Risk of clinical events in virologically suppressed people with HIV switching to a two-drug regimen vs. remaining on a three-drug regimen: a target trial emulation

Cristina Mussini,[a,r,*] Andrea Giacomelli,[b,r] Eugenia Quiros-Roldan,[c] Valentina Mazzotta,[d] Antonio Di Biagio,[e] Andrea De Vito,[f] Andrea Costantini,[g] Gabriella D'Ettorre,[h] Andrea Giacometti,[i] Alessandra Vergori,[d] Alessandro Tavelli,[j,k] Vincenzo Malagnino,[l] Antonella Castagna,[m,p] Johanna Chester,[n] Andrea Antinori,[d] Antonella d'Arminio Monforte,[j] and Alessandro Cozzi-Lepri,[o] on behalf of the Icona Foundation Cohort Study for the Icona Foundation Study Group[q]

[a]Infectious Diseases Unit, Azienda Ospedaliero-Universitaria Policlinico di Modena; University of Modena and Reggio Emilia, School of Medicine, Modena, Italy
[b]Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, III Infectious Disease Unit, ASST-Fatebenefratelli Sacco Milano, Italy
[c]Department of Clinical and Experimental Sciences, Unit of Infectious and Tropical Diseases, University of Brescia and ASST Spedali Civili di Brescia, Brescia, Italy
[d]Clinical and Research Infectious Diseases Department, National Institute for Infectious Diseases, Lazzaro Spallanzani IRCCS, Rome, Italy
[e]Department of Specialist Medicine, Infectious Disease Clinic, IRCCS Ospedale Policlinico San Martino, Genoa, Italy
[f]Unit of Infectious Diseases, Department of Medicine, Surgery and Pharmacy, University of Sassari, Sassari, Italy
[g]Clinical Immunology Unit, Azienda Ospedaliero Universitaria delle Marche, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy
[h]Department of Public Health and Infectious Diseases, Umberto I Hospital, Sapienza University of Rome, Rome, Italy
[i]Infectious Diseases Unit, Azienda Ospedaliero Universitaria delle Marche, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy
[j]Icona Foundation, Milan, Italy
[k]National PhD Programme in One Health Approaches to Infectious Diseases and Life Science Research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy
[l]Policlinico Tor Vergata, University of Rome "Tor Vergata", Rome, Italy
[m]Infectious Diseases Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[n]Department of Surgical, Medical, Dental and Morphological Sciences Related to Transplant Oncology and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy
[o]Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CREME), Institute for Global Health, UCL, London, UK
[p]IRCCS Ospedale San Raffaele Università Vita-Salute, Milan, Italy

Summary

Background **Guidelines support the switch to a two-drug regimen (2DR) in virologically suppressed people with HIV (PWH) on a three-drug regimen (3DR). Randomized clinical trials have not included clinical outcomes in study endpoints. We provide estimates of 3-year clinical risk by means of a target trial emulation using the data of a large cohort of PWH in Italy.**

Methods **PWH from the Icona Foundation Study who were virologically suppressed (HIV-RNA ≤50 copies/mL) for ≥6 months on a 3DR on or after November 2014, were enrolled (database closure on July 31, 2024). PWH were classified according to therapeutic strategies: switching to 2DR (protease inhibitors or dolutegravir plus lamivudine or dolutegravir plus rilpivirine) or remaining on 3DR (any combination). The primary endpoint was the time to the first clinical composite event (cardiovascular disease [CVD], cancer [AIDS and non-AIDS related], or death). We calculated the difference in 3-year risk between therapeutic strategies, estimated using a weighted non-parametric Kaplan–Meier estimator.**

eClinicalMedicine 2025;86: 103368

Published Online 24 July 2025
https://doi.org/10.1016/j.eclinm.2025.103368

*Corresponding author. Department of Surgical, Medical, Dental and Morphological Sciences, University of Modena and Reggio Emilia, Largo del Pozzo, 71 41124, Modena, Italy.
E-mail address: cristina.mussini@unimore.it (C. Mussini).
[q]Full list in the Supplementary Material section.
[r]Cristina Mussini and Andrea Giacomelli shared the first authorship.
The abstract has been presented as an oral abstract at the Italian Conference on AIDS and antiviral Research (ICAR) 2024, Rome and it was published in the special issue of Sexually Transmitted Infections 2024; 100:A34-A35.

Findings 7672 participants entered the analysis: 629 (8.2%) switching to 2DR and 7043 (91.8%) remaining on 3DR. Over the 3-year follow-up, 408 events were registered (64 CVD, 234 cancer, and 110 deaths). The 3-year adjusted risk estimate was 2.55 (95% CI 1.72, 5.33) in 2DR vs. 4.69 (95% CI 4.48, 6.17) in 3DR. The difference (−2.15% [95% CI −3.56%, −0.20%]) in favor of 2DR was mainly driven by events of non-AIDS related cancer and mortality.

Interpretation This study provides evidence that virologically suppressed PWH can be safely switched to 2DR, and may slightly reduce the 3-year risk of a composite clinical outcome.

Funding The Icona Foundation Study is supported by unrestricted grants from Gilead Sciences, ViiV Healthcare, Merck Sharpe & Dohme.

Keywords: Antiretroviral therapy; Two drug regimens; HIV; Clinical outcomes

Research in context

Evidence before this study

We conducted a search on PubMed/Medline, Web of Science and Scopus for English-language clinical trials and/or cohort studies and/or review articles, published up to January 22, 2024. Studies reporting a comparison of the effectiveness between 2-drug regimens (2DRs) and 3-drug regimens (3DRs) on clinical outcomes in virologically suppressed people with HIV virus (PWH) who switched or maintained antiretroviral therapy (ART). We searched for studies with clinical endpoints using combinations, abbreviations, and variations of the following search terms: "HIV", "dual therapy", "triple therapy", "two-drug regimens", "three-drug regimens", "clinical outcome", "cancer", "cardiovascular disease", "death", and "switch".

