CURRENT OPINION

Impact of antiretroviral drugs on the microbiome: unknown answers to important questions

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Purpose of review
Little is known on how different antiretroviral (ARV) drugs affect the gut microbiome in HIV infection; and conflicting data exists on the effect of ARV drugs on residual inflammation/immune activation and microbial translocation.

Recent findings
Gut microbiome involvement in the transmission and pathogenesis of HIV infection is increasingly being recognized. Various studies have shown that antiretroviral therapy (ART) is unable to restore gut health despite effective suppression of plasma HIV viremia. Indeed, the resolution of residual inflammation and gut microbial translocation is partial under ART. Very recent studies have provided new evidence that ARV combinations can differentially affect the gut microbiome, immune activation and microbial translocation. Furthermore, a recent article uncovered a link between drug metabolism and specific microbial species indicating that microbes can directly metabolically degrade ARV drugs when administered topically.

Summary
There are still many unanswered questions regarding ARVs and the gut microbiome. It is, therefore, critical for researchers to address the effect of distinct ARV drugs on the microbiome and vice versa: the effects of the microbiome on ARV drug metabolism, and speculate about possible therapeutic avenues.

Keywords
antimicrobial properties, antiretroviral drugs, butyrate-producing bacteria, gut microbiome, microbial dysbiosis

INTRODUCTION

Over 19 million people living with HIV worldwide are currently on antiretroviral therapy (ART) [1]. The main objective of ART is to suppress HIV viral replication to levels that are undetectable in peripheral circulation (i.e. virally suppressed) and improve immune function (in the best case scenario; normalization of the CD4$^+$ T-cell count in blood to near-normal levels). Certainly, ART has single-handedly changed the face of HIV infection from a progressive deadly infection to a chronic, mostly manageable condition. To date, six distinct classes of antiretroviral (ARV) drugs are currently approved by the Food and Drug Administration (FDA) for the treatment of HIV-infected individuals and these are: nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, integrase strand transfer inhibitors (INSTIs), chemokine receptor antagonists and fusion inhibitors (Tables 1 and 2, [2■]). Each ARV drug targets a specific phase of the HIV replication cycle from entry to reverse transcription to integration and finally maturation. Current guidelines for ART initiation can be found at www.aidsinfo.nih.gov; briefly, all HIV-infected individuals should start ART immediately upon HIV diagnosis independently of their circulating CD4$^+$ T-cell counts [3,4], and the recommended first line ART regimens are based on INSTIs or boosted protease inhibitor regimens with two NRTIs (Tables 1 and 2). Alternate options are available and are based on NNRTIs or boosted protease inhibitors with two NRTIs. Despite these advances, HIV-associated morbidities remain a great concern in the post-ART era; in particular, with the onset of noncommunicable diseases (NCD);

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KEY POINTS

- ART does not completely reverse the damage caused to the gut mucosa epithelia during early HIV infection nor does it completely restore HIV-associated microbial dysbiosis.

- ARV drug combinations, commonly used as first line ART regimens in most countries, have differential effects on the gut microbiome and markers of microbial translocation, inflammation/immune activation and gut epithelial barrier damage; with ART-experienced individuals on INSTIs being favored and on protease inhibitors being worst off. Short-term and long-term clinical implications are still to be determined and warrant further investigation.

- Despite effective ART and across ARV drug combinations, key commensal species including butyrate-producing bacteria remain depleted in treated HIV-infected individuals; possibly contributing to the lack of epithelial barrier repair and delaying the return of gut homeostasis; and allowing for continued microbial translocation and residual immune activation.

- Research should aim to incorporate systems biology approaches to fully understand the compositional and functional changes associated with HIV infection itself, with ART initiation and different ARV combinations, whilst controlling for all confounding factors, so as to identify the immunologically and inflammatory relevant microbiome and biomarkers of genuine dysbiosis that could be intervened against.

specifically, kidney and liver disease, cardiovascular complications, neurologic disorders, and malignancies (non-AIDS cancers); these ultimately affect not only the quality of life of treated HIV-infected individuals but also reduce their life expectancy [5,6[-],7].

Much interest and considerable advances have been made in understanding the contribution of microbial translocation, the passage of microbes and microbial products to the systemic circulation; and the gut microbiome, the microbial communities that inhabit our intestines and their genes and metabolites, to HIV-associated comorbidities [8–11]. Interestingly, strong associations have been made between dysbiotic or altered microbiomes and a range of diseases fueled by chronic inflammation, echoing what is seen in HIV infection [12[-],13]. Indeed, the microbiome involvement in the transmission and pathogenesis of HIV infection is being acknowledged [8,14,15,16[--],17], although findings and interpretation of results diverge quite significantly between studies [18[--]], in part because of cohort effects, sampling biogeography and perhaps most importantly because of the lack of adjustment for confounding factors, such as sexual practices [19] and diet [20]. Several recent reviews have provided up-to-date information on gut dysbiosis reported in untreated HIV infection [18[--],21]. Concerning treated HIV infection, several studies seem to indicate that ART by itself does not restore the gut microbiome of HIV-infected individuals to healthy communities comparable to HIV-uninfected

