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HIV persistence in tissues on dolutegravir-based therapy is not associated with resistance mutations to dolutegravir

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Abstract:

Background: Relatively few studies have investigated HIV-1 persistence in tissues, especially in healthy people-living-with-HIV-1 (PLWH) on a successful antiretroviral regimen containing second generation integrase inhibitors.
Methods: In the ANRS EP64 DOLUVOIR, we explore HIV-1 persistence in five accessible anatomical sites in 20 PLWH on an efficient first-line ART regimen containing dolutegravir with virological load<50 copies/mL: PBMCs, rectum, adipose tissue, lymph node and sperm. We quantify total HIV-DNA and cell-associated HIV-1 RNA in different compartments. We sequence HIV-1 DNA for searching drug resistance mutations (DRM) (in RT and INT) and for studying HIV diversity within tissues (ENV). Intact proviral DNA is estimated in PBMCs with an adapted IPDA assay.
Results: Broad ranges of total HIV-DNA and transcripts levels are detected in lymph nodes, PBMCs, adipose tissue and rectum with the highest levels being found in lymph nodes (2.77 log copies HIV-1-DNA/10^6 cells and 1.50 log copies of HIV-1 cell-associated-RNA/µg RNA). HIV-1 DNA is undetected in all sperm samples (n=19) except for one (1.52 log copies HIV-1-DNA/106 cells). No difference is noted between the diversity in the four compartments. DRMs to the current regimen are found archived in compartments of six participants. Only two major DRMs to dolutegravir (G118R and R263K) are found archived in two participants. They are the results of APOBEC hypermutations.
Conclusions: Despite ongoing transcriptional activity, persistence of HIV-1 in deep tissues is not associated with the selection of DRMs to dolutegravir on intact proviruses. Our results suggest that the detectable transcriptional activity stems predominantly from defective proviral DNA.

Keywords: HIV-1 persistence, tissue reservoir, HIV-1 reservoir, dolutegravir, quasispecies diversity

Plain language summary:

The main obstacle for eradication of Human immunodefiency Virus (HIV-1) is that the virus persists deep in the human body. In this present study, we explore this persistence by measuring the level of infection and expression of viral genes in five parts of the body: blood, rectum, lymph nodes, sperm and fat. We look in 20 People-living-with-HIV-1 on successful treatment with a combination of medicines including one called Dolutegravir. We demonstrate that the levels of infection are highest in lymph nodes. By testing HIV-1 DNA for drug resistance, we show that this persistence in the body does not lead to major resistance to Dolutegravir.

Introduction

Even though combined antiretroviral therapy (cART) has improved drastically the quality of life of people living with HIV-1 (PLWH), it is unable to eradicate the virus due to the establishment of the HIV-1 reservoir. This reservoir is rapidly established during acute

infection and is mainly present in tissues (1,2). HIV-1 reservoir persists in tissues, mainly in resting memory CD4 T cells(3) through cellular survival and proliferation [2,3]. Some of the tissue reservoirs are protected by an anatomical barrier that limits the diffusion of antiretroviral drugs with certain tissues, such as testis and lymph nodes (LN), being considered as pharmacological sanctuaries (1). Due to this insufficient drug diffusion, a residual replication has been previously reported in LN of three PLWH despite complete viral suppression in plasma(4). This has also been reported by other studies in the lymphoid and the central nervous systems (5–7). Due to the error-prone HIV-1's reverse transcriptase (RT), a residual replication in the presence of low drug levels could result in the selection of drug resistance mutations (DRMs) in deep tissues, which may lead to therapeutic failure. Moreover, some studies have shown compartmentalization of HIV-1 sequences in PLWH on cART indicating an autonomous replication in some anatomical sectors such as LNs and testis (8–10). However, this has not been confirmed by other recent studies, particularly the compelling findings from autopsies of deceased PLWH, which showed that HIV-1 clones are predominantly shared across multiple tissues (11–15).

While the HIV-1 reservoir in peripheral blood mononuclear cells (PBMCs) has been extensively studied, HIV-1 persistence in tissues remains insufficiently explored due to limited accessibility. Little is known about HIV-1 persistence in LNs and gut-associated lymphoid tissue (GALT), which harbor most of the body's CD4 T cells, as well as in the genital tract, a major source of HIV-1 transmission (9,16–18). In addition, adipose tissue (AT) is an often neglected HIV-1 reservoir that plays a significant role in the physiopathology of the infection (19–22). Relatively few studies have explored multiple anatomical compartments in PLWH under ART, they were limited by the number of participants included, who received diverse cART, and some were conducted on autopsy samples from deceased PLWH(23–25). Particularly, very few have investigated the potential selection of DRMs under cART in deep tissues in PLWH under Dolutegravir (4,14).

