTIs, weight gain, and the gut microbiome. Future research should investigate the causal role of the identified taxa to improve our understanding of microbiome-drug interactions and further support personalized antiretroviral strategies.

Trial registration: Eudra-CT 2020-001103-17 (registration date: 2020–12-01).

Keywords Microbiota, Dolutegravir, Bictegravir, Tenofovir alafenamide, Weight gain, *Dysosmobacter welbionis*

Human immunodeficiency virus (HIV) infection has become a chronic and manageable condition with the advent of combined antiretroviral therapy (cART). cART generally comprises one or two backbone antiretroviral agents from the nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) drug class, combined with one anchor antiretroviral agent from another drug class. Integrase strand transfer inhibitors (INSTIs) are currently a key first-line anchor drug class. Among them, dolutegravir (DTG) and bictegravir (BIC) are second-generation

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INSTIs and both have been largely recommended as first-line agents^{1,2}. Despite their good safety profile, abnormal weight gain has emerged as an important concern for both DTG and BIC³. Exact causality and underlying mechanisms for this observed weight gain remain largely unknown. Besides INSTIs, the backbone NRTI drug tenofovir, especially when administered as tenofovir alafenamide (TAF) could play a significant role herein⁴. Moreover, several factors including sex have been shown to influence the treatment-associated weight gain^{5,6}.

Despite growing evidence of complex and multidirectional interactions between drugs and the gut microbiome^{7,8}, the latter remains a poorly explored player in DTG- and BIC-related adverse drug reactions. However, these interactions deserve specific attention, as on one hand, antiretroviral agents have been shown to influence patient's gut microbiome, with differential effects according to drug class^{9,10} and, on the other hand, cross talk between gut microbiome and the brain, the endocrine system and the adipose tissue has been well demonstrated¹¹. Moreover, gut microbiome composition has been linked to overweight, obesity and related metabolic disorders^{12,13}.

We conducted a prospective phase IV study encompassing pharmacokinetic, pharmacogenetic and microbiome analysis, aiming at better understanding the interindividual variability of response to DTG and BIC at both clinical efficacy and adverse drug reaction levels. Here, we summarize the findings of a cross sectional gut microbiome analysis, deciphering complex interactions between cART, patients' characteristics and gut microbiome diversity as well as taxa composition.

Methods

Study design and population

The study recruited patients from three different subgroups during their regular follow-up at the HIV/AIDS Reference Center of Cliniques universitaires Saint-Luc, a tertiary academic hospital located in Brussels, Belgium: (a) BIC-treated patients, (b) DTG-treated patients and (c) patients having previously discontinued DTG due to the occurrence of neuropsychiatric adverse events. The latter subgroup was included in the parent phase IV study with the aim of exploring factors associated with the occurrence of neuropsychiatric adverse events leading to DTG discontinuation, as previously reported¹⁴. Two other subgroups were originally planned, consisting of patients shifting from another drug class to DTG- and BIC-containing regimens, but were abandoned due to slow recruitment. Exclusion criteria were: (a) pregnancy at the time of inclusion or expected pregnancy within 12 months, for patients treated by DTG or BIC during the study, and (b) liver failure (Child–Pugh A, B or C). All participants gave written informed consent before taking part in the study.

This sub-study reports the association between gut microbiome composition and different relevant parameters (other sub-studies including a pharmacogenetics and pharmacokinetics/pharmacodynamics analysis will be reported separately). All study participants were requested to bring one stool sample on a single occasion, at least 6 months after the initiation of their ongoing anchor drug (DTG, BIC or other). 180 participants were included in total, of which 131 provided a stool sample within the expected timeframe for this microbiota sub-study. Ten samples were not included as they met some predefined exclusion criteria for analysis (further details on Fig. 1).

The study received approval by the local Ethical committee (2020/30NOV/593) and regulatory authorities (Eudra-CT Number 2020-001103-17; registration date 2020-12-01), and is registered with ClinicalTrials.gov (NCT04805944; registration date 2021-03-16).

All methods were performed in accordance with the relevant guidelines and regulations.

Demographic and clinical characteristics

Sex, age, ethnicity, sexual orientation at HIV diagnosis, past medical history and ongoing treatments were obtained from electronic medical records. The CD4 count was measured by flow cytometry (Navios, Beckman Coulter).

Weight was regularly measured during follow-up visits, which were scheduled every 3 to 6 months according to clinical need, following the HIV standards. Body mass index (BMI) of patients was calculated at the time of stool collection. Estimates of weight gain after anchor treatment initiation, i.e., before study inclusion was based on weight measurements which are part of standard evaluation during routine visits. When weight was not available at the date of drug initiation, the weight was estimated by interpolation, using the last weight before initiation, and the first weight measure after drug initiation. We studied the relative weight gain normalized over time during the first year after anchor initiation, i.e., the weight gain calculated between treatment initiation and the closest visit to one year after treatment initiation, reported over the baseline weight, divided by the duration of follow-up (again defined as the duration between treatment initiation and the closest visit to one year after treatment initiation):

$$\frac{\frac{(\Delta \text{Weight during year one after anchor start})}{(\text{Weight at anchor start})} (\%)}{\Delta \text{Time (days)}}.$$

This enabled to compare patients taking into account (a) the uneven duration of routine follow-up from one patient to the other (as visits were not planned to occur precisely 12 months after treatment initiation), and (b) the variability in baseline weight. When not specified otherwise, the terms “weight gain” are used to describe the relative weight gain reported over time. Importantly, the cross-sectional design and logistical constraints in patients recruitment and sample collection of this study led to a timing discrepancy between the measure of weight gain after treatment initiation and the stool collection, as reported in the results section.

