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Class of antiretroviral drugs and anemia risk in the current treatment era

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Ms. Harding reports grants from NHLBI during the conduct of the study; Ms. Whitney reports grants from NIH during the conduct of the study; Ms. Nance reports grants from NIH during the conduct of the study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH, grants from ViiV and grants from PCORI outside the submitted work; Dr. Burkholder reports grants from NIH during the conduct of the study, other from Amgen, Inc outside the submitted work; Dr. Moore reports grants from NHLBI during the conduct of the study; Dr. Mathews reports grants from NHLBI during the conduct of the study; Dr. Eron reports grants from National Institutes of Health, during the conduct of the study, grants and personal fees from Gilead Sciences, grants and personal fees from ViiV Healthcare, grants and personal

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Contributions:

Ms. Harding, has conducted all analyses, contributed to methodologic approach, and written the manuscript. In addition, Ms. Whitney and Ms. Nance contributed to data preparation and analysis, Dr. Delaney contributed to the analysis, Drs. Heckbert, Crane and Delaney contributed to conception and design of the work and all authors contributed to the interpretation of data and critically revising the manuscript for important intellectual content.

Transparency declaration:

Ms. Harding affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Ethical approval:

Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols.

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Abstract: (276 words)

OBJECTIVES: Anemia is common among people living with HIV (PLWH) and has been associated with certain, often older, antiretroviral medications. Information on current antiretroviral therapy (ART) and anemia is limited. The objectives were to compare associations between anemia incidence or hemoglobin change with core ART classes in the current ART era.

DESIGN: Retrospective cohort study.

SETTING: U.S.-based prospective clinical cohort of PLWH aged 18 and above receiving care at 8 sites between 1/2010-3/2018.

PARTICIPANTS: 16,505 PLWH were included in this study.

MAIN OUTCOME MEASURES: Anemia risk and hemoglobin change were measured for person-time on a protease inhibitor (PI) or an integrase strand transfer inhibitor (INSTI), relative to a non-nucleoside reverse transcriptase inhibitor (NNRTI) reference. We also examined PLWH on multiple core classes. Cox proportional hazards regression analyses were conducted to measure associations between time-updated ART classes and incident anemia or severe anemia. Linear mixed effects models were used to examine relationships between ART classes and hemoglobin change.

RESULTS: During a median of 4.9 years of follow-up, 1,040 developed anemia and 488 developed severe anemia during. Compared to NNRTI use, INSTI-based regimens were associated with an increased risk of anemia (adjusted hazard ratio [aHR] 1.17, 95% confidence interval [CI] 0.94-1.47) and severe anemia (aHR1.55 95%CI 1.11-2.17), and a decrease in hemoglobin level. Time on multiple core classes was also associated with increased anemia risk (aHR 1.30, 95%CI 1.06-1.60) and severe anemia risk (aHR 1.35, 95%CI 0.99-1.85), while no associations were found for PI use.

CONCLUSION: These findings suggest INSTI use may increase the risk of anemia. If confirmed, screening for anemia development in users of INSTIs may be beneficial. Further research into underlying mechanisms is warranted.

Strengths and limitations of this study:

- This study utilized a large and geographically diverse population of PLWH in care across the U.S.
- This study leveraged comprehensive clinical data, including information on diagnoses, medication use, laboratory test results, demographic information, and medical history.
- This study investigated associations between specific types of ART core regimens and anemia risk.
- This observational study is subject to residual confounding.
- This study focused on anemia assessed from hemoglobin lab values taken at regular medical care visits without excluding participants with conditions strongly associated with hemoglobin level through non-traditional HIV mechanisms.

Introduction:

Anemia (hemoglobin [Hb]<10 g/dL) and severe anemia (Hb<7.5 g/dL) [1] are common among people living with HIV (PLWH) [2]. The prevalence of anemia is elevated in PLWH compared to the general population. One study reported that among non-pregnant American women living with HIV, the prevalence of anemia was 28.1% compared to 15.1% among women without HIV [3]. Estimates vary by age, sex, HIV disease stage, use of antiretroviral therapy (ART) and injection drug use status [2, 4]. Among PLWH, associations have been found between anemia and mortality [5-10] health-related quality-of- life [2], morbidity, dementia [11], and treatment failure [12]. In

addition, anemia is an independent prognostic indicator associated with HIV disease progression [2, 13, 14], including development of AIDS [8].

Research shows that ART impacts anemia risk among PLWH. In the early treatment era, use of zidovudine (AZT) was a cause of bone marrow suppression leading to anemia [15]. However, in recent years, AZT use has decreased substantially as other, better tolerated ART medications have become available. Despite the impact of specific agents such as AZT, ART use in general is associated with reduced anemia incidence [16, 17] likely due to inhibition of HIV disease progression. Since worsening HIV disease parameters are associated with anemia, better disease control with ART reduces the risk of anemia [18]. Current ART regimens typically include a pair of nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone plus a core agent. Common core classes include non-nucleoside reverse-transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitor (INSTIs), and protease inhibitors (PIs). While ART use overall reduces anemia, little is known about whether anemia risk differs between commonly used ART classes in the current treatment era, particularly the newer INSTI class. Some studies found a possible increased rate of anemia among PI users [19], and in a randomized controlled trial, some participants discontinued INSTI due to anemia adverse events [20]. However, many studies included few participants or were mostly from an earlier ART era when older ART medications were predominantly used. The objective of this study was to compare rates of anemia and severe anemia development

as well as changes in Hb overtime based on classes of ART used in the current treatment era.

Methods:

Overview and setting:

The present study included PLWH in care in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort during the period of January 1, 2010 to March 31, 2018. The CNICS cohort has been described in detail elsewhere [21]. Briefly, CNICS is a dynamic prospective clinical cohort of >32,000 adult PLWH receiving care at eight participating sites across the U.S. Comprehensive clinical data, including diagnoses, ART and other medications, laboratory test results, demographic information, and historical information, including ART use before enrollment, is collected through electronic medical records and other institutional data systems at each site and harmonized in the CNICS data repository. Medication data including ART use are entered into the electronic medical records by clinicians or prescription fill/refill data are uploaded directly from pharmacy systems and verified through medical record review. Participants entered the current study on January 1, 2010 and the earliest date that they met the following enrollment criteria (cohort entry date): a) enrollment in CNICS for ≥6 months to allow time for covariate ascertainment and b) use of an ART regimen containing a PI, NNRTI, or INSTI. In addition, all

participants were required to have at least 2 available hemoglobin lab values during study follow-up. Figure 1 shows inclusion criteria and exclusions made. Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols.

Exposure:

The exposure of interest was the ART core drug class (NNRTI, INSTI or PI) prescribed as part of an ART regimen. Participants switching to different core drugs within the same class were considered to be continually exposed to the same core drug class. Individuals with a gap in ART use of 6 or more months were censored at the start of the gap and did not re-enter the study.

Person-time on INSTIs or PIs was compared to the reference of NNRTI use. In addition, some PLWH in this cohort had prescriptions for multiple core classes simultaneously. Participants with regimens containing more than 1 core class were categorized separately as users of "multiple core classes" in analyses. Boosting agents (e.g. boosted ritonavir, or cobicistat) were not considered a 2nd core agent.

Outcome ascertainment:

Hb levels, expressed in grams per deciliter (g/dl), were ascertained using inpatient and outpatient laboratory data obtained as part of clinical care. Outcomes

included incident anemia (first Hb measure below 10 g/dl), incident severe anemia (first Hb measure below 7.5 g/d) and changes in hemoglobin level. Another outcome, chronic anemia, defined as anemia lasting for ≥6 months was also examined. Chronic anemia was defined as Hb lab results on two separate occasions at least 6 months apart which were consistently in the anemic range without any Hb values above the anemia range during this 6-month period.

Participant characteristics:

Characteristics that were analyzed as confounders of the association between ART core drugs and incident anemia, severe anemia or change in hemoglobin overtime included: age, sex, race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection, kidney function measured using estimated glomerular filtration rate (eGFR, categorized as <30, 30-59, or ≥60 mL/minute/1.73 m^2) [22], CD4 count (categorized as ≥500, 350-499, 200-399, 100-199 or <100 cells/mm^3), viral load (VL, assessed as $\log_{10}$(VL+1)), and time in care at CNICS sites, defined as time from cohort entry date until the last available CNICS activity: either last lab date or last visit. HCV, eGFR, CD4 count and VL were assessed as part of clinical care visits and were time-updated as repeated measures occurred. All covariates were selected *a priori*, based on review of the literature and clinical knowledge. In addition, assessment of self-reported ART adherence was available for a subset of ~55% of the study population who were in care after each

individual site initiated a clinical assessment of patient reported outcomes including adherence [23].

Statistical analysis:

Baseline characteristics are presented for all participants at the cohort entry date. Median and interquartile range (IQR) are displayed for continuous variables and frequencies and proportions are displayed for categorical variables.

Two multivariable Cox proportional hazards regression analyses were conducted, one among the subset of PLWH who were anemia-free at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of anemia, and another among the subset of participants who were free of severe anemia at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of severe anemia.

Participants were censored at a) the time they developed the outcome of interest, or b) at the time of last activity in CNICS, or c) at the time of death, or d) at the time of administrative censoring per site, or e) at the time they no longer were prescribed one of the ART core classes, whichever came first. The timescale for the models was time since cohort entry. Complete case analysis methods were used (<2% had missing data).

In a sensitivity analysis, we examined ART-naïve PLWH who initiated a drug in one of the ART classes of interest during study follow-up. Follow-up in this analysis

began when a person began their initial ART regimen and extended until the earliest time of anemia occurrence, last activity in CNICS, time of death, administrative censoring, or at the time their initial regimen ended. PI or INSTI use were compared to the reference, NNRTI use. We also examined the change in Hb overtime using mixed models among this ART-naïve population.

Linear mixed effects models with random slopes for time were used to examine the association of ART core classes with Hb levels among all PLWH after adjustment for the same characteristics as in the incident anemia and severe anemia analyses. Mixed-effects models utilize random slopes and intercepts at the participant level to handle irregular patterns of repeated measures over follow-up [24]. All analyses were performed using Stata version 14.2.

Patient and public involvement:

There was no patient or public participation in the present study.

Results:

In total, 16,505 PLWH met inclusion criteria and were included in these analyses (Figure 1). Participants had an average of 11 outpatient hemoglobin values measured during a median follow-up of 4.9 (IQR 3.0-7.2) years. A total of 12,626 (76%) were free of anemia at baseline, and 15,357 (93%) were free of severe anemia at baseline. Table 1

provides baseline characteristics for all study participants, and additionally shows characteristics for the 1,040 participants who developed anemia during follow-up as well as the 488 participants who developed severe anemia during follow-up. The mean age of study participants was 46 years at cohort entry, 20% were female, and 19% were co-infected with HCV. At baseline, 18% were prescribed NNRTIs, 53% were prescribed PIs, 14% were prescribed INSTIs and 16% used multiple cores. INSTI core agents were increasingly used over the last few years of the enrollment period (Figure 2), and the proportion of those prescribed multiple cores were comprised of INSTI plus another core class with increasing frequency as study years progressed.

The incidence of anemia was 2.1/100 person-years and the incidence of severe anemia was 0.8/100 person-years. INSTI use was associated with an increased risk of anemia (aHR 1.17, 95%CI 0.94-1.47) compared to NNRTIs, though this was not statistically significant (Table 2). Use of multiple core classes together was also associated with an increased risk of anemia (aHR 1.30, 95%CI 1.06-1.60)) while no associations were found between PI use and anemia (aHR of 1.00, 95%CI 0.83-1.21). In adjusted analyses restricted to participants free of severe anemia at baseline (Table 2), INSTI use was associated with an increased risk of severe anemia (aHR 1.55, 95%CI 1.11-2.17) compared to NNRTI use. Time on multiple ART core classes was associated with an increased risk of anemia (aHR 1.35 (0.99-1.85), though this was not statistically significant, and no association was found for PIs (aHR 1.01 (0.74-1.36) Among the 12,626

PLWH who were free of anemia at baseline, 225 developed chronic anemia (lasting for ≥6 months), during follow-up. For chronic anemia, results were similar to those in the primary analysis. Relative to NNRTI use, person-time on multiple core classes was associated with an aHR 2.26 (95%CI 0.98-5.23), person-time on an INSTI with an aHR of 1.94 (95%CI 0.80-4.68) and person-time on a PI with an aHR of 1.29 (95%CI 0.55-3.06).

Average hemoglobin levels remained steady during follow-up; the mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0 (IQR 12.6-15.2) g/dL at the last available measurement per person. Relative to NNRTI use, a decrease in hemoglobin level over time was associated with both INSTI use (-0.06 g/dL per year, 95%CI -0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -0.18, -0.11). No association was found for PI use (-0.01, 95%CI -0.04, 0.03). (Table 3).

The sensitivity analysis restricted to ART-naïve PLWH included 6,426 PLWH who were free of prevalent anemia at baseline, of whom 378 developed anemia. Compared to NNRTI initiators, those initiating a PI had an aHR of 0.78 (0.56-1.08) while those initiating an INSTI had an aHR of 1.15 (95%CI 0.92-1.45) (Supplemental Table 1). The mixed model examining change in Hb overtime among ART-naïve PLWH initiating one of the ART core classes of interest included 7,264 participants. Compared to NNRTI initiators, a decrease in Hb was found for PI use (-0.8g/dL per year, 95%CI -0.16, -0.01), while INSTI use was associated with a larger decrease in Hb level overtime (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental Table 2).

Discussion:

In this study of 16,505 PLWH in care within the United States in the current treatment era (2010 and after), we observed that INSTI use, or time on multiple core ART classes used was associated with decreases in hemoglobin levels during follow-up compared to NNRTI use. We found that INSTI use was associated with an elevated aHR for anemia (aHR 1.17, 95%CI 0.94-1.47) and a significantly elevated aHR for severe anemia (1.55, 95%CI 1.11-2.17) as well as a significant decrease in hemoglobin levels over time. Furthermore, the naïve user analysis had nearly identical findings, although not significant with larger CIs. These findings could have implications for the treatment approach that should be used in people with risk factors for anemia.

This study's strengths include its large and geographically diverse study population and longitudinal data structure. However, there are limitations of this study to consider including the observational nature of the data, which may be subject to residual confounding. Additionally, we did not exclude participants with conditions strongly associated with anemia or hemoglobin level through non-traditional HIV mechanisms, including those on dialysis, receiving erythropoietin, or with severe bleeding, which likely caused some of the anemia cases in this analysis. However, in the sensitivity analysis focusing on factors associated with chronic anemia (less likely due to bleeding), findings for INSTI vs. NNRTI core regimens were similar to those

including all PLWH who became anemic. A final limitation is that ART medication use comes from prescriptions written which may not be filled or may be filled but never taken, although self-reported adherence was high (approximately 98%) in the subset for whom adherence information was available. CNICS participants who provided adherence information have been shown to be representative of the overall population of PLWH in CNICS [23, 25]. Finally, the fact that this study was conducted among PLWH in care in the U.S. who are on ART may limit the generalizability of findings to PLWH who are not on ART or who live outside of the U.S.