This is particularly relevant as Dolutegravir (DTG) isa potent second-generation integrase strand transfer inhibitor (INSTI), and is widely used worldwide, with an estimated 20 million PLWH on a cART regimen containing DTG. Recent data in humans and in animal

models have shown that its diffusion is variable among tissues (26,27). Understanding HIV-1 tissue reservoirs dynamics under ART containing DTG is thus crucial.

In this light, we conduct a prospective study that aims to explore HIV-1 persistence in deep tissues in PLWH who arevirologically suppressed on an efficient first-line therapy containing DTG. To this end, we explore i) the level of infection and transcriptional activity of HIV-1 reservoirs, ii) the selection of DRMs and iii) the viral genetic diversity in five matched anatomical sites: blood, LN, rectum, AT, and sperm. Broad ranges of total HIV-1 DNA and viral transcripts levels are detected in anatomical compartments with the highest levels are in in lymph nodes. Despite ongoing transcriptional activity, no difference is found between the proviral diversity between anatomical sites and no major DRMs to the current regimen are archived on intact proviral DNA in compartments.

Methods

Study design

ANRS EP64 DOLUVOIR is a prospective study aiming to map HIV-1 tissue reservoirs in virologically suppressed, chronically HIV-1–infected men receiving first-line antiretroviral therapy including DTG. This study was conducted in France from February 2020 to June 2024. Participants were on daily antiretroviral therapy for at least 18 months, either with DTG plus two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) abacavir/lamivudine (ABC/3TC) or tenofovir/emtricitabine (TDF/FTC) throughout the entire period or had been switched to a double therapy of DTG+3TC for at least six months after 18 months of triple therapy with DTG. All participants had a plasma HIV-1 RNA <50 copies/mL six months after treatment initiation, confirmed at 12 months. Inclusion criteria are detailed in supplementary document 1. Blood samples, semen samples by self-masturbation, rectal biopsies during anoscopy, inguinal LN (to obtain peripheral and germinal lymph node tissue) and subcutaneous AT biopsies by ultrasound-guided fine-needling were collected during a single day hospitalization. LN and subcutaneous AD were extracted during the same sampling after local anesthesia. PBMCs and sperm cell pellets were isolated by lymphocyte separation medium (Eurobio ref: CMSMSL01-01). All participants gave their written informed consent to participate.

This study was approved by the National Agency for Medicines and Health Products Safety (ANSM) and by the ethics committee of Sud Méditerranée III and was registered in http://clinicaltrials.gov/ (NCT04133012).

Nucleic acid extraction

Biopsies were pretreated with RLT or RNA later and were mechanically dissociated with MagnaLyser. DNA was then extracted using QIAamp DNA mini kit (Qiagen, Germany) and RNA using Rneasy plus mini kit (Qiagen) after treatment with DNAse I (Qiagen). Nucleospin® miRNA (Macherey-Nagel, Germany) kit was used for intracellular RNA extraction and QIAamp DNA mini kit® (Qiagen) for DNA extraction from PBMCs. Sperm pellets were used for DNA extraction (QIAamp DNA micro kit, QIAGEN).

Virological parameters quantification

Total HIV-1 DNA was quantified in all sample types using ultrasensitive-real time PCR with Generic HIV-1 DNA Cell Kit (Biocentric, France) as previously described (28). Results were reported as the log_{10} HIV-1 DNA copies /10^6 PBMCs or /10^6 cells in biopsies. Cell-associated unspliced HIV-1 RNA (HIV-1 ca-RNA) was quantified using an in-house ultrasensitive-real time RT-qPCR targeting the Psi region (Ψ) in rectal biopsies, PBMCs and LN. Reverse transcription was performed using SuperScript IV VILO Master Mix (Invitrogen) containing random hexamers and oligo dT primers with the following program: 10 min at 25°C, 10 min at 50°C and 5 min at 80°C according to the manufacturer's instructions. The reaction volume was 50µl, containing 25µl of Platinum qPCR Supermix-UDG with ROX (Invitrogen), 0.4µM of each primer (forward primer: CAGGACTCGGCTTGCTGARG, reverse primer: GCACCCATCTCTCCTTAGC) and 0.4µM of probe (FAM-TTTTGGCGTACTCACCAGT-MGB). Cycling conditions were 50°C for 2 min and 95°C for 10 min followed by 50 cycles at 95°C for 15 sec and 60°C for 1 min.HIV-1 transcripts from 8E5 cells were used as a quantification standard.

