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

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

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12 the conduct of the study; Dr. Heckbert reports grants from National Institutes of Health during
13 the conduct of the study; Dr. Delaney reports grants from NHLBI during the conduct of the
14 study.
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16
17

18 **Contributions:**

19
20 Ms. Harding, has conducted all analyses, contributed to methodologic approach, and
21 written the manuscript. In addition, Ms. Whitney and Ms. Nance contributed to data
22 preparation and analysis, Dr. Delaney contributed to the analysis, Drs. Heckbert, Crane and
23 Delaney contributed to conception and design of the work and all authors contributed to the
24 interpretation of data and critically revising the manuscript for important intellectual content.
25
26

27 **Transparency declaration:**

28 Ms. Harding affirms that the manuscript is an honest, accurate, and transparent account of the
29 study being reported; that no important aspects of the study have been omitted; and that any
30 discrepancies from the study as planned (and, if relevant, registered) have been explained.
31
32

33 **Ethical approval:**

34 Informed consent was obtained from all participants and institutional review boards at each site
35 approved CNICS protocols.
36
37

38 **Funding:**

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40 provided an unrestricted grant and we are completely independent from the study sponsors.
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42

43 **Data sharing:** No additional data available.
44
45

46 **Dissemination declaration:** Dissemination of the study findings are not applicable to
47 participants or patient organizations.
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11 **Abstract:** (276 words)
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14 **OBJECTIVES:** Anemia is common among people living with HIV (PLWH) and has been
15 associated with certain, often older, antiretroviral medications. Information on current
16 antiretroviral therapy (ART) and anemia is limited. The objectives were to compare
17 associations between anemia incidence or hemoglobin change with core ART classes in
18 the current ART era.
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27 **DESIGN:** Retrospective cohort study.
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30 **SETTING:** U.S.-based prospective clinical cohort of PLWH aged 18 and above receiving
31 care at 8 sites between 1/2010-3/2018.
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36 **PARTICIPANTS:** 16,505 PLWH were included in this study.
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39 **MAIN OUTCOME MEASURES:** Anemia risk and hemoglobin change were measured
40 for person-time on a protease inhibitor (PI) or an integrase strand transfer inhibitor
41 (INSTI), relative to a non-nucleoside reverse transcriptase inhibitor (NNRTI) reference.
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46 We also examined PLWH on multiple core classes. Cox proportional hazards regression
47 analyses were conducted to measure associations between time-updated ART classes
48 and incident anemia or severe anemia. Linear mixed effects models were used to
49 examine relationships between ART classes and hemoglobin change.
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3 RESULTS: During a median of 4.9 years of follow-up, 1,040 developed anemia and 488
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5 developed severe anemia during. Compared to NNRTI use, INSTI-based regimens were
6
7 associated with an increased risk of anemia (adjusted hazard ratio [aHR] 1.17, 95%
8
9 confidence interval [CI] 0.94-1.47) and severe anemia (aHR1.55 95%CI 1.11-2.17), and a
10
11 decrease in hemoglobin level. Time on multiple core classes was also associated with
12
13 increased anemia risk (aHR 1.30, 95%CI 1.06-1.60) and severe anemia risk (aHR 1.35,
14
15 95%CI 0.99-1.85), while no associations were found for PI use.
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22 CONCLUSION: These findings suggest INSTI use may increase the risk of anemia. If
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24 confirmed, screening for anemia development in users of INSTIs may be beneficial.
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27 Further research into underlying mechanisms is warranted.
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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

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3 addition, anemia is an independent prognostic indicator associated with HIV disease
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6 progression [2, 13, 14], including development of AIDS [8].
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9 Research shows that ART impacts anemia risk among PLWH. In the early
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11 treatment era, use of zidovudine (AZT) was a cause of bone marrow suppression
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13 leading to anemia [15]. However, in recent years, AZT use has decreased substantially
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15 as other, better tolerated ART medications have become available. Despite the impact of
16
17 specific agents such as AZT, ART use in general is associated with reduced anemia
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19 incidence [16, 17] likely due to inhibition of HIV disease progression. Since worsening
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21 HIV disease parameters are associated with anemia, better disease control with ART
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23 reduces the risk of anemia [18]. Current ART regimens typically include a pair of
24
25 nucleoside reverse transcriptase inhibitors (NRTIs) as a backbone plus a core agent.
26
27 Common core classes include non-nucleoside reverse-transcriptase inhibitors (NNRTIs),
28
29 integrase strand transfer inhibitor (INSTIs), and protease inhibitors (PIs). While ART
30
31 use overall reduces anemia, little is known about whether anemia risk differs between
32
33 commonly used ART classes in the current treatment era, particularly the newer INSTI
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35 class. Some studies found a possible increased rate of anemia among PI users [19], and
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37 in a randomized controlled trial, some participants discontinued INSTI due to anemia
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39 adverse events [20]. However, many studies included few participants or were mostly
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41 from an earlier ART era when older ART medications were predominantly used. The
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43 objective of this study was to compare rates of anemia and severe anemia development
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3 as well as changes in Hb overtime based on classes of ART used in the current
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6 treatment era.
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11 12 **Methods:**

13 Overview and setting:

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18 The present study included PLWH in care in the Centers for AIDS Research
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20 (CFAR) Network of Integrated Clinical Systems (CNICS) cohort during the period of
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22 January 1, 2010 to March 31, 2018. The CNICS cohort has been described in detail
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24 elsewhere [21]. Briefly, CNICS is a dynamic prospective clinical cohort of >32,000 adult
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26 PLWH receiving care at eight participating sites across the U.S. Comprehensive clinical
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28 data, including diagnoses, ART and other medications, laboratory test results,
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30 demographic information, and historical information, including ART use before
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32 enrollment, is collected through electronic medical records and other institutional data
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34 systems at each site and harmonized in the CNICS data repository. Medication data
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36 including ART use are entered into the electronic medical records by clinicians or
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38 prescription fill/refill data are uploaded directly from pharmacy systems and verified
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40 through medical record review. Participants entered the current study on January 1,
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42 2010 and the earliest date that they met the following enrollment criteria (cohort entry
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44 date): a) enrollment in CNICS for ≥ 6 months to allow time for covariate ascertainment
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46 and b) use of an ART regimen containing a PI, NNRTI, or INSTI. In addition, all
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3 participants were required to have at least 2 available hemoglobin lab values during
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6 study follow-up. Figure 1 shows inclusion criteria and exclusions made. Informed
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9 consent was obtained from all participants and institutional review boards at each site
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11 approved CNICS protocols.
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13 14 15 16 17 Exposure:

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19 The exposure of interest was the ART core drug class (NNRTI, INSTI or PI)
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21 prescribed as part of an ART regimen. Participants switching to different core drugs
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23 within the same class were considered to be continually exposed to the same core drug
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25 class. Individuals with a gap in ART use of 6 or more months were censored at the start
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27 of the gap and did not re-enter the study.
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33 Person-time on INSTIs or PIs was compared to the reference of NNRTI use. In
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35 addition, some PLWH in this cohort had prescriptions for multiple core classes
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37 simultaneously. Participants with regimens containing more than 1 core class were
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39 categorized separately as users of “multiple core classes” in analyses. Boosting agents
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41 (e.g. boosted ritonavir, or cobicistat) were not considered a 2nd core agent.
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49 Outcome ascertainment:

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52 Hb levels, expressed in grams per deciliter (g/dl), were ascertained using
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54 inpatient and outpatient laboratory data obtained as part of clinical care. Outcomes
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3 included incident anemia (first Hb measure below 10 g/dl), incident severe anemia (first
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5 Hb measure below 7.5 g/d) and changes in hemoglobin level. Another outcome, chronic
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7 anemia, defined as anemia lasting for ≥ 6 months was also examined. Chronic anemia
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9 was defined as Hb lab results on two separate occasions at least 6 months apart which
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11 were consistently in the anemic range without any Hb values above the anemia range
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13 during this 6-month period.
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22 Participant characteristics:

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24 Characteristics that were analyzed as confounders of the association between
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26 ART core drugs and incident anemia, severe anemia or change in hemoglobin overtime
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28 included: age, sex, race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection,
29
30 kidney function measured using estimated glomerular filtration rate (eGFR, categorized
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32 as <30 , 30-59, or ≥ 60 mL/minute/1.73 m²) [22], CD4 count (categorized as ≥ 500 , 350-499,
33
34 200-399, 100-199 or <100 cells/mm³), viral load (VL, assessed as $\log_{10}(\text{VL}+1)$), and time in
35
36 care at CNICS sites, defined as time from cohort entry date until the last available
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38 CNICS activity: either last lab date or last visit. HCV, eGFR, CD4 count and VL were
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40 assessed as part of clinical care visits and were time-updated as repeated measures
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42 occurred. All covariates were selected *a priori*, based on review of the literature and
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44 clinical knowledge. In addition, assessment of self-reported ART adherence was
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46 available for a subset of ~55% of the study population who were in care after each
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3 individual site initiated a clinical assessment of patient reported outcomes including
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5 adherence [23].
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10 Statistical analysis:

11 Baseline characteristics are presented for all participants at the cohort entry date.

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14 Median and interquartile range (IQR) are displayed for continuous variables and
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16 frequencies and proportions are displayed for categorical variables.
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22 Two multivariable Cox proportional hazards regression analyses were
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24 conducted, one among the subset of PLWH who were anemia-free at baseline to
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26 determine associations between time-updated NNRTI, PI, and INSTI use and
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28 development of anemia, and another among the subset of participants who were free of
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30 severe anemia at baseline to determine associations between time-updated NNRTI, PI,
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32 and INSTI use and development of severe anemia.
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38 Participants were censored at a) the time they developed the outcome of interest,
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40 or b) at the time of last activity in CNICS, or c) at the time of death, or d) at the time of
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42 administrative censoring per site, or e) at the time they no longer were prescribed one of
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44 the ART core classes, whichever came first. The timescale for the models was time since
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46 cohort entry. Complete case analysis methods were used (<2% had missing data).
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51 In a sensitivity analysis, we examined ART-naïve PLWH who initiated a drug in
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53 one of the ART classes of interest during study follow-up. Follow-up in this analysis
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3 began when a person began their initial ART regimen and extended until the earliest
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6 time of anemia occurrence, last activity in CNICS, time of death, administrative
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9 censoring, or at the time their initial regimen ended. PI or INSTI use were compared to
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11 the reference, NNRTI use. We also examined the change in Hb overtime using mixed
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14 models among this ART-naïve population.
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17 Linear mixed effects models with random slopes for time were used to examine
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19 the association of ART core classes with Hb levels among all PLWH after adjustment for
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21 the same characteristics as in the incident anemia and severe anemia analyses. Mixed-
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23 effects models utilize random slopes and intercepts at the participant level to handle
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25 irregular patterns of repeated measures over follow-up [24]. All analyses were
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28 performed using Stata version 14.2.
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36 Patient and public involvement:

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38 There was no patient or public participation in the present study.
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44 **Results:**

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46 In total, 16,505 PLWH met inclusion criteria and were included in these analyses
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48 (Figure 1). Participants had an average of 11 outpatient hemoglobin values measured
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50 during a median follow-up of 4.9 (IQR 3.0-7.2) years. A total of 12,626 (76%) were free of
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52 anemia at baseline, and 15,357 (93%) were free of severe anemia at baseline. Table 1
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3 provides baseline characteristics for all study participants, and additionally shows
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5 characteristics for the 1,040 participants who developed anemia during follow-up as
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7 well as the 488 participants who developed severe anemia during follow-up. The mean
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9 age of study participants was 46 years at cohort entry, 20% were female, and 19% were
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11 co-infected with HCV. At baseline, 18% were prescribed NNRTIs, 53% were prescribed
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13 PIs, 14% were prescribed INSTIs and 16% used multiple cores. INSTI core agents were
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15 increasingly used over the last few years of the enrollment period (Figure 2), and the
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17 proportion of those prescribed multiple cores were comprised of INSTI plus another
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19 core class with increasing frequency as study years progressed.
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28 The incidence of anemia was 2.1/100 person-years and the incidence of severe
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30 anemia was 0.8/100 person-years. INSTI use was associated with an increased risk of
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32 anemia (aHR 1.17, 95%CI 0.94-1.47) compared to NNRTIs, though this was not
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34 statistically significant (Table 2). Use of multiple core classes together was also
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36 associated with an increased risk of anemia (aHR 1.30, 95%CI 1.06-1.60)) while no
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38 associations were found between PI use and anemia (aHR of 1.00, 95%CI 0.83-1.21). In
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40 adjusted analyses restricted to participants free of severe anemia at baseline (Table 2),
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42 INSTI use was associated with an increased risk of severe anemia (aHR 1.55, 95%CI
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44 1.11-2.17) compared to NNRTI use. Time on multiple ART core classes was associated
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46 with an increased risk of anemia (aHR 1.35 (0.99-1.85), though this was not statistically
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48 significant, and no association was found for PIs (aHR 1.01 (0.74-1.36) Among the 12,626
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3 PLWH who were free of anemia at baseline, 225 developed chronic anemia (lasting for
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5 ≥ 6 months), during follow-up. For chronic anemia, results were similar to those in the
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7 primary analysis. Relative to NNRTI use, person-time on multiple core classes was
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9 associated with an aHR 2.26 (95%CI 0.98-5.23), person-time on an INSTI with an aHR of
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11 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).
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17 Average hemoglobin levels remained steady during follow-up; the mean level
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19 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
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21 available measurement per person. Relative to NNRTI use, a decrease in hemoglobin
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23 level over time was associated with both INSTI use (-0.06 g/dL per year, 95%CI -0.10, -
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25 0.03) and use of multiple core classes (-0.14, 95%CI -0.18, -0.11). No association was
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27 found for PI use (-0.01, 95%CI -0.04, 0.03). (Table 3).
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33 The sensitivity analysis restricted to ART-naïve PLWH included 6,426 PLWH
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35 who were free of prevalent anemia at baseline, of whom 378 developed anemia.
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37 Compared to NNRTI initiators, those initiating a PI had an aHR of 0.78 (0.56-1.08) while
38
39 those initiating an INSTI had an aHR of 1.15 (95%CI 0.92-1.45) (Supplemental Table 1).
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42 The mixed model examining change in Hb overtime among ART-naïve PLWH
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44 initiating one of the ART core classes of interest included 7,264 participants. Compared
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46 to NNRTI initiators, a decrease in Hb was found for PI use (-0.8g/dL per year, 95%CI -
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48 0.16, -0.01), while INSTI use was associated with a larger decrease in Hb level overtime
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50 (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental Table 2).
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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

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2
3 including all PLWH who became anemic. A final limitation is that ART medication use
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5 comes from prescriptions written which may not be filled or may be filled but never
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7 taken, although self-reported adherence was high (approximately 98%) in the subset for
8
9 whom adherence information was available. CNICS participants who provided
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11 adherence information have been shown to be representative of the overall population
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13 of PLWH in CNICS [23, 25]. Finally, the fact that this study was conducted among
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15 PLWH in care in the U.S. who are on ART may limit the generalizability of findings to
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17 PLWH who are not on ART or who live outside of the U.S.
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25 One study, conducted during the newer-era of HIV treatment with drugs other
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27 than AZT, (during 2008-2012) presented findings for AZT versus non-AZT regimens,
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29 finding an increased risk of anemia among AZT compared to non-AZT regimens
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31 (HR=2.84, 95%CI 1.52-5.31) [26]. However, anemia risk was not analyzed separately for
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33 the use of specific classes of ART, resulting in the inability of comparison to the present
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35 study's findings and a lack of generalizability to PLWH who are treated with newer
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37 ART core agents.
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44 It is possible that PLWH in our study whose HIV is progressing due to resistance
45
46 or other complications may get switched to an INSTI. This, in addition to prior
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48 knowledge that poorly controlled HIV parameters are on their own a risk factor for
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50 anemia [6, 27, 28], could result in confounding by indication. However, the switch to
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52 INSTI core regimens since their approval in 2007 has been widespread in this
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3 population (Figure 2) and INSTIs are recommended for use as initial regimens [29]. In
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6 addition, we rigorously controlled for many of the important HIV-related factors that
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9 correspond to poorly-controlled HIV, and our sensitivity analysis examining new users
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12 of ART medications failed to reinforce the notion that an increased risk of anemia
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15 among INSTI core regimen users could be entirely explained by sicker participants
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17
18 getting switched to these therapies.

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20 PLWH on multiple core classes were in a different category in our analyses.
21
22 There are several reasons PLWH may be prescribed multiple core classes. For example,
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25 sometimes PLWH are prescribed multiple core classes to ensure they receive a complete
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28 regimen while awaiting approval for specific agents from their insurance company.
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31 However, the primary concern was that they were receiving multiple core classes due to
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34 provider concerns such as prior failed regimens which may also increase their risk of
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37 anemia.

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

^aCohort 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.