One study, conducted during the newer-era of HIV treatment with drugs other than AZT, (during 2008-2012) presented findings for AZT versus non-AZT regimens, finding an increased risk of anemia among AZT compared to non-AZT regimens (HR=2.84, 95%CI 1.52-5.31) [26]. However, anemia risk was not analyzed separately for the use of specific classes of ART, resulting in the inability of comparison to the present study's findings and a lack of generalizability to PLWH who are treated with newer ART core agents.

It is possible that PLWH in our study whose HIV is progressing due to resistance or other complications may get switched to an INSTI. This, in addition to prior knowledge that poorly controlled HIV parameters are on their own a risk factor for anemia [6, 27, 28], could result in confounding by indication. However, the switch to INSTI core regimens since their approval in 2007 has been widespread in this

population (Figure 2) and INSTIs are recommended for use as initial regimens [29]. In addition, we rigorously controlled for many of the important HIV-related factors that correspond to poorly-controlled HIV, and our sensitivity analysis examining new users of ART medications failed to reinforce the notion that an increased risk of anemia among INSTI core regimen users could be entirely explained by sicker participants getting switched to these therapies.

PLWH on multiple core classes were in a different category in our analyses. There are several reasons PLWH may be prescribed multiple core classes. For example, sometimes PLWH are prescribed multiple core classes to ensure they receive a complete regimen while awaiting approval for specific agents from their insurance company. However, the primary concern was that they were receiving multiple core classes due to provider concerns such as prior failed regimens which may also increase their risk of anemia.

In conclusion, in this large, diverse, multicenter cohort of PLWH, we found that INSTI use and time on multiple ART core classes were associated with progression to anemia and a lowering of Hb level. INSTI use was also associated with severe anemia risk. Our findings suggest that careful selection of ART regimen could mitigate anemia development, although this anemia risk needs to be balanced with the possibility of improvement in overall HIV care [30]. Further research is needed to replicate the finding of INSTI core regimen use and anemia risk and to understand the underlying

mechanisms. If confirmed, screening for anemia development in users of INSTIs may be beneficial.

Figure Legends

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 hemoglobin levels, and those missing baseline covariates.

Figure 2. Proportion of study population using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

Table 1. Baseline characteristics of PLWH in CNICS who were receiving an ART core agent of interest[a]

	All participants (N=16,505)	Developed anemia during follow-up (n=1,040)	Developed severe anemia during follow-up (n=488)
Age (median, IQR)	46 (38-52)	47 (40-54)	46 (39-54)
Female	3,265 (20)	276 (27)	158 (32)
Race/ethnicity			
White	7,263 (44)	396 (38)	157 (32)
Black	6,386 (39)	499 (48)	254 (52)
Hispanic	2,129 (13)	106 (10)	58 (12)
Other/missing	727 (4)	39 (4)	19 (4)
Years in CNICS at cohort entry [a] (median, IQR)	5.6 (2.5-9.1)	5.8 (2.4-9.5)	5.5 (2.8-9.1)
Viral load ≥400 copies/ml	3,706 (23)	283 (27)	172 (35)
CD4 count (cells/mm^3)			
<100	1,225 (7)	112 (11)	90 (18)
100-199	1,497 (9)	96 (9)	63 (13)
200-349	3,027 (18)	216 (21)	113 (23)
350-499	3,462 (21)	226 (22)	80 (16)
≥500	7,294 (44)	390 (38)	142 (29)
Hepatitis C virus coinfection	3,161 (19)	303 (29)	139 (28)
Kidney function (eGFR)			
<30	295 (2)	36 (3)	42 (9)
30-59	921 (6)	80 (8)	51 (10)
≥60	15,289 (92)	924 (89)	395 (81)
Hemoglobin (g/dL) (median, IQR)	14.1 (12.8-15.2)	13.3 (12.2-14.4)	12.4 (10.8-13.8)

BMI (kg/m^2)			
<18.5	516 (3)	36 (4)	30 (6)
18.5 to <25.0	6,809 (43)	426 (42)	211 (45)
25.0 to <30.0	5,297 (33)	301 (30)	117 (25)
≥30.0	3,233 (20)	245 (24)	115 (24)
ART class			
Non-nucleoside reverse-Transcriptase inhibitor	2,901 (18)	177 (17)	70 (14)
Protease inhibitor	8,713 (53)	558 (71)	251 (51)
Integrase strand transfer inhibitor	2,318 (14)	93 (9)	46 (10)
Multiple core classes	2,573 (16)	212 (20)	121 (25)
Self-reported adherence (on a 100-point scale) (median, IQR)[b]	98 (92-100)	98 (91-100)	96 (89-99)

[a]Cohort entry date was defined as the earliest date during January 1, 2010- March 31, 2018 that a person had 6+ months in CNICS care and were using an ART core agent of interest.

[b]For the 55% of the population who reported medication adherence

Abbreviations: PLWH: people living with HIV, CNICS: Centers for AIDS Research Network of Integrated Clinical Systems, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate

Table 2: Association of ART classes with incident anemia (hemoglobin<10 g/dL), severe anemia (hemoglobin<7.5 g/dL) or chronic anemia (6+ months of anemia)

ART Regimen	Hazard Ratio of incident anemia (n=12,626)		Hazard Ratio of incident severe anemia (n=15,357)		Hazard Ratio of incident chronic anemia (n=12,626)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.00 (0.83-1.21)	1.37 (1.02-1.83)	1.01 (0.74-1.36)	1.43 (0.61-3.35)	1.29 (0.55-3.06)
INSTI	1.39 (1.11-1.75)	1.17 (0.94-1.47)	1.96 (1.40-2.750	1.55 (1.11-2.17)	2.05 (0.85-4.94)	1.94 (0.80-4.68)
Multiple core classes	2.02 (1.65-3.48)	1.30 (1.06-1.60)	2.70 (1.99-3.67)	1.35 (0.99-1.85)	3.46 (1.51-7.93)	2.26 (0.98-5.23)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGF)

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 3. Association of ART classes with change in hemoglobin level during follow-up in adjusted analyses (linear mixed-effect model); N=16,505

ART class	Coefficient[a]	95% CI	P-value
NNRTI (REF)			
PI	-0.01	-0.04, 0.03	0.675
INSTI	-0.06	-0.10, -0.03	<0.001
Multiple core classes	-0.14	-0.18, -0.11	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.
Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

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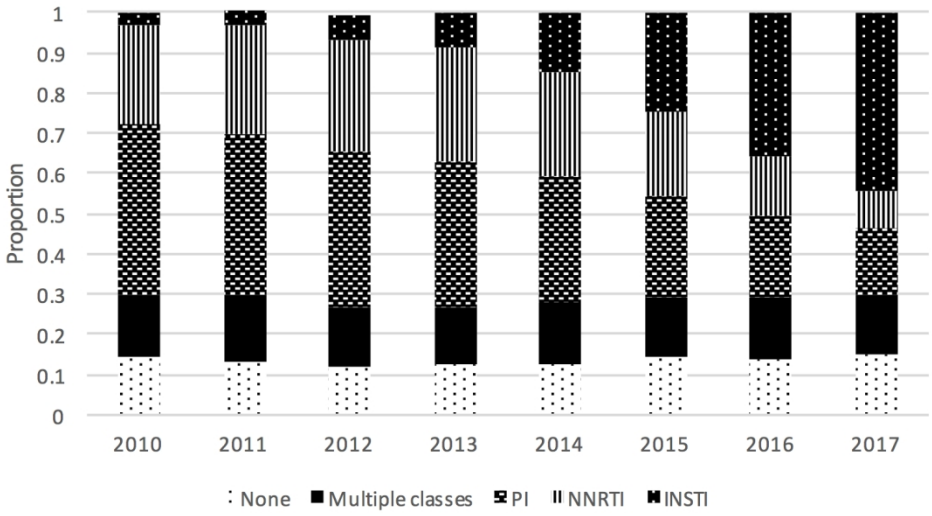
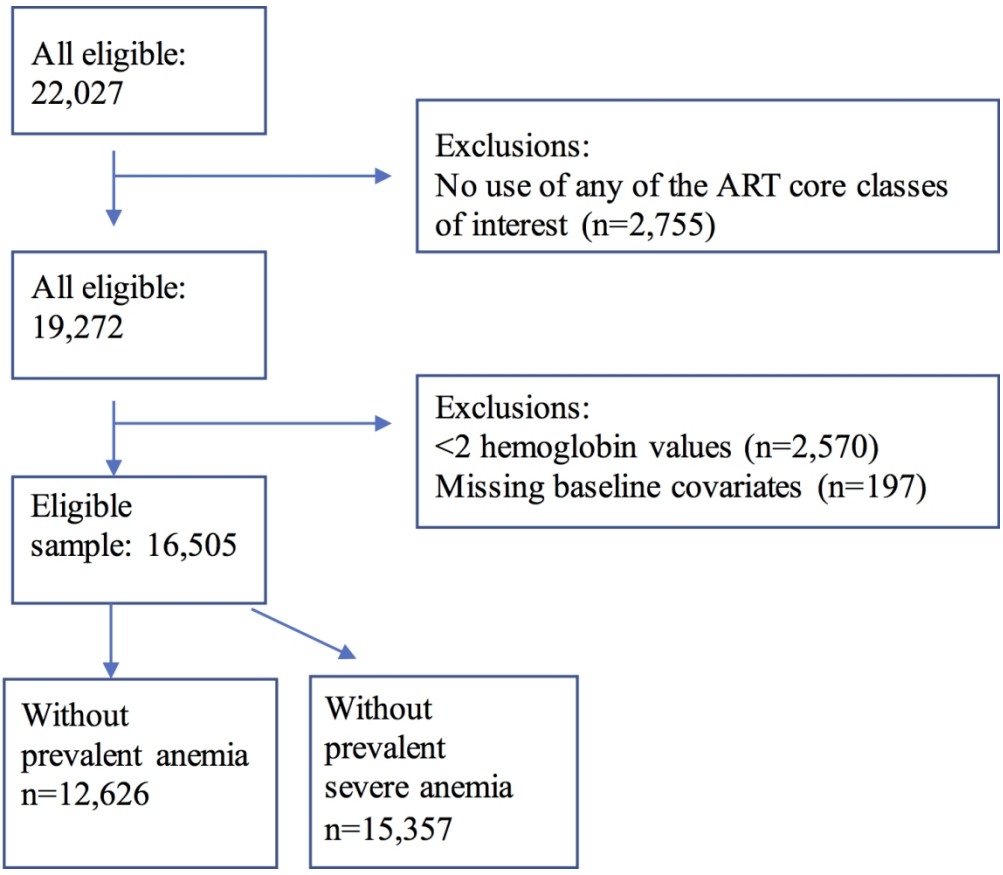


Figure 2. Proportion of study population using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

142x79mm (300 x 300 DPI)



Caption : Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 hemoglobin levels, and those missing baseline covariates.

125x108mm (300 x 300 DPI)

Supplemental Table 1: Association of ART classes with incident anemia among naïve users

ART class	Hazard Ratio of incident anemia (n=6,426)	
	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00
PI	0.92 (0.67-1.27)	0.78 (0.56-1.08)
INSTI	1.73 (1.39-2.15)	1.15 (0.92-1.45)

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 2. Association of ART classes with change in hemoglobin level during follow-up in adjusted analyses among naïve users (linear mixed-effect model); N=7,264

ART class	Coefficient[a]	95% CI	P-value
NNRTI (REF)			
PI	-0.08	-0.16, -0.01	0.031
INSTI	-0.15	-0.22, -0.09	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
✔**Title and abstract** (page 1-title, page 3 abstract)	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
✔Background/rationale (pages 5, 6)	2	Explain the scientific background and rationale for the investigation being reported
✔Objectives (page 6)	3	State specific objectives, including any prespecified hypotheses
Methods		
✔Study design (page 6)	4	Present key elements of study design early in the paper
✔Setting (page 6)	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✔Participants (page 6)	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
✔Variables (pages 7, 8)	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✔Data sources/ measurement (pages 7, 8)	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
✔Bias (page 9)	9	Describe any efforts to address potential sources of bias
✔Study size (page 6)	10	Explain how the study size was arrived at
✔Quantitative variables (pages 7, 8)	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✔Statistical methods (pages 8, 9)	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
✔Participants (figure 1, page 9)	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✔Descriptive data (pages 9, 10)	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)

✔Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
✔Main results (pages 10, 11)	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
✔Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
✔Key results (page 11)	18	Summarise key results with reference to study objectives
✔Limitations (page 12)	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
✔Interpretation (page 13)	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
✔Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
Other information		
✔Funding (page 2)	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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A study of antiretroviral drug class and anaemia risk in the current treatment era among people living with HIV in the United States: A clinical cohort study

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A study of antiretroviral drug class and anaemia risk in the current treatment era among people living with HIV in the United States: A clinical cohort study

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Competing interest statement:
Dr. Harding reports grants from National Heart, Lung and Blood Institute(NHLBI) during the conduct of the study; Ms. Whitney reports grants from the National Institutes of Health (NIH) during the conduct of the study; Ms. Nance reports grants from NIH during the conduct of the study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH, grants from ViiV healthcare and grants from PCORI outside the submitted work; Dr. Burkholder reports grants from NIH during the conduct of the study, other from Amgen, Inc outside the submitted work; Dr. Moore reports grants from NHLBI during the conduct of the study; Dr. Mathews reports grants from NHLBI during the conduct of the study; Dr. Eron reports grants from NIH, during the conduct of the study, grants and personal fees from Gilead Sciences, grants and personal fees from ViiV Healthcare, grants and personal fees from Janssen and personal fees

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Contributions:
Dr. Harding, has conducted all analyses, contributed to methodologic approach, and written the manuscript. In addition, Ms. Whitney and Ms. Nance contributed to data preparation and analysis, Dr. Delaney contributed to the analysis, Drs. Heckbert, Crane and Delaney contributed to conception and design of the work, Drs. Crane, Burkholder, Moore, Mathews, Eron, Rodriguez, Mayer, Saag and Kitahata contributed to data collection and all authors contributed to the interpretation of data and critically revising the manuscript for important intellectual content.