Currently, no randomized clinical trials (RCT) of ART in PWH include clinical outcomes as a study end-point. The RESPOND cohort, currently the largest available study of real-world data (RWD) evaluating over 9000 PWH, reported no difference in clinical outcomes between 2DR and 3DR regimens. However, results were not generated according to the European Medical Agency's methodological recommendation for the comparison of treatment strategies in an observational setting: target trial emulation (TTE). The differential risk of developing clinical outcomes (cardiovascular events [CVD], cancer [AIDS and non-AIDS-related], or death) in virologically suppressed PWH switching from 2DR compared to remaining on 3DR remains unclear. Further research of observational data assessed with TTE may assist in guiding clinicians in their clinical decision making.

Added value of this study

Our study utilized RWD data of PWH enrolled in the ICONA Foundation Study; the largest cohort of PWH in Italy. A TTE was conducted on virologically suppressed PWH switching to 2DR or remaining on 3DR. The primary aim of the evaluation was the risk of a composite clinical event of CVD, cancer, and death. The 3-year causal effects of treatment strategies were estimated using a weighted non-parametric Kaplan–Meier estimator.

Our study found a small but significantly lower 3-year clinical event risk for PWH who switched to 2DR. After adjustment for immortal-time and confounding bias, the estimated difference in risk was 2.15% in favor of switching to 2DR vs remaining on 3DR. Our findings provide clinicians with evidence that virologically suppressed PWH switching to 2DR are at less risk of clinical events at 3 years.

The major strength of our analysis is the estimate of causal effect with TTE analysis with RWD. Similar estimates are unlikely to be produced by RCTs in the near future.

Implications of All the Available Evidence

Our findings reassure clinicians that switching to 2DR in virologically suppressed PWH is safe and may slightly reduce the risk of clinical events at 3 years. As prospective randomized comparisons with clinical endpoints are unlikely to be conducted soon, and as most current and future ART combinations are based on a 2DR strategy, RWD are crucial for guiding current clinical decisions.

Introduction

Dual therapy (2DR) is the current recommended by guidelines as a possible antiretroviral therapy (ART) strategy for people with HIV (PWH), either as first-line or switch.[1–3] The most prescribed 2DR therapy strategy is dolutegravir/lamivudine (DTG/3TC). Current guidelines were developed based on evidence from several trials proving virological non-inferiority of 2DR regimens compared to standard care with triple regimens (3DR).[4–8] The rationale for implementing 2DR for

virologically suppressed PWH is based on reducing drug exposure and long-term side effects, whilst preserving virological efficacy.

3DR ART was responsible for transformed HIV into a chronic condition. Compared to people without HIV, PWH now enjoy a similar life expectancy,[9] but more years with comorbidities.[10] It has been hypothesized that the anticipated onset of comorbidities in PWH may be due to a state of chronic inflammation, maintained despite ART-related plasma viral suppression.[11] However, studies on circulating markers correlated to chronic inflammation among PWH in 2DR and 3DR showed conflicting results, ranging from no evidence of a difference to potentially higher transient levels of inflammation among those treated with 2DR.[12–15] Nevertheless, whether prolonged ART with 2DR may be associated with a higher risk of clinical endpoints, such as cardiovascular events and non-AIDS-related cancer, is to be established. As analysis of clinical outcomes require large sample sizes and prolonged follow-up, randomized clinical HIV trials do not include clinical outcomes among the study endpoints. Therefore, real-world data (RWD) are crucial for risk assessment of clinical events over the follow-up.

The RESPOND retrospective observational study evaluated >10,000 PWH treated with either 2DR or 3DR and reported no difference in the incidences of clinical events. The authors concluded that despite the use of a large patient cohort, further research on clinical outcomes was necessary.[16]

The European Medical Agency recommends retrospective analysis of RWD with a target trial emulation (TTE) methodology. We conducted a TTE of virologically suppressed PWH, randomized either to switching to 2DR or remaining on 3DR, according to retrospective therapy strategies undertaken. The primary outcome was the 3-year risk of a new clinical event, defined as a composite outcome of cardiovascular disease (CVD), cancer or death.

Methods

Study design

We performed a TTE according to an established framework.[17,18] The analysis was designed with the TANGO and SALSA trials as reference, with some small differences in overall design.[4,5]

Data from the Icona Foundation Study, an Italian prospective observational cohort of ART-naïve, PWH adults, were accessed.[19] Cohort enrolment began in January 1997, and is ongoing. Data include laboratory parameters, dates of initiation and interruption of ARTs and the incidence of clinical outcomes.[19]

Ethics

The institutional review boards of all participating centers approved the Icona Foundation Study. Each participant signed an informed consent according to committee ethical standards and the Helsinki Declaration (October 2013). Our current study proposal was shared with patient representatives at the annual Icona Foundation Study meeting (2023). The latest amendment of the ICONA Foundation Study was approved centrally by the Lazio Area 4 Territorial Ethics Committee on 01 July 2024 (approval 158 no. 83-2024). Reporting of the study follows the strengthening of the reporting of observational studies in epidemiology (STROBE) guidelines.[20]

Eligibility criteria and treatment strategies

We considered all virologically suppressed (≥6 months of HIV-RNA ≤50 cp/mL) participants, registered after Italian health authorities recommended a switch strategy to protease-inhibitor [PI]-based 2DR, in virologically suppressed PWH (on or after November 1, 2014). We excluded PWH with contraindications for 2DR (positive HBsAg and pregnancy, Supplementary Figure S1).