Table 1. Food and Drug Administration-approved individual antiretroviral drugs [1,2[-]]

Class of ARV drugs	NRTIs	NNRTIs	Protease inhibitors	Fusion Inhibitors	Entry Inhibitors	INSTIs
Phase of HIV replication cycle it blocks	Reverse transcription	Reverse transcription	Maturation	Entry	Entry	Integration
Protein target	HIV reverse transcriptase	HIV reverse transcriptase	HIV protease	HIV gp41	Host CCR5 co-receptor	HIV integrase
ARV drug generic name (and known acronyms)	Abacavir (ABC)	Efavirenz (EFV)	Atazanavir (ATV)	Enfuvirtide (T-20)	Maraviroc (MVC)	Dolutegravir (DTG)
	Didanosine (ddI, ddI EC)	Etravirine (ETR)	Darunavir (DRV)			Elvitegravir (EVG)
	Emtricitabine (FTC)	Nevirapine (NVP)	Fosamprenavir (FPV)			Raltegravir (RAL)
	Lamivudine (3TC)	Rilpivirine (RPV)	Indinavir (IDV)			
	Stavudine (d4T)		Nelfinavir (NFV)			
	Tenofovir disoproxil fumarate (TDF)		Ritonavir (RTV)			
	Zidovudine (AZT, ZDV)		Saquinavir (SQV)			
	Tenofovir alafenamide (TAF)		Tipranavir (TPV)			

ARV, antiretroviral; CCR5, C-C chemokine receptor type 5; FDA, Food and Drug Administration; gp41, glycoprotein 41; INSTIs, integrase strand transfer inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors.

Table 2. Current recommended antiretroviral therapy regimens[a]

ARV drug class	NRTIs	NNRTIs	INSTIs	Protease inhibitors	Pharmacokinetic enhancer	Comments
Recommended options						
INSTI-based	ABC + 3TC	–	DTG	–	–	If HLA-B∗5701 negative
INSTI-based	TDF + FTC	–	DTG	–	–	
INSTI-based	TDF + FTC	–	EVG	–	Cobicistat	
INSTI-based	TAF + FTC	–	EVG	–	Cobicistat	
INSTI-based	TDF + FTC	–	RAL	–	–	
Protease inhibitor-based	TDF + FTC	–	–	DRV + RTV	–	
Protease inhibitor-based	TAF + FTC	–	–	DRV + RTV	–	
Alternative options						
NNRTI-based	TDF + FTC	EFV	–	–	–	
NNRTI-based	TAF + FTC	EFV	–	–	–	
NNRTI-based	TDF + FTC	RPV	–	–	–	If pVL < 100 000 copies/ml and $CD4^+ > 200$ cells/mm^3
NNRTI-based	TAF + FTC	RPV	–	–	–	If pVL < 100 000 copies/ml and $CD4^+ > 200$ cells/mm^3
Protease inhibitor-based	TDF + FTC	–	–	ATV + RTV	–	
Protease inhibitor-based	TAF + FTC	–	–	ATV + RTV	–	
Protease inhibitor-based	TDF + FTC	–	–	ATV	Cobicistat	
Protease inhibitor-based	TAF + FTC	–	–	ATV	Cobicistat	
Protease inhibitor-based	ABC + 3TC	–	–	DRV + RTV	–	If HLA-B∗5701 negative
Protease inhibitor-based	ABC + 3TC	–	–	DRV	Cobicistat	If HLA-B∗5701 negative
Protease inhibitor-based	TDF + FTC	–	–	DRV	Cobicistat	
Protease inhibitor-based	TAF + FTC	–	–	DRV	Cobicistat	

[a]Based on the DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents Guidelines: https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/11/what-to-start.
INSTIs, integrase strand transfer inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors.

individuals [19,22–34]. Also, ARV drugs could promote further dysbiosis [18▪▪] and distinct ARV combinations could have dramatic effects on the gut microbiome as ARVs themselves have antimicrobial properties and conversely, specific microbes (mainly bacteria) could catabolize ARV drugs [35,36,37▪▪]. Furthermore, a nonnegligible proportion of ART-treated individuals suffer from gastrointestinal discomfort, commonly diarrhea, mostly associated with protease inhibitor-based ART regimens [38–40]. Indeed, despite vast improvements in drug tolerability and overall drug safety of contemporary drugs, different ARV combinations have different side effects and this could differentially affect how the gut microbiome responds: reduced diversity versus increased diversity, restoration versus further dysbiosis, and so on. Surprisingly, however, only a handful of articles have addressed the differential effects of ARV drugs on the gut microbiome, microbial translocation, enterocyte damage and inflammation/immune activation [29,30▪▪,31▪▪]. Improving our understanding of the impact of ART and different

ARV combinations is needed to draw a complete picture as more HIV-infected individuals will have access to ART and become ART-experienced, and as more people are using ART as preexposure prophylaxis (PrEP) in the absence of HIV infection. Here we will review the current knowledge regarding the impact of ARV drugs on the gut microbiome and what is known on understanding the mechanisms that could be at the heart of ART-induced dysbiosis.