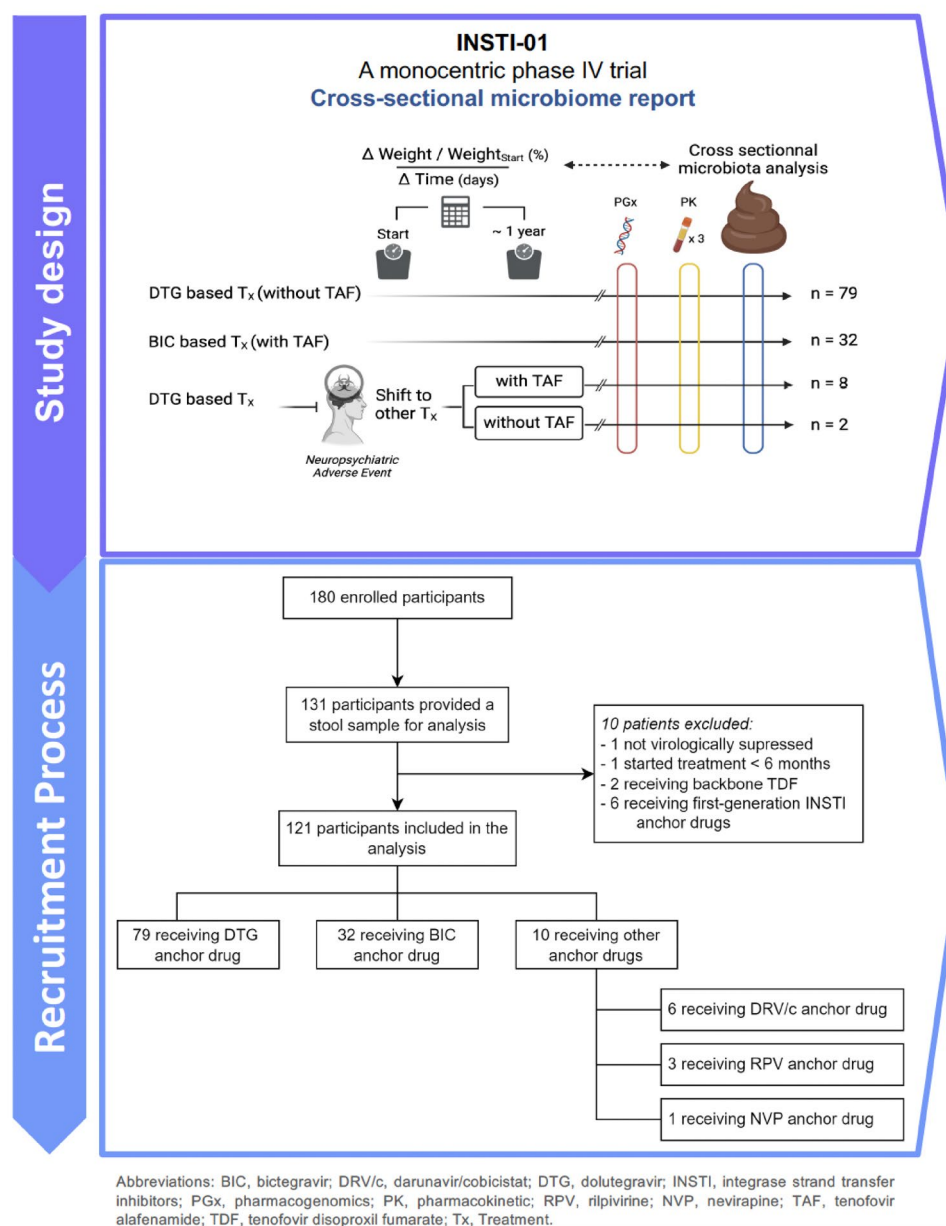


Fig. 1. Description of the study design and participants enrollment flowchart.

Fecal sample collection, DNA extraction and 16S rRNA gene sequencing

Participants received a stool collection kit with DNA stabilizer at inclusion (Invitex Diagnostics, Germany). On the day before their next appointment, they were reminded by phone to collect and bring the stool sample. Collected samples were frozen at -80°C until DNA extraction. DNA was extracted with an Invisorb Spin Universal Kit (Invitex Diagnostics, Germany), and its concentration and purity were assessed using a NanoDrop2000 (Thermo Fisher Scientific, USA). With all samples of this study included in the same run, 16S rRNA gene sequencing was performed by MrDNA Lab (www.mrdnalab.com, USA) using dual-barcoded primers targeting the V4 region (515F 5'-GTGYCAGCMGCCGCGGTAA-3', and 806R 5'-GGACTACNVTGGGTWCTCTAAT-3').

Further information of microbiome analysis from data processing and advanced statistical methods used in this study are detailed in the [Supplementary method](#). Brief descriptions are presented as follow.

Data processing

Raw sequencing data was pre-processed using the MrDNA pipeline (www.mrdnalab.com, USA) to obtain Amplicon Sequence Variants (ASVs). Taxonomy was assigned to ASVs by importing representative sequences into Qiime2¹⁵, using the qiime feature-classifier (classify-sklearn)¹⁶, against the pre-trained Silva v138 database for the V4 region (515F/806R) via RESCRIPt¹⁷. Low abundance and extremely rare ASVs present in fewer than 3 counts in at least 10% of all samples were excluded.

Ultimately, our samples exhibited exceptionally high coverage values, exceeding 99.27%. This high quality was evident in the number of reads, with a median of 26,866 and a mean of 26,856 reads after all quality controls and filtering steps (Table S1). A total of 766 taxa in the form of ASVs were identified. Count data were transformed in proportion form and center-log-ratio (clr) transformed data, managed by the Phyloseq¹⁸ and microbiome¹⁹ packages, respectively. In metadata, BMI category was defined as 'obese' for BMIs over 30, 'normal' for BMIs 25 or below, and 'overweight' for values in between. Age categories were labelled 'old' for ages above the median and 'young' for those below. Missing values for weight gain in patients 37.MV and 69.MV were replaced with the cohort's median value.

Statistical analysis

R (version 4.2.0; www.r-project.org), Python 3.9.6 and JMP were used in statistical analysis, and data visualization operated in Rstudio (version 1.4.1106) and Visual studio Code 1.84.2 on MacOS. Graphical representations were primarily created with the ggplot2²⁰, ggpubr²¹, Matplotlib packages²². For comparison of demographic and clinical characteristics of the study participants, continuous variables were compared across groups using one-way ANOVA or the Kruskal–Wallis test, as appropriate. Proportions were compared using Fisher's Exact Test. Prior to analysis, all linear regression models were evaluated for linearity and independence assumptions. In microbiome-related linear models, only clr transformed data were utilized. The Benjamini–Hochberg procedure was applied to control the false discovery rate (FDR) across all taxa-level tests when conducting multiple linear regressions across microbial outcomes. A p-value of less than 0.05 and a false discovery rate (FDR)–adjusted p-value (q) of less than 0.05 were set as thresholds for statistical significance.