^bFor 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 (1.40-2.75)	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)

^aAdjusted 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

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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

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3^aCoefficient is the mean difference per year in Hb (g/dL) for each core regimen relative
4 to the NNRTI core regimen, after adjustment for site, age, sex, race/ethnicity, hepatitis C
5 virus coinfection, CD4 cell count, viral load, eGFR and years in study.
6

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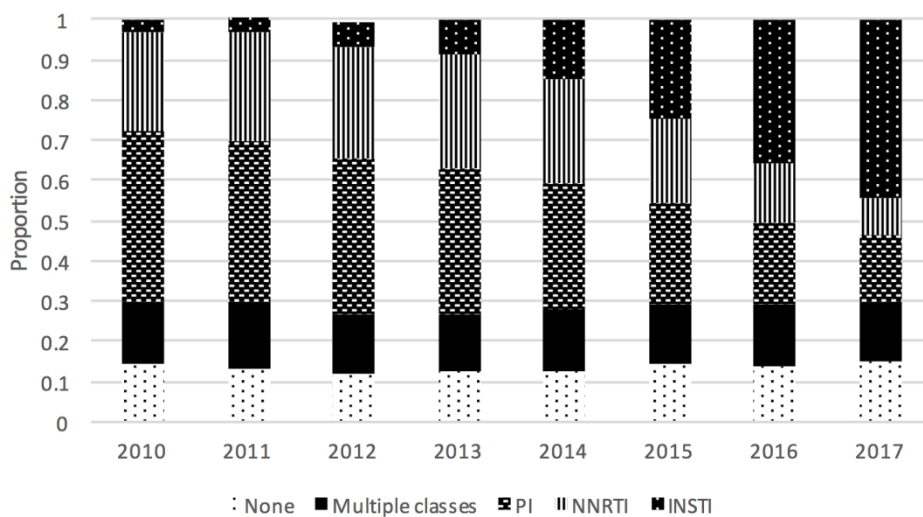
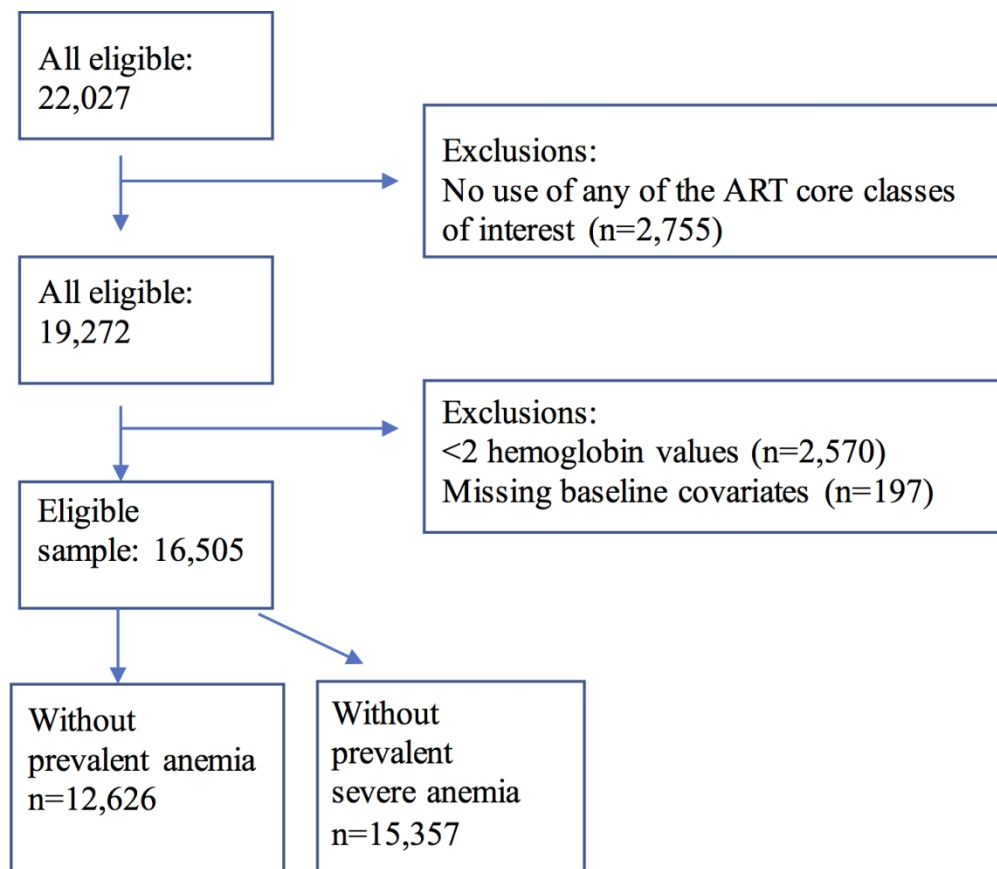


Figure 2. Proportion of study population using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

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

^aCoefficient 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)

1	✓Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
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4	✓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
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7			(b) Report category boundaries when continuous variables were categorized
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9			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
10			
11	✓Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
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14	Discussion		
15	✓Key results (page 11)	18	Summarise key results with reference to study objectives
16			
17			
18	✓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
19			
20	✓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
21			
22			
23	✓Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
24			
25			
26	Other information		
27	✓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
28			
29			

*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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3 1 **A study of antiretroviral drug class and anaemia risk in the**
4 2 **current treatment era among people living with HIV in the United**
5 3 **States: A clinical cohort study**
6 4

7
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29 24 **Keywords:** antiretroviral agents, cohort, anaemia, integrase
30 25 inhibitors, protease inhibitors, non-nucleoside reverse
31 26 transcriptase inhibitors
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13 **Contributions:**

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

22 **Transparency declaration:**

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

27 **Ethical approval:**

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

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41 **Data sharing:**

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

46 **Dissemination declaration:**

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

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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 (aHR 1.51, 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.

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

12 Introduction:

13 Anaemia and severe anaemia are common among people living
14 with HIV (PLWH) [1]. The prevalence of anaemia is elevated in
15 PLWH compared to the general population. One study reported that
16 among non-pregnant American women living with HIV, the
17 prevalence of anaemia was 28.1% compared to 15.1% among women
18 without HIV [2]. Estimates vary by age, sex, HIV disease stage,
19 use of antiretroviral therapy (ART) and injection drug use
20 status [1, 3]. Among PLWH, associations have been found between
21 anaemia and mortality [4-9], health-related quality-of-life
22 [1], morbidity, dementia [10], and ART failure [11]. In
23 addition, anaemia is an independent prognostic indicator
24 associated with HIV disease progression [1, 12, 13], including
25 development of AIDS [7].

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

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

11 **Methods:**

12 Overview and setting:

13 The present study included PLWH in care in the Centers for
14 AIDS Research (CFAR) Network of Integrated Clinical Systems
15 (CNICS) cohort during the period of January 1, 2010 to March 31,
16 2018 (the date through which each site had complete data
17 [administrative censor date] varied somewhat, median date:
18 October 31, 2017). The CNICS cohort has been described in detail
19 elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical
20 cohort of >32,000 adult PLWH receiving care at eight
21 participating sites across the U.S. Comprehensive clinical data,
22 including diagnoses, ART and other medications, laboratory test
23 results, demographic information, and historical information,
24 including ART use before enrollment, is collected through
25 electronic medical records and other institutional data systems
26 at each site and harmonized in the CNICS data repository.
27 Medication data including ART use are entered into the
28 electronic medical records by clinicians or prescription
29 fill/refill data are uploaded directly from pharmacy systems and
30 verified through medical record review. Participants entered the
31 current study on January 1, 2010 or the earliest date after
32 January 1, 2010 that they met the following enrollment criteria
33 (cohort entry date): a) enrollment in CNICS for ≥ 6 months to
34 allow time for covariate ascertainment, and b) use of an ART
35 regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or
36 INSTI. In addition, all participants were required to have at
37 least 2 available haemoglobin lab values during study follow-up.
38 Figure 1 shows inclusion criteria and exclusions made. Informed
39 consent was obtained from all participants and institutional
40 review boards at each site approved CNICS protocols.

42 Exposure:

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

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

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

11 Outcome ascertainment:

12 Hb levels, expressed in grams per deciliter (g/dL), were
13 ascertained using inpatient and outpatient laboratory data
14 obtained as part of clinical care. Outcomes included incident
15 anaemia (first post-baseline Hb measure below 10 g/dL), incident
16 severe anaemia (first post-baseline Hb measure below 7.5 g/dL)
17 [21] and changes in Hb level. Another outcome, chronic anaemia,
18 defined as anaemia lasting for ≥ 6 months, was also examined.
19 Chronic anaemia was defined as post-baseline Hb lab results on
20 two separate occasions at least 6 months apart which were
21 consistently in the anemic range without any Hb values above the
22 anaemia range during this 6-month period.

23 Participant characteristics:

24 Characteristics that were analyzed as confounders of the
25 association between ART core drugs and incident anaemia, severe
26 anaemia or change in Hb over time included: age, sex,
27 race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection
28 defined as a detectable HCV RNA level or HCV genotype or HCV
29 antibody, kidney function measured using estimated glomerular
30 filtration rate (eGFR, categorized as <30 , $30-59$, or ≥ 60
31 mL/minute/1.73 m²) [22], CD4 count (categorized as ≥ 500 , $350-499$,
32 $200-399$, $100-199$ or <100 cells/mm³), viral load (VL, assessed as
33 $\log_{10}(\text{VL}+1)$), baseline Hb, and time in care at CNICS sites,
34 defined as time from cohort entry date until the last available
35 CNICS activity: either last lab date or last visit. HCV, eGFR,
36 CD4 count and VL were assessed as part of clinical care visits
37 and were time-updated as repeated measures occurred. All
38 covariates were selected *a priori*, based on review of the
39 literature and clinical knowledge. In addition, assessment of
40 self-reported ART adherence was available for a subset of ~55%
41 of the study population who were in care after each individual
42 site initiated a clinical assessment of patient reported
43 outcomes, including adherence [23].

44 Statistical analysis:

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3 1 Baseline characteristics are presented for all participants
4 2 at the cohort entry date. Median and interquartile range (IQR)
5 3 are displayed for continuous variables and frequencies and
6 4 proportions are displayed for categorical variables.

