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.

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

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

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.
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

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**Table 2. Current recommended antiretroviral therapy regimens**

| ARV drug class   | NRTIs | NNRTIs | INSTIs | Protease inhibitors | Pharmacokinetic enhancer | Comments                                      |
|------------------|-------|--------|--------|---------------------|--------------------------|-----------------------------------------------|
| 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 | –     | 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/mm³ |
| NNRTI-based      | TAF + FTC | RPV   | –      | –                   | –                        |                                               |

Protease inhibitor-based

| 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               |                                               |

*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.
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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 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

| Type of cohort | Novak et al. [29] | Pinto-Cardoso et al., 2017 [30**] | Villanueva-Millán et al. [31**] |
|---------------|------------------|-------------------------------|----------------------------------|
| Sampling      | Feces and blood (plasma) | Feces and blood (plasma) | Feces and blood (plasma and serum) |
| Cohort        | 26 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 = 9) | 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 | IFABP | ICAM V-CAM |
| Markers of systemic inflammation–immune activation | IL-6 D-Dimer | IL-6 | IL-6 |
| Effect of ARVs on alpha diversity (number of species) | No differences were observed between NNRTIs and protease inhibitors *** Number bacteria taxa in ART patients compared with baseline *** | Protease inhibitors versus controls Protease inhibitors versus NNRTIs Protease inhibitors versus INSTIs | Protease inhibitors versus controls NNRTIs versus controls INSTIs versus controls Protease inhibitors versus NNRTIs versus INSTIs |
| Effect of ARVs on microbial translocation | Negative correlation between ILPS, LBP, sCD14, sCD163 and CD4+/CD8+ | sCD14 protease inhibitors versus controls NNRTIs versus controls Protease inhibitors versus INSTIs | Protease inhibitors versus controls NNRTIs versus controls Protease inhibitors versus INSTIs |
| Effect of ARVs on gut microbiome | In Firmicutes: Lachnospira spp. Oralibacterium spp. Oscillospira spp. in proteobacteria (Sutterella spp.) and L. in bacteriaeetes (Peptivellette spp.) after ART initiation | Differential clustering of gut microbiota with ART regimens (Adonis R2 = 10.37%+ Doublet microbiome 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 Protease inhibitors versus controls Protea Proteobacteria versus Instis Proteobacteria versus Instis Proteobacteria versus controls Proteobacteria versus controls |
| Effect 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 ** |
| Effect of ARVs on endothelial damage/turnover/activation | Not assessed | IFABP protease inhibitors versus controls Protease inhibitors versus INSTIs | ICAM INSTIs versus controls ICAM INSTIs versus controls ICAM 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 BPP are depleted in treated HIV infection Absence of BPP 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 ethnic 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 Pvalues, P < 0.05, P < 0.01 and P < 0.001, respectively, as reported in the individual manuscripts.**

ART, antiretroviral therapy; ARVs, antiretroviral drugs; ART, antiretroviral therapy; ARVs, antiretroviral drugs; BPP, butyrate-producing bacteria; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV−, HIV-uninfected; hsCRP, high-sensitivity C-reactive protein; iCAM, intercellular adhesion molecule; IFABP, intestinal fatty acid-binding protein; IL-6, interleukin 6; INSTIs, integrase strand transfer inhibitors; LBP, LPS-binding protein; LPS, lipopolysaccharide; 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.

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Conflicts of interest
S.P.C. and N.K.R. conceived and wrote the manuscript, N.K.R. and G.R.T. critically revised the manuscript. All authors read and approved the final manuscript. There are no conflicts of interest.

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