Cellular RNA input was estimated with Qubit 4 (Qubit RNA BR assay kit, Invitrogen). Results were reported as HIV-1 ca-RNA copies/µg of total RNA.

HIV-1 RNA was quantified in seminal fluid using ALINITY m HIV-1 assay (ABBOTT, USA). Results were reported as HIV-1 RNA copies/mL of semen fluid.

The quantification limits of the above-described virological parameters depended on the input of cellular DNA or RNA. When HIV-1 DNA or RNA were not detected, measurements were excluded from analysis if the low input of cellular DNA or RNA, did not allow to obtain a quantification limit less than 100 copies per million cells for HIV-1 DNA or per µg of total RNA.

The levels of intact and defective HIV-1 proviruses in PBMCs were estimated with multiplex droplet digital PCR (ddPCR) performed on a QX200 ddPCR system (Bio-Rad) using an in-house adapted version of Intact Proviral DNA assay (IPDA) (26). Following genomic DNA extraction from RLT cell pellets using Allprep DNA/RNA kit (Qiagen), the first ddPCR measurement targeted two regions of RPP30 gene using the following mixture: 1X ddPCR Supermix for probes (Bio-Rad), 800 nm of each primer, 400 nmM of each probe and qsp H20 for a total mixture volume of 15 µL. 5 µL of genomic DNA at a concentration of 1.6 ng/ µL is then added to the ddPCR mixture. For the second ddPCR, targeting HIV-1 Ψ and env, the following mixture was used: ddPCR Supermix for probes (1X), 800 nM of each primer, 250 nM Ψ HIV-1 Probe and Env HIV-1 hypermut DG , 400 nM Env HIV-1 intact probe DG and H20 qsp for a total volume of mixture of 15 µL. 5 µL of genomic DNA is added to the mixture at a maximum concentration of 160 ng/µl. The following PCR program was used for both PCRs: 10 min at 95°C, 50 cycles of 30 sec at 94°C and 2 min at 54°C, and 10 min at 98°C. Samples with more than 10000 accepted droplets, less than 30% of positive droplets and a DNA Shearing Index (DSI) estimated from RRP30 ddPCR ≤ 0,5. The list of primers are detailed in supplementary table 1.

Ultra-deep sequencing of *RT, INT* and *ENV*

Sequencing of the RT, IN and ENV (V3-V4) genes was performed using DNA extracts from LN, rectum, PBMCs and AT, provided total HIV-1 DNA was detected and sufficient biological material was available. We used an amplicon approach with Illumina Miseq technology (Miseq v3 kit - 2x300). Indexing was performed with Nextera XT Index Kit v2 Set A (96 indexes, 384 samples). Before sequencing, a nested PCR using KAPA HiFi HotStart ReadyMix (Roche®, USA) was realized to amplify *RT* and *IN* in two fragments (RTA1 and RTA2 and INTA1 and INTA2). For the *ENV,* we proceeded to a single nested PCR. Primers are exposed in supplementary table 2. For the first PCR, we used the following program: 95ºC for 3 min followed by 45 cycles of 95ºC for 30 sec, 55ºC for 30

sec and 72 ºC for 2 min and at the end 72ºC for 7 min. For the nested PCR, we used the following program: 95ºC for 3 min followed by 45 cycles of 98ºC for 20 sec, 63ºC for 30 sec and 72 ºC for 40 sec and at the end 72ºC for 5 min.

Quality control and bioinformatic analysis of NGS data

For *RT* and *IN*, paired-end reads were filtered using fastp with default parameters to remove low-quality reads. Reads were further filtered based on mapping quality: unmapped reads and those with a mapping quality score below 60 are discarded. Additionally, reads that lost their mates during this process were removed to maintain properly paired reads. Merged reads were used to reconstruct haplotypes, and those shorter than 250 bp were discarded to ensure reliability in downstream analyses. DRMs were interpreted according to the algorithm of the ANRS MIE (French national Agency for AIDS, hepatitis, tuberculosis Research and Emerging Infectious Diseases; version 35, April 2024). Those with a frequency above 5% were reported. Variants harboring G-to-A DRMs were analyzed with Hypermut 2 to detect hypermutations (30).