Follow up, outcomes, and definitions

Baseline for the main analysis we used as time zero the last time a participant was eligible for the target trial (see criteria above) while in a key sensitivity analysis we used as time zero the first of these eligible times (on or after November 1, 2014). Eligible patients were included in the analysis until the registration of a composite clinical endpoint, confirmed HIV-RNA rebound (two consecutive values > 50 copies/mL), last cohort visit or an administrative censoring date (the date of database closure on July 31, 2024), whichever occurred first.

We used a procedure of cloning (i.e. the creation of a copy for each of the participants), censoring and weighting for treatment strategies that are initiated during a grace period after study entry, as previously described.[21] This analytical procedure with adequate adjustment was chosen to estimate the per-protocol effect.[22] A complete description of the implementation target trial study procedure, comprehensive of cloning, survival time definition, handling of vital status and estimation of the censoring weights, are reported in Supplementary materials.

Briefly, after applying the cloning, artificial censoring was applied for clones allocated to 3DR when switching to 2DR within 6 months from baseline (the "grace period"). Follow-up was censored at the time of starting 2DR. For clones assigned to 2DR if they did not follow the trial strategy (i.e. they did not switch to 2DR with DRV/r/c+3TC or ATV/r/c+3TC or DTG + RPV or DTG+3TC within 6 months from baseline) their follow-up was censored at 6 months (see Supplementary material for further details).

The primary outcome was a composite clinical endpoint, defined as the time to newly develop CVD (myocardial infarction, stroke, or coronary revascularization), cancer (both AIDS and non-AIDS-defining

cancer) or death events from baseline. Secondary outcomes were the time to CVD or CVD-associated-death, and cancer and cancer-associated death. Clinical events were collected using a dedicated clinical case form and causes of death were verified by two independent clinicians (AG and ADV) when data were missing in the database. In case of any discrepancy, a third senior researcher (ADM) reviewed the clinical case for a final decision.

Statistical analysis

We emulated a parallel trial in which virologically suppressed PWH (for ≥6 months) were assigned either to switching to 2DR or remaining on 3DR within a grace period of 6 months. All regimens of any combination of 3 drugs defined the 3DR strategy. In contrast, only switches to combinations which included darunavir/ritonavir (DRV/r) or cobicistat (DRV/c) + lamivudine (3TC); atazanavir/ritonavir (ATV/r) or cobicistat (ATV/c) + 3TC; dolutegravir (DTG) + rilpivirine (RPV), and DTG + 3TC within the grace period (6 months from study date baseline) defined the 2DR strategy.

Survival curves were estimated using a weighted non-parametric Kaplan–Meier estimator. The 95% confidence intervals for the difference in 3-year risk of outcomes by treatment strategy were obtained using non-parametric bootstrap with 100 replicates. To assess the extent of immortal and confounding bias, we compared TTE results to unweighted estimates with and without cloning.

We used a "disjunctive cause" criterion for selecting covariates for propensity scores models.[23] Specifically, variables used to construct the weights to control for informative censoring induced by the cloning were: calendar year of baseline, age, sex at birth, nadir and CD4 counts at baseline, zenith and baseline CD8 counts, peak in HIV-RNA before baseline, duration of viral suppression before baseline, nationality (Italian vs. foreign), mode of HIV transmission, number of PI and of NNRTI previously virologically failed, total duration of ART, use of abacavir and class of anchor drug used at baseline, gap in care (defined as >18 months with no clinical visits) prior to baseline (yes/no), participating clinical site, and traditional risk factors for CVD and cancer collected at baseline (BMI, hypertension, smoking, total cholesterol and diabetes). All continuous variables were fitted with a single parameter (linear assumption). Arterial hypertension was defined as a systolic blood pressure >140 mm Hg or a diastolic blood pressure >90 mm Hg registered at ≥ 2 cohort visits or receiving antihypertensive drugs. Diabetes was defined as a confirmed glycemia value ≥ 126 mg/dL or receiving antidiabetic medication. When incorporating variables with missing values (smoking 13% and blood pressure 17%) in the propensity score models, a missing indicator method was used.

Sensitivity analyses

Potential selection bias in the main analysis was controlled for with key sensitivity analyses: 1) applying the date of the first episode of virological suppression; 2) selecting 3TC/DTG as the only 2DR-defining strategy; 3) restricting participants to those using 2NRTI + 1 anchor drug at baseline; 4) removing participants with a history of virological failure to 3TC at baseline, and 5) excluding participants with an AIDS diagnosis prior to baseline. Finally, we also performed a sensitivity analysis after removing from the cohort participants who use 2DR-based regimens after the end of the grace period. To test the robustness to violations of the causal model assumptions, additional sensitivity analysis with a nonparametric approach was adopted. Specifically, the minimum strength of association between unmeasured confounders, treatment assignment, and the primary outcome that would be needed to bring the risk difference between treatment assignment and outcome to zero, was calculated by an e-value estimate.[24,25] For example, an e-value of 2 means that an unmeasured confounder must have an RR of at least 2.0 with both the intervention and the outcome, to fully explain the treatment strategy–outcome association, conditional on all measured covariates. All statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA) and Stata (StataCorp. 2023. *Stata Statistical Software: Release 18*. College Station, TX: StataCorp LLC).

Role of funding source

The Icona Foundation Study is supported by unrestricted grants from Gilead Sciences, ViiV Healthcare, Merck Sharpe & Dohme. The supporters have no role in the study design and data interpretation.

Results

The main study population characteristics are summarized in Table 1. 7672 participants entered the analysis: 629 (8.2%) switched to 2DR and 7043 (91.8%) continued/switched 3DR.