EFFECTS OF ANTIRETROVIRAL THERAPY ON THE GUT MICROBIOME

Overall, in untreated HIV infection, most studies report an enrichment in the genus *Prevotella* and *Enterobacteriaceae*, a Gram-negative family of the phylum Proteobacteria. Importantly, these bacteria are known to be involved in microbial translocation [41] and contributing to residual immune activation [8,41,42]. Indeed, the enrichment or depletion in some key species within the microbial communities' structure of HIV-infected individuals is associated

with markers of HIV disease progression [43▪▪]. Of particular interest, the depletion of butyrate-producing bacteria (BPB) has been associated with increased microbial translocation and immune activation [43▪▪]. We recommend several recent systemic reviews for up-to-date information on the gut microbiome alterations in untreated HIV infection as it is outside the scope of this review [8,14,17,18▪▪,21,44▪]. Studies on the short-term and long-term impact of ART on the gut microbiome have convincingly demonstrated that ART is unable to consistently restore gut health. The gut microbiome of treated HIV-infected individuals shows a shift away from viremic HIV-infected individuals, but yet display a distinct community structure from HIV-uninfected individuals [19,22–34]. Findings and interpretation of results are often conflicting and vary by type of sampling used (fecal versus swabs versus mucosal biopsies), time on ART and potential methods used to extract and sequence the microbial DNA [18▪▪,19,22,24–29,34,45]. Conflicting data are further complicated by the need to control for sexual practices [19], and overall power issues driven by sample size and the use of appropriate control groups. For example, most but not all authors reported a decrease in microbial diversity, which is an independent indicator of gut microbiome restoration, and has been proposed by Nowak et al. [29] to reflect immune reconstitution. The impact of ART on the microbiome has been mostly studied in cross-sectional cohorts and have included HIV-infected individuals with many years on ART. Going forward, we need to disentangle two separate notions: ART can to some degree reverse HIV-associated gut dysbiosis as shown in most but not all studies. But the initiation of ART can also lead to a separate dysbiosis, which may be confounded by the HIV-associated gut dysbiosis and go unreported and unstudied. This could lead to differential outcomes: non-AIDS communicable disease such as cardiovascular disease, accelerated aging, cognitive defects, diabetes, elevated liver enzymes and alterations of fat deposits. Furthermore, almost all studies have exclusively focused on the bacterial component of the gut microbiome. Interestingly, it is worth mentioning that virome expansion seems to be more indicative of the immune dysfunction and could be used as a biomarker for immune reconstitution [34]. Future work should include longitudinal cohorts of HIV-individuals before and after ART initiation.

EFFECT OF INDIVIDUAL ANTIRETROVIRAL DRUGS AND ANTIRETROVIRAL COMBINATIONS ON THE GUT MICROBIOME

To date, three articles have purposefully evaluated the effects of distinct ARV combinations on the gut microbiome [29,30▪▪,31▪▪] rather than ART as a whole. The main study endpoints were to investigate the effects of distinct ART regimens on the gut (fecal) microbiome and markers of microbial translocation, inflammation/immune activation and endothelial damage with one specific question in mind: which ART combinations were best to restore the HIV-associated dysbiosis (Table 3). 16S profiles were generated and conclusions were drawn by the authors based on the analysis of a cohort of ART-treated HIV-infected individuals on different ART regimens. Interestingly, Villanueva-Millán et al. [31▪▪] showed that ART combinations tested: protease inhibitors, NNRTIs and INSTIs with NRTIs backbone, increased significantly the plasma levels of endothelial damage markers compared with HIV-uninfected controls; with protease inhibitor-based regimen showing the most endothelial damage compared with both NNRTIs and INSTIs. On the order hand, Pinto-Cardoso et al. [30▪▪] showed that ritonavir-boosted protease inhibitor-ART regimen increased endothelial damage compared with both NNRTIs and HIV-uninfected controls; whilst NNRTI-based ART damage was significantly increased compared with HIV-uninfected controls. Both authors included soluble CD14 (sCD14), a marker that is released after monocyte activation in response to lipopolysaccharide (LPS) [46] and has been shown to independently predict mortality in HIV infection [47]. Villanueva-Millán et al. [31▪▪] also used the LPS-binding protein as a surrogate marker of microbial translocation but no differences were observed. Both articles concluded that protease inhibitor-based ART combinations were more detrimental because of both microbial translocation and endothelial damage, and this was associated with increased inflammation in individuals with protease inhibitor-based regimens only [31▪▪], accentuating the idea that the least favorable ART combination was protease inhibitor-based. Both articles had limitations; mainly lack of adjustment for known confounding factors (Table 3). Taken together, however, these results provide strong evidence that ARV combinations promote differential dysbiosis leading to inflammation. One possible explanation for the differential effects of ARV drugs on the gut microbiome and markers of microbial translocation, inflammation and immune activation would be the differential penetration of ARV drugs on the gastrointestinal tissue and their pharmacokinetics. Raltegravir has been shown to penetrate faster in the gastrointestinal tract (GIT) [48]. Indeed, multiple dosing administration of Raltegravir in a cohort of 14 HIV-uninfected men penetrated rapidly into the gut-associated-lymphoid tissue (GALT; terminal ileum) and reached concentrations higher than that