ENV haplotypes were inferred using CliqueSNV (31) with the following criteria: mean depth >100 read and frequency >5%. HIV-1 diversity was assessed by calculating the mean pairwise genetic distances between *ENV* sequences inside each compartment. As a validation control, *ENV was* sequenced from the clonal 8E5 cell line using distinct PCR products across separate sequencing runs. No diversity was seen between generated sequences.

Statistical analysis:

Continuous variables are expressed as median and interquartile range (IQR). Detectable signals under the limit of quantification were reported at the value of limit of quantification and undetectable signals were reported as half of it for statistical analysis. The highest limits of quantification were 1.90 $\log_{10}$ copies and 1.65 $\log_{10}$ copies for total HIV-1 DNA and HIV-1 ca-RNA respectively in PBMCs, AT, LN and rectum. For HIV-1 DNA in semen of aviremic PLWH, results were analyzed qualitatively due to the low frequency of cells. As for HIV RNA in seminal fluid, the highest limit of quantification was 2.1 log copies/mL of seminal fluid. For paired continuous variables, Wilcoxon matched pairs signed rank

test and Friedman test were used for comparison. Correlations between continuous variables were evaluated using Spearman's rank correlation test. p values <0.05 were considered significant. The statistical analyses were performed using GraphPad Prism 10.4.1 (GraphPad Software LLC) (532).

Results:

Participants' characteristics and samples

A total of 20 PLWH were included in our study. They were on successful ART with undetectable plasma viral loads (VL) at inclusion. 70% of participants were on a 2 NRTIs + DTG regimen and 30% were on double therapy of 3TC+ DTG. They had all been diagnosed during the chronic phase and were treated for a median period of 55 months [IQR: 43; 75]. All participants were virologically controlled throughout their treatment with a median duration of undetectability of 52 [38; 68] months (calculated as the time between the date of the first VL<50 copies/mL and the day of inclusion). There was no difference in time on treatment or duration of undetectability between PLWH on triple therapy and those on double therapy (63 months [47; 78] for PLWH on triple therapy vs 49 months [43; 64] for double therapy p=0.303, and 53 months [42; 69] and 44 months [36; 60] p=0.506, respectively). Participants' treatment and biological characteristics are detailed in table 1.

We explored 20 PBMC samples, 19 sperm samples, 19 rectal biopsies, 14 LN biopsies and 13 AT biopsies (supplementary table 3). HIV-1 RNA was undetectable in sperm fluid in all samples analyzed, except for one in which a detectable HIV-1 RNA signal was found below the limit of quantification (<1.6 log copies/mL).

Total HIV DNA levels differ between anatomical compartments

Total HIV-1 DNA was detectable in most individuals in PBMCs (19 out of 20), LN (14 out of 14), rectum (18 out of 19) and AT (11 out of 11). HIV-1 DNA was undetectable in all tested sperm pellets (n=19) except for one with a low total HIV-1 load of 1.52 log copies

of total HIV-1-DNA/10^6 cells. The proportion of detectable HIV-1 DNA was significantly lower in sperm pellets (5%) compared with PBMCs (95%), LN (100%), AT (85%) and rectum (95%) (Fisher exact test, p<0.0001 for all of beforementioned comparisons). Globally, levels of infection were different between the explored compartments (Friedman test, *p*=0.03, n=11) (Figure 1). The highest levels of infection were found in LN (median of 2.8 log copies/10^6 cells [interquartile range 2.0; 3.1] followed by rectum (2.6 [2.0; 2.9 log], PBMCs (2.3 [1.8; 2.6] log) and then AT (1.9 [1.7; 2,6] log copies/10^6 cells). When performing pairwise comparisons, we showed significant differences between the levels in LN vs PBMCs (p<0.001, n=14), LN and AT (p=0.007, n=12) and a trend of significance between LN and rectum (p=0.057, n=13). No difference was observed with pairwise comparisons between the other compartments (supplementary table 4).