Switching to 2DR was less likely in female PWHs (18% vs. 21%, p = 0.05), and more likely in PWH men who have sex with men (MSM) (49% vs. 44%, p = 0.01), are of Italian nationality (88%, vs. 84%, p = 0.008), and receive an INSTI-based regimen at baseline (p < 0.001). The median calendar year of baseline in participants who switched to 2DR was 2016 vs. 2018 in 3DR, p < 0.001. Participants on a 3DR strategy had longer periods from HIV diagnosis to baseline (74 vs. 53 months, p < 0.001) and ART duration (56 vs. 39 months, p < 0.001). PWH switched to 2DR were less likely to have experienced a gap in care (5% vs. 9%, p = 0.002). There was no evidence for a difference in classic CVD and cancer risk factors, except for diabetes,

Characteristics at baseline[a]	Treatment strategy			
	2DR	3DR	p-value[b]	Total
	N = 629	N = 7043		N = 7672
Age, years			0.134	
Median (IQR)	45 (38, 53)	46 (38, 54)		46 (38, 54)
Gender, n (%)			0.051	
Female	111 (17.6%)	1475 (20.9%)		1586 (20.7%)
Mode of HIV Transmission, n (%)			0.013	
PWID	51 (8.1%)	678 (9.6%)		729 (9.5%)
MSM	305 (48.5%)	3110 (44.2%)		3415 (44.5%)
Heterosexual	225 (35.8%)	2853 (40.5%)		3078 (40.1%)
Other/Unknown	48 (7.6%)	402 (5.7%)		450 (5.9%)
Nationality, n (%)			0.008	
Italian	553 (87.9%)	5911 (83.9%)		6464 (84.3%)
Current CD4 count, cells/mmc			0.650	
Median (IQR)	677 (493, 905)	692 (499, 902)		690 (499, 903)
Nadir CD4 count, cells/mmc			0.799	
Median (IQR)	289 (164, 432)	297 (163, 427)		296 (163, 427)
Peak viral load, log10 copies/mL			0.469	
Median (IQR)	4.85 (4.20, 5.41)	4.83 (4.22, 5.34)		4.83 (4.22, 5.34)
Number of previous ART failures			0.834	
Median (IQR)	3 (3, 4)	3 (3, 4)		3 (3, 4)
Previous PI failures, n (%)			0.064	
Yes	36 (5.7%)	491 (7.0%)		527 (6.9%)
Previous NNRTI failures			0.236	
Yes	27 (4.3%)	359 (5.1%)		386 (5.0%)
Time from baseline to 2DR switch, days				
Median (IQR)	92 (31, 153)			92 (31, 153)
Time from ART initiation to baseline, months			<.001	
Median (IQR)	39 (13, 92)	56 (24, 101)		55 (23, 100)
Time from HIV diagnosis to baseline, months			<.001	
Median (IQR)	53 (17, 126)	74 (33, 141)		73 (31, 140)
Class of previous anchor, n (%)			<.001	
INSTI	296 (47.1%)	2537 (36.0%)		2833 (36.9%)
NNRTI	171 (27.2%)	2859 (40.6%)		3030 (39.5%)
PI	149 (23.7%)	1491 (21.2%)		1640 (21.4%)
Other	13 (2.1%)	156 (2.2%)		169 (2.2%)
Gap in care before baseline >18 months, n (%)			0.002	
Yes	34 (5.4%)	634 (9.0%)		668 (8.7%)
Year of enrolment			<.001	
Median (IQR)	2016 (2016, 2019)	2018 (2016, 2020)		2018 (2016, 2020)
BMI			0.031	
Median (IQR)	24 (22, 26)	24 (22, 27)		24 (22, 26)
Hypertension, n (%)			0.626	
No	183 (29.1%)	2073 (29.4%)		2256 (29.4%)
Yes	331 (52.6%)	3788 (53.8%)		4119 (53.7%)
Smoking, n (%)			0.216	
No	303 (48.2%)	3457 (49.1%)		3760 (49.0%)
Yes	232 (36.9%)	2703 (38.4%)		2935 (38.3%)
Total cholesterol			0.202	
Median (IQR)	185 (158, 210)	186 (161, 214)		186 (161, 214)
Use of abacavir, n (%)			<.001	
Yes	200 (31.8%)	799 (11.3%)		999 (13.0%)
Duration of last episode of VL suppression			<.001	
Median (IQR)	28 (11, 66)	41 (15, 75)		40 (14, 74)

(Table 1 continues on next page)

Characteristics at baseline[a]	Treatment strategy			
	2DR	3DR	p-value[b]	Total
	N = 629	N = 7043		N = 7672
(Continued from previous page)				
Zenith CD8 count, cells/mmc			0.099	
Median (IQR)	1292 (926, 1737)	1316 (974, 1768)		1315 (970, 1764)
Diabetes, n (%)			0.064	
Yes	22 (3.5%)	365 (5.2%)		387 (5.0%)

List of abbreviations: 2DR, two drug regimens; 3DR, three drug regimens; IQR, Inter Quartile Range; PWID, people who inject drugs; MSM, men who have sex with men; VL, viral load; CD, cluster of differentiation; BMI, body mass index; ART, antiretroviral therapy; PI, protease inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; INSTI, integrase strand transfer inhibitors. [a]At least one VL $\leq$ 50 copies/mL after November 2014. [b]Chi-square and Mann–Whitney test, as appropriate.

Table 1: **Main characteristics of the study population according to baseline trial intervention strategy.**

which was slightly more frequent in participants in the 3DR strategy (5% vs 4% p = 0.06).

The plot of the standardised mean differences confirms that the largest imbalance in characteristics by intervention was observed within the first year from baseline. However, for all key potential confounding variables, after weighting the standardised difference was <10% (shown as the negligible difference region in grey in Supplementary Figure S2A).

Fig. 1 shows the 2DR switch strategy prescribed after baseline. The graph shows both the switches within the 6-month grace period (red bars) and the overall switches (at any time point during therapy; blue bars). The most frequent 2DR switch strategy was the 3TC/DTG combination (507/629; 77%), followed by RPV/DTG (7%).