Table 3. Differential effects of ARV drugs on the gut microbiome: summary of the main findings

	Nowak *et al.* [29]	Pinto-Cardoso *et al.*, 2017 [30▪▪]	Villanueva-Millán *et al.* [31▪▪]
Type of cohort	Longitudinal	Cross-sectional	Cross-sectional
Sampling	Feces and blood (plasma)	Feces and blood (plasma)	Feces and blood (plasma and serum)
Cohort	28 viremic HIV+ with pVL 3 elite controllers 9 HIV- controls	33 HIV+ on ART 10 HIV- controls	45 HIV+ on ART 5 untreated HIV+ 21 HIV- controls
Type of ART combinations and number of individuals (number per group)	NNRTIs with NRTIs (*n* = 8) RTV-protease inhibitors with NRTIs (*n* = 11)	NNRTIs with NRTIs (*n* = 18) RTV-protease inhibitors with NRTIs (*n* = 15)	NNRTIs with NRTIs (*n* = 22) Protease inhibitors with NRTIs (*n* = 15) INSTIs with NRTIs (*n* = 8)
Time on ART	10 months	5 years	13 years
Effective ART suppression	At 10-month follow-up: pVL < 40 (*n* = 15) pVL: median 60 (29–224; *n* = 4)	pVL < 40 for all ART patients	ART for at least 1 year and pVL less than 20 for at least 6 months
Markers of microbial translocation	sCD14 LPS LBP	sCD14	sCD14 LBP
Markers of endothelial damage/turnover/activation	Not included	I-FABP	I-CAM V-CAM
Markers of systemic inflammation–immune activation	IL-6 D-Dimer	IL-6 D-Dimer hsCRP % HLADR$^+$ CD38$^+$ CD8$^+$ T cell	IL-6
Effect of ARVs on alpha diversity (number of species)	No differences were observed between NNRTIs and protease inhibitors ↓↓↓ Number bacterial taxa in ART patients compared with baseline***	↓ Protease inhibitors versus controls* ↓ NNRTIs versus controls* ≈ Protease inhibitors versus NNRTIs	↓↓ Protease inhibitors versus controls ** ↓ NNRTIs versus controls * ≈ INSTIs versus controls ≈ Protease inhibitors versus NNRTIs versus INSTIs
Effects of ARVs on microbial translocation	Not assessed Negative correlation between LPS, LBP, sCD14, sCD163 and CD4$^+$/CD8$^+$ ratio	↑ sCD14 protease inhibitors versus controls* ≈ NNRTIs versus controls ≈ Protease inhibitors versus NNRTIs	↑ sCD14 protease inhibitors versus controls * ↑ sCD14 NNRTIs versus controls * ≈ sCD14 INSTIs versus controls
Effects of ARVs on gut microbiome	↓↓ In Firmicutes: *Lachnospira* spp.***, *Oribacterium* spp.***, *Oscillospira* spp.**); ↓↓ in proteobacteria (*Sutturella* spp.**) and ↓↓ in bacteroidetes (*Prevotella* spp.***) after ART initiation	Differential clustering of gut microbiome with ART regimens (Adonis R2 = 10.37%***) ↓↓↓ *Ruminococcaceae* family (including *Faecalibacterium prausnitzii*) OTUs in HIV+ on ART versus controls	↑ Proteobacteria in ART versus controls ↓ Firmicutes in protease inhibitors versus controls* ↓ Number of bacterial species in protease inhibitors versus controls* 13 genera depleted (↓↓↓) in protease inhibitors versus controls, against 7 for NNRTIs and 6 for INSTIs INSTIs cluster inside the control cluster ↓↓↓ F. prausnitzii in protease inhibitors versus controls*** ↑↑↑ *Desulfovibrio* spp. and *Blautia* spp. in INSTIs versus controls*** ↑↑ *Pseudomonas* spp. in NNRTIs versus controls**
Effects of ARVs on systemic inflammation and immune activation	No correlation between IL-6 and D-dimer and observed bacterial species	≈ Protease inhibitors versus NNRTIs ≈ Protease inhibitors versus controls ≈ NNRTIs versus controls	↑ IL-6 protease inhibitors versus controls**
Effects of ARVs on endothelial damage/turnover/activation	Not assessed	↑↑↑ I-FABP protease inhibitors versus controls *** ↑↑ I-FABP protease inhibitors versus NNRTIs ** ≈ NNRTIs versus controls	↑ I-CAM NNRTIs versus controls* ↑ I-CAM INSTIs versus controls* ↑↑ I-CAM protease inhibitors versus controls** ↑↑ V-CAM protease inhibitors versus controls***
Main findings and conclusions	Bacterial diversity correlated positively with CD4$^+$ T-cell counts and negatively with markers of microbial translocation and monocyte activation	Long-term ART does not restore richness of the gut microbiome BPB are depleted in treated HIV infection Absence of BPB correlates with increased endothelial barrier damage	INSTIs with NRTIs ART combination restores the richness of the gut microbiome to normal levels (control group)
Strengths	Longitudinal study	Dietary assessment	Inclusion of INSTIs in ART cohort Co-infection with HCV and HBV
Limitations acknowledged by authors	Did not control for diet Lack of intestinal biopsies to corroborate findings in feces Control group not matched for ethnical background	Did not control for sexual practices Absence of untreated HIV+ individuals Small number of HIV- individuals	Did not control for confounding factors (HIV acquisition, diet)