Transparency declaration:
Dr. Harding affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Ethical approval:
Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols for patient protection and provided general approval for secondary data analysis.

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Data sharing:
The Centers for AIDS research (CFAR) Network of Integrated Clinical Systems (CNICS) data may be accessed with an approved concept proposal. Instructions for data access and concept proposal forms may be found at https://www.uab.edu/ cnics/submit-proposal.

Dissemination declaration:
Dissemination of the study findings are not applicable to participants or patient organizations.

Abstract: (276 words)

OBJECTIVES: Anaemia is common among people living with HIV (PLWH) and has been associated with certain, often older, antiretroviral medications. Information on current antiretroviral therapy (ART) and anaemia is limited. The objectives were to compare associations between anaemia incidence or haemoglobin change with core ART classes in the current ART era.

DESIGN: Retrospective cohort study.

SETTING: U.S.-based prospective clinical cohort of PLWH aged 18 and above receiving care at 8 sites between 1/2010-3/2018.

PARTICIPANTS: 16,505 PLWH were included in this study.

MAIN OUTCOME MEASURES: Anaemia risk and haemoglobin change were estimated among PLWH for person-time on a protease inhibitor (PI) or an integrase strand transfer inhibitor (INSTI)-based regimen, relative to a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based reference. We also examined PLWH on regimens containing multiple core classes. Cox proportional hazards regression analyses were conducted to measure associations between time-updated ART classes and incident anaemia or severe anaemia. Linear mixed effects models were used to examine relationships between ART classes and haemoglobin change.

RESULTS: During a median of 4.9 years of follow-up, 1,040 developed anaemia and 488 developed severe anaemia. Compared to NNRTI use, INSTI-based regimens were associated with an increased risk of anaemia (adjusted hazard ratio [aHR] 1.26, 95% confidence interval [CI] 1.00-1.58) and severe anaemia (aHR1.51 95%CI 1.07-2.11), and a decrease in haemoglobin level. Time on multiple core classes was also associated with increased anaemia risk (aHR 1.39, 95%CI 1.13-1.70) and severe anaemia risk (aHR 1.30, 95%CI 0.95-1.78), while no associations were found for PI use.

CONCLUSION: These findings suggest INSTI use may increase the risk of anaemia. If confirmed, screening for anaemia development in users of INSTIs may be beneficial. Further research into underlying mechanisms is warranted.

Strengths and limitations of this study:

- This study utilized a large and geographically diverse population of PLWH in care across the U.S.

- This study leveraged comprehensive clinical data, including information on diagnoses, medication use, laboratory test results, demographic information, and medical history.
- This study investigated associations between specific types of ART core regimens and anaemia risk.
- This observational study is subject to residual confounding.
- This study focused on anaemia assessed from haemoglobin lab values taken at regular medical care visits without excluding participants with conditions strongly associated with haemoglobin level through mechanisms unrelated to HIV infection.

Introduction:

Anaemia and severe anaemia are common among people living with HIV (PLWH) [1]. The prevalence of anaemia is elevated in PLWH compared to the general population. One study reported that among non-pregnant American women living with HIV, the prevalence of anaemia was 28.1% compared to 15.1% among women without HIV [2]. Estimates vary by age, sex, HIV disease stage, use of antiretroviral therapy (ART) and injection drug use status [1, 3]. Among PLWH, associations have been found between anaemia and mortality [4-9], health-related quality-of- life [1], morbidity, dementia [10], and ART failure [11]. In addition, anaemia is an independent prognostic indicator associated with HIV disease progression [1, 12, 13], including development of AIDS [7].

Research shows that ART impacts anaemia risk among PLWH. In the early treatment era, use of zidovudine (AZT) was a cause of bone marrow suppression leading to anaemia [14]. However, in recent years, AZT use has decreased substantially as other, better tolerated ART medications have become available. Despite the impact of specific agents such as AZT, ART use in general is associated with reduced anaemia incidence [15, 16], likely due to inhibition of HIV disease progression [17]. Current ART regimens typically include a pair of nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone plus a core agent. Common core classes include non-nucleoside reverse-transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitors (INSTIs), and protease inhibitors (PIs). While ART use overall reduces anaemia, little is known about whether anaemia risk differs between commonly used ART classes in the current treatment era, particularly the newer INSTI class. From clinical safety data of trials, 36-49% of participants using PIs had haemoglobin (Hb) levels <10g/dL, indicating anaemia [18],

and in a randomized controlled trial, two participants discontinued INSTI use due to anaemia adverse events [19]. However, many studies included few participants or were mostly from an earlier ART era when older ART medications were predominantly used or from trials that may be less generalizable to the diverse population of PLWH in clinical care. The objective of this study was to compare rates of anaemia and severe anaemia development as well as changes in Hb over time based on classes of ART used in the current treatment era.

Methods:

Overview and setting:

The present study included PLWH in care in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort during the period of January 1, 2010 to March 31, 2018 (the date through which each site had complete data [administrative censor date] varied somewhat, median date: October 31, 2017). The CNICS cohort has been described in detail elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical cohort of >32,000 adult PLWH receiving care at eight participating sites across the U.S. Comprehensive clinical data, including diagnoses, ART and other medications, laboratory test results, demographic information, and historical information, including ART use before enrollment, is collected through electronic medical records and other institutional data systems at each site and harmonized in the CNICS data repository. Medication data including ART use are entered into the electronic medical records by clinicians or prescription fill/refill data are uploaded directly from pharmacy systems and verified through medical record review. Participants entered the current study on January 1, 2010 or the earliest date after January 1, 2010 that they met the following enrollment criteria (cohort entry date): a) enrollment in CNICS for ≥6 months to allow time for covariate ascertainment, and b) use of an ART regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or INSTI. In addition, all participants were required to have at least 2 available haemoglobin lab values during study follow-up. Figure 1 shows inclusion criteria and exclusions made. Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols.

Exposure:

The exposure of interest was the ART core drug class (NNRTI, INSTI or PI) prescribed as part of an ART regimen (a backbone of two NRTIs plus a core drug). Participants switching to different core drugs within the same class were considered to be continually exposed to the same core drug class. Individuals

with a gap of 6 or more months in use of the ART core drug classes of interest were censored at the start of the gap and did not re-enter the study.

Person-time on INSTI or PI-based regimens was compared to the NNRTI reference. In addition, some PLWH in this cohort had prescriptions for multiple core classes simultaneously. Participants with regimens containing more than 1 core class were categorized separately in analyses as users of "multiple core classes". Boosting agents (e.g. boosted ritonavir, or cobicistat) were not considered a 2nd core agent.

Outcome ascertainment:

Hb levels, expressed in grams per deciliter (g/dL), were ascertained using inpatient and outpatient laboratory data obtained as part of clinical care. Outcomes included incident anaemia (first post-baseline Hb measure below 10 g/dL), incident severe anaemia (first post-baseline Hb measure below 7.5 g/dL) [21] and changes in Hb level. Another outcome, chronic anaemia, defined as anaemia lasting for ≥6 months, was also examined. Chronic anaemia was defined as post-baseline Hb lab results on two separate occasions at least 6 months apart which were consistently in the anemic range without any Hb values above the anaemia range during this 6-month period.

Participant characteristics:

Characteristics that were analyzed as confounders of the association between ART core drugs and incident anaemia, severe anaemia or change in Hb over time included: age, sex, race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection defined as a detectable HCV RNA level or HCV genotype or HCV antibody, kidney function measured using estimated glomerular filtration rate (eGFR, categorized as <30, 30-59, or ≥60 mL/minute/1.73 m^2) [22], CD4 count (categorized as ≥500, 350-499, 200-399, 100-199 or <100 cells/mm^3), viral load (VL, assessed as $\log_{10}$(VL+1)), baseline Hb, and time in care at CNICS sites, defined as time from cohort entry date until the last available CNICS activity: either last lab date or last visit. HCV, eGFR, CD4 count and VL were assessed as part of clinical care visits and were time-updated as repeated measures occurred. All covariates were selected *a priori*, based on review of the literature and clinical knowledge. In addition, assessment of self-reported ART adherence was available for a subset of ~55% of the study population who were in care after each individual site initiated a clinical assessment of patient reported outcomes, including adherence [23].

Statistical analysis:

Baseline characteristics are presented for all participants at the cohort entry date. Median and interquartile range (IQR) are displayed for continuous variables and frequencies and proportions are displayed for categorical variables.

Two multivariable Cox proportional hazards regression analyses were conducted, one among the subset of PLWH who were anaemia-free at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of anaemia, and another among the subset of participants who were free of severe anaemia at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of severe anaemia.

Participants were censored at a) the time they developed the outcome of interest, b) at the time of last activity in CNICS, c) at the time of death, d) at the date of administrative censoring at each site or e) at the time they no longer were prescribed one of the ART core classes of interest, whichever came first. The timescale for the models was time since cohort entry. Complete case analysis methods were used (<2% had missing data).

In a sensitivity analysis, we examined ART-naïve PLWH who initiated a regimen including one of the core ART classes of interest during study follow-up. Follow-up in this analysis began when a person began their initial ART regimen and extended until the earliest time of anaemia occurrence, last activity in CNICS, time of death, administrative censoring, or at the time their initial regimen ended. PI or INSTI use were compared to the reference, NNRTI use. We also examined the change in Hb over time using mixed models among this ART-naïve population. We also conducted a sensitivity analysis including baseline NRTI backbone adjustment for abacavir versus tenofovir in an analysis with incident anemia.

Linear mixed effects models with random slopes for time were used to examine the association of ART core classes with Hb levels among all PLWH after adjustment for the same characteristics as in the incident anaemia and severe anaemia analyses. Mixed-effects models utilize random slopes and intercepts at the participant level to handle irregular patterns of repeated measures over follow-up [24]. All analyses were performed using Stata version 14.2.

Patient and public involvement:

There was no patient or public participation in the present study.

Results:

In total, 16,505 PLWH met inclusion criteria and were

included in these analyses (Figure 1). Participants had an average of 11 outpatient Hb values measured during a median follow-up of 4.9 (IQR 3.0-7.2) years. A total of 12,626 (76%) were free of anaemia at baseline, and 15,357 (93%) were free of severe anaemia at baseline. Table 1 provides baseline characteristics for study participants in the analyses of incident anaemia and incident severe anaemia. Overall, the mean age of study participants was 46 years at cohort entry, 20% were female, and 19% were co-infected with HCV. At baseline, 18% were prescribed regimens with an NNRTI, 53% with a PI, 14% an INSTI, and 16% regimens with multiple cores. INSTIs were increasingly used over the last few years of the study period (Figure 2), and among those simultaneously prescribed multiple core medications, the proportion comprised of INSTI plus another core class increased as study years progressed. Zidovudine was used by 4% of participants during follow-up.

The overall incidence of anaemia was 2.1/100 person-years and the overall incidence of severe anaemia was 0.8/100 person-years. The unadjusted incidence rates of anaemia and severe anaemia based on ART core class are provided in Table 2. In adjusted analyses, INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs (Table 3). Use of multiple core classes together was also associated with an increased risk of anaemia (aHR 1.39, 95%CI 1.13-1.70)) while no associations were found between PI use and anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses restricted to participants free of severe anaemia at baseline (Table 3), INSTI use was associated with an increased risk of severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI use. Although the HR appeared elevated, there was no association between time on multiple ART core classes and an increased risk of severe anaemia (aHR 1.30 (0.95-1.78), and no association was found for PIs (aHR 1.09 (0.81-1.47). Among the 12,626 PLWH who were free of anaemia at baseline, 225 developed chronic anaemia (lasting for ≥6 months), during follow-up. For chronic anaemia, results were similar to those in the primary analysis. Relative to NNRTI use, person-time on multiple core classes was associated with an aHR for chronic anaemia of 2.21 (95%CI 0.94-5.18), person-time on an INSTI with an aHR of 1.90 (95%CI 0.76-4.64) and person-time on a PI with an aHR of 1.27 (95%CI 0.54-3.04).

Average Hb levels remained steady during follow-up; the mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0 g/dL (IQR 12.6-15.2) at the last available measurement per person. Relative to NNRTI use, a decrease in Hb level over time was associated with both INSTI use (-0.06 g/dL per year, 95%CI -0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -

0.18, -0.11). No association was found for PI use (-0.01, 95%CI -0.04, 0.03). (Table 4).

The sensitivity analysis restricted to ART-naïve participants included 6,426 PLWH who were free of prevalent anaemia at baseline, of whom 378 developed anaemia. Compared to NNRTI initiators, those initiating a PI had an aHR of 0.69 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10 (95%CI 0.84-1.44) (Supplemental Table 1). The mixed model examining change in Hb over time among ART-naïve PLWH initiating one of the ART core classes of interest included 7,264 participants. Compared to NNRTI initiators, a decrease in Hb was found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while INSTI use was associated with a larger decrease in Hb level over time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental Table 2). Finally, results from the sensitivity analysis including baseline NRTI backbone adjustment for abacavir) were essentially unchanged (e.g. the abacavir backbone aHR for use of an INSTI-based regimen was 1.27 (95%CI 1.01-1.60) for incident anaemia compared to the findings from the primary analysis of an aHR of 1.26 (95%CI 1.00-1.58) (data not shown).

Discussion:

In this study of 16,505 PLWH in care within the United States in the current treatment era (2010 and after), we observed that INSTI use, and time on multiple core ART classes, were associated with decreases in Hb levels during follow-up compared to using NNRTI-based regimens. We found that INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over time. Furthermore, the naïve user analysis indicated similar findings despite a smaller sample size. These findings could have implications for the treatment approach that should be used in people with risk factors for anaemia.

This study's strengths include its large and geographically diverse study population and longitudinal data structure. Nevertheless, there are limitations of this study to consider, including the observational nature of the data, which may be subject to residual confounding including confounding by indication [25]. However, anaemia is not a recognized adverse effect of NNRTIs, PIs, or INSTIs. Thus, it is unlikely that ART core class was selected based on prescriber concern about anaemia risk. Additionally, we did not exclude participants with conditions strongly associated with anaemia or Hb level, including those on dialysis, receiving erythropoietin, or with severe bleeding, which likely caused some of the anaemia cases in this analysis. However, in the sensitivity analysis focusing on factors associated with chronic anaemia (less likely due to bleeding), findings for INSTI vs. NNRTI core regimens were similar to those including all PLWH who became anemic.

Information on ART medication use was from prescription data which does not necessarily indicate medications were taken, although self-reported adherence was high (~98%) in the subset for whom adherence information was available. CNICS participants who provided adherence information have been shown to be representative of the overall population of PLWH in CNICS [23, 26]. Finally, the fact that this study was conducted among PLWH in care in the U.S. who are on ART may limit the generalizability of findings to PLWH who live outside of the U.S.