Main composite outcome analysis

Table 2 outlines the descriptive analysis' composite clinical endpoint by treatment strategy. Within 3 years, 408 (5.3%) events were registered (64 [0.8%] CVD, 234 [3.1%] cancers [36 (0.5%) AIDS related, and 198 (2.6%) non-AIDS related] and 110 (1.4%) deaths. A lower overall crude risk of clinical events was observed in the 2DR strategy compared to the 3DR strategy (2.9% vs. 5.5%), mainly due to a lower frequency of non-AIDS cancers in 2DR strategy (1.4% vs. 2.7%) and deaths (0.5% vs. 1.5%). The distribution of the specific causes of death is described in Table 2. Supplementary Table S1 shows the types of cancers registered by treatment strategy.

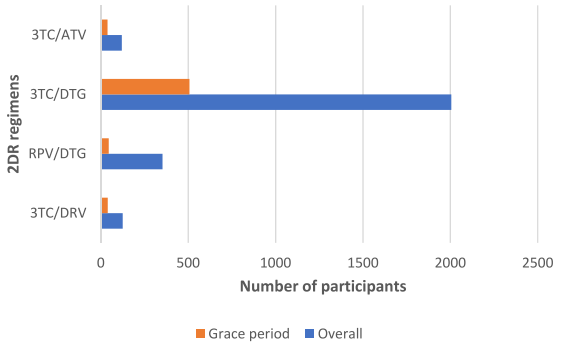

Fig. 1: Overall and baseline 2DR switches. Legend 2DR switches occurred within the grace period in red and in the observational cohort in blue.

Table 3 shows the results of the main survival analysis. The estimated 3-year cumulative risk of the composite clinical event in the unweighted Kaplan–Meier analysis was slightly lower than the crude risks calculated in the descriptive analysis, not accounting for time, amounting to 2.8% in 2DR vs. 5.11% in 3DR (risk difference of −2.31% [95% CI −3.34%, −1.29%]) (Table 3, Supplementary Figure S3 [log-rank p-value = 0.004]). This unweighted Kaplan–Meier plot showed a very low incidence of events in the switch to 2DR group over the first 3–9 months. Results were similar after weighting (Supplementary Figure S4). In the fully adjusted analysis (controlling for both immortal time and confounding bias), the 3-year risk difference in the main composite clinical outcome remained in favour of the 2DR treatment strategy (−2.15% [95% CI −3.56%, −0.20%]). The sensitivity analysis for the possible effect of unmeasured confounding produced an e-value of 3.28 [Supplementary Figure S7].

Sensitivity analyses for the main composite outcome

The main characteristics of the study population included in the sensitivity analyses are reported in Supplementary Table S2. The plot of the standardised mean differences for the main and sensitivity analyses are depicted in Supplementary Figure S2B. Compared to the main analysis, the difference in the 3-year risk estimated in the sensitivity analyses 1–5 was attenuated: −1.37% (95% CI −2.49%, −1.26%) analysis 1 (Table 4); −1.93% (95% CI −3.86%; −0.35%) analysis 2 (Supplementary Table S3), −2.06% (95% CI −3.70%; −0.22%) analysis 3 (Supplementary Table S4), and −1.64 (95% CI −2.57%, −0.41%) analysis 4 (Supplementary Table S5). Results were similar

Events (% of total)	Treatment strategy		
	2DR (n = 629)	3DR (n = 7043)	Total (n = 7662)
Total	18 (2.9%)	390 (5.5%)	408 (5.3%)
AIDS cancer	1 (0.2%)	35 (0.5%)	36 (0.5%)
Non-AIDS cancer	9 (1.4%)	189 (2.7%)	198 (2.6%)
CVD	5 (0.8%)	59 (0.8%)	64 (0.8%)
Death	3 (0.5%)	107 (1.5%)	110 (1.4%)
Causes of death			
AIDS	0	10 (9.4%)	10 (9.1%)
Other infections (COVID-19, sepsi)	1 (33.3%)	16 (14.9%)	17 (15.5%)
Respiratory disease	0	3 (2.8%)	3 (2.7%)
Liver disease	1 (33.3%)	8 (7.5%)	9 (8.2%)
Violent death (suicide, accident)	1 (33.3%)	13 (12.1%)	14 (12.7%)
Cancer	0	8 (7.5%)	8 (7.3%)
CVD	0	18 (16.8%)	18 (16.4%)
Renal disease	0	2 (1.9%)	2 (1.8%)
Other/Unknown	0	29 (27.1%)	29 (26.3%)

Table 2: **Composite clinical endpoint: events by baseline trial intervention strategy.**

to the main analysis −2.18% (95% CI −3.52%; −0.11%) for sensitivity analysis 5 (Supplementary Table S6) and in the analysis performed after removing from the data set participants who used 2DR-based regimens after the end of the grace period (Supplementary Table S7).

Secondary outcomes

The analyses of the secondary outcomes are reported in Tables 5 and 6. These analyses showed inconclusive evidence for a difference in 3-year risk between treatment strategies, both for cancer/death due to cancer [−0.57% (95% CI −1.80%, +0.83%)] and for CVD/death due to CVD [−0.71% (95% CI −1.98%, +0.95%)].

Discussion

Our TTE analyses found that ART-experienced, virologically suppressed PWH who switch to 2DR have a small but significantly lower 3-year risk of composite clinical outcomes (CVD, cancer and death) compared to remaining on 3DR.