8 5 Two multivariable Cox proportional hazards regression
9 6 analyses were conducted, one among the subset of PLWH who were
10 7 anaemia-free at baseline to determine associations between time-
11 8 updated NNRTI, PI, and INSTI use and development of anaemia, and
12 9 another among the subset of participants who were free of severe
13 10 anaemia at baseline to determine associations between time-
14 11 updated NNRTI, PI, and INSTI use and development of severe
15 12 anaemia.

17 13 Participants were censored at a) the time they developed
18 14 the outcome of interest, b) at the time of last activity in
19 15 CNICS, c) at the time of death, d) at the date of administrative
20 16 censoring at each site or e) at the time they no longer were
21 17 prescribed one of the ART core classes of interest, whichever
22 18 came first. The timescale for the models was time since cohort
23 19 entry. Complete case analysis methods were used (<2% had missing
24 20 data).

26 21 In a sensitivity analysis, we examined ART-naïve PLWH who
27 22 initiated a regimen including one of the core ART classes of
28 23 interest during study follow-up. Follow-up in this analysis
29 24 began when a person began their initial ART regimen and extended
30 25 until the earliest time of anaemia occurrence, last activity in
31 26 CNICS, time of death, administrative censoring, or at the time
32 27 their initial regimen ended. PI or INSTI use were compared to
33 28 the reference, NNRTI use. We also examined the change in Hb over
34 29 time using mixed models among this ART-naïve population. We also
35 30 conducted a sensitivity analysis including baseline NRTI
36 31 backbone adjustment for abacavir versus tenofovir in an analysis
37 32 with incident anemia.

39 33 Linear mixed effects models with random slopes for time
40 34 were used to examine the association of ART core classes with Hb
41 35 levels among all PLWH after adjustment for the same
42 36 characteristics as in the incident anaemia and severe anaemia
43 37 analyses. Mixed-effects models utilize random slopes and
44 38 intercepts at the participant level to handle irregular patterns
45 39 of repeated measures over follow-up [24]. All analyses were
46 40 performed using Stata version 14.2.

42 Patient and public involvement:

43 43 There was no patient or public participation in the present
44 44 study.

46 **Results:**

47 47 In total, 16,505 PLWH met inclusion criteria and were

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

17 The overall incidence of anaemia was 2.1/100 person-years
18 and the overall incidence of severe anaemia was 0.8/100 person-
19 years. The unadjusted incidence rates of anaemia and severe
20 anaemia based on ART core class are provided in Table 2. In
21 adjusted analyses, INSTI use was associated with an increased
22 risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs
23 (Table 3). Use of multiple core classes together was also
24 associated with an increased risk of anaemia (aHR 1.39, 95%CI
25 1.13-1.70) while no associations were found between PI use and
26 anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses
27 restricted to participants free of severe anaemia at baseline
28 (Table 3), INSTI use was associated with an increased risk of
29 severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI
30 use. Although the HR appeared elevated, there was no association
31 between time on multiple ART core classes and an increased risk
32 of severe anaemia (aHR 1.30 (0.95-1.78), and no association was
33 found for PIs (aHR 1.09 (0.81-1.47)). Among the 12,626 PLWH who
34 were free of anaemia at baseline, 225 developed chronic anaemia
35 (lasting for ≥ 6 months), during follow-up. For chronic anaemia,
36 results were similar to those in the primary analysis. Relative
37 to NNRTI use, person-time on multiple core classes was
38 associated with an aHR for chronic anaemia of 2.21 (95%CI 0.94-
39 5.18), person-time on an INSTI with an aHR of 1.90 (95%CI 0.76-
40 4.64) and person-time on a PI with an aHR of 1.27 (95%CI 0.54-
41 3.04).

42 Average Hb levels remained steady during follow-up; the
43 mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0
44 g/dL (IQR 12.6-15.2) at the last available measurement per
45 person. Relative to NNRTI use, a decrease in Hb level over time
46 was associated with both INSTI use (-0.06 g/dL per year, 95%CI -
47 0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -

1 0.18, -0.11). No association was found for PI use (-0.01, 95%CI
2 -0.04, 0.03). (Table 4).

3 The sensitivity analysis restricted to ART-naïve
4 participants included 6,426 PLWH who were free of prevalent
5 anaemia at baseline, of whom 378 developed anaemia. Compared to
6 NNRTI initiators, those initiating a PI had an aHR of 0.69
7 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10
8 (95%CI 0.84-1.44) (Supplemental Table 1). The mixed model
9 examining change in Hb over time among ART-naïve PLWH initiating
10 one of the ART core classes of interest included 7,264
11 participants. Compared to NNRTI initiators, a decrease in Hb was
12 found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while
13 INSTI use was associated with a larger decrease in Hb level over
14 time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental
15 Table 2). Finally, results from the sensitivity analysis including baseline NRTI backbone
16 adjustment for abacavir were essentially unchanged (e.g. the abacavir backbone aHR for use of
17 an INSTI-based regimen was 1.27 (95%CI 1.01-1.60) for incident anaemia compared to the
18 findings from the primary analysis of an aHR of 1.26 (95%CI 1.00-1.58) (data not shown).

20 Discussion:

21 In this study of 16,505 PLWH in care within the United
22 States in the current treatment era (2010 and after), we
23 observed that INSTI use, and time on multiple core ART classes,
24 were associated with decreases in Hb levels during follow-up
25 compared to using NNRTI-based regimens. We found that INSTI use
26 was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-
27 1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over
28 time. Furthermore, the naïve user analysis indicated similar
29 findings despite a smaller sample size. These findings could
30 have implications for the treatment approach that should be used
31 in people with risk factors for anaemia.

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

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3 1 Information on ART medication use was from prescription data
4 2 which does not necessarily indicate medications were taken,
5 3 although self-reported adherence was high (~98%) in the subset
6 4 for whom adherence information was available. CNICS participants
7 5 who provided adherence information have been shown to be
8 6 representative of the overall population of PLWH in CNICS [23,
9 7 26]. Finally, the fact that this study was conducted among PLWH
10 8 in care in the U.S. who are on ART may limit the
11 9 generalizability of findings to PLWH who live outside of the
12 10 U.S.

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

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

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

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

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

16 17 **Figure Legends**

18
19 Figure 1: Flow chart for inclusion/exclusion criteria for 22,027
20 PLWH in care at CNICS after 1/2010. Exclusions were made for
21 those not exposed to any of the ART core classes, those with
22 fewer than 2 haemoglobin levels, and those missing baseline
23 covariates, resulting in 16,505 PLWH who were included in these
24 analyses.

25 Figure 2. Proportion of study population (N=16,505) using
26 various ART classes during complete years of study follow-up.
27 This figure shows the trends in use of the ART core classes
28 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 \geq 400 copies/ml	2441 (21)	283 (27)	3259 (22)	172 (35)
CD4 count (cells/mm ³)				
<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)
\geq 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/m²)				
<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)

^aCohort 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.

^bFor 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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5 2 Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL),
6 3 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)

4 ^aAdjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count,
5 viral load, kidney function (eGFR), baseline haemoglobin

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

1 Table 4. Association of ART core classes with change in
 2 haemoglobin level during follow-up in adjusted analyses (linear
 3 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

4 ^aCoefficient is the mean difference per year in Hb (g/dL) for
 5 each core regimen relative to the NNRTI core regimen, after
 6 adjustment for site, age, sex, race/ethnicity, hepatitis C virus
 7 coinfection, CD4 cell count, viral load, eGFR and years in
 8 study.

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

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10 5 **References :**

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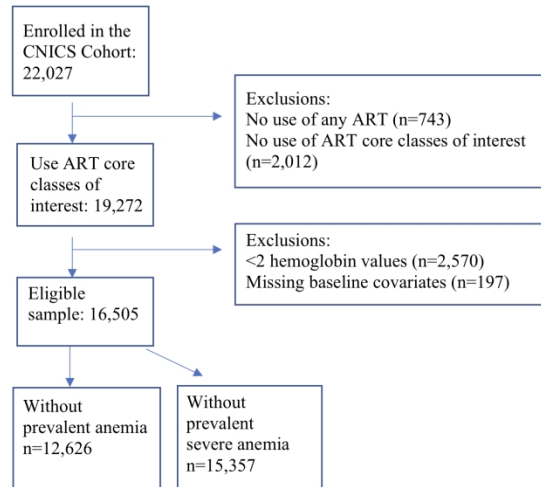


Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included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

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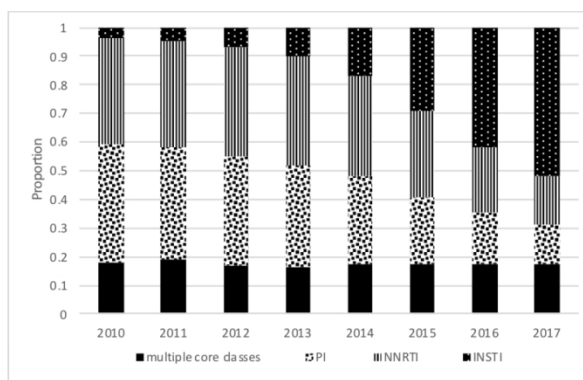


Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

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)

^aAdjusted 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

^aCoefficient 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)