Results

Participant characteristics

We studied a cohort of 121 persons living with HIV (PLHIV), as shown in Fig. 1. Patients were grouped according to the anchor drug in their ongoing cART: 79 received DTG, 32 received BIC and 10 received non-INSTI based regimens. Among DTG-treated patients, 69 (87%) received dual, 9 (11%) triple, and 1 (1%) quadruple cART. Patients from the two other anchor drug groups all received triple cART. None of the patients on DTG-based regimen was receiving TAF. On the contrary, all patients under BIC-based regimens received TAF. Among non-INSTI-based regimens, 8 out of 10 included TAF among backbone drugs. Further details of received treatments are provided in Table S2.

Main demographic and clinical characteristics were similar across the different groups, except for age, previously received cART regimen and time elapsed since the start of the current anchor drug. A vast majority of participants had received previous cART before the start of the current anchor, with only 9% in the BIC group, 13% in the DTG group and none in the non-BIC-non-DTG group being treatment-naïve.

Overall, 54% of participants experienced an increase in weight after approximately one year of treatment: 49% of DTG-treated, 74% of BIC-treated, and 30% of patients treated with non-INSTI regimens ($p=0.015$). Further details on study participants and on weight gain after treatment initiation are provided in Table 1.

This monocentric phase IV trial recruited persons living with Human Immunodeficiency Virus grouped according to the anchor drug of their combined antiretroviral therapy. Persons living with Human immunodeficiency virus not receiving bictegavir nor dolutegravir were grouped together and were recruited among a cohort of patients that previously discontinued dolutegravir due to neuropsychiatric adverse events. Blood samples were collected for pharmacogenetic and pharmacogenomic analysis, while fecal samples were used to evaluate microbiome by 16S rRNA gene sequencing. Only the cross-sectional microbiota analysis will be reported in this paper. Fecal samples were collected, DNA extracted and sequenced before analysis to obtain raw count data of microbiome for downstream biostatistical analyses.

Overall association of weight gain, microbiome, treatments and host

To estimate the overall association between relative weight gain during the first year of current anchor (further referred as weight gain), microbiome, treatments, and hosts' characteristics, we first simplified the microbiome community by calculating beta-diversity (Figure S1), and we further generated Pco1 and Pco2 which can explain about 35% of the variability of the data using principal coordinate analysis. Using two separate linear regression models, we then studied the association of sex, age category, BMI category and the anchor drug (DTG, BIC or other) with weight gain, and with simplified microbiome (Fig. 2A). We found that weight gain was different between anchor treatment groups, being highest for BIC (BIC vs DTG: $p=0.028$ and BIC vs 'other': $p=0.016$; Fig. 2A,B; Table S3), while simplified microbiome was not. On the contrary, regardless of the effect of anchor drugs, male and female participants were different in the microbiome-Pco1 ($p < 1 \times 10^{-6}$) (Fig. 2B; Table S3) but not the extent of weight gain. These differences are further illustrated in the taxonomic composition stacked bar plots (Figure S2 and S3; Table S4).

Associations between weight gain, bacterial diversities, treatments and host

The overall analyses suggest a link between treatment and weight gain, as well as sex and simplified microbiome profile, but the link between weight gain, host's characteristics and microbiome diversities or bacterial clusters remains undetermined. To explore this, we initially performed Spearman correlation analysis, calculating a matrix to examine the association between weight gain, principal coordinate analysis variables (Pco1 & Pco2 calculated using Bray–Curtis dissimilarity index) of the beta diversity, various alpha diversity indices, diversity associated-bacterial ratios, and host characteristics (age, BMI and CD4 + T-cell count (CD4)), as depicted in Fig. 2C. Surprisingly, weight gain showed no significant association with any of the variables tested.

Considering the limitations of correlation analysis, we subsequently performed multiple linear regression analyses. These allowed for simultaneous testing of associations between dependent variables (bacterial

	BIC (n = 32)	DTG (n = 79)	Other (n = 10)	P value
Female sex, n (%)	10 (31.3)	28 (35.4)	5 (50.0)	0.58
Age, mean (SD)	43.0 (9.7)	51.6 (11.6)	53.2 (7.9)	0.0007
Caucasian ethnicity, n (%)	15 (46.9)	47 (59.5)	3 (30.0)	0.15
MSM, n (%)	15 (46.9)	37 (46.8)	2 (20.0)	0.31
BMI, median (IQR)	26.2 (24.0–29.8)	26.9 (23.9–29.4)	30.0 (23.9–31.5)	0.61
Treated diabetes, n (%)	0 (0)	6 (7.6)	0 (0)	0.29
Treated dyslipidemia, n (%)	3 (9.4)	10 (12.7)	4 (40.0)	0.062
CD4+ T-cell count, median (IQR) (cells/mm ³)	633 (561–834)	726 (565–877)	691 (468–851)	0.28
Time since HIV diagnosis, mean (SD) (years)	15.5 (8.8)	16.2 (7.7)	15.8 (10.8)	0.92
AIDS diagnosis history, n (%)	19 (59.4)	37 (47.4)	5 (50.0)	0.54
Nadir CD4+ T-cell count, median (IQR) (cells/mm ³)	202 (89–336)	237 (125–339)	217 (123–425)	0.87
Days since start of current anchor drug, median (IQR)	803 (721–929)	1876 (637–2239)	1118 (589–1810)	<0.0002
ART naïve at start current anchor, n (%)	3 (9.4)	10 (12.7)	0	0.72
Treatment regimen before current anchor, n (%)				0.0004
None ^a	3 (9.1)	11 (13.9)	0	
NNRTI-based	1 (3.0)	18 (22.8)	0	
PI-based	1 (3.0)	17 (21.5)	3 (30.0)	
INSTI-based	27 (84.4)	33 (41.8)	7 (70.0)	
Change in weight during first year after anchor initiation (kg) (median, IQR)	2.25 (–0.06–3.25)	0 (–1.67–2.14)	–3.4 (–5.59–1.27)	0.0038

Table 1. Demographic and clinical characteristics of the study participants. All variables are reported at the time of stool collection, unless otherwise stated. AIDS, Acquired Immune Deficiency Syndrome; ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitors; IQR, interquartile range; MSM, men who have sex with men; NNRTI, Non-Nucleoside Reverse Transcriptase Inhibitor; PI, protease inhibitors; SD, standard deviation. ^aOne non-naïve patient had interrupted treatment for 3y and 9 months before start of the DTG based regimen. Significant values are in bold.

diversities, and ratios) and predictors including weight gain, treatments, and host factors. For these analyses, individuals were categorized into a “gain” group if their weight gain was greater than 0, with the remainder classified into the “lose” group (including 6 patients with unchanged weight). We hypothesize that individuals in the “gain” group might show distinct microbiome characteristics compared to those in the “lose” group.

