

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	The electronic data capture system used was REDcap
Data analysis	Analyses were performed using R project v4.5.2 (https://www.r-project.org/) and GraphPad Prism v11.0.0 for Windows (GraphPad Software, https://www.graphpad.com).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Individual-level clinical, virological, pharmacokinetic and tissue-imaging data are not publicly available because the small clinical cohort creates a potential risk of participant reidentification. De-identified data may be made available to qualified researchers for scientifically and ethically appropriate research that is consistent with the study objectives and applicable data-protection requirements. Requests should be submitted to the corresponding author, and should include a research

proposal, analysis plan, requested variables and information about the requesting investigators and institution. Requests will be reviewed by the DUALITY study investigators and the relevant institutional bodies. Access will require approval of the proposal and execution of a data-use agreement that prohibits participant reidentification and unauthorized data sharing. The study protocol is provided in the Supplementary Information.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	All participants (n=39) were male at birth. No gender- or sex-based analyses were performed in this study. We were unable to recruit a more heterogeneous and diverse population.
Reporting on race, ethnicity, or other socially relevant groupings	No reporting or analyses based on race, ethnicity or other socially-relevant groupings were performed in this study.
Population characteristics	The study population consisted of young men with HIV diagnosis and no prior exposure to antiretroviral treatment. Median (IQR) plasma viral load of 23,828 (4,921–50,045) copies/mL and CD4+ T cell count of 460 (364–568) cells/mm ³ at study entry.
Recruitment	Participants were recruited at Infectious Diseases Department of the Hospital Germans Trias i Pujol (HUGTIP), Badalona, Spain. The first study visit was conducted on 15th Oct 2019. Study recruitment was interrupted temporarily in 2020 due to the disruptions caused by the COVID-19 pandemic. The last participant's last visit was 21 Sept 2022
Ethics oversight	All participants provided written informed consent. The study was approved by the institutional ethical review board (reference number AC-19-073-HGT-CEIM) and by the Spanish Regulatory Authorities and was conducted in accordance with the principles of the Helsinki Declaration and local personal data protection law (LOPD 15/1999). The study was registered at https://www.clinicaltrialsregister.eu (Eudra-CT number 2019-002733-10).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	DUALITY was an exploratory clinical trial with a planned sample size of 44 participants. Although no formal sample-size requirement was specified, this sample size was estimated to provide 80% power at a two-sided alpha level of 0.05 to detect a minimum twofold between-group difference in proviral HIV-1 DNA in peripheral blood CD4+ T cells at week 48, based on previously reported reservoir dynamics during the first year of ART. For the lymph-node substudy, 40 participants were planned, with four participants from each treatment group assigned to each of five biopsy timepoints. No separate formal power calculation was performed for the lymph-node analyses; this sample size was selected on the basis of the exploratory objectives and the ethical and logistical constraints associated with excisional lymph-node biopsy. One participant declined biopsy, resulting in 39 participants. All available evaluable samples were included, and assay-specific sample sizes are reported in the tables and figure legends.
Data exclusions	One participant from the 2DR group declined the procedure and was not replaced; therefore, 39 participants were included in the final analysis. Two participants were sampled <20 hours after drug intake and were excluded from pharmacokinetic analyses, since drug concentrations were not representative of trough concentrations. Lymph node tissue from one participant (3DR, week 12) was damaged during cryosectioning and excluded from HIV DNA and RNA imaging but retained for drug distribution analyses.
Replication	Reproducibility was supported by the use of prespecified study procedures, standardized sample collection and processing, validated LC–MS/MS assays, calibration standards for IR-MALDESI MSI, predefined detection thresholds and consistent image-analysis workflows across all samples. Subjacent lymph-node sections were used for ISH, IHC and MSI to support spatial comparison across methods. Each participant contributed one biologically independent lymph-node sample, and technical replicates were not treated as independent observations. Formal replication within participants was not possible because lymph-node excision was performed once per participant. One sample damaged during cryosectioning was excluded from HIV-1 DNA and RNA imaging, and TFV could not be spatially quantified because concentrations were below the MSI limit of quantification; both limitations are reported in the manuscript. All other planned analyses were completed using the available evaluable samples.
Randomization	After inclusion, participants were randomized (1:1) to initiate antiretroviral treatment with DTG 50 mg plus 3TC 300 mg (2DR group), or with DTG 50 mg plus FTC/TAF 200/25 mg (3DR group).
Blinding	Does not apply since this was an open-label study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☐	☒ Clinical data		
☒	☐ Dual use research of concern		
☒	☐ Plants		

Antibodies

Antibodies used	Primary Antibodies: [anti-Collagen-I, Mouse mAb, Sigma-Aldrich, cat# C2456, lot# 175587, clone COL1A1] [anti-CD20, Goat pAb, Invitrogen, cat# PA1-9024, lot# xe3586331, clone N/A] Secondary Antibodies: [anti-Mouse IgG, Donkey pAb, Invitrogen, cat# A31571, lot# 2420713, clone N/A] [anti-Goat IgG, Donkey pAb, Invitrogen, cat# A11055, lot# 2465077, clone N/A]
Validation	Anti-Collagen-I was validated by the manufacturer by dot blot and ELISA for use in human tissue sections and further verified in this study by the characteristic staining of connective tissue fibers histologically consistent with the known distribution of collagen-I. Anti-CD20 was validated by the manufacturer by Western blot and immunohistochemistry for use in human tissue sections and further verified in this study by the characteristic cell membrane staining of enriched populations of B-cells in areas consistent with known lymph node histology.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	DUALITY trial is registered at https://www.clinicaltrialsregister.eu (Eudra-CT number 2019-002733-10).
Study protocol	Version 2.0, 9th Jul 2020 (last version)- submitted as Supplementary Data S1,
Data collection	The electronic data capture system used was REDcap
Outcomes	Primary endpoint: Change in proviral HIV-1 DNA and RNA in CD4+ Tcells from baseline to week 48 within peripheral lymph node. Secondary endpoints: antiretroviral drugs penetration and distribution within peripheral lymph node; Spatial association between drug distribution and cell-associated HIV-1 DNA and RNA within peripheral lymph node.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>