Previous RCTs have reported no evidence for a difference in virological outcome between 2DR and 3DR, but clinical endpoints of morbidity and mortality were not evaluated.[4–8] However, some RCTs and observational studies have underlined a potential increase in inflammation in 2DR compared to 3DR. The Icona Foundation Study reported a higher increase in

Strategy	3-year risk of composite clinical endpoint[b] (%)	95% CI
Original cohort		
2DR	2.80	2.49, 4.30
3DR	5.11	4.77, 6.61
Difference	−2.31	−3.34, −1.29
Emulated cohort		
2DR	2.80	2.02, 4.85
3DR	4.43	4.21, 5.75
Difference	−1.63	−2.93, −0.24
Adjusted[a] emulated cohort		
2DR	2.55	1.72, 5.33
3DR	4.69	4.48, 6.17
Difference	−2.15	−3.56, −0.20

[a]Weighted for age, sex, mode of transmission, age, AIDS, nadir CD4, most recent CD4, peak HIV-RNA, duration of ART, class of anchor drug at baseline, zenith CD8, risk factors for CVD, duration of VL suppression, number of PI/NNRTI failures, adherence score and clinical site. [b]CVD, Cancer or death.

Table 3: **Kaplan–Meier estimates of the 3-year risk of the composite clinical endpoint by trial intervention strategy, and risk difference with 95% CI.**

Strategy	3-year risk of composite clinical endpoint[b] (%)	95% CI
Original cohort		
2DR	1.03	0.84, 1.94
3DR	1.96	1.75, 3.16
Difference	−0.93	−1.75, −0.50
Emulated cohort		
2DR	0.35	0.23, 0.48
3DR	1.66	1.51, 2.67
Difference	−1.31	−2.30, −1.19
Adjusted[a] emulated cohort		
2DR	0.26	0.14, 0.42
3DR	1.63	1.48, 2.78
Difference	−1.37	−2.49, −1.26

[a]Weighted for age, sex, mode of transmission, age, AIDS, nadir CD4, most recent CD4, peak HIV-RNA, duration of ART, class of anchor drug at baseline, zenith CD8, risk factors for CVD, duration of VL suppression, number of PI/NNRTI failures and adherence score. [b]CVD, Cancer or death.

Table 4: **Kaplan–Meier estimates of the 3-year risk of the composite clinical endpoint by trial intervention strategy and risk difference with 95% CI (first viral suppression episode).**

Strategy	3-year risk of cancer/death due to cancer (%)	95% CI
Original cohort		
2DR	1.93	1.52, 2.87
3DR	2.41	2.06, 3.10
Difference	−0.48	−1.26, 0.37
Emulated cohort		
2DR	1.64	0.70, 2.75
3DR	2.28	2.07, 2.95
Difference	−0.64	−1.80, 0.29
Adjusted[a] emulated cohort		
2DR	1.85	0.88, 3.61
3DR	2.42	2.15, 3.16
Difference	−0.57	−1.80, 0.83

[a]Weighted for age, sex, mode of transmission, age, nadir CD4, most recent CD4, peak HIV-RNA, duration of ART, class of anchor drug at baseline and adherence score.

Table 5: **Kaplan–Meier estimates of the 3-year risk of cancer and cancer related death by trial intervention strategy and risk difference with 95% CI.**

inflammation (CD8 count) after switching to 2DRs vs. remaining on 3DR.[12] The Spanish AIDS research network, a large observational study, proved a significant increase in IL-6, hrCRP and d-Dimer in virologically suppressed PWH under 2DR, but only after 3 years from the date of switching. The study included both PI and INSTI-based ART.[13] Among RCTs, the TANGO study reported a significantly higher concentration of IL-6 in the DTG/3TC compared to the 3DR arm,[14] while in the DEBATE trial, there was a higher expression of CD4 lymphocyte count of activation markers and non-classical monocytes in the DTG/3TC compared to the bictegravir/emtricitabine/tenofovir alafenamide arm, 6 months after switch. This difference was attenuated by 12 months.[15]

Strategy	3-year risk of CVD/death due to CVD (%)	95% CI
Original cohort		
2DR	1.97	1.62, 2.94
3DR	2.44	2.10, 3.22
Difference	−0.48	−1.27, 0.26
Emulated cohort		
2DR	1.56	0.84, 2.80
3DR	2.32	2.11, 3.02
Difference	−0.76	−1.79, 0.17
Adjusted[a] emulated cohort		
2DR	1.76	0.79, 3.80
3DR	2.47	2.26, 3.27
Difference	−0.71	−1.98, 0.95

[a]Weighted for age, sex, mode of transmission, age, AIDS, nadir CD4, most recent CD4, peak HIV-RNA, duration of ART, class of anchor drug at baseline, zenith CD8, risk factors for CVD, duration of VL suppression, number of PI/NNRTI failures and adherence score.

Table 6: **Kaplan–Meier estimates of the 3-year risk of the CVD events of death due to CVD by trial intervention strategy and risk difference with 95% CI.**

Our clinical study did not measure inflammation. Nevertheless, the major inflammation driver is considered viral replication.[26] In our study, there was no difference in zenith viral load among groups. The only difference was observed in the prevalence in gap in care, which was more frequent among the 3DR group. It has been shown that a gap in care, especially those of long duration and with treatment discontinuation, determines a return of viral load towards the set-point level, potentially increasing inflammation.[26,27] After adjusting for confounders, including previous gaps in care, switching to a 2DR strategy was associated with a lower rate of clinical events compared to those remaining on 3DR. Compared to the main analysis of composite clinical 3-year risk, when secondary endpoints were measured (cancer/death due to cancer and CVD/death due to CVD) results were inconclusive: (1) the point estimates of difference in favor of 2DR was largely attenuated, (2) a potential 1% worse outcome with the 2DR switch strategy cannot be excluded.

The only other study reporting clinical outcome of PWH on ART switching to 2DR, is the retrospective, observational RESPOND study, published in 2021. The authors reported no difference in adjusted composite outcomes after a median follow up of 2.6 years.[16] Our study cannot be compared to the RESPOND study, as the populations and outcomes are very different; the RESPOND study also enrolled groups of ART-naïve, not virologically suppressed PWH, and the analysis was conducted using standard regression modelling conditioned on the covariates with no adjustment for immortal-time bias.