There have been few epidemiologic studies of anaemia risk among users of newer ART core regimens. From clinical safety data reported from multiple trials, approximately 36-49% of participants using PIs had Hb levels <10g/dL, indicating anaemia [18], and in another trial, two participants discontinued INSTI use due to anaemia adverse events [19], however the strict inclusion criteria applied in clinical trials makes it difficult to generalize these findings to more diverse populations of PLWH in clinical care. Another study, conducted during the newer-era of HIV treatment with drugs other than AZT, (during 2008-2012) presented findings for AZT versus non-AZT regimens, finding an increased risk of anaemia among AZT compared to non-AZT regimens (HR=2.84, 95%CI 1.52-5.31) [27]. However, anaemia risk was not analyzed separately for the use of specific classes of ART, resulting in the inability of comparison to the present study's findings and a lack of generalizability to PLWH who are treated with newer ART core agents.

It is possible that PLWH in our study whose HIV is progressing due to resistance or other complications may get switched to an INSTI. This, in addition to prior knowledge that poorly controlled HIV parameters are on their own a risk factor for anaemia [5, 28, 29], could result in confounding by indication. However, the switch to INSTI core regimens since their approval in 2007 has been widespread in this population (Figure 2) and INSTIs are recommended for use as initial regimens [30]. In addition, we rigorously controlled for many of the important HIV-related factors that correspond to poorly-controlled HIV, and our sensitivity analysis examining new users of ART medications failed to reinforce the notion that an increased risk of anaemia among INSTI core regimen users could be entirely explained by sicker participants getting switched to these therapies.

PLWH on multiple core classes were in a different category in our analyses. There are several reasons PLWH may be prescribed multiple core classes. For example, sometimes PLWH are prescribed multiple core classes to ensure they receive a complete regimen while awaiting approval for specific agents

from their insurance company. However, the primary concern was that they were receiving multiple core classes due to provider concerns such as prior failed regimens which may also increase their risk of anaemia.

In conclusion, in this large, diverse, multicenter cohort of PLWH, we found that INSTI use and time on multiple ART core classes were associated with progression to anaemia and a lower Hb level. INSTI use was also associated with severe anaemia risk. Our findings suggest that careful selection of ART regimen could mitigate anaemia development, although this anaemia risk needs to be balanced with the possibility of improvement in overall HIV care [31]. Further research is needed to replicate the finding of INSTI core regimen use and anaemia risk and to understand the underlying mechanisms. If confirmed, screening for anaemia development in users of INSTIs may be beneficial.

Figure Legends

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

Table 1. Baseline characteristics of PLWH in CNICS who were receiving an ART core agent of interest (N=16,505)[a]

	Incident anaemia analysis (n=12,626)		Incident severe anaemia analysis (n=15,357)	
	Do not develop anaemia (n=11,586)	**Develop anaemia (n=1,040)**	**Do not develop severe anaemia (n=14,896)**	**Develop severe anaemia (n=488)**
Age (median, IQR)	45 (37, 51)	47 (40, 54)	45 (37-52)	46 (39, 54)
Female	1574 (14)	276 (27)	2681 (18)	158 (32)
Race/ethnicity				
White	5782 (50)	396 (38)	6840 (46)	157 (32)
Black	3720 (32)	499 (48)	5442 (37)	254 (52)
Hispanic	1537 (13)	106 (10)	1920 (13)	58 (12)
Other/missing	547 (5)	39 (4)	667 (4)	19 (4)
Years in CNICS at cohort entry a (median, IQR)	5.2 (2.3, 8.8)	5.8 (2.8, 6.9)	5.5 (2.4, 9.0)	5.56 (2.8, 9.1)
Viral load ≥400 copies/ml	2441 (21)	283 (27)	3259 (22)	172 (35)
CD4 count (cells/mm3)				
<100	528 (5)	112 (11)	915 (6)	90 (18)
100-199	870 (8)	96 (9)	1256 (8)	63 (13)
200-349	1974 (17)	216 (21)	2675 (18)	113 (23)
350-499	2497 (21)	226 (22)	3160 (21)	80 (16)
≥500	5717 (49)	390 (38)	6863 (46)	142 (29)
Hepatitis C virus coinfection	1816 (16)	303 (29)	2711 (18)	139 (28)

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Kidney function (eGFR) (mL/min/1.73 m^2)				
<30	32 (<1)	36 (3)	142 (1)	42 (9)
30-59	459 (4)	80 (8)	731 (5)	51 (10)
≥60	11095 (96)	924 (89)	13996 (94)	395 (81)
Baseline haemoglobin (g/dL) (median, IQR)	14.5 (13.5, 15.4)	13.3 (12.2, 14.4)	14.3 (13.1, 15.2)	12.4 (10.8, 13.8)
BMI (kg/m^2)				
<18.5	229 (2)	36 (4)	377 (3)	30 (6)
18.5 to <25.0	4806 (43)	426 (42)	6120 (43)	211 (45)
25.0 to <30.0	3929 (35)	301 (30)	4885 (345)	117 (25)
≥30.0	2142 (19)	245 (24)	2893 (20)	115 (24)
ART core class				
NNRTI	2109 (18)	117 (17)	2633 (18)	70 (14)
PI	6135 (53)	558 (54)	7935 (53)	251 (51)
INSTI	1803 (16)	93 (9)	2126 (14)	46 (9)
Multiple core classes	1539 (13)	212 (20)	2175 (15)	121 (25)
Self-reported adherence (on a 100-point scale) (median, IQR)[b]	98 (93, 100)	98 (91-99)	98 (92, 100)	97 (90-99)

[a]Cohort entry date was defined as the earliest date during January 1, 2010- March 31, 2018 that a person had ≥6 months in CNICS and was receiving an ART regimen with a core agent of interest.

[b]For the 55% of the population who reported medication adherence

Abbreviations: PLWH: people living with HIV, CNICS: Centers for AIDS Research Network of Integrated Clinical Systems, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 2. Incidence rate of anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) by ART core drug class

ART Regimen	Follow-up time (person-years)	Events	Rate (per 100 person-years)
Anaemia			
NNRTI	9,964	150	1.50
PI	24,710	485	1.96
INSTI	7,389	155	2.10
Multiple core classes	8,172	250	3.06
Severe anaemia			
NNRTI	12,113	57	0.47
PI	31,156	204	0.65
INSTI	9,132	84	0.92
Multiple core classes	11,258	143	1.27

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL), severe anaemia (hemoglobin<7.5 g/dL) or chronic anaemia (>6 months of anaemia)

ART Regimen	Hazard Ratio of incident anaemia (n=12,626)		Hazard Ratio of incident severe anaemia (n=15,357)		Hazard Ratio of incident chronic anaemia (n=12,626)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.09 (0.90, 1.32)	1.37 (1.02-1.83)	1.09 (0.81, 1.47)	1.43 (0.61-3.35)	1.27 (0.54-3.04)
INSTI	1.39 (1.11-1.75)	1.26 (1.00, 1.58)	1.96 (1.40-2.75)	1.51 (1.07, 2.11)	2.05 (0.85-4.94)	1.90 (0.76-4.64)
Multiple core classes	2.02 (1.65-3.48)	1.39 (1.13, 1.70)	2.70 (1.99-3.67)	1.30 (0.95, 1.78)	3.46 (1.51-7.93)	2.21 (0.94-5.18)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR), baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 4. Association of ART core classes with change in haemoglobin level during follow-up in adjusted analyses (linear mixed-effect model); N=16,505

ART class	Coefficient [a]	95% CI	P-value
NNRTI (REF)			
PI	-0.01	-0.04, 0.03	0.675
INSTI	-0.06	-0.10, -0.03	<0.001
Multiple core classes	-0.14	-0.18, -0.11	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

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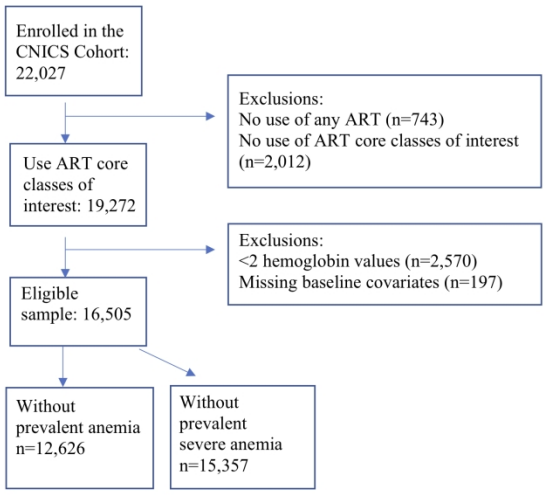

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

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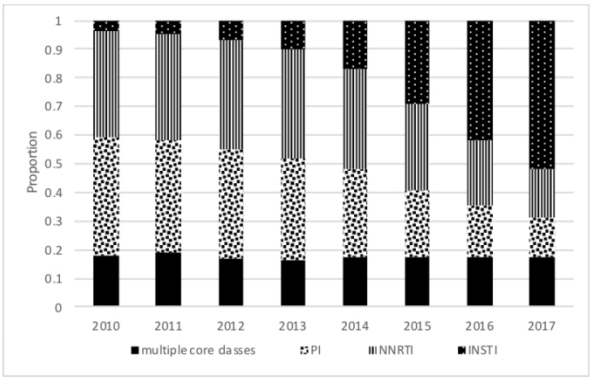

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

215x279mm (300 x 300 DPI)

Supplemental Table 1: Association of ART classes with incident anaemia among naïve users

ART class	Hazard ratio of incident anaemia (n=6,426)	
	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00
PI	0.92 (0.67-1.247)	0.6978 (0.4556-1.068)
INSTI	1.73 (1.39-2.15)	1.105 (0.8492, 1.445)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR) and baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 2. Association of ART classes with change in haemoglobin level during follow-up in adjusted analyses among naïve users (linear mixed-effect model); N=7,264

ART class	Coefficient[a]	95% CI	P-value
NNRTI (REF)			
PI	-0.08	-0.16, -0.01	0.031
INSTI	-0.15	-0.22, -0.09	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study. Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
✔**Title and abstract** (page 1-title, page 3 abstract)	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
✔Background/rationale (pages 5, 6)	2	Explain the scientific background and rationale for the investigation being reported
✔Objectives (page 6)	3	State specific objectives, including any prespecified hypotheses
Methods		
✔Study design (page 6)	4	Present key elements of study design early in the paper
✔Setting (page 6)	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✔Participants (page 6)	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
✔Variables (pages 7, 8)	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✔Data sources/ measurement (pages 7, 8)	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
✔Bias (page 9)	9	Describe any efforts to address potential sources of bias
✔Study size (page 6)	10	Explain how the study size was arrived at
✔Quantitative variables (pages 7, 8)	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✔Statistical methods (pages 8, 9)	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
✔Participants (figure 1, page 9)	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✔Descriptive data (pages 9, 10)	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)

✔Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
✔Main results (pages 10, 11)	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
✔Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
✔Key results (page 11)	18	Summarise key results with reference to study objectives
✔Limitations (page 12)	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
✔Interpretation (page 13)	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
✔Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
Other information		
✔Funding (page 2)	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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A study of antiretroviral drug class and anaemia risk in the current treatment era among people living with HIV in the United States: A clinical cohort study

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Dr. Harding reports grants from National Heart, Lung and Blood Institute(NHLBI) during the conduct of the study; Ms. Whitney reports grants from the National Institutes of Health (NIH) during the conduct of the study; Ms. Nance reports grants from NIH during the conduct of the study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH, grants from ViiV healthcare and grants from PCORI outside the submitted work; Dr. Burkholder reports grants from NIH during the conduct of the study, other from Amgen, Inc outside the submitted work; Dr. Moore reports grants from NHLBI during the conduct of the study; Dr. Mathews reports grants from NHLBI during the conduct of the study; Dr. Eron reports grants from NIH, during the conduct of the study, grants and personal fees from Gilead Sciences, grants and personal fees from ViiV Healthcare, grants and personal fees from Janssen and personal fees

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Contributions:
BNH has conducted all analyses, contributed to methodologic approach, and written the manuscript. In addition, BMW and RMN contributed to data preparation and analysis, JACD contributed to the analysis, HMC, SRH and JACD contributed to conception and design of the work, HMC, GB, RDM, WCM, JJE, BR, KHM, MSS and MMK contributed to data collection and BNH, BMW, RMN, HMC, GB, RDM, WCM, JJE, PWH, PV, BR, KHM, MSS, MMK, SRH and JACD contributed to the interpretation of data and critically revising the manuscript for important intellectual content.

Transparency declaration:
Dr. Harding affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Ethical approval:
Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols for patient protection and provided general approval for secondary data analysis. The University of Washington Human Subjects Division served as the institutional review board for the centralized deidentified CNICS Data Repository (IRB approval number 27674-D).

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Data sharing:

The Centers for AIDS research (CFAR) Network of Integrated Clinical Systems (CNICS) data may be accessed with an approved concept proposal. Instructions for data access and concept proposal forms may be found at https://www.uab.edu/ cnics/submit-proposal.

Dissemination declaration:
Dissemination of the study findings are not applicable to participants or patient organizations.

Abstract: (276 words)
OBJECTIVES: Anaemia is common among people living with HIV (PLWH) and has been associated with certain, often older, antiretroviral medications. Information on current antiretroviral therapy (ART) and anaemia is limited. The objectives were to compare associations between anaemia incidence or haemoglobin change with core ART classes in the current ART era.
DESIGN: Retrospective cohort study.
SETTING: U.S.-based prospective clinical cohort of PLWH aged 18 and above receiving care at 8 sites between 1/2010-3/2018.
PARTICIPANTS: 16,505 PLWH were included in this study.
MAIN OUTCOME MEASURES: Anaemia risk and haemoglobin change were estimated among PLWH for person-time on a protease inhibitor (PI) or an integrase strand transfer inhibitor (INSTI)-based regimen, relative to a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based reference. We also examined PLWH on regimens containing multiple core classes. Cox proportional hazards regression analyses were conducted to measure associations between time-updated ART classes and incident anaemia or severe anaemia. Linear mixed effects models were used to examine relationships between ART classes and haemoglobin change.
RESULTS: During a median of 4.9 years of follow-up, 1,040 developed anaemia and 488 developed severe anaemia. Compared to NNRTI use, INSTI-based regimens were associated with an increased risk of anaemia (adjusted hazard ratio [aHR] 1.26, 95% confidence interval [CI] 1.00-1.58) and severe anaemia (aHR1.51 95%CI 1.07-2.11), and a decrease in haemoglobin level. Time on multiple core classes was also associated with increased anaemia risk (aHR 1.39, 95%CI 1.13-1.70), while no associations were found for PI use.
CONCLUSION: These findings suggest INSTI use may increase the risk of anaemia. If confirmed, screening for anaemia development in users of INSTIs may be beneficial. Further research into underlying mechanisms is warranted.