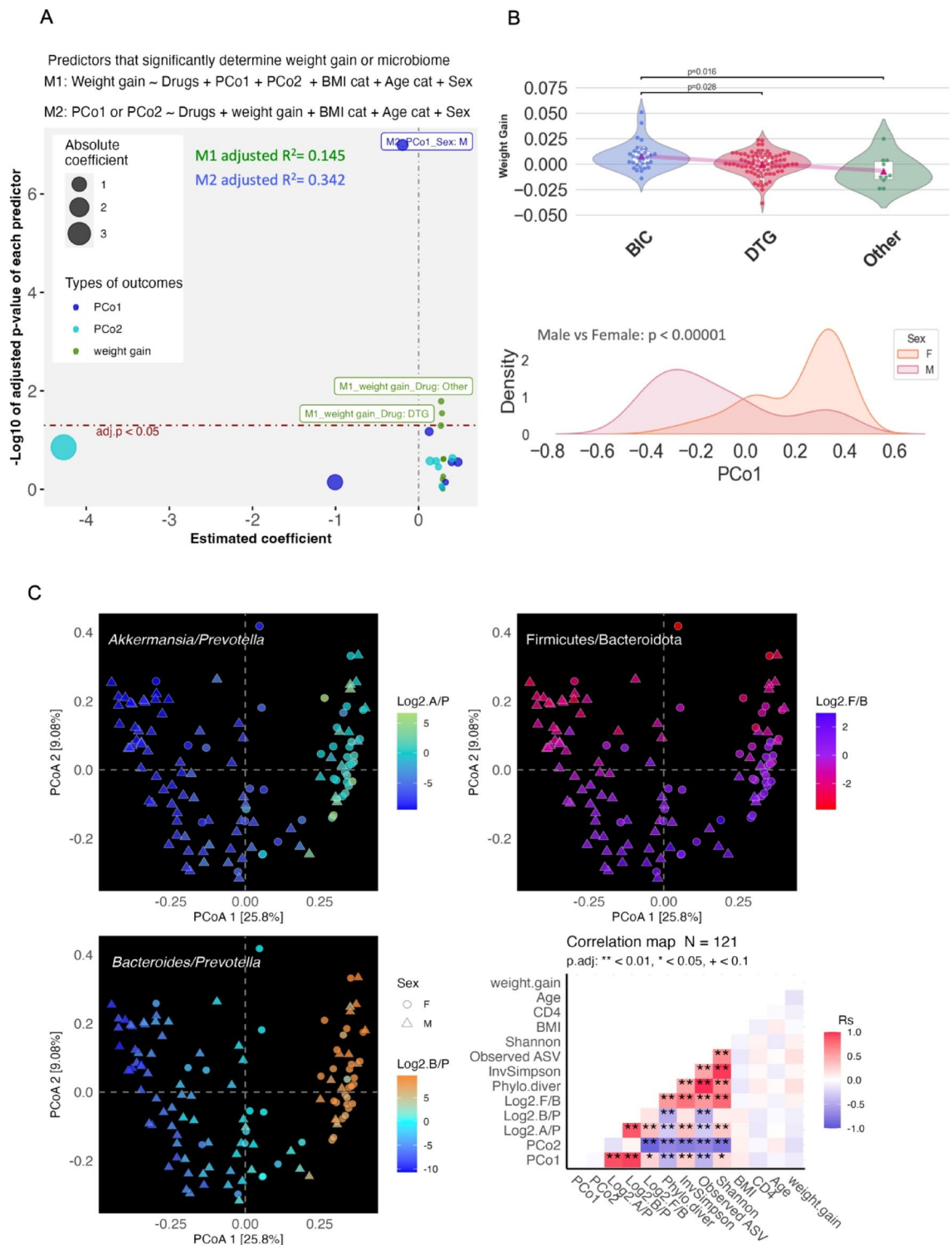
While constructing the models with anchor treatment categories, BMI, age categories, sex, and ethnicity, we noticed a high collinearity between sex and ethnicity. This necessitated the separation of these variables into different models, which in turn allowed us to compare their respective impacts on the microbiome (Figure S3A, Table S5). We found no difference in microbiome diversity either between “gain” and “lose” groups or between different drugs or age groups; however, both sex and ethnicity showed significant effects (Figure S3B, Table S3 and S5). Notably, sex was a better predictor of outcomes than ethnicity as indicated by its inclusion in a greater number of models with superior Akaike information Criterion value (Figure S3A, Table S5). Further evaluation using PERMANOVA analysis on the Bray–Curtis distance of the microbiome underscored the significance of both sex and ethnicity ($p = 0.001$ and $p = 0.007$, respectively); however, sex explained the variability of microbiome 4 times more effectively than ethnicity, based on their respective R^2 value (0.0857 vs 0.02) (Figure S1, Table S6). Consequently, we decided to exclude ethnicity in the subsequent analyses, and conclude that weight gain did not show significant association with either bacterial community composition or key bacterial ratios.

Associations between weight gain or anchor drug and individual taxa

Although weight gain and anchor drugs did not show any association to bacterial diversities, we postulated that they might be associated with specific taxa. Therefore, we applied multiple linear regression models to determine whether some individual taxa (at the ASV and genus levels) were associated either with the use of DTG or BIC (first model), or with weight gain (second model). Due to its limited size, the group consisting of non-DTG non-BIC containing regimens was excluded from the analysis.

In the first model, after correction for weight gain, sex, age and BMI categories, we found three taxa to be significantly overrepresented under specific anchor drugs. *Christensenellaceae R-7 group* (asv.199) and an uncultured bacterium (asv.925) were found to be significantly (nearly 8 to tenfold) higher in relative abundance between DTG- and BIC-treated participants (taxa further referred as DTG-taxa). Oppositely, *Haemophilus* sp. (asv.116) was about two-fold higher in relative abundance on average (BIC vs DTG) (taxa further referred as BIC-taxa) (Fig. 3A, Figure S4A, and Table S7).