1	✓Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
2			
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4	✓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
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6			
7			(b) Report category boundaries when continuous variables were categorized
8			
9			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
10			
11	✓Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
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14	Discussion		
15	✓Key results (page 11)	18	Summarise key results with reference to study objectives
16			
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18	✓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
19			
20	✓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
21			
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23	✓Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
24			
25			
26	Other information		
27	✓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
28			
29			

*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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3 1 **A study of antiretroviral drug class and anaemia risk in the**
4 2 **current treatment era among people living with HIV in the United**
5 3 **States: A clinical cohort study**
6 4

7
8 5 **Authors:** B.N. Harding¹, B.M. Whitney¹, R.M. Nance¹, H.M. Crane¹,
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29 24 **Keywords:** antiretroviral agents, cohort, anaemia, integrase
30 25 inhibitors, protease inhibitors, non-nucleoside reverse
31 26 transcriptase inhibitors
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44 38 **Competing interest statement:**

45 39 Dr. Harding reports grants from National Heart, Lung and Blood Institute(NHLBI) during the
46 40 conduct of the study; Ms. Whitney reports grants from the National Institutes of Health (NIH)
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48 42 study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH,
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52 46 Mathews reports grants from NHLBI during the conduct of the study; Dr. Eron reports grants
53 47 from NIH, during the conduct of the study, grants and personal fees from Gilead Sciences, grants
54 48 and personal fees from ViiV Healthcare, grants and personal fees from Janssen and personal fees
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2 of the study, personal fees from Gilead, personal fees from ViiV Healthcare, personal fees from
3 Janssen and non-financial support from Merck outside the submitted work; Dr. Volberding
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5 submitted work; Dr. Rodriguez reports grants from NIH during the conduct of the study,
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8 / NIH during the conduct of the study, grants from Gilead, Merck, and ViiV Healthcare outside
9 the submitted work; Dr. Kitahata reports grants from NHLBI during the conduct of the study; Dr.
10 Heckbert reports grants from NIH during the conduct of the study; Dr. Delaney reports grants
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13 **Contributions:**

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

23 **Transparency declaration:**

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

28 **Ethical approval:**

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

35 **Funding:**

36 This project was funded by R01HL126538-01A1/National Heart, Lung and Blood Institute. They
37 provided an unrestricted grant and we are completely independent from the study sponsors.
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39 at the National Institutes of Health [CNICS R24 AI067039, UW CFAR NIAID Grant P30
40 AI027757; UNC CFAR grant P30 AI50410, JHU CFAR grant P30 AI094189, and UAB CFAR
41 grant P30 AI027767]. Dr. Harding is supported by a National Heart, Lung and Blood Institute
42 grant T32HL007828.

44 **Data sharing:**

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

4
5 **Dissemination declaration:**

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

7
8 **Abstract:** (276 words)

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

16 DESIGN: Retrospective cohort study.

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

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

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

31 RESULTS: During a median of 4.9 years of follow-up, 1,040
32 developed anaemia and 488 developed severe anaemia. Compared to
33 NNRTI use, INSTI-based regimens were associated with an
34 increased risk of anaemia (adjusted hazard ratio [aHR] 1.26, 95%
35 confidence interval [CI] 1.00-1.58) and severe anaemia (aHR 1.51
36 95%CI 1.07-2.11), and a decrease in haemoglobin level. Time on
37 multiple core classes was also associated with increased anaemia
38 risk (aHR 1.39, 95%CI 1.13-1.70), while no associations were
39 found for PI use.

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

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45
46 **Strengths and limitations of this study:**

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

14 Introduction:

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

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

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

12 **Methods :**

13 Overview and setting:

14 The present study included PLWH in care in the Centers for
15 AIDS Research (CFAR) Network of Integrated Clinical Systems
16 (CNICS) cohort during the period of January 1, 2010 to March 31,
17 2018 (the date through which each site had complete data
18 [administrative censor date] varied somewhat, median date:
19 October 31, 2017). The CNICS cohort has been described in detail
20 elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical
21 cohort of >32,000 adult PLWH receiving care at eight
22 participating sites across the U.S. Comprehensive clinical data,
23 including diagnoses, ART and other medications, laboratory test
24 results, demographic information, and historical information,
25 including ART use before enrollment, is collected through
26 electronic medical records and other institutional data systems
27 at each site and harmonized in the CNICS data repository.
28 Medication data including ART use are entered into the
29 electronic medical records by clinicians or prescription
30 fill/refill data are uploaded directly from pharmacy systems and
31 verified through medical record review. Participants entered the
32 current study on January 1, 2010 or the earliest date after
33 January 1, 2010 that they met the following enrollment criteria
34 (cohort entry date): a) enrollment in CNICS for ≥6 months to
35 allow time for covariate ascertainment, and b) use of an ART
36 regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or
37 INSTI. In addition, all participants were required to have at
38 least 2 available haemoglobin lab values during study follow-up.
39 Figure 1 shows inclusion criteria and exclusions made. Informed
40 consent was obtained from all participants and institutional
41 review boards at each site approved CNICS protocols (University
42 of Washington Human Subjects Division served as the institutional review
43 board for the centralized deidentified CNICS Data Repository).

44 Exposure:

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3 1 The exposure of interest was the ART core drug class
4 2 (NNRTI, INSTI or PI) prescribed as part of an ART regimen (a
5 3 backbone of two NRTIs plus a core drug). Participants switching
6 4 to different core drugs within the same class were considered to
7 5 be continually exposed to the same core drug class. Individuals
8 6 with a gap of 6 or more months in use of the ART core drug
9 7 classes of interest were censored at the start of the gap and
10 8 did not re-enter the study.

11 9 Person-time on INSTI or PI-based regimens was compared to
12 10 the NNRTI reference. In addition, some PLWH in this cohort had
13 11 prescriptions for multiple core classes simultaneously.
14 12 Participants with regimens containing more than 1 core class
15 13 were categorized separately in analyses as users of "multiple
16 14 core classes". Boosting agents (e.g. boosted ritonavir, or
17 15 cobicistat) were not considered a 2nd core agent.

17 Outcome ascertainment:

18 18 Hb levels, expressed in grams per deciliter (g/dL), were
19 19 ascertained using inpatient and outpatient laboratory data
20 20 obtained as part of clinical care. Outcomes included incident
21 21 anaemia (first post-baseline Hb measure below 10 g/dL), incident
22 22 severe anaemia (first post-baseline Hb measure below 7.5 g/dL)
23 23 [21] and changes in Hb level. Another outcome, chronic anaemia,
24 24 defined as anaemia lasting for ≥ 6 months, was also examined.
25 25 Chronic anaemia was defined as post-baseline Hb lab results on
26 26 two separate occasions at least 6 months apart which were
27 27 consistently in the anemic range without any Hb values above the
28 28 anaemia range during this 6-month period.

30 Participant characteristics:

31 31 Characteristics that were analyzed as confounders of the
32 32 association between ART core drugs and incident anaemia, severe
33 33 anaemia or change in Hb over time included: age, sex,
34 34 race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection
35 35 defined as a detectable HCV RNA level or HCV genotype or HCV
36 36 antibody, kidney function measured using estimated glomerular
37 37 filtration rate (eGFR, categorized as <30 , $30-59$, or ≥ 60
38 38 mL/minute/1.73 m²) [22], CD4 count (categorized as ≥ 500 , $350-499$,
39 39 $200-399$, $100-199$ or <100 cells/mm³), viral load (VL, assessed as
40 40 $\log_{10}(\text{VL}+1)$), baseline Hb (in incident anemia, severe anemia and
41 41 chronic anemia only analyses only), and time in care at CNICS
42 42 sites, defined as time from cohort entry date until the last
43 43 available CNICS activity: either last lab date or last visit.
44 44 HCV, eGFR, CD4 count and VL were assessed as part of clinical
45 45 care visits and were time-updated as repeated measures occurred.
46 46 All covariates were selected *a priori*, based on review of the
47 47 literature and clinical knowledge. In addition, assessment of

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

5 6 Statistical analysis:

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

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

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

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

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

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

6 Patient and public involvement:

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

9 **Results:**

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

26 The overall incidence of anaemia was 2.1/100 person-years
27 and the overall incidence of severe anaemia was 0.8/100 person-
28 years. The unadjusted incidence rates of anaemia and severe
29 anaemia based on ART core class are provided in Table 2. In
30 adjusted analyses, INSTI use was associated with an increased
31 risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs
32 (Table 3). Use of multiple core classes together was also
33 associated with an increased risk of anaemia (aHR 1.39, 95%CI
34 1.13-1.70) while no associations were found between PI use and
35 anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses
36 restricted to participants free of severe anaemia at baseline
37 (Table 3), INSTI use was associated with an increased risk of
38 severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI
39 use. Although the HR appeared elevated, there was no association
40 between time on multiple ART core classes and an increased risk
41 of severe anaemia (aHR 1.30 (0.95-1.78), and no association was
42 found for PIs (aHR 1.09 (0.81-1.47). Among the 12,626 PLWH who
43 were free of anaemia at baseline, 225 developed chronic anaemia
44 (lasting for ≥ 6 months), during follow-up. For chronic anaemia,
45 results were similar to those in the primary analysis although
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47

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

6 Average Hb levels remained steady during follow-up; the
7 mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0
8 g/dL (IQR 12.6-15.2) at the last available measurement per
9 person. Relative to NNRTI use, a decrease in Hb level over time
10 was associated with both INSTI use (-0.06 g/dL per year, 95%CI -
11 0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -
12 0.18, -0.11). No association was found for PI use (-0.01, 95%CI
13 -0.04, 0.03). (Table 4).