Strengths and limitations of this study:

- This study utilized a large and geographically diverse population of PLWH in care across the U.S.
- This study leveraged comprehensive clinical data, including information on diagnoses, medication use, laboratory test results, demographic information, and medical history.
- This study investigated associations between specific types of ART core regimens and anaemia risk.
- This observational study is subject to residual confounding.
- This study focused on anaemia assessed from haemoglobin lab values taken at regular medical care visits without excluding participants with conditions strongly associated with haemoglobin level through mechanisms unrelated to HIV infection.

Introduction:

Anaemia and severe anaemia are common among people living with HIV (PLWH) [1]. The prevalence of anaemia is elevated in PLWH compared to the general population. One study reported that among non-pregnant American women living with HIV, the prevalence of anaemia was 28.1% compared to 15.1% among women without HIV [2]. Estimates vary by age, sex, HIV disease stage, use of antiretroviral therapy (ART) and injection drug use status [1, 3]. Among PLWH, associations have been found between anaemia and mortality [4-9], health-related quality-of-life [1], morbidity, dementia [10], and ART failure [11]. In addition, anaemia is an independent prognostic indicator associated with HIV disease progression [1, 12, 13], including development of AIDS [7].

Research shows that ART impacts anaemia risk among PLWH. In the early treatment era, use of zidovudine (AZT) was a cause of bone marrow suppression leading to anaemia [14]. However, in recent years, AZT use has decreased substantially as other, better tolerated ART medications have become available. Despite the impact of specific agents such as AZT, ART use in general is associated with reduced anaemia incidence [15, 16], likely due to inhibition of HIV disease progression [17]. Current ART regimens typically include a pair of nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone plus a core agent. Common core classes include non-nucleoside reverse-transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitors (INSTIs), and protease inhibitors (PIs). While ART use overall reduces anaemia, little is known about whether anaemia risk differs between commonly used ART classes in the current treatment era, particularly the newer INSTI class. From

clinical safety data of trials, 36-49% of participants using PIs had haemoglobin (Hb) levels <10g/dL, indicating anaemia [18], and in a randomized controlled trial, two participants discontinued INSTI use due to anaemia adverse events [19]. However, many studies included few participants or were mostly from an earlier ART era when older ART medications were predominantly used or from trials that may be less generalizable to the diverse population of PLWH in clinical care. The objective of this study was to compare rates of anaemia and severe anaemia development as well as changes in Hb over time based on classes of ART used in the current treatment era.

Methods:

Overview and setting:

The present study included PLWH in care in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort during the period of January 1, 2010 to March 31, 2018 (the date through which each site had complete data [administrative censor date] varied somewhat, median date: October 31, 2017). The CNICS cohort has been described in detail elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical cohort of >32,000 adult PLWH receiving care at eight participating sites across the U.S. Comprehensive clinical data, including diagnoses, ART and other medications, laboratory test results, demographic information, and historical information, including ART use before enrollment, is collected through electronic medical records and other institutional data systems at each site and harmonized in the CNICS data repository. Medication data including ART use are entered into the electronic medical records by clinicians or prescription fill/refill data are uploaded directly from pharmacy systems and verified through medical record review. Participants entered the current study on January 1, 2010 or the earliest date after January 1, 2010 that they met the following enrollment criteria (cohort entry date): a) enrollment in CNICS for ≥6 months to allow time for covariate ascertainment, and b) use of an ART regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or INSTI. In addition, all participants were required to have at least 2 available haemoglobin lab values during study follow-up. Figure 1 shows inclusion criteria and exclusions made. Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols (University of Washington Human Subjects Division served as the institutional review board for the centralized deidentified CNICS Data Repository).

Exposure:

The exposure of interest was the ART core drug class (NNRTI, INSTI or PI) prescribed as part of an ART regimen (a backbone of two NRTIs plus a core drug). Participants switching to different core drugs within the same class were considered to be continually exposed to the same core drug class. Individuals with a gap of 6 or more months in use of the ART core drug classes of interest were censored at the start of the gap and did not re-enter the study.

Person-time on INSTI or PI-based regimens was compared to the NNRTI reference. In addition, some PLWH in this cohort had prescriptions for multiple core classes simultaneously. Participants with regimens containing more than 1 core class were categorized separately in analyses as users of "multiple core classes". Boosting agents (e.g. boosted ritonavir, or cobicistat) were not considered a 2nd core agent.

Outcome ascertainment:

Hb levels, expressed in grams per deciliter (g/dL), were ascertained using inpatient and outpatient laboratory data obtained as part of clinical care. Outcomes included incident anaemia (first post-baseline Hb measure below 10 g/dL), incident severe anaemia (first post-baseline Hb measure below 7.5 g/dL) [21] and changes in Hb level. Another outcome, chronic anaemia, defined as anaemia lasting for ≥6 months, was also examined. Chronic anaemia was defined as post-baseline Hb lab results on two separate occasions at least 6 months apart which were consistently in the anemic range without any Hb values above the anaemia range during this 6-month period.

Participant characteristics:

Characteristics that were analyzed as confounders of the association between ART core drugs and incident anaemia, severe anaemia or change in Hb over time included: age, sex, race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection defined as a detectable HCV RNA level or HCV genotype or HCV antibody, kidney function measured using estimated glomerular filtration rate (eGFR, categorized as <30, 30-59, or ≥60 mL/minute/1.73 m^2) [22], CD4 count (categorized as ≥500, 350-499, 200-399, 100-199 or <100 cells/mm^3), viral load (VL, assessed as $\log_{10}$(VL+1)), baseline Hb (in incident anemia, severe anemia and chronic anemia only analyses only), and time in care at CNICS sites, defined as time from cohort entry date until the last available CNICS activity: either last lab date or last visit. HCV, eGFR, CD4 count and VL were assessed as part of clinical care visits and were time-updated as repeated measures occurred. All covariates were selected *a priori*, based on review of the literature and clinical knowledge. In addition, assessment of

self-reported ART adherence was available for a subset of ~55% of the study population who were in care after each individual site initiated a clinical assessment of patient reported outcomes, including adherence [23].

Statistical analysis:

Baseline characteristics are presented for all participants at the cohort entry date. Median and interquartile range (IQR) are displayed for continuous variables and frequencies and proportions are displayed for categorical variables.

Two multivariable Cox proportional hazards regression analyses were conducted, one among the subset of PLWH who were anaemia-free at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of anaemia, and another among the subset of participants who were free of severe anaemia at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of severe anaemia.

Participants were censored at a) the time they developed the outcome of interest, b) at the time of last activity in CNICS, c) at the time of death, d) at the date of administrative censoring at each site or e) at the time they no longer were prescribed one of the ART core classes of interest, whichever came first. The timescale for the models was time since cohort entry. Complete case analysis methods were used (<2% had missing data).

In a sensitivity analysis, we examined those who were ART-naïve at baseline and who initiated a regimen including one of the core ART classes of interest during study follow-up. Follow-up in this analysis began when a person began their initial ART regimen and extended until the earliest time of anaemia occurrence, last activity in CNICS, time of death, administrative censoring, or at the time their initial regimen ended. PI or INSTI use were compared to the reference, NNRTI use. We also examined the change in Hb over time using mixed models among this ART-naïve population. We also conducted sensitivity analyses including time-updated NRTI backbone adjustment for abacavir in analyses of incident anaemia and incident severe anaemia risk. These sensitivity analyses addressed possible concerns that the NRTI backbone may influence anaemia risk rather than the core agent. Finally, we conducted a sensitivity analysis that excluded users of AZT because of concerns that AZT has been found strongly associated with anaemia.

Linear mixed effects models with random slopes for time were used to examine the association of ART core classes with Hb levels among all PLWH after adjustment for the same

characteristics as in the incident anaemia and severe anaemia analyses. Mixed-effects models utilize random slopes and intercepts at the participant level to handle irregular patterns of repeated measures over follow-up [24]. All analyses were performed using Stata version 14.2.

Patient and public involvement:
There was no patient or public participation in the present study.

Results:

In total, 16,505 PLWH met inclusion criteria and were included in these analyses (Figure 1). Participants had an average of 11 outpatient Hb values measured during a median follow-up of 4.9 (IQR 3.0-7.2) years. A total of 12,626 (76%) were free of anaemia at baseline, and 15,357 (93%) were free of severe anaemia at baseline. Table 1 provides baseline characteristics for study participants in the analyses of incident anaemia and incident severe anaemia. Overall, the mean age of study participants was 46 years at cohort entry, 20% were female, and 19% were co-infected with HCV. At baseline, 18% were prescribed regimens with an NNRTI, 53% with a PI, 14% an INSTI, and 16% regimens with multiple cores. INSTIs were increasingly used over the last few years of the study period (Figure 2), and among those simultaneously prescribed multiple core medications, the proportion comprised of INSTI plus another core class increased as study years progressed.

The overall incidence of anaemia was 2.1/100 person-years and the overall incidence of severe anaemia was 0.8/100 person-years. The unadjusted incidence rates of anaemia and severe anaemia based on ART core class are provided in Table 2. In adjusted analyses, INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs (Table 3). Use of multiple core classes together was also associated with an increased risk of anaemia (aHR 1.39, 95%CI 1.13-1.70)) while no associations were found between PI use and anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses restricted to participants free of severe anaemia at baseline (Table 3), INSTI use was associated with an increased risk of severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI use. Although the HR appeared elevated, there was no association between time on multiple ART core classes and an increased risk of severe anaemia (aHR 1.30 (0.95-1.78), and no association was found for PIs (aHR 1.09 (0.81-1.47). Among the 12,626 PLWH who were free of anaemia at baseline, 225 developed chronic anaemia (lasting for ≥6 months), during follow-up. For chronic anaemia, results were similar to those in the primary analysis although

confidence intervals overlapped one. Relative to NNRTI use, person-time on multiple core classes was associated with an aHR for chronic anaemia of 2.21 (95%CI 0.94-5.18), person-time on an INSTI with an aHR of 1.90 (95%CI 0.76-4.64) and person-time on a PI with an aHR of 1.27 (95%CI 0.54-3.04).

Average Hb levels remained steady during follow-up; the mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0 g/dL (IQR 12.6-15.2) at the last available measurement per person. Relative to NNRTI use, a decrease in Hb level over time was associated with both INSTI use (-0.06 g/dL per year, 95%CI -0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -0.18, -0.11). No association was found for PI use (-0.01, 95%CI -0.04, 0.03). (Table 4).

The sensitivity analysis restricted to ART-naïve participants included 6,426 PLWH who were free of prevalent anaemia at baseline, of whom 378 developed anaemia. Compared to NNRTI initiators, those initiating a PI had an aHR of 0.69 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10 (95%CI 0.84-1.44) (Supplemental Table 1). The mixed model examining change in Hb over time among ART-naïve PLWH initiating one of the ART core classes of interest included 7,264 participants. Compared to NNRTI initiators, a decrease in Hb was found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while INSTI use was associated with a larger decrease in Hb level over time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental Table 2). Results from the sensitivity analyses including time-updated NRTI backbone adjustment for abacavir were essentially unchanged (Supplemental Table 3). Finally, results from the sensitivity analysis excluding participants with AZT use indicated similar findings to those from the primary analyses, although confidence intervals were wider (Supplemental Table 4, 5).

Discussion:

In this study of 16,505 PLWH in care within the United States in the current treatment era (2010 and after), we observed that INSTI use, and time on multiple core ART classes, were associated with decreases in Hb levels during follow-up compared to using NNRTI-based regimens. We found that INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over time. Furthermore, the naïve user analysis indicated similar findings despite a smaller sample size. These findings could have implications for the treatment approach that should be used in people with risk factors for anaemia.

This study's strengths include its large and geographically diverse study population and longitudinal data structure. Nevertheless, there are limitations of this study to consider, including the observational nature of the data, which may be

subject to residual confounding including confounding by indication [25]. However, anaemia is not a recognized adverse effect of NNRTIs, PIs, or INSTIs. Thus, it is unlikely that ART core class was selected based on prescriber concern about anaemia risk. Additionally, we did not exclude participants with conditions strongly associated with anaemia or Hb level, including those on dialysis, receiving erythropoietin, or with severe bleeding, which likely caused some of the anaemia cases in this analysis. However, in the sensitivity analysis focusing on factors associated with chronic anaemia (less likely due to bleeding), findings for INSTI vs. NNRTI core regimens were similar to those including all PLWH who became anemic. Information on ART medication use was from prescription data which does not necessarily indicate medications were taken, although self-reported adherence was high (~98%) in the subset for whom adherence information was available. CNICS participants who provided adherence information have been shown to be representative of the overall population of PLWH in CNICS [23, 26]. Finally, the fact that this study was conducted among PLWH in care in the U.S. who are on ART may limit the generalizability of findings to PLWH who live outside of the U.S.

There have been few epidemiologic studies of anaemia risk among users of newer ART core regimens. From clinical safety data reported from multiple trials, approximately 36-49% of participants using PIs had Hb levels <10g/dL, indicating anaemia [18], and in another trial, two participants discontinued INSTI use due to anaemia adverse events [19], however the strict inclusion criteria applied in clinical trials makes it difficult to generalize these findings to more diverse populations of PLWH in clinical care. Another study, conducted during the newer-era of HIV treatment with drugs other than AZT, (during 2008-2012) presented findings for AZT versus non-AZT regimens, finding an increased risk of anaemia among AZT compared to non-AZT regimens (HR=2.84, 95%CI 1.52-5.31) [27]. However, anaemia risk was not analyzed separately for the use of specific classes of ART, resulting in the inability of comparison to the present study's findings and a lack of generalizability to PLWH who are treated with newer ART core agents.

It is possible that PLWH in our study whose HIV is progressing due to resistance or other complications may get switched to an INSTI. This, in addition to prior knowledge that poorly controlled HIV parameters are on their own a risk factor for anaemia [5, 28, 29], could result in confounding by indication. However, the switch to INSTI core regimens since their approval in 2007 has been widespread in this population (Figure 2) and INSTIs are recommended for use as initial

regimens [30]. In addition, we rigorously controlled for many of the important HIV-related factors that correspond to poorly-controlled HIV, and our sensitivity analysis examining PLWH initiating their initial regimen failed to reinforce the notion that an increased risk of anaemia among INSTI core regimen users could be entirely explained by sicker participants getting switched to these therapies.