Symbols to denote a significant increase (↑) or decrease (↓) or no differences (≈) were used. The asterisks (*), (**), (***) are used according to the *P*-values, *P* < 0.05, *P* < 0.01 and *P* < 0.001, respectively, as reported in the individual manuscripts.

ART, antiretroviral therapy; ARV, antiretroviral; BPB, butyrate-producing bacteria; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV+, HIV-infected; HIV-, HIV-uninfected; hsCRP, high-sensitivity C-reactive protein; I-CAM, intercellular adhesion molecule; I-FABP, intestinal-fatty acid-binding protein; IL-6, interleukin 6; INSTIs, integrase strand transfer inhibitors; LBP, LPS-binding protein; LPS, lipopolysaccharide; NNRTIs, nonnucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; pVL, plasma viral load (copies/ml); RTV, ritonavir; sCD14, soluble CD14; V-CAM, vascular cell adhesion molecule.

of blood and plasma. Furthermore, novel work by Hladik et al. [49] demonstrated that ARV drugs may have direct effects on inducing inflammation and epithelial damage at mucosal sites. Interestingly, INSTI-based regimens have shown greater propensity to decrease inflammation compared with NNRTIs [50]. On the other hand, bacteria that maintain epithelial health and immune homeostasis, for example, by providing short-chain fatty acids (SCFAs) such as butyrate, have been consistently found to be depleted in HIV-infected individuals on ART [30▪▪,31▪▪,43▪▪]. Butyrate is a metabolite produced in the colon by a subset of gut commensal bacteria, the BPB, through the fermentation of non-digestible carbohydrates [51]. Butyrate is utilized by the host, and is the main energy source for the colonocytes. Of the many SCFAs, butyrate and propionate have been shown to have the most health-promoting functions [52▪]. Interestingly Dillon et al. [43▪▪] confirmed these observations and further showed that butyrate is essential for the prevention and repair of the intestinal epithelial barrier in the context of HIV infection. Collectively, these studies indicate that interventional therapies to prevent and recover disrupted homeostasis should include the repopulation of the gut with BPB.

PHARMACOMICROBIOMICS: STUDY OF DRUG–MICROBIOME INTERACTIONS

Microbiome composition at mucosal sites where HIV is first encountered may have significant impact on early HIV infection, and therefore, disease progression. Indeed, despite very early ART treatment in HIV-infected individuals, dysbiosis still occurs and persists [53]. Although it is accepted and well studied that bacteria can metabolize dietary products and produce key metabolites such as vitamins and SCFAs, metabolism of other compounds, such as drugs, has not overtly been studied, despite the remarkable ability for bacteria to metabolize many xenobiotic compounds [36]. Many studies have demonstrated that several subgroups of bacteria possess enzymes, or enzyme analogs, that are known to play a role in drug pharmacokinetics and metabolism [54–60]. Metabolism and/or biodegradation, however, of drugs by bacteria and how this contributes to human health has remained understudied. Recent studies have begun to provide important information demonstrating that gut microbes can affect the efficacy of several drugs [61]. Klatt et al. [37▪▪] recently demonstrated that the microbiota in the female reproductive tract (FRT) can directly metabolize the ARV, tenofovir, and the presence of these bacteria (Gardnerella vaginalis) was associated with decreased efficacy of topical PrEP in women [62▪]. Furthermore, they showed that classes of bacteria in the FRT that are also commonly found in the gut, such as Prevotella spp. and Escherichia coli, can metabolize tenofovir, indicating that ARVs may be impacted by gut bacteria [37▪▪]. Interestingly, oral PrEP efficacy does not seem to be affected in adherent women with vaginal dysbiosis as defined by Nugent scoring, however, this should be further investigated in larger studies [63▪▪]. This highlights the need to better understand the pharmacokinetics of oral and non-oral ARV drugs, fully characterize the microbiome (in particular, the rectal, penile and vaginal microbiome for non-oral ARV drugs) and understand how microbial communities affect the ARV drug metabolism either locally (genital microbiome) or systemically (gut microbiome). This is of a particular interest for microbicide-based HIV prevention strategies [64,65]. For the moment, however, these important questions remain unanswered, and studies examining the role of microbiome on ARV drug metabolism in HIV-infected individuals or in the context of PrEP are warranted.