14 The sensitivity analysis restricted to ART-naïve
15 participants included 6,426 PLWH who were free of prevalent
16 anaemia at baseline, of whom 378 developed anaemia. Compared to
17 NNRTI initiators, those initiating a PI had an aHR of 0.69
18 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10
19 (95%CI 0.84-1.44) (Supplemental Table 1). The mixed model
20 examining change in Hb over time among ART-naïve PLWH initiating
21 one of the ART core classes of interest included 7,264
22 participants. Compared to NNRTI initiators, a decrease in Hb was
23 found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while
24 INSTI use was associated with a larger decrease in Hb level over
25 time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental
26 Table 2). Results from the sensitivity analyses including time-updated NRTI backbone
27 adjustment for abacavir were essentially unchanged (Supplemental Table 3). Finally, results
28 from the sensitivity analysis excluding participants with AZT use indicated similar findings to
29 those from the primary analyses, although confidence intervals were wider (Supplemental Table
30 4, 5).

31 Discussion:

32 In this study of 16,505 PLWH in care within the United
33 States in the current treatment era (2010 and after), we
34 observed that INSTI use, and time on multiple core ART classes,
35 were associated with decreases in Hb levels during follow-up
36 compared to using NNRTI-based regimens. We found that INSTI use
37 was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-
38 1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over
39 time. Furthermore, the naïve user analysis indicated similar
40 findings despite a smaller sample size. These findings could
41 have implications for the treatment approach that should be used
42 in people with risk factors for anaemia.

43 This study's strengths include its large and geographically
44 diverse study population and longitudinal data structure.
45 Nevertheless, there are limitations of this study to consider,
46 including the observational nature of the data, which may be
47

1
2
3 1 subject to residual confounding including confounding by
4 2 indication [25]. However, anaemia is not a recognized adverse
5 3 effect of NNRTIs, PIs, or INSTIs. Thus, it is unlikely that ART
6 4 core class was selected based on prescriber concern about
7 5 anaemia risk. Additionally, we did not exclude participants with
8 6 conditions strongly associated with anaemia or Hb level,
9 7 including those on dialysis, receiving erythropoietin, or with
10 8 severe bleeding, which likely caused some of the anaemia cases
11 9 in this analysis. However, in the sensitivity analysis focusing
12 10 on factors associated with chronic anaemia (less likely due to
13 11 bleeding), findings for INSTI vs. NNRTI core regimens were
14 12 similar to those including all PLWH who became anemic.
15 13 Information on ART medication use was from prescription data
16 14 which does not necessarily indicate medications were taken,
17 15 although self-reported adherence was high (~98%) in the subset
18 16 for whom adherence information was available. CNICS participants
19 17 who provided adherence information have been shown to be
20 18 representative of the overall population of PLWH in CNICS [23,
21 19 26]. Finally, the fact that this study was conducted among PLWH
22 20 in care in the U.S. who are on ART may limit the
23 21 generalizability of findings to PLWH who live outside of the
24 22 U.S.

25 23 There have been few epidemiologic studies of anaemia risk among users of newer ART
26 24 core regimens. From clinical safety data reported from multiple trials, approximately 36–49%
27 25 of participants using PIs had Hb levels <10g/dL, indicating
28 26 anaemia [18], and in another trial, two participants
29 27 discontinued INSTI use due to anaemia adverse events [19],
30 28 however the strict inclusion criteria applied in clinical trials
31 29 makes it difficult to generalize these findings to more diverse
32 30 populations of PLWH in clinical care. Another study, conducted
33 31 during the newer-era of HIV treatment with drugs other than AZT,
34 32 (during 2008–2012) presented findings for AZT versus non-AZT
35 33 regimens, finding an increased risk of anaemia among AZT
36 34 compared to non-AZT regimens (HR=2.84, 95%CI 1.52–5.31) [27].
37 35 However, anaemia risk was not analyzed separately for the use of
38 36 specific classes of ART, resulting in the inability of
39 37 comparison to the present study's findings and a lack of
40 38 generalizability to PLWH who are treated with newer ART core
41 39 agents.

42 40 It is possible that PLWH in our study whose HIV is
43 41 progressing due to resistance or other complications may get
44 42 switched to an INSTI. This, in addition to prior knowledge that
45 43 poorly controlled HIV parameters are on their own a risk factor
46 44 for anaemia [5, 28, 29], could result in confounding by
47 45 indication. However, the switch to INSTI core regimens since
48 46 their approval in 2007 has been widespread in this population
49 47 (Figure 2) and INSTIs are recommended for use as initial

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

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

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

28 29 **Figure Legends**

30
31 Figure 1: Flow chart for inclusion/exclusion criteria for 22,027
32 PLWH in care at CNICS after 1/2010. Exclusions were made for
33 those not exposed to any of the ART core classes, those with
34 fewer than 2 haemoglobin levels, and those missing baseline
35 covariates, resulting in 16,505 PLWH who were included in these
36 analyses.

37 Figure 2. Proportion of study population (N=16,505) using
38 various ART classes during complete years of study follow-up.
39 This figure shows the trends in use of the ART core classes
40 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 \geq 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)
\geq 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 m²)				
<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/m²)				
<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)

^aCohort 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.

^bFor 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)

^aAdjusted 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

1 Table 4. Association of ART core classes with change in
 2 haemoglobin level during follow-up in adjusted analyses (linear
 3 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

4 ^aCoefficient is the mean difference per year in Hb (g/dL) for
 5 each core regimen relative to the NNRTI core regimen, after
 6 adjustment for site, age, sex, race/ethnicity, hepatitis C virus
 7 coinfection, CD4 cell count, viral load, eGFR and years in
 8 study.

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

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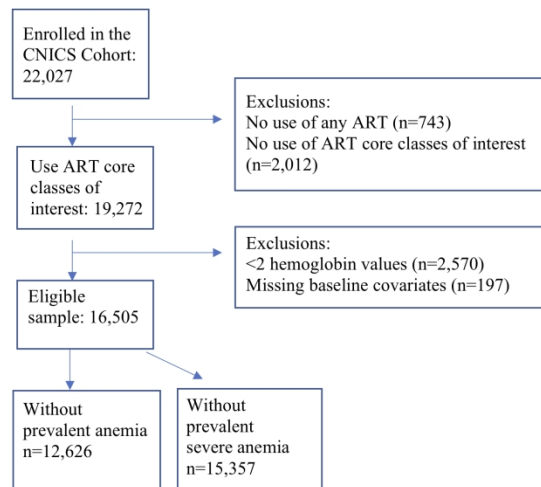


Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included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

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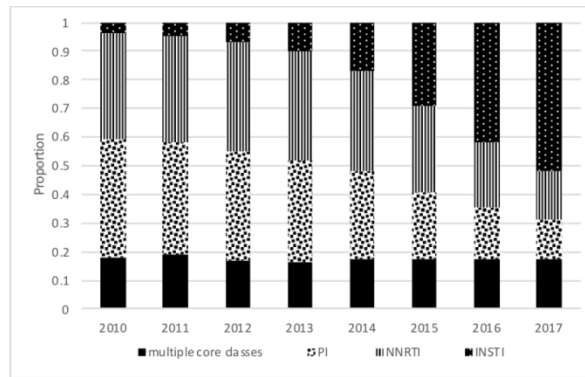


Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

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Supplemental Table 1: 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)

^aAdjusted 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

^aCoefficient 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

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

4 ^aMinimally adjusted model includes adjustment for: age, sex, race, site, hepatitis C co-infection,
5 CD4 category, VL, eGFR category and haemoglobin at baseline.