Residual HIV transcriptional activity persists under ART

Due to the lack of sufficient material, HIV-1 ca-RNA was not quantified in adipose tissues. It was detectable for 14 PLWH in PBMCs (n=19), for seven in LNs (n=10), and for 14 in the rectum (n=19). Generally, levels of HIV-1 transcripts were low (Figure 1). Similarly to the levels of total HIV-1 DNA, globally the levels of transcriptional activity of HIV-1 reservoir differed between compartments (Friedman test, *p value* =0.026 N=9) with the highest levels being found in LNs (1.50 [1.30; 2.30] log copies of HIV-1 ca-RNA/µg of total RNA (supplementary table 4). PBMCs harbored higher levels of transcriptional activity than the rectum (1.28 [1.11; 1.95] *vs* 1.05 [0.7; 1.18] log copies of HIV-1 ca-RNA/µg of total RNA (p<0.001), while LN harbored higher levels than PBMCs at the limit of significancy (p=0.061, n=10) and significantly higher than Rectum (p=0.008, n=9).

HIV persistence markers in PBMCs reflect the levels in tissues

Total HIV-1 DNA levels were correlated across different compartments, with the levels in PBMCs serving as a reliable reflection of those in tissues (Figure 2a). Similarly, levels of HIV-1 transcriptional activity in PBMCs were a reliable reflection of those in LN and the

rectum. A tendency for correlation was observed between HIV-1 transcript levels in LN and rectum (r = 0.67, p = 0.054). (Figure 2b). The details of Spearman's correlation coefficient (r) and p values are represented in supplementary table 5. HIV-1 ca-RNA was positively correlated with total HIV-1 DNA in LNs (r=0.79, p=0.011) but not in PBMCs (p=0.129, r=0.38) and rectum (r=0.37, p=0.122) (figure 2, supplementary table 5).

Clinical and biological determinants of HIV persistence markers

We were then interested in studying the effect of CD4 T cell count nadir, plasma VL zenith, time on ART and duration of undetectability on total HIV-1 DNA and HIV-1 ca-RNA. Among these factors, CD4 T cell count nadir was negatively correlated to HIV-1 ca-RNA in LNs (r=-0.65, p=0.049) (Figure 2d, supplementary table 6). A trend towards a positive correlation was observed between plasma VL zenith and total HIV-1 DNA in PBMCs (r=0.40, p=0.089) and in LN (r=0.55, p=0.052) (supplementary table 6).

The study of DRM in different compartments under ART

To study whether the higher levels of HIV DNA or ca-RNA in some tissues could be associated with the selection of DRMs in aviremic PLWH, we sequenced *RT* and *IN* on HIV-1 DNA with NGS when HIV-1 DNA was detectable and sufficient biological material was available. We sequenced *RT* in 44 compartments from 18 participants and *IN* in 56 compartments from 19 participants (supplemental table 3). DRMs were found archived in six participants (figure 3). Among them, three mutations confer high level of resistance to the current regimen found in participants receiving tri-therapy of DTC/ABC/3TC: G118R on *IN* (ID3 12% in PBMCs, not found in rectum and LN), R263K on *IN* (ID8 9% in AT, not found in PBMCs, LN and rectum) and M184I on *RT (* ID1 30% in rectum, not found in PBMCs). These three DRMs are G-to-A mutations. When analyzing reads harboring these mutations, G118R and R263K were associated with hypermutations and stop codons on the same variants indicating that they are probably the result of APOBEC-induced hypermutations and harbored by defective proviruses. M184I in *RT* can also be an APOBEC hypermutation. Apart from those high-level DRMs, L74M in *IN* was found at a frequency of almost 100% in all sequenced sites in two participants under tri-therapy

indicating that it is a transmitted DRM. Alone, it does not confer resistance to second generation INSTIs. M41L in *RT* (ID16 at 100% in rectum, no other *RT* sequences from other compartments were retrieved) and T97A in *IN* (ID12 at 50% in rectum, not found in LN) also do not confer alone any resistance to current ART regimen.

HIV-1 quasispecies diversity

We analyzed the genetic diversity of HIV-1 proviruses based on the sequences of a hypervariable region containing V3 loop of *ENV*. Pairwise genetic distance was calculated within compartments (figure 4). The median diversity was not significantly different between blood and tissues (median diversity in PBMCs 0.01 [0.003; 0.03], in LN 0.02; [0.01; 0.04] and in rectum 0.02 [0.004; 0.02]) (PBMC vs rectum, p=0.438; PBMC vs LN p=0.625; LN vs rectum p>0.999). Due to the few available cells and the low levels of HIV-1 DNA, we were unable to sequence *ENV* in AT.