PLWH on multiple core classes were in a different category in our analyses. There are several reasons PLWH may be prescribed multiple core classes. For example, sometimes PLWH are prescribed multiple core classes to ensure they receive a complete regimen while awaiting approval for specific agents from their insurance company. However, the primary concern was that they were receiving multiple core classes due to provider concerns such as prior failed regimens which may also increase their risk of anaemia.

In conclusion, in this large, diverse, multicenter cohort of PLWH, we found that INSTI use and time on multiple ART core classes were associated with progression to anaemia and a lower Hb level. INSTI use was also associated with severe anaemia risk. Our findings suggest that careful selection of ART regimen could mitigate anaemia development, although this anaemia risk needs to be balanced with the possibility of improvement in overall HIV care [31]. Further research is needed to replicate the finding of INSTI core regimen use and anaemia risk and to understand the underlying mechanisms. If confirmed, screening for anaemia development in users of INSTIs may be beneficial.

Figure Legends

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

Table 1. Baseline characteristics of PLWH in CNICS who were receiving an ART core agent of interest (N=16,505)[a]

	Incident anaemia analysis (n=12,626)		Incident severe anaemia analysis (n=15,357)	
	Do not develop anaemia (n=11,586)	**Develop anaemia (n=1,040)**	**Do not develop severe anaemia (n=14,869)**	**Develop severe anaemia (n=488)**
Age (median, IQR)	45 (37, 51)	47 (40, 54)	45 (37, 52)	46 (39, 54)
Female	1574 (14)	276 (27)	2681 (18)	158 (32)
Race/ethnicity				
White	5782 (50)	396 (38)	6840 (46)	157 (32)
Black	3720 (32)	499 (48)	5442 (37)	254 (52)
Hispanic	1537 (13)	106 (10)	1920 (13)	58 (12)
Other/missing	547 (5)	39 (4)	667 (4)	19 (4)
Years in CNICS at cohort entry[a] (median, IQR)	5.2 (2.3, 8.8)	5.8 (2.4, 9.5)	5.5 (2.4, 9.0)	5.5 (2.8, 9.1)
Viral load ≥400 copies/ml	2441 (21)	283 (27)	3259 (22)	172 (35)
CD4 count (cells/mm^3)				
<100	528 (5)	112 (11)	915 (6)	90 (19)
100-199	870 (8)	96 (9)	1256 (8)	63 (13)
200-349	1974 (17)	216 (21)	2675 (18)	113 (23)
350-499	2497 (21)	226 (22)	3160 (21)	80 (16)
≥500	5717 (49)	390 (38)	6863 (46)	142 (29)

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Hepatitis C virus coinfection	1816 (16)	303 (29)	2711 (18)	139 (28)
Kidney function (eGFR) (mL/min/1.73 m^2)				
<30	32 (<1)	36 (3)	142 (1)	42 (9)
30-59	459 (4)	80 (8)	731 (5)	51 (10)
≥60	11095 (96)	924 (89)	13996 (94)	395 (81)
Baseline haemoglobin (g/dL) (median, IQR)	14.5 (13.5, 15.4)	13.3 (12.2, 14.4)	14.3 (13.1, 15.2)	12.4 (10.8, 13.8)
BMI (kg/m^2)				
<18.5	229 (2)	36 (3)	377 (3)	30 (6)
18.5 to <25.0	4806 (41)	426 (41)	6120 (41)	211 (43)
25.0 to <30.0	3929 (34)	301 (29)	4885 (33)	117 (24)
≥30.0	2622 (23)	277 (27)	3487 (23)	130 (27)
ART core class				
NNRTI	2109 (18)	177 (17)	2633 (18)	70 (14)
PI	6135 (53)	558 (54)	7935 (53)	251 (51)
INSTI	1803 (16)	93 (9)	2126 (14)	46 (9)
Multiple core classes	1539 (13)	212 (20)	2175 (15)	121 (25)
Self-reported adherence (on a 100-point scale) (median, IQR)[b]	98 (93, 100)	98 (91, 99)	98 (92, 100)	97 (90, 99)

[a]Cohort entry date was defined as the earliest date during January 1, 2010- March 31, 2018 that a person had ≥6 months in CNICS and was receiving an ART regimen with a core agent of interest.

[b]For the 55% of the population who reported medication adherence

Abbreviations: PLWH: people living with HIV, CNICS: Centers for AIDS Research Network of Integrated Clinical Systems, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 2. Incidence rate of anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) by ART core drug class

ART Regimen	Follow-up time (person-years)	Events	Rate (per 100 person-years)
Anaemia			
NNRTI	9,964	150	1.50
PI	24,710	485	1.96
INSTI	7,389	155	2.10
Multiple core classes	8,172	250	3.06
Severe anaemia			
NNRTI	12,113	57	0.47
PI	31,156	204	0.65
INSTI	9,132	84	0.92
Multiple core classes	11,258	143	1.27

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL), severe anaemia (haemoglobin<7.5 g/dL) or chronic anaemia (>6 months of anaemia)

ART Regimen	Hazard Ratio of incident anaemia (n=12,626)		Hazard Ratio of incident severe anaemia (n=15,357)		Hazard Ratio of incident chronic anaemia (n=12,626)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.09 (0.90, 1.32)	1.37 (1.02-1.83)	1.09 (0.81, 1.47)	1.43 (0.61-3.35)	1.27 (0.54-3.04)
INSTI	1.39 (1.11-1.75)	1.26 (1.00, 1.58)	1.96 (1.40-2.75)	1.51 (1.07, 2.11)	2.05 (0.85-4.94)	1.90 (0.76-4.64)
Multiple core classes	2.02 (1.65-3.48)	1.39 (1.13, 1.70)	2.70 (1.99-3.67)	1.30 (0.95, 1.78)	3.46 (1.51-7.93)	2.21 (0.94-5.18)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR), baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 4. Association of ART core classes with change in haemoglobin level during follow-up in adjusted analyses (linear mixed-effect model); N=16,505

ART class	Coefficient [a]	95% CI	P-value
NNRTI (REF)			
PI	-0.01	-0.04, 0.03	0.675
INSTI	-0.06	-0.10, -0.03	<0.001
Multiple core classes	-0.14	-0.18, -0.11	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

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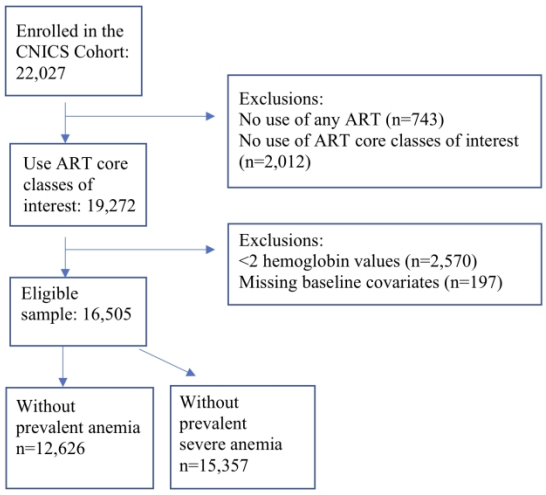

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

215x279mm (300 x 300 DPI)

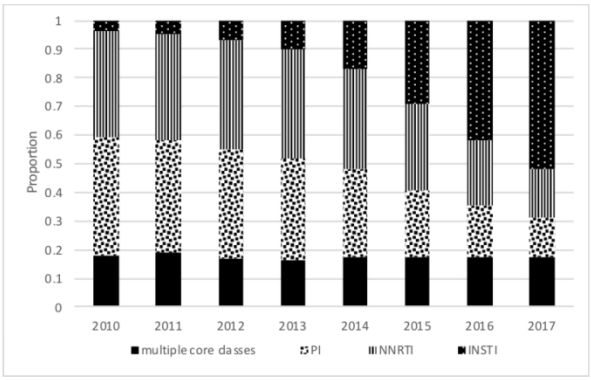

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

215x279mm (300 x 300 DPI)

Supplemental Table 1: Association of ART classes with incident anaemia among naïve users

ART class	Hazard ratio of incident anaemia (n=6,426)	
	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00
PI	0.92 (0.67-1.247)	0.6978 (0.4556-1.068)
INSTI	1.73 (1.39-2.15)	1.105 (0.8492, 1.445)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR) and baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 2. Association of ART classes with change in haemoglobin level during follow-up in adjusted analyses among naïve users (linear mixed-effect model); N=7,264

ART class	Coefficient[a]	95% CI	P-value
NNRTI (REF)			
PI	-0.08	-0.16, -0.01	0.031
INSTI	-0.15	-0.22, -0.09	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study. Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) including time-updated adjustment for abacavir

ART Regimen	Unadjusted	Minimally adjusted[a]	With time-updated abacavir adjustment[b]
		Incident anaemia	
NNRTI (REF)	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.09 (0.90, 1.32)	1.07 (0.88-1.30)
INSTI	1.39 (1.11-1.75)	1.26 (1.00, 1.58)	1.23 (0.97, 1.56)
Multiple core classes	2.02 (1.65-3.48)	1.39 (1.13, 1.70)	1.36 (1.09, 1.68)
		Incident severe anaemia	
NNRTI (REF)	1.00	1.00	1.00
PI	1.37 (1.02-1.83)	1.09 (0.81, 1.47)	1.10 (0.80-1.50)
INSTI	1.96 (1.40-2.75)	1.51 (1.07, 2.11)	1.52 (1.07, 2.15)
Multiple core classes	2.70 (1.99-3.67)	1.30 (0.95, 1.78)	1.31 (0.95 1.81)

[a]Minimally adjusted model includes adjustment for: age, sex, race, site, hepatitis C co-infection, CD4 category, VL, eGFR category and haemoglobin at baseline.

[b]Model includes adjustment variables in minimally adjusted model plus a term for time-updated abacavir use

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Supplemental Table 4. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) excluding people with AZT use

ART Regimen	Hazard Ratio of incident anaemia (n=11,187)		Hazard Ratio of incident severe anaemia (n=13,513)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00
PI	1.30 (1.05,1.60)	1.11 (0.90,1.38)	1.34 (0.97, 1.86)	1.05 (0.75, 1.47)
INSTI	1.54 (1.20, 1.97)	1.37 (1.07,1.77)	1.90 (1.31, 2.76)	1.45 (1.00, 2.11)
Multiple core classes	2.23 (1.76, 2.82)	1.55 (1.22,1.97)	2.90 (2.06, 4.08)	1.34 (0.94, 1.90)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR), baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, AZT: zidovudine, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 5. Association of ART core classes with change in haemoglobin level during follow-up in adjusted analyses (linear mixed-effect model) excluding people with AZT use; N=14,486

ART class	Coefficient[a]	95% CI
NNRTI (REF)		
PI	0.00	-0.04, 0.04
INSTI	-0.05	-0.09, -0.01
Multiple core classes	-0.13	-0.17, -0.09

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.
Abbreviations: ART: antiretroviral therapy, AZT: zidovudine, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
✔**Title and abstract** (page 1-title, page 3 abstract)	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
✔Background/rationale (pages 5, 6)	2	Explain the scientific background and rationale for the investigation being reported
✔Objectives (page 6)	3	State specific objectives, including any prespecified hypotheses
Methods		
✔Study design (page 6)	4	Present key elements of study design early in the paper
✔Setting (page 6)	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✔Participants (page 6)	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
✔Variables (pages 7, 8)	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✔Data sources/ measurement (pages 7, 8)	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
✔Bias (page 9)	9	Describe any efforts to address potential sources of bias
✔Study size (page 6)	10	Explain how the study size was arrived at
✔Quantitative variables (pages 7, 8)	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✔Statistical methods (pages 8, 9)	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
✔Participants (figure 1, page 9)	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✔Descriptive data (pages 9, 10)	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)

✔Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
✔Main results (pages 10, 11)	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
✔Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
✔Key results (page 11)	18	Summarise key results with reference to study objectives
✔Limitations (page 12)	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
✔Interpretation (page 13)	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
✔Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
Other information		
✔Funding (page 2)	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

A study of antiretroviral drug class and anaemia risk in the current treatment era among people living with HIV in the United States: A clinical cohort study

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A study of antiretroviral drug class and anaemia risk in the current treatment era among people living with HIV in the United States: A clinical cohort study

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Dr. Harding reports grants from National Heart, Lung and Blood Institute(NHLBI) during the conduct of the study; Ms. Whitney reports grants from the National Institutes of Health (NIH) during the conduct of the study; Ms. Nance reports grants from NIH during the conduct of the study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH, grants from ViiV healthcare and grants from PCORI outside the submitted work; Dr. Burkholder reports grants from NIH during the conduct of the study, other from Amgen, Inc outside the submitted work; Dr. Moore reports grants from NHLBI during the conduct of the study; Dr. Mathews reports grants from NHLBI during the conduct of the study; Dr. Eron reports grants from NIH, during the conduct of the study, grants and personal fees from Gilead Sciences, grants and personal fees from ViiV Healthcare, grants and personal fees from Janssen and personal fees

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Contributions:
BNH has conducted all analyses, contributed to methodologic approach, and written the manuscript. In addition, BMW and RMN contributed to data preparation and analysis, JACD contributed to the analysis, HMC, SRH and JACD contributed to conception and design of the work, HMC, GB, RDM, WCM, JJE, BR, KHM, MSS and MMK contributed to data collection and BNH, BMW, RMN, HMC, GB, RDM, WCM, JJE, PWH, PV, BR, KHM, MSS, MMK, SRH and JACD contributed to the interpretation of data and critically revising the manuscript for important intellectual content.

Transparency declaration:
Dr. Harding affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Ethical approval:
Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols for patient protection and provided general approval for secondary data analysis. The University of Washington Human Subjects Division served as the institutional review board for the centralized deidentified CNICS Data Repository (IRB approval number 27674-D).

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Data sharing:

The Centers for AIDS research (CFAR) Network of Integrated Clinical Systems (CNICS) data may be accessed with an approved concept proposal. Instructions for data access and concept proposal forms may be found at https://www.uab.edu/ cnics/submit-proposal.

Dissemination declaration:
Dissemination of the study findings are not applicable to participants or patient organizations.