In the second model, after correction for anchor drug (DTG or BIC), sex, age and BMI categories, we found 14 taxa to be associated either positively or negatively with weight gain, and they did not overlap with taxa identified in the first model. *Dialister* (genus.995), *UCG-002* sp. (asv.32), an uncultured organism (asv.74), *UCG-002* sp. (asv.109), metagenome (asv.186), and an uncultured bacterium (asv.397) were found to be positively associated to weight gain (taxa further referred as positive weight-gain taxa), while *incertae sedis* (genus.369),



Colidextribacter (Genus.362), *Ruminococcus* (genus.348), *Dysosmobacter* (Genus.62), *Dysosmobacter* sp. (asv.62), *Parabacteroides* sp. (asv.510), *Ruminococcus torques* group sp. (asv.631), and gut metagenome (asv.612) were found to be negatively associated with weight gain (taxa further referred as negative weight-gain taxa) (Fig. 3B and Table S7).

Considering that *Dysosmobacter* sp. (asv.62) is a likely health-beneficial bacteria which could be negatively associated with BMI^{23,24}, we tested this association by linear regression. We found that *Dysosmobacter* sp. (asv.62) is negatively associated to BMI ($p < 0.001$). Additionally, we validated our model by confirming a significant negative association between *Dysosmobacter* sp. (asv.62) and BMI ($p = 0.023$, $R^2 = 0.145$) even after excluding from the analysis participants with the three highest abundances of *Dysosmobacter* sp. (asv.62), whose inclusion could have disproportionately driven the relationship (Figure S4B).

These preliminary findings indicate that while anchor drugs and weight gain may not significantly impact overall microbiome diversity, they could be associated with specific taxa. To delve deeper into their effects on

◀ **Fig. 2.** Exploring the relationship between weight gain, microbiome, treatments and host characteristics: Sex, Age, and BMI. (A) Multiple linear regressions were performed to determine the association between weight gain, microbiome (beta-diversity: Pco1 & Pco2), drugs (DTG, BIC, Other) Sex, BMI category (normal, overweight, obese), Age category (old > median, young ≤ median; median = 51 years old). A volcano plot visualizes all predictors based on their adjusted p-value ($-\log_{10}$) and coefficients. Significant threshold is adjusted p-value < 0.05. (B) Significant results from volcano plot are further illustrated in the violin plot (weight gain in different treatments) and density plots. Pco1 & Pco2 in Bray–Curtis distance were used to represent the variability of microbiome. Difference between male & female in only Pco1 are presented in density plots. (C) Principal coordinate analyses of all samples (n = 121) based on Bray–Curtis distance metrics (Pco1 & Pco2) showing different clusters of bacteria regardless of treatment groups. Male and female samples are different in shape while color gradients represent the continuous data of log2 ratios of *Akkermansia/Prevotella*, *Firmicutes/Bacteroidota*, *Bacteroides/Prevotella* respectively. Spearman's correlation (Rs) heatmap between host's characteristics (BMI, CD4 + T-cell count, age, weight gain), bacterial diversities (Pco1, Pco2, alpha diversity indices: Phylo.diver, InvSimpson, Observed ASV, Shannon) and diversity associated-bacterial ratios.

the host microbiome, we computed Spearman's correlation inter-species matrices. These matrices assessed the relationships between DTG-taxa, BIC-taxa, weight gain, positive weight-gain taxa and negative weight-gain taxa (Figure S4C). DTG-taxa and BIC-taxa exhibited significant correlations with some specific taxa from both positive weight-gain taxa and negative weight-gain taxa groups. Given that weight gain was different between BIC and DTG treatment groups and drug-associated taxa formed a complex but clear pattern of association with both positive and negative weight-gain taxa, we hypothesized that these taxa together could determine the weight-gain level. To test this hypothesis, we trained linear prediction models with cross-validation on 75% of all samples and tested the performance of 2 different models: model 1 included all DTG-, BIC-, positive and negative weight-gain taxa except asv.74 and genus.62 because of collinearity, while model 2 included all taxa in model 1 plus sex, age, and BMI categories. Our results show that model 1 using only taxa predicted the weight gain better with a lower root mean square error (RMSE) of 0.0126 and a higher R^2 of 0.159 (Fig. 3C). Predicted weight gain determined by the second model did not significantly correlate to the observed values ($p = 0.0502$), suggesting that sex, age and BMI categories are not very informative to predict weight gain. Individual contribution of each taxon in the model 1 was illustrated in Fig. 3D.

In summary, our analyses have revealed contrasting interactions between four groups of bacteria, each characterized by their associations with either anchor drugs or weight gain. More importantly, our findings suggest that a set of core taxa could help to predict the magnitude of weight gain of one year taking DTG or BIC for PLHIV.

Identifying contributing factors to the abundance of microbial taxa associated with weight gain.

In our previous analyses, we found that while some taxa have a positive effect on weight gain, others have a negative impact, and not every individual experience weight gain. Despite this variation, our bacterial collection can successfully predict the level of weight gain. Therefore, understanding the factors that influence the abundance of these taxa is crucial. To this end, we employed LASSO regression for feature selection, aiming to identify key determinants of the abundance of positive and negative weight-gain taxa. This approach included the following predictors: weight gain, sex, age category, BMI category and DTG- and BIC-taxa. The significant predictors and their coefficients for each positively and negatively weight-gain-associated taxa are detailed in Table S8.

For visualization purposes, only taxa with coefficient larger than 10% of the largest absolute value of coefficient in the linear prediction model will be visualised using their coefficients (size effect) (Fig. 4). Overall, besides weight gain, DTG- and BIC-taxa played an important role in influencing the abundance of all dependent variables. Our model indicates a trend where all predictors are negatively associated with the abundance of negative weight-gain taxa while positively associated with the abundance of positive weight-gain taxa. It appears that DTG-taxa decrease the abundance of all negative weight-gain taxa. However, BIC-taxa (asv.116) not only contributed in a lesser proportion but also do not always follow this pattern; in some cases, it shows a positive contribution to negative weight-gain taxa such as *Ruminococcus* (genus.348) and *incertae sedis* (genus.369). Concerning *Dysosmobacter* sp. (asv.62), our analysis showed that DTG- and BIC-taxa, together with weight gain, have a negative impact on its abundance. Moreover, comparing to those with normal BMI, being overweight seem to have negative impact on the abundance of *Dysosmobacter* sp. And this finding is in line with the non-HIV population²³. These findings provide information to understand the link between host characteristic and specific bacteria which are likely to contribute to the weight gain.