Our analysis has several limitations. Cohort studies capture routine care data from HIV or infectious disease clinics, and therefore some events may not have been precisely documented. Specific causes of deaths were unavailable in 29/408 patients (all in the 3DR arm). The lower incidence of clinical events observed in 2DR mainly appeared to be due to a very low incidence of events over 3–9 months from baseline in 2DR strategy, as shown by the unadjusted Kaplan–Meier plot. This outcome suggests that a potential selection/immortal time bias may have occurred. However, this bias was controlled in our fully adjusted analysis. Our analyses were adjusted for all known determinants of ART switch, predictors of clinical events or both; however, we cannot exclude unmeasured confounding factors. For example, although we adjusted for the number of previously failed PI- and NNRTI-based regimens, we did not gather information about drug resistance. Our sensitivity analyses under different intervention scenarios and inclusion criteria (including one attempting

to estimate the pure on-treatment effect) provided similar results and unmeasured confounders would require a 3.3-fold or greater association with treatment assignment and outcome (e-value, 3.28) to explain away the effect. Despite recent criticism of the e-value, it remains a possible, albeit basic, way of conducting sensitivity analyses in the TTE framework.[28,29] There are not many conceivable factors associated with a >3-fold higher risk for all-cause mortality and also considering the large number of measured confounding factors included in the propensity score models, we believe that our results are robust. As our analysis was underpowered for single events (CVD, cancer and death), we reported a composite outcome. Lastly, both our analysis, which is restricted to the estimation of the per-protocol effect, and the RESPOND study did not investigate possible mechanisms which led to the difference in risk among treatment groups (principally values of inflammation markers), and further research is needed to investigate this causal pathway.

In virologically suppressed PWH, our study suggests that switching to 2DR (mainly DTG/3TC), compared to remaining on 3DR, reduces the 3-year risk of a composite clinical outcome. The difference is primarily driven by events occurring within the first 3–9 months of regimen switch and mortality. As prospective randomized comparisons of clinical endpoints are unlikely to be conducted soon, and most future ART regimens are based on a 2DR strategy, RWD are crucial to guide clinical decisions. Our data reassures the safety of the physicians' choice to switch virologically suppressed PWH to 2DR in a real-world setting.

Contributors

Cristina Mussini conceptualisation, writing—original draft, and writing—review & editing, Andrea Giacomelli writing—original draft, and writing—review & editing, Eugenia Quiros-Roldan investigation and writing—review & editing, Valentina Mazzotta investigation and writing—review & editing, Antonio Di Biagio investigation and writing—review & editing, Andrea De Vito investigation and writing—review & editing, Andrea Costantini investigation and writing—review & editing, Gabriella D'Ettorre investigation and writing—review & editing, Andrea Giacometti investigation and writing—review & editing, Alessandra Vergori, Alessandro Tavelli accessed and verified data and data curation, Vincenzo Malagnino investigation and writing—review & editing, Antonella Castagna investigation and writing—review & editing, Johanna Chester writing—review & editing, Andrea Antinori supervision and writing—review & editing, Antonella d'Arminio Monforte supervision and writing—review & editing and funding acquisition, Alessandro Cozzi-Lepri accessed and verified data and formal analysis and methodology.

Data sharing statement

The datasets generated during the current study are not publicly available because they contain sensitive data to be treated under data protection laws and regulations. Appropriate agreement of data sharing can be arranged after a reasonable request to the corresponding author.

Declaration of interests

CM has received research grants from Gilead Sciences, Speaker honoraria from Gilead Sciences, ViiV Healthcare, MSD, Johnson & Johnson, travel grants from Gilead; AGiacomelli received consultancy fees from ViiV Healthcare, Gilead Sciences, MSD and Jannsen travel grant from Gilead Sciences; EQR received travel grants from Gilead Sciences and ViiV Healthcare; AV received consultacy fees from ViiV Healthcare, Gilead Sciences and Astrazeneca; ADV received consultancy fees from ViiV Healthcare; ACostantini has received consultancy fees from Gilead Sciences; ADB received speakers' honoraria from Gilead Sciences, ViiV Healthcare and Janssen-Cilag, has been an advisor for ViiV Healthcare, Gilead Sciences, travel reimbursement by Gilead Sciences, and has received grant for research from Gilead Sciences and ViiV healthcare; GDE received Speaker honoraria and has been an advisor for Gilead Sciences, MSD, AbbVie, Janssen-Cilag and ViiV Healthcare, and research grant from GSK and ViiV Healthcare; VMazzotta received institutional research grant from Gilead Science, speaking honoraria for congress from ViiV Healthcare and consultation fees for ViiV Healtcare, Pfizer, and Gilead Science; A. Castagna received fees from ViiV Healthcare, Gilead Sciences, MSD and Janssen-Cilag; AA served as a paid consultant to Astra Zeneca, Bavarian Nordic, Gilead Sciences, GSK, Janssen-Cilag, MSD, Moderna, Pfizer, and ViiV Healthcare and received institutional research grants from Astra Zeneca, Gilead Sciences and ViiV Healthcare; ACL declared research grant or contract for his institute (UCL) by Icona Foundation, and by European Union's Horizon2020: Grant Agreement No 101046016 "EuCARE: European Cohorts of Patients and Schools to Advance Response to Epidemics" and grant Agreement No 101194735 "VIROMARKERS Consortium Agreement"; AG, VMalagnino AdM, JC, AT have nothing to disclose.

Acknowledgements

The Icona Foundation Study is supported by unrestricted grants from Gilead Sciences, ViiV Healthcare, Merck Sharpe & Dohme.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2025.103368.