Abstract: (276 words)
OBJECTIVES: Anaemia is common among people living with HIV (PLWH) and has been associated with certain, often older, antiretroviral medications. Information on current antiretroviral therapy (ART) and anaemia is limited. The objectives were to compare associations between anaemia incidence or haemoglobin change with core ART classes in the current ART era.
DESIGN: Retrospective cohort study.
SETTING: U.S.-based prospective clinical cohort of PLWH aged 18 and above receiving care at 8 sites between 1/2010-3/2018.
PARTICIPANTS: 16,505 PLWH were included in this study.
MAIN OUTCOME MEASURES: Anaemia risk and haemoglobin change were estimated among PLWH for person-time on a protease inhibitor (PI) or an integrase strand transfer inhibitor (INSTI)-based regimen, relative to a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based reference. We also examined PLWH on regimens containing multiple core classes. Cox proportional hazards regression analyses were conducted to measure associations between time-updated ART classes and incident anaemia or severe anaemia. Linear mixed effects models were used to examine relationships between ART classes and haemoglobin change.
RESULTS: During a median of 4.9 years of follow-up, 1,040 developed anaemia and 488 developed severe anaemia. Compared to NNRTI use, INSTI-based regimens were associated with an increased risk of anaemia (adjusted hazard ratio [aHR] 1.26, 95% confidence interval [CI] 1.00-1.58) and severe anaemia (aHR1.51 95%CI 1.07-2.11), and a decrease in haemoglobin level. Time on multiple core classes was also associated with increased anaemia risk (aHR 1.39, 95%CI 1.13-1.70), while no associations were found for PI use.
CONCLUSION: These findings suggest INSTI use may increase the risk of anaemia. If confirmed, screening for anaemia development in users of INSTIs may be beneficial. Further research into underlying mechanisms is warranted.

Strengths and limitations of this study:

- This study utilized a large and geographically diverse population of PLWH in care across the U.S.
- This study leveraged comprehensive clinical data, including information on diagnoses, medication use, laboratory test results, demographic information, and medical history.
- This study investigated associations between specific types of ART core regimens and anaemia risk.
- This observational study is subject to residual confounding.
- This study focused on anaemia assessed from haemoglobin lab values taken at regular medical care visits without excluding participants with conditions strongly associated with haemoglobin level through mechanisms unrelated to HIV infection.

Introduction:

Anaemia and severe anaemia are common among people living with HIV (PLWH) [1]. The prevalence of anaemia is elevated in PLWH compared to the general population. One study reported that among non-pregnant American women living with HIV, the prevalence of anaemia was 28.1% compared to 15.1% among women without HIV [2]. Estimates vary by age, sex, HIV disease stage, use of antiretroviral therapy (ART) and injection drug use status [1, 3]. Among PLWH, associations have been found between anaemia and mortality [4-9], health-related quality-of-life [1], morbidity, dementia [10], and ART failure [11]. In addition, anaemia is an independent prognostic indicator associated with HIV disease progression [1, 12, 13], including development of AIDS [7].

Research shows that ART impacts anaemia risk among PLWH. In the early treatment era, use of zidovudine (AZT) was a cause of bone marrow suppression leading to anaemia [14]. However, in recent years, AZT use has decreased substantially as other, better tolerated ART medications have become available. Despite the impact of specific agents such as AZT, ART use in general is associated with reduced anaemia incidence [15, 16], likely due to inhibition of HIV disease progression [17]. Current ART regimens typically include a pair of nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone plus a core agent. Common core classes include non-nucleoside reverse-transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitors (INSTIs), and protease inhibitors (PIs). While ART use overall reduces anaemia, little is known about whether anaemia risk differs between commonly used ART classes in the current treatment era, particularly the newer INSTI class. From

clinical safety data of trials, 36-49% of participants using PIs had haemoglobin (Hb) levels <10g/dL, indicating anaemia [18], and in a randomized controlled trial, two participants discontinued INSTI use due to anaemia adverse events [19]. However, many studies included few participants or were mostly from an earlier ART era when older ART medications were predominantly used or from trials that may be less generalizable to the diverse population of PLWH in clinical care. The objective of this study was to compare rates of anaemia and severe anaemia development as well as changes in Hb over time based on classes of ART used in the current treatment era.

Methods:

Overview and setting:

The present study included PLWH in care in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort during the period of January 1, 2010 to March 31, 2018 (the date through which each site had complete data [administrative censor date] varied somewhat, median date: October 31, 2017). The CNICS cohort has been described in detail elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical cohort of >32,000 adult PLWH receiving care at eight participating sites across the U.S. Comprehensive clinical data, including diagnoses, ART and other medications, laboratory test results, demographic information, and historical information, including ART use before enrollment, is collected through electronic medical records and other institutional data systems at each site and harmonized in the CNICS data repository. Medication data including ART use are entered into the electronic medical records by clinicians or prescription fill/refill data are uploaded directly from pharmacy systems and verified through medical record review. Participants entered the current study on January 1, 2010 or the earliest date after January 1, 2010 that they met the following enrollment criteria (cohort entry date): a) enrollment in CNICS for ≥6 months to allow time for covariate ascertainment, and b) use of an ART regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or INSTI. In addition, all participants were required to have at least 2 available haemoglobin lab values during study follow-up. Figure 1 shows inclusion criteria and exclusions made. Informed consent was obtained from all participants and institutional review boards at each site approved CNICS protocols (University of Washington Human Subjects Division served as the institutional review board for the centralized deidentified CNICS Data Repository).

Exposure:

The exposure of interest was the ART core drug class (NNRTI, INSTI or PI) prescribed as part of an ART regimen (a backbone of two NRTIs plus a core drug). Participants switching to different core drugs within the same class were considered to be continually exposed to the same core drug class. Individuals with a gap of 6 or more months in use of the ART core drug classes of interest were censored at the start of the gap and did not re-enter the study.

Person-time on INSTI or PI-based regimens was compared to the NNRTI reference. In addition, some PLWH in this cohort had prescriptions for multiple core classes simultaneously. Participants with regimens containing more than 1 core class were categorized separately in analyses as users of "multiple core classes". Boosting agents (e.g. boosted ritonavir, or cobicistat) were not considered a 2nd core agent.

Outcome ascertainment:

Hb levels, expressed in grams per deciliter (g/dL), were ascertained using inpatient and outpatient laboratory data obtained as part of clinical care. Outcomes included incident anaemia (first post-baseline Hb measure below 10 g/dL), incident severe anaemia (first post-baseline Hb measure below 7.5 g/dL) [21] and changes in Hb level. Another outcome, chronic anaemia, defined as anaemia lasting for ≥6 months, was also examined. Chronic anaemia was defined as post-baseline Hb lab results on two separate occasions at least 6 months apart which were consistently in the anemic range without any Hb values above the anaemia range during this 6-month period.

Participant characteristics:

Characteristics that were analyzed as confounders of the association between ART core drugs and incident anaemia, severe anaemia or change in Hb over time included: age, sex, race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection defined as a detectable HCV RNA level or HCV genotype or HCV antibody, kidney function measured using estimated glomerular filtration rate (eGFR, categorized as <30, 30-59, or ≥60 mL/minute/1.73 m^2) [22], CD4 count (categorized as ≥500, 350-499, 200-399, 100-199 or <100 cells/mm^3), viral load (VL, assessed as $\log_{10}$(VL+1)), baseline Hb (in incident anemia, severe anemia and chronic anemia analyses only), and time in care at CNICS sites, defined as time from cohort entry date until the last available CNICS activity: either last lab date or last visit. HCV, eGFR, CD4 count and VL were assessed as part of clinical care visits and were time-updated as repeated measures occurred. All covariates were selected *a priori*, based on review of the literature and clinical knowledge. In addition, assessment of

self-reported ART adherence was available for a subset of ~55% of the study population who were in care after each individual site initiated a clinical assessment of patient reported outcomes, including adherence [23].

Statistical analysis:

Baseline characteristics are presented for all participants at the cohort entry date. Median and interquartile range (IQR) are displayed for continuous variables and frequencies and proportions are displayed for categorical variables.

Two multivariable Cox proportional hazards regression analyses were conducted, one among the subset of PLWH who were anaemia-free at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of anaemia, and another among the subset of participants who were free of severe anaemia at baseline to determine associations between time-updated NNRTI, PI, and INSTI use and development of severe anaemia.

Participants were censored at a) the time they developed the outcome of interest, b) at the time of last activity in CNICS, c) at the time of death, d) at the date of administrative censoring at each site or e) at the time they no longer were prescribed one of the ART core classes of interest, whichever came first. The timescale for the models was time since cohort entry. Complete case analysis methods were used (<2% had missing data).

In a sensitivity analysis, we examined those who were ART-naïve at baseline and who initiated a regimen including one of the core ART classes of interest during study follow-up. Follow-up in this analysis began when a person began their initial ART regimen and extended until the earliest time of anaemia occurrence, last activity in CNICS, time of death, administrative censoring, or at the time their initial regimen ended. PI or INSTI use were compared to the reference, NNRTI use. We also examined the change in Hb over time using mixed models among this ART-naïve population. We also conducted sensitivity analyses including time-updated NRTI backbone regimen adjustment in analyses of incident anaemia and incident severe anaemia risk. These sensitivity analyses addressed possible concerns that the NRTI backbone may influence anaemia risk rather than the core agent. Finally, we conducted a sensitivity analysis that excluded users of AZT from comparisons of NNRTI, PI and INSTI use versus the referent category of NNRTI use because of concerns that AZT has been found strongly associated with anaemia.

Linear mixed effects models with random slopes for time were used to examine the association of ART core classes with Hb

levels among all PLWH after adjustment for the same characteristics as in the incident anaemia and severe anaemia analyses. Mixed-effects models utilize random slopes and intercepts at the participant level to handle irregular patterns of repeated measures over follow-up [24]. All analyses were performed using Stata version 14.2.

Patient and public involvement:

There was no patient or public participation in the present study.

Results:

In total, 16,505 PLWH met inclusion criteria and were included in these analyses (Figure 1). Participants had an average of 11 outpatient Hb values measured during a median follow-up of 4.9 (IQR 3.0-7.2) years. A total of 12,626 (76%) were free of anaemia at baseline, and 15,357 (93%) were free of severe anaemia at baseline. Table 1 provides baseline characteristics for study participants in the analyses of incident anaemia and incident severe anaemia. Overall, the mean age of study participants was 46 years at cohort entry, 20% were female, and 19% were co-infected with HCV. At baseline, 18% were prescribed regimens with an NNRTI, 53% with a PI, 14% an INSTI, and 16% regimens with multiple cores. INSTIs were increasingly used over the last few years of the study period (Figure 2), and among those simultaneously prescribed multiple core medications, the proportion comprised of INSTI plus another core class increased as study years progressed. In the analytic study sample, backbone regimens mainly consisted of emtricitabine/lamivudine (3TC) plus tenofovir or 3TC plus abacavir (Supplemental Table 1).

The overall incidence of anaemia was 2.1/100 person-years and the overall incidence of severe anaemia was 0.8/100 person-years. The unadjusted incidence rates of anaemia and severe anaemia based on ART core class are provided in Table 2. In adjusted analyses, INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs (Table 3). Use of multiple core classes together was also associated with an increased risk of anaemia (aHR 1.39, 95%CI 1.13-1.70)) while no associations were found between PI use and anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses restricted to participants free of severe anaemia at baseline (Table 3), INSTI use was associated with an increased risk of severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI use. Although the HR appeared elevated, there was no association between time on multiple ART core classes and an increased risk of severe anaemia (aHR 1.30 (0.95-1.78), and no association was found for PIs (aHR 1.09 (0.81-1.47). Among the 12,626 PLWH who

were free of anaemia at baseline, 225 developed chronic anaemia (lasting for ≥6 months), during follow-up. For chronic anaemia, results were similar to those in the primary analysis although confidence intervals overlapped 1. Relative to NNRTI use, person-time on multiple core classes was associated with an aHR for chronic anaemia of 2.21 (95%CI 0.94-5.18), person-time on an INSTI with an aHR of 1.90 (95%CI 0.76-4.64) and person-time on a PI with an aHR of 1.27 (95%CI 0.54-3.04).

Average Hb levels remained steady during follow-up; the mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0 g/dL (IQR 12.6-15.2) at the last available measurement per person. Relative to NNRTI use, a decrease in Hb level over time was associated with both INSTI use (-0.06 g/dL per year, 95%CI -0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -0.18, -0.11). No association was found for PI use (-0.01, 95%CI -0.04, 0.03). (Table 4).

The sensitivity analysis restricted to ART-naïve participants included 6,426 PLWH who were free of prevalent anaemia at baseline, of whom 378 developed anaemia. Compared to NNRTI initiators, those initiating a PI had an aHR of 0.69 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10 (95%CI 0.84-1.44) (Supplemental Table 2). The mixed model examining change in Hb over time among ART-naïve PLWH initiating one of the ART core classes of interest included 7,264 participants. Compared to NNRTI initiators, a decrease in Hb was found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while INSTI use was associated with a larger decrease in Hb level over time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental Table 3). Results from the sensitivity analyses including time-updated NRTI backbone regimen adjustment were essentially unchanged (Supplemental Table 4). Finally, results from the sensitivity analysis excluding participants with AZT use indicated similar findings to those from the primary analyses, although confidence intervals were wider (Supplemental Table 5, 6).

Discussion:

In this study of 16,505 PLWH in care within the United States in the current treatment era (2010 and after), we observed that INSTI use, and time on multiple core ART classes, were associated with decreases in Hb levels during follow-up compared to using NNRTI-based regimens. We found that INSTI use was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over time. Furthermore, the naïve user analysis indicated similar findings despite a smaller sample size. These findings could have implications for the treatment approach that should be used in people with risk factors for anaemia.

This study's strengths include its large and geographically diverse study population and longitudinal data structure.

Nevertheless, there are limitations of this study to consider, including the observational nature of the data, which may be subject to residual confounding including confounding by indication [25]. However, anaemia is not a recognized adverse effect of NNRTIs, PIs, or INSTIs. Thus, it is unlikely that ART core class was selected based on prescriber concern about anaemia risk. Additionally, we did not exclude participants with conditions strongly associated with anaemia or Hb level, including those on dialysis, receiving erythropoietin, or with severe bleeding, which likely caused some of the anaemia cases in this analysis. However, in the sensitivity analysis focusing on factors associated with chronic anaemia (less likely due to bleeding), findings for INSTI vs. NNRTI core regimens were similar to those including all PLWH who became anemic. Information on ART medication use was from prescription data which does not necessarily indicate medications were taken, although self-reported adherence was high (~98%) in the subset for whom adherence information was available. CNICS participants who provided adherence information have been shown to be representative of the overall population of PLWH in CNICS [23, 26]. Finally, the fact that this study was conducted among PLWH in care in the U.S. who are on ART may limit the generalizability of findings to PLWH who live outside of the U.S.