CONCLUSION

In the past 5 years, the importance of the gut microbiome in HIV infection has been recognized, with several studies describing distinct levels of dysbiotic gut microbial communities associated with HIV infection and ART. Little attention, however, has been paid to the impact of distinct classes of ARV drugs on the microbiome and how gut microbes could impact ARV drug metabolism. Recent articles seem to indicate that both issues are important but many critical questions are still answered. Differential effects of ARV drugs on the gut microbiome, if confirmed and further studied, could warrant specific microbiome-targeted therapies depending on the ART regimen administered.

Conflicts of interest

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest
■■ of outstanding interest

1. UNAIDS, Global AIDS update − 2016. Joint United Nations Programme on HIV/AIDS (UNAIDS); Geneva, Switzerland: UNAIDS; 2016.
2. Cihlar T, Fordyce M. Current status and prospects of HIV treatment. Curr Opin
■ Virol 2016; 18:50−56.
This review summarizes the current guidelines for ART initiation and discusses future directions in ART drug development.
3. INSIGHT START Study Group. Lundgren JD, Babiker AG, *et al.* Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med 2015; 373:795−807.
4. TEMPRANO ANRS 12136 Study Group. Danel C, Moh R, *et al.* A trial of early antiretrovirals and isoniazid preventive therapy in Africa. N Engl J Med 2015; 373:808−822.
5. Tenorio AR, Zheng Y, Bosch RJ, *et al.* Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis 2014; 210: 1248−1259.
6. Bandera A, Colella E, Rizzardini G, *et al.* Strategies to limit immune-activation
■ in HIV patients. Expert Rev Anti Infect Ther 2017; 15:43−54.
This review discusses the current strategies to reduce residual immune activation, from timing of ART initiation and ART intensification to reducing microbial translocation. This research area is of great importance as residual immune activation remains linked to non-AIDS comorbidities associated with aging and more and more HIV-infected individuals are starting ART early and living longer.
7. Castilho JL, Shepherd BE, Koethe J, *et al.* CD4+/CD8+ ratio, age, and risk of serious noncommunicable diseases in HIV-infected adults on antiretroviral therapy. AIDS 2016; 30:899−908.
8. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Curr Opin HIV AIDS 2016; 11:182−190.
9. Tincati C, Douek DC, Marchetti G. Gut barrier structure, mucosal immunity and intestinal microbiota in the pathogenesis and treatment of HIV infection. AIDS Res Ther 2016; 13:19.
10. Mudd JC, Brenchley JM. Gut mucosal barrier dysfunction, microbial dysbiosis, and their role in HIV-1 disease progression. J Infect Dis 2016; 214(Suppl 2):S58−S66.
11. Hunt PW, Sinclair E, Rodriguez B, *et al.* Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis 2014; 210:1228−1238.
12. Blander JM, Longman RS, Iliev ID, *et al.* Regulation of inflammation by
■ microbiota interactions with the host. Nat Immunol 2017; 18:851−860.
Excellent overview of the interplay between the host and the commensal microbiota, focusing on the effect of the microbiome on the innate and adaptive immune system and how this latter can drive inflammation.
13. Netea MG, Balkwill F, Chonchol M, *et al.* A guiding map for inflammation. Nat Immunol 2017; 18:826−831.
14. Dillon SM, Frank DN, Wilson CC. The gut microbiome and HIV-1 pathogenesis: a two-way street. AIDS 2016; 30:2737−2751.
15. Gootenberg DB, Paer JM, Luevano JM, Kwon DS. HIV-associated changes in the enteric microbial community: potential role in loss of homeostasis and development of systemic inflammation. Curr Opin Infect Dis 2017; 30: 31−43.
16. Williams B, Mirmonsef P, Boucher CA, *et al.* A summary of the first HIV
■■ microbiome workshop. AIDS Res Hum Retroviruses 2016; 32:935−941.
This systematic review discusses the main conclusions and future directions in the field of HIV and microbiome from the first HIV microbiome workshop, which took place in 2015 at the National Institute of Health. It was the first time, officially that scientists from different countries and disciplines gathered purposefully to discuss microbiome research in HIV infection. After the success of this first workshop, organizers announced follow-up 2-day yearly meetings.
17. Zilberman-Schapira G, Zmora N, Itav S, *et al.* The gut microbiome in human immunodeficiency virus infection. BMC Med 2016; 14:83.
18. Li SX, Armstrong A, Neff CP, *et al.* Complexities of gut microbiome dysbiosis
■■ in the context of HIV Infection and antiretroviral therapy. Clin Pharmacol Ther 2016; 99:600−611.
This systemic review gives an excellent overview of the current knowledge in the field of HIV and microbiome.