References

1 European AIDS Clinical Society (EACS) guidelines. Available at: http://www.eacsociety.org; 2024. Accessed December 19, 2024. v12.1.
2 Gandhi RT, Landovitz RJ, Sax PE, et al. Antiretroviral drugs for treatment and prevention of HIV in adults: 2024 recommendations of the international antiviral society–USA panel. *JAMA*. 2025;333:609–628.
3 Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Department of health and human services. Available at: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new-guidelines. Accessed January 19, 2024.
4 De Wit S, Bonnet F, Osiyemi O, et al. Durable efficacy of switching from a 3- or 4-drug Tenofovir Alafenamide-Based regimen to the 2-drug regimen Dolutegravir/lamivudine in the TANGO study through week 196. *J Acquir Immune Defic Syndr*. 2024;96:156–160.
5 Llibre JM, Brites C, Cheng CY, et al. Efficacy and safety of switching to the 2-drug regimen Dolutegravir/Lamivudine versus continuing a 3- or 4-drug regimen for maintaining Virologic suppression in adults living with Human Immunodeficiency Virus 1 (HIV-1): week 48 results from the phase 3, non-inferiority SALSA randomized trial. *Clin Infect Dis*. 2023;76:720–729.
6 Aboud M, Orkin C, Podzamczer D, et al. Efficacy and safety of dolutegravir-rilpivirine for maintenance of virological suppression in adults with HIV-1: 100-week data from the randomised, open-label, phase 3 SWORD-1 and SWORD-2 studies. *Lancet HIV*. 2019;6:e576–e587.
7 Spinner CD, Kümmerle T, Schneider J, et al. Efficacy and safety of switching to Dolutegravir with boosted darunavir in virologically suppressed adults with HIV-1: a randomized, open-label, multicenter, phase 3, noninferiority trial: the DUALIS study. *Open Forum Infect Dis*. 2020;7:ofaa356.
8 Ramgopal MN, Castagna A, Cazanave C, et al. Efficacy, safety, and tolerability of switching to long-acting cabotegravir plus rilpivirine versus continuing fixed-dose bictegravir, emtricitabine, and tenofovir alafenamide in virologically suppressed adults with HIV, 12-month results (SOLAR): a randomised, open-label, phase 3b, non-inferiority trial. *Lancet HIV*. 2023;10:e566–e577.

9 Trickey A, Sabin CA, Burkholder G, et al. Life expectancy after 2015 of adults with HIV on long-term antiretroviral therapy in Europe and North America: a collaborative analysis of cohort studies. *Lancet HIV*. 2023;10:e295–e307.
10 Marcus JL, Leyden WA, Alexeeff SE, et al. Comparison of overall and comorbidity-free life expectancy between insured adults with and without HIV infection, 2000-2016. *JAMA Netw Open*. 2020;3:e207954.
11 Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. *Lancet*. 2013;382:1525–1533.
12 Mussini C, Lorenzini P, Cozzi-Lepri A, et al. Switching to dual/monotherapy determines an increase in CD8+ in HIV-infected individuals: an observational cohort study. *BMC Med*. 2018;16:79.
13 Serrano-Villar S, López-Huertas MR, Jiménez D, et al. Long-term changes of inflammatory biomarkers in individuals on suppressive three-drug or two-drug antiretroviral regimens. *Front Immunol*. 2022;13:848630.
14 Llibre JM, Cahn PE, Lo J, et al. Changes in inflammatory and atherogenesis biomarkers with the 2-drug regimen dolutegravir plus lamivudine in antiretroviral therapy-experienced, virologically suppressed people with HIV-1: a systematic literature review. *Open Forum Infect Dis*. 2022;9:ofac068.
15 Cossarizza A, Cozzi-Lepri A, Mattioli M, et al. Evaluating immunological and inflammatory changes of treatment-experienced people living with HIV switching from first-line triple cART regimens to DTG/3TC *vs*. B/F/TAF: the DEBATE trial. *Front Immunol*. 2023;14:1279390.
16 Greenberg L, Ryom L, Neesgaard B, et al. RESPOND (International Cohort Consortium of Infectious Diseases) study group. Clinical outcomes of 2-drug regimens vs 3-drug regimens in antiretroviral treatment-experienced people living with human immunodeficiency virus. *Clin Infect Dis*. 2021;73:e2323–e2333.
17 https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-use-real-world-data-non-interventional-studies-generate-real-world-evidence_en.pdf.
18 Hernán MA, Sauer BC, Hernández-Díaz S, Platt R, Shrier I. Specifying a target trial prevents immortal time bias and other self-inflicted injuries in observational analyses. *J Clin Epidemiol*. 2016;79:70–75.
19 d'Arminio Monforte A, Cozzi–Lepri A, Rezza G, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naive patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naive Patients. *AIDS*. 2000;14:499–507.
20 von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for re- porting observational studies. *Ann Intern Med*. 2007;147:573–577.
21 Robins JM, Orellana L, Rotnitzky A. Estimation and extrapolation of optimal treatment and testing strategies. *Stat Med*. 2008;27:4678–4721.
22 Hernan M, Robins J. *Causal Inference: What If*. Chapman and Hall; 2023.
23 VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol*. 2019;34:211–219.
24 Mathur MB, Ding P, Riddell CA, VanderWeele TJ. Website and R package for computing E-values. *Epidemiology*. 2018;29:e45–e47.
25 VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. *Ann Intern Med*. 2017;167:268–274.
26 Borges ÁH, O'Connor JL, Phillips AN, et al. INSIGHT SMART and ESPRIT study groups and the SILCAAT scientific committee. Factors associated with plasma IL-6 levels during HIV infection. *J Infect Dis*. 2015;212:585–595.
27 El-Sadr WM, Lundgren J, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*. 2006;355:2283–2296.
28 Ioannidis JPA, Tan YJ, Blum MR. Limitations and misinterpretations of E-values for sensitivity analyses of observational studies. *Ann Intern Med*. 2019;170:108–111.
29 Greenland S. Commentary: an argument against E-values for assessing the plausibility that an association could be explained away by residual confounding. *Int J Epidemiol*. 2020;49:1501–1503.