Discussion

Recent studies have established a link between the use of second-generation INSTIs, such as DTG and BIC, and weight gain in PLHIV^{4,25}. A noteworthy percentage of individuals in the current study experienced weight gain within the first year of starting these medications. The mechanisms driving this weight gain are not fully understood but are believed to involve complex interactions with other medications, particularly TAF, which may independently contribute to weight changes^{26,27}. In addition to drug-related factors, HIV-specific considerations play a role in weight dynamics. The 'return-to-health' effect, a phenomenon observed after the initiation of an effective HIV treatment, leading to viral suppression and immune recovery, can result in weight gain. This is particularly evident in individuals with advanced HIV status, where weight increase follows the improvement of

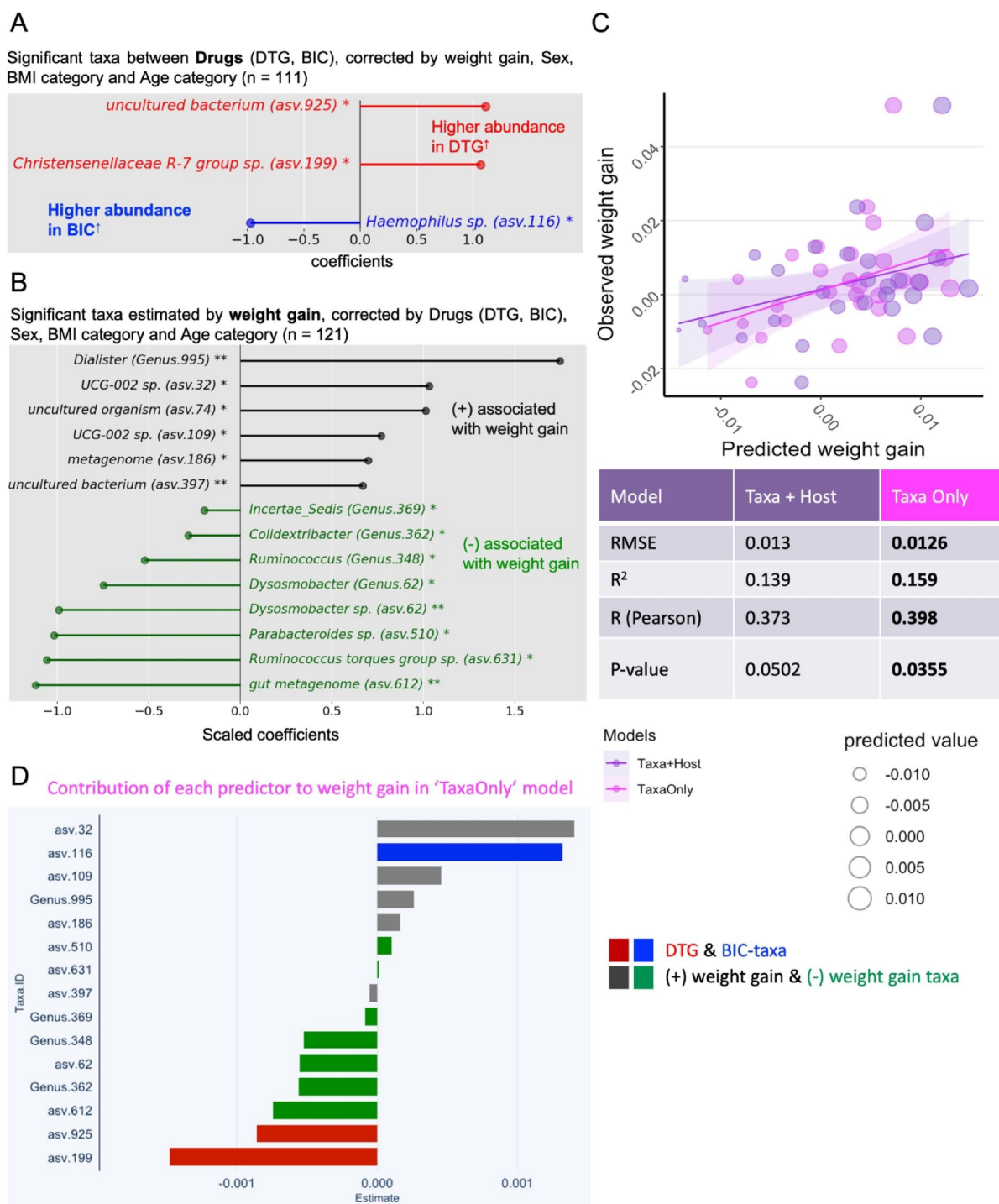


Fig. 3. Interactions between Integrase Strand Transfer Inhibitors-associated weight gain and specific taxa of gut microbiome. Lollipop plots of significant taxa and their coefficient (**A**) or scaled coefficient (**B**) obtained from multiple-linear regression models in which confounders (sex, BMI and age categories) were controlled. Ten subjects taking “other” drugs than DTG or BIC were excluded in the analysis resulting the sample size of 111 in (**A**). (**C**) a scatter plot of linear regression between predicted weight gain and its actual observed value. Purple and pink colors represent 2 different prediction linear models: (1) Taxa only model includes all taxa found in lollipop plots (**A** & **B**) excluded asv.74 and genus.62 because of multi-collinearity; (2) Taxa + Host model includes all taxa in the first model and host’s characteristics (sex, BMI and age categories). RMSE and the R² estimated for each model are provided. Pearson’s correlation between the observed and predicted value for each model are calculated (N = 28). (**D**) Illustrating the contributing effect of each taxa in predicting weight gain (supporting information for Fig. 2D). Estimates (effect size) obtained from trained model with 93 samples (~75%). Different colors present the groups of different taxa.