There have been few epidemiologic studies of anaemia risk among users of newer ART core regimens. From clinical safety data reported from multiple trials, approximately 36-49% of participants using PIs had Hb levels <10g/dL, indicating anaemia [18], and in another trial, two participants discontinued INSTI use due to anaemia adverse events [19], however the strict inclusion criteria applied in clinical trials makes it difficult to generalize these findings to more diverse populations of PLWH in clinical care. Another study, conducted during the newer-era of HIV treatment with drugs other than AZT, (during 2008-2012) presented findings for AZT versus non-AZT regimens, finding an increased risk of anaemia among AZT compared to non-AZT regimens (HR=2.84, 95%CI 1.52-5.31) [27]. However, anaemia risk was not analyzed separately for the use of specific classes of ART, resulting in the inability of comparison to the present study's findings and a lack of generalizability to PLWH who are treated with newer ART core agents.

It is possible that PLWH in our study whose HIV is progressing due to resistance or other complications may get switched to an INSTI. This, in addition to prior knowledge that poorly controlled HIV parameters are on their own a risk factor for anaemia [5, 28, 29], could result in confounding by indication. However, the switch to INSTI core regimens since

their approval in 2007 has been widespread in this population (Figure 2) and INSTIs are recommended for use as initial regimens [30]. In addition, we rigorously controlled for many of the important HIV-related factors that correspond to poorly-controlled HIV, and our sensitivity analysis examining PLWH initiating their initial regimen failed to reinforce the notion that an increased risk of anaemia among INSTI core regimen users could be entirely explained by sicker participants getting switched to these therapies.

PLWH on multiple core classes were in a different category in our analyses. There are several reasons PLWH may be prescribed multiple core classes. For example, sometimes PLWH are prescribed multiple core classes to ensure they receive a complete regimen while awaiting approval for specific agents from their insurance company. However, the primary concern was that they were receiving multiple core classes due to provider concerns such as prior failed regimens which may also increase their risk of anaemia.

In conclusion, in this large, diverse, multicenter cohort of PLWH, we found that INSTI use and time on multiple ART core classes were associated with progression to anaemia and a lower Hb level. INSTI use was also associated with severe anaemia risk. Our findings suggest that careful selection of ART regimen could mitigate anaemia development, although this anaemia risk needs to be balanced with the possibility of improvement in overall HIV care [31]. Further research is needed to replicate the finding of INSTI core regimen use and anaemia risk and to understand the underlying mechanisms. If confirmed, screening for anaemia development in users of INSTIs may be beneficial.

Figure Legends

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

Table 1. Baseline characteristics of PLWH in CNICS who were receiving an ART core agent of interest (N=16,505)[a]

	Incident anaemia analysis (n=12,626)		Incident severe anaemia analysis (n=15,357)	
	Do not develop anaemia (n=11,586)	**Develop anaemia (n=1,040)**	**Do not develop severe anaemia (n=14,869)**	**Develop severe anaemia (n=488)**
Age (median, IQR)	45 (37, 51)	47 (40, 54)	45 (37, 52)	46 (39, 54)
Female	1574 (14)	276 (27)	2681 (18)	158 (32)
Race/ethnicity				
White	5782 (50)	396 (38)	6840 (46)	157 (32)
Black	3720 (32)	499 (48)	5442 (37)	254 (52)
Hispanic	1537 (13)	106 (10)	1920 (13)	58 (12)
Other/missing	547 (5)	39 (4)	667 (4)	19 (4)
Years in CNICS at cohort entry[a] (median, IQR)	5.2 (2.3, 8.8)	5.8 (2.4, 9.5)	5.5 (2.4, 9.0)	5.5 (2.8, 9.1)
Viral load ≥400 copies/ml	2441 (21)	283 (27)	3259 (22)	172 (35)
CD4 count (cells/mm^3)				
<100	528 (5)	112 (11)	915 (6)	90 (19)
100-199	870 (8)	96 (9)	1256 (8)	63 (13)
200-349	1974 (17)	216 (21)	2675 (18)	113 (23)
350-499	2497 (21)	226 (22)	3160 (21)	80 (16)

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≥500	5717 (49)	390 (38)	6863 (46)	142 (29)
Hepatitis C virus coinfection	1816 (16)	303 (29)	2711 (18)	139 (28)
Kidney function (eGFR) (mL/min/1.73 m^2)				
<30	32 (<1)	36 (3)	142 (1)	42 (9)
30-59	459 (4)	80 (8)	731 (5)	51 (10)
≥60	11095 (96)	924 (89)	13996 (94)	395 (81)
Baseline haemoglobin (g/dL) (median, IQR)	14.5 (13.5, 15.4)	13.3 (12.2, 14.4)	14.3 (13.1, 15.2)	12.4 (10.8, 13.8)
BMI (kg/m^2)				
<18.5	229 (2)	36 (3)	377 (3)	30 (6)
18.5 to <25.0	4806 (41)	426 (41)	6120 (41)	211 (43)
25.0 to <30.0	3929 (34)	301 (29)	4885 (33)	117 (24)
≥30.0	2622 (23)	277 (27)	3487 (23)	130 (27)
ART core class				
NNRTI	2109 (18)	177 (17)	2633 (18)	70 (14)
PI	6135 (53)	558 (54)	7935 (53)	251 (51)
INSTI	1803 (16)	93 (9)	2126 (14)	46 (9)
Multiple core classes	1539 (13)	212 (20)	2175 (15)	121 (25)
Self-reported adherence (on a 100-point scale) (median, IQR)[b]	98 (93, 100)	98 (91, 99)	98 (92, 100)	97 (90, 99)

[a]Cohort entry date was defined as the earliest date during January 1, 2010- March 31, 2018 that a person had ≥6 months in CNICS and was receiving an ART regimen with a core agent of interest.

[b]For the 55% of the population who reported medication adherence

Abbreviations: PLWH: people living with HIV, CNICS: Centers for AIDS Research Network of Integrated Clinical Systems, ART: antiretroviral therapy, eGFR: estimated glomerular filtration rate, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 2. Incidence rate of anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) by ART core drug class

ART Regimen	Follow-up time (person-years)	Events	Rate (per 100 person-years)
Anaemia			
NNRTI	9,964	150	1.50
PI	24,710	485	1.96
INSTI	7,389	155	2.10
Multiple core classes	8,172	250	3.06
Severe anaemia			
NNRTI	12,113	57	0.47
PI	31,156	204	0.65
INSTI	9,132	84	0.92
Multiple core classes	11,258	143	1.27

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

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Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL), severe anaemia (haemoglobin<7.5 g/dL) or chronic anaemia (>6 months of anaemia)

ART Regimen	Hazard Ratio of incident anaemia (n=12,626)		Hazard Ratio of incident severe anaemia (n=15,357)		Hazard Ratio of incident chronic anaemia (n=12,626)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.09 (0.90, 1.32)	1.37 (1.02-1.83)	1.09 (0.81, 1.47)	1.43 (0.61-3.35)	1.27 (0.54-3.04)
INSTI	1.39 (1.11-1.75)	1.26 (1.00, 1.58)	1.96 (1.40-2.75)	1.51 (1.07, 2.11)	2.05 (0.85-4.94)	1.90 (0.76-4.64)
Multiple core classes	2.02 (1.65-3.48)	1.39 (1.13, 1.70)	2.70 (1.99-3.67)	1.30 (0.95, 1.78)	3.46 (1.51-7.93)	2.21 (0.94-5.18)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR), baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Table 4. Association of ART core classes with change in haemoglobin level during follow-up in adjusted analyses (linear mixed-effect model); N=16,505

ART class	Coefficient [a]	95% CI	P-value
NNRTI (REF)			
PI	-0.01	-0.04, 0.03	0.675
INSTI	-0.06	-0.10, -0.03	<0.001
Multiple core classes	-0.14	-0.18, -0.11	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

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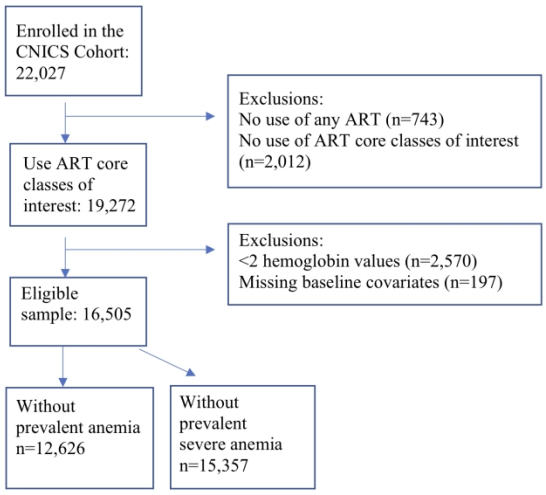

Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included in these analyses.

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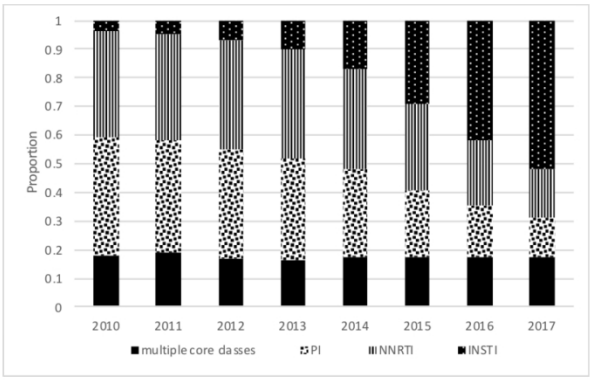

Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

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Supplemental Table 1: Backbone component drugs at baseline for participants in analytic sample (N=15,505)

Backbone Regimen	Drug 1	Drug 2	Percentage
1	Emtricitabine/Lamivudine	Tenofovir	69
2	Emtricitabine/Lamivudine	Abacavir	10
3	Emtricitabine/Lamivudine	Tenofovir/Abacavir	4
4	Emtricitabine/Lamivudine	Zidovudine	4
5	Other[a]		13

[a] Other backbone combinations include complex backbone combinations, NRTI-sparing regimens and salvage therapies.

Supplemental Table 2: Association of ART classes with incident anaemia among those ART naïve at baseline

ART class	Hazard ratio of incident anaemia (n=6,426)	
	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00
PI	0.92 (0.67-1.247)	0.69 (0.45-1.06)
INSTI	1.73 (1.39-2.15)	1.10 (0.84, 1.44)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR) and baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 3. Association of ART classes with change in haemoglobin level during follow-up in adjusted analyses among those ART naïve at baseline (linear mixed-effect model); N=7,264

ART class	Coefficient[a]	95% CI	P-value
NNRTI (REF)			
PI	-0.08	-0.16, -0.01	0.031
INSTI	-0.15	-0.22, -0.09	<0.001

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study. Abbreviations: ART: antiretroviral therapy, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 4. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) including time-updated adjustment for for regimens including abacavir/3TC.

ART Regimen	Unadjusted	Minimally adjusted[a]	With time-updated abacavir adjustment[b]
		Incident anaemia	
NNRTI (REF)	1.00	1.00	1.00
PI	1.26 (1.05-1.52)	1.09 (0.90, 1.32)	1.07 (0.88-1.30)
INSTI	1.39 (1.11-1.75)	1.26 (1.00, 1.58)	1.23 (0.97, 1.56)
Multiple core classes	2.02 (1.65-3.48)	1.39 (1.13, 1.70)	1.36 (1.09, 1.68)
		Incident severe anaemia	
NNRTI (REF)	1.00	1.00	1.00
PI	1.37 (1.02-1.83)	1.09 (0.81, 1.47)	1.10 (0.80-1.50)
INSTI	1.96 (1.40-2.75)	1.51 (1.07, 2.11)	1.52 (1.07, 2.15)
Multiple core classes	2.70 (1.99-3.67)	1.30 (0.95, 1.78)	1.31 (0.95 1.81)

[a]Minimally adjusted model includes adjustment for: age, sex, race, site, hepatitis C co-infection, CD4 category, VL, eGFR category and haemoglobin at baseline.

[b]Model includes adjustment variables in minimally adjusted model plus a term for time-updated abacavir/3TC backbone regimen use. The other backbone regimens largely consisted of tenofovir /3TC or other complex backbone combinations, NRTI-sparing regimens and salvage therapies.

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Supplemental Table 5. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL) and severe anaemia (haemoglobin<7.5 g/dL) excluding people with AZT use

ART Regimen	Hazard Ratio of incident anaemia (n=11,187)		Hazard Ratio of incident severe anaemia (n=13,513)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
NNRTI (REF)	1.00	1.00	1.00	1.00
PI	1.30 (1.05,1.60)	1.11 (0.90,1.38)	1.34 (0.97, 1.86)	1.05 (0.75, 1.47)
INSTI	1.54 (1.20, 1.97)	1.37 (1.07,1.77)	1.90 (1.31, 2.76)	1.45 (1.00, 2.11)
Multiple core classes	2.23 (1.76, 2.82)	1.55 (1.22,1.97)	2.90 (2.06, 4.08)	1.34 (0.94, 1.90)

[a]Adjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count, viral load, kidney function (eGFR), baseline haemoglobin

Abbreviations: ART: antiretroviral therapy, AZT: zidovudine, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

Supplemental Table 6. Association of ART core classes with change in haemoglobin level during follow-up in adjusted analyses (linear mixed-effect model) excluding people with AZT use; N=14,486

ART class	Coefficient[a]	95% CI
NNRTI (REF)		
PI	0.00	-0.04, 0.04
INSTI	-0.05	-0.09, -0.01
Multiple core classes	-0.13	-0.17, -0.09

[a]Coefficient is the mean difference per year in Hb (g/dL) for each core regimen relative to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C virus coinfection, CD4 cell count, viral load, eGFR and years in study.

Abbreviations: ART: antiretroviral therapy, AZT: zidovudine, NNRTI: non-nucleoside reverse-transcriptase inhibitor, PI: protease inhibitor, INSTI: integrase strand transfer inhibitor

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
✔**Title and abstract** (page 1-title, page 3 abstract)	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
✔Background/rationale (pages 5, 6)	2	Explain the scientific background and rationale for the investigation being reported
✔Objectives (page 6)	3	State specific objectives, including any prespecified hypotheses
Methods		
✔Study design (page 6)	4	Present key elements of study design early in the paper
✔Setting (page 6)	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
✔Participants (page 6)	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
✔Variables (pages 7, 8)	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
✔Data sources/ measurement (pages 7, 8)	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
✔Bias (page 9)	9	Describe any efforts to address potential sources of bias
✔Study size (page 6)	10	Explain how the study size was arrived at
✔Quantitative variables (pages 7, 8)	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
✔Statistical methods (pages 8, 9)	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
✔Participants (figure 1, page 9)	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
✔Descriptive data (pages 9, 10)	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)

✔Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
✔Main results (pages 10, 11)	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
✔Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
✔Key results (page 11)	18	Summarise key results with reference to study objectives
✔Limitations (page 12)	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
✔Interpretation (page 13)	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
✔Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
Other information		
✔Funding (page 2)	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.