19. Noguera-Julian M, Rocafort M, Guillén Y, *et al.* Gut microbiota linked to sexual preference and HIV Infection. EBioMedicine 2016; 5:135−146.
20. Shanahan F, van Sinderen D, O'Toole PW, Stanton C. Feeding the microbiota: transducer of nutrient signals for the host. Gut 2017; 66:1709−1717.
21. Liu J, Williams B, Frank D, *et al.* Inside out: HIV, the gut microbiome, and the mucosal immune system. J Immunol 2017; 198:605−614.
22. Lozupone CA, Li M, Campbell TB, *et al.* Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe 2013; 14:329−339.
23. Lozupone CA, Rhodes ME, Neff CP, *et al.* HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut Microbes 2014; 5:562−570.
24. McHardy IH, Li X, Tong M, *et al.* HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome 2013; 1:26.
25. Mutlu EA, Keshavarzian A, Losurdo J, *et al.* A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog 2014; 10:e1003829.
26. Vujkovic-Cvijin I, Dunham RM, Iwai S, *et al.* Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med 2013; 5:193ra91.
27. Yu G, Fadrosh D, Ma B, *et al.* Anal microbiota profiles in HIV-positive and HIV-negative MSM. AIDS 2014; 28:753−760.
28. Dinh DM, Volpe GE, Duffalo C, *et al.* Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. J Infect Dis 2015; 211:19−27.
29. Nowak P, Troseid M, Avershina E, *et al.* Gut microbiota diversity predicts immune status in HIV-1 infection. AIDS 2015; 29:2409−2418.
30. Pinto-Cardoso S, Lozupone C, Briceño O, *et al.* Fecal bacterial communities
■■ in treated HIV infected individuals on two antiretroviral regimens. Sci Rep 2017; 7:43741.
One of two recent articles presenting data on the differential effects of two first line ARV combinations on fecal microbiome, microbial translocation, gut epithelial and immune activation. The study shows that ART-experienced HIV-infected individuals lack specific bacteria, important for the return of gut homeostasis. The study also highlights that protease inhibitor-based regimen has more microbial translocation and gut endothelial damage as compared with NNRTI-based regimen.
31. Villanueva-Millán MJ, Pérez-Matute P, Recio-Fernández E, *et al.* Differential
■■ effects of antiretrovirals on microbial translocation and gut microbiota composition of HIV-infected patients. J Int AIDS Soc 2017; 20:1−13.
This article is among one of the first to evaluate the differential effects of antiretroviral drugs and ARV combinations on the fecal microbiome of ART-experienced HIV-infected individuals and includes patients on INSTIs; showing that HIV-infected individuals on integrase inhibitors fully restore their gut microbiome richness (to level comparable with HIV-uninfected). It is also one of the first HIV-associated gut microbiome study to compare individuals with or without coinfections (HCV and HBV).
32. Sun Y, Ma Y, Lin P, *et al.* Fecal bacterial microbiome diversity in chronic HIV-infected patients in China. Emerg Microbes Infect 2016; 5:e31.
33. Vazquez-Castellanos JF, Serrano-Villar S, Latorre A, *et al.* Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. Mucosal Immunol 2015; 8:760−772.
34. Monaco CL, Gootenberg DB, Zhao G, *et al.* Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe 2016; 19:311−322.
35. Shilaih M, Angst DC, Marzel A, *et al.* Antibacterial effects of antiretrovirals, potential implications for microbiome studies in HIV. Antivir Ther 2017; doi: 10.3851/IMP3173. [Epub ahead of print]
36. ElRakaiby M, Dutilh BE, Rizkallah MR, *et al.* Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics. OMICS 2014; 18:402−414.
37. Klatt NR, Cheu R, Birse K, *et al.* Vaginal bacteria modify HIV tenofovir
■■ microbicide efficacy in African women. Science 2017; 356:938−945.
First article to put forward evidence that microbial communities in the reproductive tract of women can affect ARV drug metabolism by directly degrading the ARV drug (1% tenofovir, locally administered) before it can be metabolized by target cells. This was linked to reduced efficacy of the tenofovir microbicide gel in the CAPRISA 004 study in women with non-*Lactobacillus* spp. vaginal microbiomes. This article is extremely relevant to all microbicide-related HIV-prevention strategies.
38. Logan C, Beadsworth MB, Beeching NJ. HIV and diarrhoea: what is new? Curr Opin Infect Dis 2016; 29:486−494.
39. Dikman AE, Schonfeld E, Srisarajivakul NC, Poles MA. Human immunodeficiency virus-associated diarrhea: still an issue in the era of antiretroviral therapy. Dig Dis Sci 2015; 60:2236−2245.
40. Clay PG, Crutchley RD. Noninfectious diarrhea in HIV seropositive individuals: a review of prevalence rates, etiology, and management in the era of combination antiretroviral therapy. Infect Dis Ther 2014; 3:103−122.

41. Klase Z, Ortiz A, Deleage C, et al. Dysbiotic bacteria translocate in progressive SIV infection. Mucosal Immunol 2015; 8:1009–1020.