6 ^bModel includes adjustment variables in minimally adjusted model plus a term for time-updated
7 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)

^aAdjusted 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

^aCoefficient 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)

1	✓Outcome data	15*	Report numbers of outcome events or summary measures over time
2	(page 10)		
3	✓Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates
4	(pages 10, 11)		and their precision (eg, 95% confidence interval). Make clear which confounders
5			were adjusted for and why they were included
6			(b) Report category boundaries when continuous variables were categorized
7			(c) If relevant, consider translating estimates of relative risk into absolute risk for
8			a meaningful time period
9	✓Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and
10	(page 11)		sensitivity analyses
11	Discussion		
12	✓Key results	18	Summarise key results with reference to study objectives
13	(page 11)		
14	✓Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or
15	(page 12)		imprecision. Discuss both direction and magnitude of any potential bias
16	✓Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations,
17	(page 13)		multiplicity of analyses, results from similar studies, and other relevant evidence
18	✓Generalisability	21	Discuss the generalisability (external validity) of the study results
19	(pages 12, 13)		
20	Other information		
21	✓Funding	22	Give the source of funding and the role of the funders for the present study and, if
22	(page 2)		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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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Epidemiology, Haematology (incl blood transfusion)
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, integrase inhibitors, antiretroviral therapy, cohort, Anaemia < HAEMATOLOGY

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3 1 **A study of antiretroviral drug class and anaemia risk in the**
4 2 **current treatment era among people living with HIV in the United**
5 3 **States: A clinical cohort study**
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8 5 **Authors:** B.N. Harding¹, B.M. Whitney¹, R.M. Nance¹, H.M. Crane¹,
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29 24 **Keywords:** antiretroviral agents, cohort, anaemia, integrase
30 25 inhibitors, protease inhibitors, non-nucleoside reverse
31 26 transcriptase inhibitors
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44 38 **Competing interest statement:**

45 39 Dr. Harding reports grants from National Heart, Lung and Blood Institute(NHLBI) during the
46 40 conduct of the study; Ms. Whitney reports grants from the National Institutes of Health (NIH)
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48 42 study; Dr. Crane reports grants from NHLBI during the conduct of the study, grants from NIH,
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10 Heckbert reports grants from NIH during the conduct of the study; Dr. Delaney reports grants
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13 **Contributions:**

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

23 **Transparency declaration:**

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

28 **Ethical approval:**

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

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41 grant P30 AI027767]. Dr. Harding is supported by a National Heart, Lung and Blood Institute
42 grant T32HL007828.

44 **Data sharing:**

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

4
5 **Dissemination declaration:**

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

7
8 **Abstract:** (276 words)

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

16 DESIGN: Retrospective cohort study.

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

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

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

31 RESULTS: During a median of 4.9 years of follow-up, 1,040
32 developed anaemia and 488 developed severe anaemia. Compared to
33 NNRTI use, INSTI-based regimens were associated with an
34 increased risk of anaemia (adjusted hazard ratio [aHR] 1.26, 95%
35 confidence interval [CI] 1.00-1.58) and severe anaemia (aHR 1.51
36 95%CI 1.07-2.11), and a decrease in haemoglobin level. Time on
37 multiple core classes was also associated with increased anaemia
38 risk (aHR 1.39, 95%CI 1.13-1.70), while no associations were
39 found for PI use.

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

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45
46 **Strengths and limitations of this study:**

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

14 Introduction:

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

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

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

12 **Methods :**

13 Overview and setting:

14 The present study included PLWH in care in the Centers for
15 AIDS Research (CFAR) Network of Integrated Clinical Systems
16 (CNICS) cohort during the period of January 1, 2010 to March 31,
17 2018 (the date through which each site had complete data
18 [administrative censor date] varied somewhat, median date:
19 October 31, 2017). The CNICS cohort has been described in detail
20 elsewhere [20]. Briefly, CNICS is a dynamic prospective clinical
21 cohort of >32,000 adult PLWH receiving care at eight
22 participating sites across the U.S. Comprehensive clinical data,
23 including diagnoses, ART and other medications, laboratory test
24 results, demographic information, and historical information,
25 including ART use before enrollment, is collected through
26 electronic medical records and other institutional data systems
27 at each site and harmonized in the CNICS data repository.
28 Medication data including ART use are entered into the
29 electronic medical records by clinicians or prescription
30 fill/refill data are uploaded directly from pharmacy systems and
31 verified through medical record review. Participants entered the
32 current study on January 1, 2010 or the earliest date after
33 January 1, 2010 that they met the following enrollment criteria
34 (cohort entry date): a) enrollment in CNICS for ≥6 months to
35 allow time for covariate ascertainment, and b) use of an ART
36 regimen containing a backbone of 2 NRTIs plus a PI, NNRTI, or
37 INSTI. In addition, all participants were required to have at
38 least 2 available haemoglobin lab values during study follow-up.
39 Figure 1 shows inclusion criteria and exclusions made. Informed
40 consent was obtained from all participants and institutional
41 review boards at each site approved CNICS protocols (University
42 of Washington Human Subjects Division served as the institutional review
43 board for the centralized deidentified CNICS Data Repository).

44 Exposure:

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3 1 The exposure of interest was the ART core drug class
4 2 (NNRTI, INSTI or PI) prescribed as part of an ART regimen (a
5 3 backbone of two NRTIs plus a core drug). Participants switching
6 4 to different core drugs within the same class were considered to
7 5 be continually exposed to the same core drug class. Individuals
8 6 with a gap of 6 or more months in use of the ART core drug
9 7 classes of interest were censored at the start of the gap and
10 8 did not re-enter the study.

11 9 Person-time on INSTI or PI-based regimens was compared to
12 10 the NNRTI reference. In addition, some PLWH in this cohort had
13 11 prescriptions for multiple core classes simultaneously.
14 12 Participants with regimens containing more than 1 core class
15 13 were categorized separately in analyses as users of "multiple
16 14 core classes". Boosting agents (e.g. boosted ritonavir, or
17 15 cobicistat) were not considered a 2nd core agent.

18 17 Outcome ascertainment:

19 18 Hb levels, expressed in grams per deciliter (g/dL), were
20 19 ascertained using inpatient and outpatient laboratory data
21 20 obtained as part of clinical care. Outcomes included incident
22 21 anaemia (first post-baseline Hb measure below 10 g/dL), incident
23 22 severe anaemia (first post-baseline Hb measure below 7.5 g/dL)
24 23 [21] and changes in Hb level. Another outcome, chronic anaemia,
25 24 defined as anaemia lasting for ≥ 6 months, was also examined.
26 25 Chronic anaemia was defined as post-baseline Hb lab results on
27 26 two separate occasions at least 6 months apart which were
28 27 consistently in the anemic range without any Hb values above the
29 28 anaemia range during this 6-month period.

30 30 Participant characteristics:

31 31 Characteristics that were analyzed as confounders of the
32 32 association between ART core drugs and incident anaemia, severe
33 33 anaemia or change in Hb over time included: age, sex,
34 34 race/ethnicity, CNICS site, hepatitis C virus (HCV) coinfection
35 35 defined as a detectable HCV RNA level or HCV genotype or HCV
36 36 antibody, kidney function measured using estimated glomerular
37 37 filtration rate (eGFR, categorized as <30 , $30-59$, or ≥ 60
38 38 mL/minute/1.73 m²) [22], CD4 count (categorized as ≥ 500 , $350-499$,
39 39 $200-399$, $100-199$ or <100 cells/mm³), viral load (VL, assessed as
40 40 $\log_{10}(\text{VL}+1)$), baseline Hb (in incident anemia, severe anemia and
41 41 chronic anemia analyses only), and time in care at CNICS sites,
42 42 defined as time from cohort entry date until the last available
43 43 CNICS activity: either last lab date or last visit. HCV, eGFR,
44 44 CD4 count and VL were assessed as part of clinical care visits
45 45 and were time-updated as repeated measures occurred. All
46 46 covariates were selected *a priori*, based on review of the
47 47 literature and clinical knowledge. In addition, assessment of

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

5 6 Statistical analysis:

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

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

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

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

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

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

7 Patient and public involvement:

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

10 **Results:**

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

29 The overall incidence of anaemia was 2.1/100 person-years
30 and the overall incidence of severe anaemia was 0.8/100 person-
31 years. The unadjusted incidence rates of anaemia and severe
32 anaemia based on ART core class are provided in Table 2. In
33 adjusted analyses, INSTI use was associated with an increased
34 risk of anaemia (aHR 1.26, 95%CI 1.00-1.58) compared to NNRTIs
35 (Table 3). Use of multiple core classes together was also
36 associated with an increased risk of anaemia (aHR 1.39, 95%CI
37 1.13-1.70) while no associations were found between PI use and
38 anaemia (aHR of 1.09, 95%CI 0.90-1.32). In adjusted analyses
39 restricted to participants free of severe anaemia at baseline
40 (Table 3), INSTI use was associated with an increased risk of
41 severe anaemia (aHR 1.51, 95%CI 1.07-2.11) compared to NNRTI
42 use. Although the HR appeared elevated, there was no association
43 between time on multiple ART core classes and an increased risk
44 of severe anaemia (aHR 1.30 (0.95-1.78), and no association was
45 found for PIs (aHR 1.09 (0.81-1.47)). Among the 12,626 PLWH who
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1 were free of anaemia at baseline, 225 developed chronic anaemia
2 (lasting for ≥ 6 months), during follow-up. For chronic anaemia,
3 results were similar to those in the primary analysis although
4 confidence intervals overlapped 1. Relative to NNRTI use,
5 person-time on multiple core classes was associated with an aHR
6 for chronic anaemia of 2.21 (95%CI 0.94-5.18), person-time on an
7 INSTI with an aHR of 1.90 (95%CI 0.76-4.64) and person-time on a
8 PI with an aHR of 1.27 (95%CI 0.54-3.04).

9 Average Hb levels remained steady during follow-up; the
10 mean level was 14.1 g/dL (IQR 12.7-15.1) at baseline and 14.0
11 g/dL (IQR 12.6-15.2) at the last available measurement per
12 person. Relative to NNRTI use, a decrease in Hb level over time
13 was associated with both INSTI use (-0.06 g/dL per year, 95%CI -
14 0.10, -0.03) and use of multiple core classes (-0.14, 95%CI -
15 0.18, -0.11). No association was found for PI use (-0.01, 95%CI
16 -0.04, 0.03). (Table 4).