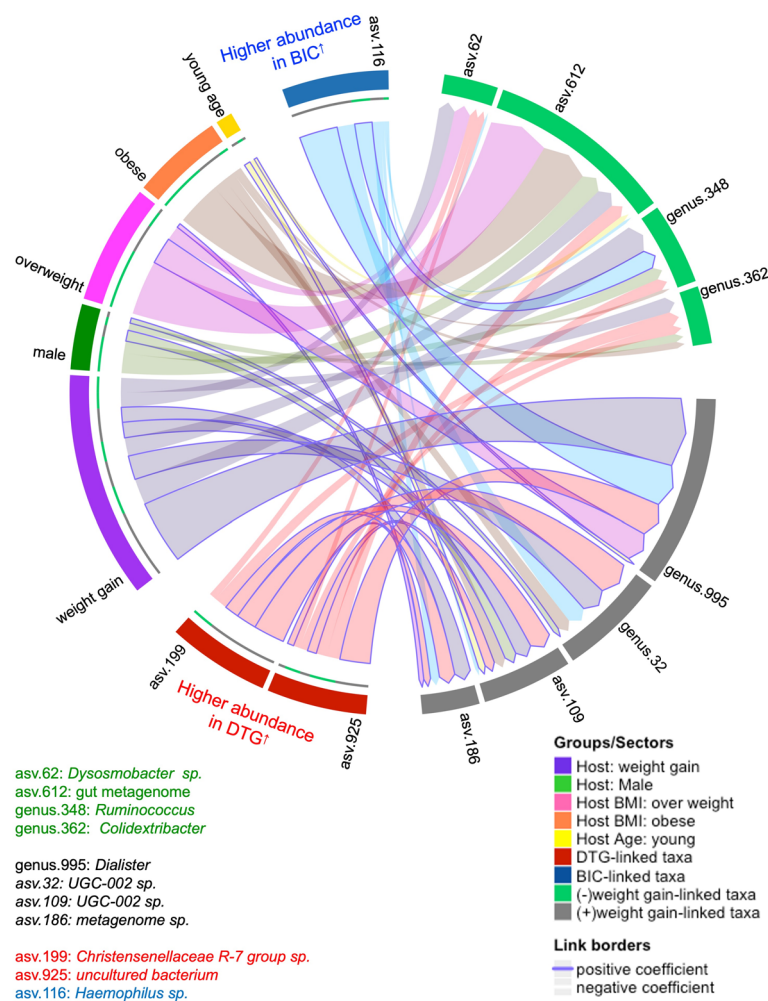


Fig. 4. A chord diagram illustrating the proposed relationship between weight-gain associated taxa and each predictor by their coefficient obtained from LASSO. The arrows distinguish a dependent variable (end-point) from their predictors. Larger width of the arrow represents a larger effect of the predictor on the dependent variable. With or without a slate blue border distinguishes positive and negative coefficient of the predictor, respectively. One dependent variable is explained by a set of best predictors selected by LASSO. There are 3 main groups/sectors of predictors: (1) Host (weight gain, sex: Male, BMI: overweight/obese, Age: young), (2) Taxa with higher abundance in dolutegravir treatment group compared to bictegravir treatment group (Red group: asv.199; asv.925), (3) Taxa with higher abundance in bictegravir treatment group compared to dolutegravir treatment group (Blue group: asv.116). There are 2 main groups/sectors of dependent variables: (1) positively weight gain-associated taxa (palegreen group: asv.62: *Dysosmobacter* sp.; asv.612: gut metagenome; genus.362: *Colidextribacter*; genus.348: *Ruminococcus*), (2) negatively weight gain-associated taxa (black group: genus.995: *Dialister*; asv.32: *UGC-002* sp.; asv.109: *UGC-002* sp.; asv.186: metagenome sp.). Only taxa with coefficient larger than 10% of the largest absolute value of coefficient in the linear prediction model will be visualised (S2-C). Abbreviations: ASV, amplicon sequence variant; BIC, bictegravir; DTG, dolutegravir; LASSO, least absolute shrinkage and selection operator; BMI, body mass index.

immune function. However, this does not fully account for the observed weight changes²⁸, and weight gain has also been demonstrated after treatment shifts in virologically suppressed²⁹.

The introduction of cART not only reverses CD4 depletion but also diminishes HIV-related immune activation. These effects might have a stronger influence on weight with INSTIs due to the potent action of these drugs^{30–32}. Furthermore, INSTIs are linked to changes in adipose tissue function, evidenced by increased adipogenesis, fibrosis, mitochondrial dysfunction, and insulin resistance^{26,33,34}. Particularly, a shift to DTG in virologically suppressed individuals has been associated with reduced levels of adiponectin, a hormone involved in regulating glucose levels and fatty acid breakdown³¹. Some emerging data from large cohort studies also suggest that INSTIs could heighten the risk of developing metabolic syndrome and diabetes, underscoring the need for ongoing research into these side effects^{35,36}. This complex interplay of drug effects, immune recovery, and metabolic changes highlights the importance of monitoring and managing weight gain and metabolic health in PLHIV undergoing INSTI-based therapy.

Therefore, this study aimed to elucidate the interplay between weight gain, microbiome, and antiretroviral drugs in PLHIV. We found that among a large cohort of virologically suppressed PLHIV, the received antiretroviral regimen comprising DTG or BIC did not appear to have a significant impact on gut bacterial communities.

In our study, we found no significant differences in gut microbiome diversity based on the anchor drug, although some compositional changes were noted. This observation is likely influenced by the close relationship between DTG and BIC. Unfortunately, our study mainly included INSTI-treated patients and may have been underpowered to comprehensively assess the effect of INSTIs compared to non-INSTI based regimens. However, previous cross-sectional studies showed that patients treated with INSTI exhibit gut microbiomes with bacterial diversity similar to that of uninfected controls, albeit with distinct compositional variations^{37,38}.

Despite those drugs having seemingly a limited effect on gut microbiome diversities, receiving either DTG or BIC was associated with an overrepresentation of several specific taxa. We also identified two other groups of taxa that were negatively and positively associated with weight gain. Putting all these fifteen microbial taxa in a machine learning model, we found them capable of prognosticating weight gain, irrespective of sex, BMI, and age. The outcomes suggest that negative weight-gain taxa and those identified as DTG-taxa might negatively influence weight accretion, whereas BIC-taxa and positive weight-gain taxa could facilitate this process.