42. Dillon SM, Lee EJ, Kotter CV, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 2014; 7:983–994.

43. Dillon SM, Kibbie J, Lee EJ, et al. Low abundance of colonic butyrate-
■■ producing bacteria in HIV infection is associated with microbial translocation and immune activation. AIDS 2017; 31:511–521.
This article demonstrates that the absence of butyrate-producing bacteria (in specific Roseburia intestinalis) is highly detrimental for gut homeostasis in ART-naïve HIV-infected individuals; higher immune activation, more gut endothelial damage and overall enteric growth is observed in the absence of these BPB and has been linked to the absence of the main produced metabolite, butyrate.

44. Williams B, Landay A, Presti RM. Microbiome alterations in HIV infection a
■ review. Cell Microbiol 2016; 18:645–651.
Excellent systemic review on the microbiome alterations associated with HIV infection discussing important aspects of this research field: methodology, results from primate studies, HIV pathogenesis (gut microbiome) and transmission (genital microbiome).

45. Perez-Santiago J, Gianella S, Massanella M, et al. Gut lactobacillales are associated with higher CD4 and less microbial translocation during HIV infection. AIDS 2013; 27:1921–1931.

46. Landmann R, Knopf HP, Link S, et al. Human monocyte CD14 is upregulated by lipopolysaccharide. Infect Immun 1996; 64:1762–1769.

47. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis 2011; 203:780–790.

48. Patterson KB, Prince HA, Stevens T, et al. Differential penetration of raltegravir throughout gastrointestinal tissue: implications for eradication and cure. AIDS 2013; 27:1413–1419.

49. Hladik F, Burgener A, Ballweber L, et al. Mucosal effects of tenofovir 1% gel. Elife 2015; 2015:4.

50. Hileman CO, Kinley B, Scharen-Guivel V, et al. Differential reduction in monocyte activation and vascular inflammation with integrase inhibitor-based initial antiretroviral therapy among HIV-infected individuals. J Infect Dis 2015; 212:345–354.

51. Chen T, Kim CY, Kaur A, et al. Dietary fibre-based SCFA mixtures promote both protection and repair of intestinal epithelial barrier function in a Caco-2 cell model. Food Funct 2017; 8:1166–1173.

52. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic
■ microbiota. Environ Microbiol 2017; 19:29–41.
This review gives an excellent overview of the bacteria and metabolic pathways contributing to butyrate and propionate formation.

53. Klatt NR, Chomont N, Douek DC, Deeks SG. Immune activation and HIV persistence: implications for curative approaches to HIV infection. Immunol Rev 2013; 254:326–342.

54. Anderson LN, Koech PK, Plymale AE, et al. Live cell discovery of microbial vitamin transport and enzyme-cofactor interactions. ACS Chem Biol 2016; 11:345–354.

55. Lindenbaum J, Tse-Eng D, Butler VP Jr, Rund DG. Urinary excretion of reduced metabolites of digoxin. Am J Med 1981; 71:67–74.

56. Mathan VI, Wiederman J, Dobkin JF, Lindenbaum J. Geographic differences in digoxin inactivation, a metabolic activity of the human anaerobic gut flora. Gut 1989; 30:971–977.

57. Schoefer L, Mohan R, Braune A, et al. Anaerobic C-ring cleavage of genistein and daidzein by Eubacterium ramulus. FEMS Microbiol Lett 2002; 208: 197–202.

58. Spatz M, Smith DW, McDaniel EG, et al. Role of intestinal microorganisms in determining cycasin toxicity. Proc Soc Exp Biol Med 1967; 124: 691–697.

59. Stingley RL, Zou W, Heinze TM, et al. Metabolism of azo dyes by human skin microbiota. J Med Microbiol 2010; 59(Pt 1):108–114.

60. Nakayama H, Kinouchi T, Kataoka K, et al. Intestinal anaerobic bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(E)-(2-bromovinyl)uracil that increases the level and toxicity of 5-fluorouracil. Pharmacogenetics 1997; 7:35–43.

61. Garcia-Gonzalez AP, Ritter AD, Shrestha S, et al. Bacterial metabolism affects the C. elegans response to cancer chemotherapeutics. Cell 2017; 169:431–441 e8.

62. Velloza J, Heffron R. The vaginal microbiome and its potential to impact
■ efficacy of HIV preexposure prophylaxis for women. Curr HIV/AIDS Rep 2017; 14:153–160.
Excellent review discussing oral and non-oral PrEP efficacy in women in the context of the vaginal microbiomes, drug formulation and drug delivery mechanisms.

63. Heffron R, McClelland RS, Balkus JE, et al. Efficacy of oral preexposure
■■ prophylaxis (PrEP) for HIV among women with abnormal vaginal microbiota: a posthoc analysis of the randomised, placebo-controlled Partners PrEP Study. Lancet HIV 2017; 4:e449–e456.
First study to demonstrate that oral PrEP is as efficacious in women with or without bacterial vaginosis; providing strong evidence that HIV prevention is achievable among women regardless of their vaginal microbiomes, with high adherence using orally administered PrEP.

64. McGowan I. The development of rectal microbicides for HIV prevention. Expert Opin Drug Deliv 2014; 11:69–82.

65. Marrazzo JM. HIV prevention: opportunities and challenges. Top Antivir Med 2017; 24:123–126.