17 The sensitivity analysis restricted to ART-naïve
18 participants included 6,426 PLWH who were free of prevalent
19 anaemia at baseline, of whom 378 developed anaemia. Compared to
20 NNRTI initiators, those initiating a PI had an aHR of 0.69
21 (0.45-1.06) while those initiating an INSTI had an aHR of 1.10
22 (95%CI 0.84-1.44) (Supplemental Table 2). The mixed model
23 examining change in Hb over time among ART-naïve PLWH initiating
24 one of the ART core classes of interest included 7,264
25 participants. Compared to NNRTI initiators, a decrease in Hb was
26 found for PI use (-0.08g/dL per year, 95%CI -0.16, -0.01), while
27 INSTI use was associated with a larger decrease in Hb level over
28 time (-0.15g/dL per year, 95%CI -0.22, -0.09) (Supplemental
29 Table 3). Results from the sensitivity analyses including time-updated NRTI backbone
30 regimen adjustment were essentially unchanged (Supplemental Table 4). Finally, results from the
31 sensitivity analysis excluding participants with AZT use indicated similar findings to those from
32 the primary analyses, although confidence intervals were wider (Supplemental Table 5, 6).

33 Discussion:

34 In this study of 16,505 PLWH in care within the United
35 States in the current treatment era (2010 and after), we
36 observed that INSTI use, and time on multiple core ART classes,
37 were associated with decreases in Hb levels during follow-up
38 compared to using NNRTI-based regimens. We found that INSTI use
39 was associated with an increased risk of anaemia (aHR 1.26, 95%CI 1.00-
40 1.58) and severe anaemia (1.51, 95%CI 1.07-2.11) as well as a decrease in Hb levels over
41 time. Furthermore, the naïve user analysis indicated similar
42 findings despite a smaller sample size. These findings could
43 have implications for the treatment approach that should be used
44 in people with risk factors for anaemia.

45 This study's strengths include its large and geographically
46 diverse study population and longitudinal data structure.
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3 1 Nevertheless, there are limitations of this study to consider,
4 2 including the observational nature of the data, which may be
5 3 subject to residual confounding including confounding by
6 4 indication [25]. However, anaemia is not a recognized adverse
7 5 effect of NNRTIs, PIs, or INSTIs. Thus, it is unlikely that ART
8 6 core class was selected based on prescriber concern about
9 7 anaemia risk. Additionally, we did not exclude participants with
10 8 conditions strongly associated with anaemia or Hb level,
11 9 including those on dialysis, receiving erythropoietin, or with
12 10 severe bleeding, which likely caused some of the anaemia cases
13 11 in this analysis. However, in the sensitivity analysis focusing
14 12 on factors associated with chronic anaemia (less likely due to
15 13 bleeding), findings for INSTI vs. NNRTI core regimens were
16 14 similar to those including all PLWH who became anemic.
17 15 Information on ART medication use was from prescription data
18 16 which does not necessarily indicate medications were taken,
19 17 although self-reported adherence was high (~98%) in the subset
20 18 for whom adherence information was available. CNICS participants
21 19 who provided adherence information have been shown to be
22 20 representative of the overall population of PLWH in CNICS [23,
23 21 26]. Finally, the fact that this study was conducted among PLWH
24 22 in care in the U.S. who are on ART may limit the
25 23 generalizability of findings to PLWH who live outside of the
26 24 U.S.

27 25 There have been few epidemiologic studies of anaemia risk among users of newer ART
28 26 core regimens. From clinical safety data reported from multiple trials, approximately 36–49%
29 27 of participants using PIs had Hb levels <10g/dL, indicating
30 28 anaemia [18], and in another trial, two participants
31 29 discontinued INSTI use due to anaemia adverse events [19],
32 30 however the strict inclusion criteria applied in clinical trials
33 31 makes it difficult to generalize these findings to more diverse
34 32 populations of PLWH in clinical care. Another study, conducted
35 33 during the newer-era of HIV treatment with drugs other than AZT,
36 34 (during 2008–2012) presented findings for AZT versus non-AZT
37 35 regimens, finding an increased risk of anaemia among AZT
38 36 compared to non-AZT regimens (HR=2.84, 95%CI 1.52–5.31) [27].
39 37 However, anaemia risk was not analyzed separately for the use of
40 38 specific classes of ART, resulting in the inability of
41 39 comparison to the present study's findings and a lack of
42 40 generalizability to PLWH who are treated with newer ART core
43 41 agents.

44 42 It is possible that PLWH in our study whose HIV is
45 43 progressing due to resistance or other complications may get
46 44 switched to an INSTI. This, in addition to prior knowledge that
47 45 poorly controlled HIV parameters are on their own a risk factor
48 46 for anaemia [5, 28, 29], could result in confounding by
49 47 indication. However, the switch to INSTI core regimens since

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

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

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

30 31 **Figure Legends**

32
33 Figure 1: Flow chart for inclusion/exclusion criteria for 22,027
34 PLWH in care at CNICS after 1/2010. Exclusions were made for
35 those not exposed to any of the ART core classes, those with
36 fewer than 2 haemoglobin levels, and those missing baseline
37 covariates, resulting in 16,505 PLWH who were included in these
38 analyses.

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

^aCohort 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.

^bFor 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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5 2 Table 3. Association of ART core classes with incident anaemia (haemoglobin<10 g/dL),
6 3 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)

4 ^aAdjusted for age, sex, race/ethnicity, CNICS site, Hepatitis C virus status, CD4 count,
5 viral load, kidney function (eGFR), baseline haemoglobin

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

1 Table 4. Association of ART core classes with change in
 2 haemoglobin level during follow-up in adjusted analyses (linear
 3 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

4 ^aCoefficient is the mean difference per year in Hb (g/dL) for
 5 each core regimen relative to the NNRTI core regimen, after
 6 adjustment for site, age, sex, race/ethnicity, hepatitis C virus
 7 coinfection, CD4 cell count, viral load, eGFR and years in
 8 study.

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

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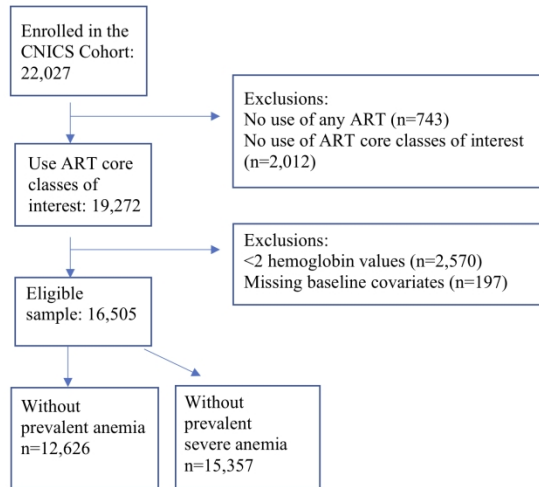


Figure 1: Flow chart for inclusion/exclusion criteria for 22,027 PLWH in care at CNICS after 1/2010. Exclusions were made for those not exposed to any of the ART core classes, those with fewer than 2 haemoglobin levels, and those missing baseline covariates, resulting in 16,505 PLWH who were included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

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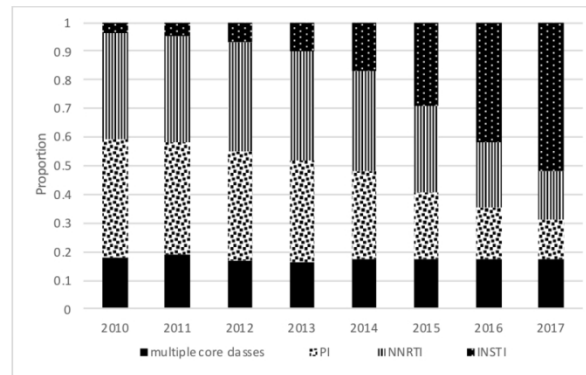


Figure 2. Proportion of study population (N=16,505) using various ART classes during complete years of study follow-up. This figure shows the trends in use of the ART core classes during 2010-2017.

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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)

^aAdjusted 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

^aCoefficient 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

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

4 ^aMinimally adjusted model includes adjustment for: age, sex, race, site, hepatitis C co-infection,
5 CD4 category, VL, eGFR category and haemoglobin at baseline.

6 ^bModel includes adjustment variables in minimally adjusted model plus a term for time-updated
7 abacavir/3TC backbone regimen use. The other backbone regimens largely consisted of tenofovir
8 /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)

^aAdjusted 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

^aCoefficient 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)

1 2 3	✓Outcome data (page 10)	15*	Report numbers of outcome events or summary measures over time
4 5 6 7 8 9 10	✓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 <hr/> (b) Report category boundaries when continuous variables were categorized <hr/> (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
11 12 13	✓Other analyses (page 11)	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
14	Discussion		
15 16 17	✓Key results (page 11)	18	Summarise key results with reference to study objectives
18 19 20	✓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
21 22	✓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
23 24 25	✓Generalisability (pages 12, 13)	21	Discuss the generalisability (external validity) of the study results
26	Other information		
27 28 29	✓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>.