Among the taxa found to be negatively associated with the weight gain under treatment, some have previously been associated with favourable host metabolism, thus supporting the hypothesis that microbiome could play a role in the weight gain, rather than being seen as a bystander or a consequence of the weight gain. Particularly, *Dysosmobacter* sp. (asv.62) was ranked second in terms of its predictive value within the model, suggesting that an increased prevalence of this taxon is inversely related to weight gain. This phenomenon might be elucidated by the observation that the DNA sequence of this ASV exhibits a complete match (100%, spanning 253 base pairs) with the V4 region of the *Dysosmobacter welbionis* J115 16S rRNA gene, a bacterium known for butyrate production which is implicated in the attenuation of brown adipose tissue whitening and the enhancement of mitochondrial activity^{23,24,39}. Furthermore, this bacterium is more abundant in prebiotic responders associated with BMI reduction and better than metformin in improving glucose tolerance in mice model²⁴. Another significant finding concerns *Haemophilus* sp. (asv.116), which emerged as the second strongest positive predictor of weight gain (Fig. 3D). This aligns with extant literature that delineates a positive correlation between the *Haemophilus* genus and parameters of adiposity and adult weight gain⁴⁰. Furthermore, elevated abundances of this genus have been more frequently associated with obesity compared to normal weight in a study focused on Chinese children⁴¹. Consequently, *Dysosmobacter* sp. and *Haemophilus* sp. warrant consideration as important components in elucidating the microbiome's contributory role to weight gain in future research endeavours.

Compiling these observations, individuals in the BIC group exhibited a pronounced weight gain and a higher prevalence of *Haemophilus* sp. (asv.116—the sole significant taxon) relative to counterparts in the DTG group. Additionally, this taxon's positive association with weight gain through our prediction model enhances our comprehension of the microbiome's influence on elevated weight gain levels in subjects administered BIC over a one-year period, compared to those receiving DTG. These insights suggest potential avenues for further investigation, particularly in assessing the predictive capability of these bacterial taxa concerning weight gain in individuals prior to the administration of DTG or BIC treatments.

The link between obesity and gut microbiome has been widely accepted⁴² paving a way to combat obesity by modulating gut microbiome. Regarding INSTI-associated weight gain, understanding the positive and negative weight-gain taxa is an important step in the comprehension of this adverse drug reaction that could eventually lead to interventional mitigation strategies. Therefore, we applied LASSO method to screen for significant factors that impact on the abundance of each taxon. In general, beside weight gain, DTG-taxa group is the only factor contributing to the abundance of all negative and positive weight-gain taxa. Host's characteristics (BMI, sex, age) either have small effect or contribute to a few numbers of taxa such as *Dialister*. This genus was found in a higher abundance in PLHIV than in HIV negative ones^{43–45}. The expansion of this genus has also been linked to adiposity in rheumatoid arthritis patients⁴⁶.

Our cohort exhibited a wide range of ethnic backgrounds, which were not evenly distributed by sex. This prompted us to investigate the effect of ethnicity on the composition of the microbiome. The influence of ethnic origin on microbiome diversity is well-documented, with environmental influences such as diet likely playing a more significant role than genetic factors^{47,48}. While our analysis identified an association between ethnicity and the microbiome, ethnicity did not emerge as the primary determinant of microbiome diversity within our cohort. Instead, we observed pronounced differences in gut microbiome diversity and composition based on sex. Despite the minimal impact on weight gain-associated taxa, sex significantly influences the bacterial community composition, a finding that might be related to the fact that 69% of males included in this study were men who have sex with men. Indeed, while we decided to study sex and ethnicity based on previous findings indicating that both can affect the degree of weight gain experienced during INSTI treatment^{4–6}, it is important to consider that sexual orientation has been well described as an important factor influencing microbiome independently of HIV⁴⁹.

Our study has limitations, including its cross-sectional design precluding causality inference; the unequal size of the different subgroups; the fact that weight was measured during routine clinical follow-up (with exact weight at drug initiation being interpolated based on available measures); the absence of a healthy control group; and a lack of dietary, fat mass and visceral fat data, all affecting the understanding of weight gain, treatments, and HIV impact on microbiome. Future longitudinal studies will be crucial to decipher HIV-related and drug class effects on the microbiome, and should take opportunity from the use of the INSTI drug cabotegravir as monotherapy for HIV pre-exposure prophylaxis. Importantly, our study focused on the influence of INSTIs and did not address the role of TAF, which will deserve specific attention in future studies. Confounding factors, including unconsidered medications like metformin and statins, and varied patient characteristics, might have influenced our findings. The variable and in some cases prolonged time elapsed between drug start and stool

collection (as detailed in Table 1) should also be taken into account. Eventually, although this subgroup was only considered in a subset of analysis, the limited size of the non-INSTI treated cohort and the fact that it consisted of patients displaying a specific phenotype (previous neuropsychiatric adverse events leading to DTG discontinuation) could have introduced some unexpected biases in the analysis. Despite these limitations, our research suggests specific taxa are linked to weight gain post-treatment.

In conclusion, this study found no significant impact of the received antiretroviral anchor agent (DTG, BIC or other) on gut bacterial communities, but identified several specific taxa differentially represented between DTG and BIC treated participants, as well as several taxa associated with the level of weight gain after initiation of those antiretroviral agents. Our findings link INSTIs, weight gain, and the gut microbiome. Future research should investigate the causal role of the identified taxa to improve our understanding of microbiome-drug interactions and could further support personalized antiretroviral strategies.

Data availability

16S rRNA gene sequencing data have been uploaded to the NCBI's Short Read Archive and are accessible at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1091927>. There is no new software or programming package developed as the results of this study. Supplemental and data source will be deposited to <https://zenodo.org/>.

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Author contributions

Conceptualization: JDG, LB, VH and PDC. Methodology: JDG, LB, VH, and PDC. Project administration: JDG, LB and VH. Funding acquisition: VH and PDC. Clinical work: JDG, LB, JCY, AV and BV. Biostatistical and Computational analysis: NKN. Management of samples and acquisition: JDG; DNA extraction: AP, MVH. Sequencing, meta, clinical data curation and data source depositing: NKN & JDG; Visualization: NKN; Writing—original draft: JDG and NKN; Writing—review and editing: NKN, JDG, MVH, LE, VH, and PDC. All authors read and approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate

The study received approval by the local Ethical committee (*Comité d'éthique Hospitalo-facultaire* from Cliniques universitaires Saint-Luc-UCLouvain; 2020/30NOV/593) and regulatory authorities (Eudra-CT Number 2020-001103-17). All participants gave written informed consent before taking part in the study. All methods were performed in accordance with the relevant guidelines and regulations.

Clinical trial

The trial is registered with ClinicalTrials.gov, NCT04805944 (registration date 2021–03–16).

Competing interests

PDC is inventor on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. PDC was co-founder of The Akkermansia Company SA and Enterosys. The

other authors declare no competing interests.

Additional information

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