Relationship between intact proviral DNA levels and HIV-1 persistence markers in PBMCs

We estimated intact and defective proviral DNA loads in PBMCs with an adapted IPDA assay. The median (IQR) levels of defective and intact HIV-1 DNA were 2.04 (1.65; 2.44) and 1.21 (0; 1.47) log copies/10^6 PBMCs respectively. Both intact and defective HIV-1 DNA were positively correlated with the levels of total HIV-1 DNA in PBMCs (r=0.53, p=0.018 and r=0.838, p<0.001 respectively). Interestingly, only the levels of defective HIV-1 DNA were correlated with those of HIV-1 ca-RNA (r=0.51, p=0.033) (figure 2e and supplementary table 5).

Discussion

HIV-1 persistence in long-lived cells within deep tissues is the main obstacle to viral eradication. Exploring the impact of new strategies on tissue reservoirs is crucial for research towards functional cure or viral clearance. (32–35). This is the largest study to extensively explore five accessible reservoirs in healthy PLWH who are virologically

suppressed under dolutegravir-based cART, by quantifying different biomarkers of HIV persistence and investigating the selection of DRMs to current cART regimen.

We detected HIV-1 infected cells at variable levels in multiple tissues, including LNs, rectum, adipose tissue (AT), and PBMCs, in PLWH under effective ART. This completes the results from previous studies, on a larger scale, in a well characterized PLWH under ART based on a second-generation INSTI(11,12,14,17–19,36). The highest levels of total HIV-1 DNA are in lymphoid tissues that harbor the majority of CD4 T cells (11,12,37). As previously suggested by our team, total HIV-1 DNA levels in PBMCs reflected well the overall burden of infection in tissues (36). Expectedly, the time on efficient cART did not impact markers of HIV-1 persistence as it had been initiated more than 18 months prior to inclusion, during chronic phase, in all participants. The minimal impact of treatment initiated during chronic phase, on HIV-1 DNA in blood has been demonstrated in previous studies (38–40).

Similar to previous studies, residual HIV-1 transcription was also noted in different anatomical compartments in most participants, independently of duration of treatment (16,41,42). Despite active transcription, neither HIV-1 proviral diversity nor the selection of DRMs differed between compartments, suggesting that unspliced transcripts are likely not associated with ongoing replication. In addition, unspliced HIV-1 RNA levels were correlated to defective HIV-1 proviral DNA but not to the intact DNA suggesting that a considerable proportion of HIV-1 ca-RNA are likely generated from defective proviruses in virologically suppressed PLWH on efficient ART containing DTG. This is concordant with studies indicating that most of the transcripts are expressed from defective genomes or are initiated but not elongated and contribute to chronic inflammation and activation of the immune system, often seen in PLWH, without complete replication (43–45). Another mechanism that could explain the detection of HIV transcripts in virologically suppressed PLWH without virion production is post-transcriptional latency (46). These two mechanisms may explain why transcription is not suppressed despite effective ART. We showed that the highest levels of transcription were in LNs, where most cells are activated and prone to transcription in addition to the high level of infection. It could be related to

differences in tissue microenvironment and to mechanisms that govern HIV-1 latency that would favor HIV-1 transcription in LNs in comparison to other tissues (24,33,47).

Despite ongoing transcription, archived DRMs in anatomical compartments were absent in most explored individuals (74%). We found some INSTI or NRTI DRMs in anatomical sites of only six individuals. However, L74M, T97A and M41L are polymorphic and do not confer alone any resistance to participants' ART regimen. L74M was present at 100% in all sequenced tissues from two participants, suggesting that it was likely a transmitted mutation. We cannot rule out that M41L (found at 100% in the rectum of one participant) was also transmitted, as our sequencing attempts of the *RT* gene in other compartments were unsuccessful. Only three major DRMs were found in three participants all on tri-therapy of DTG/ABC/3TC. Two of them (R263K, G118R), which confer high-level resistance to DTG, were associated with hypermutations, indicating that these G-to-A DRMs resulted from the activity of the cytidine deaminase APOBEC3G and were therefore present in defective proviruses (48). Thus, the impact of these mutations on virological response is uncertain. In addition, we found a M184I mutation archived in the RT of 30% of proviruses in the rectum of one PLWH on a triple therapy of DTG/ABC/3TC. However, this mutation was not detected in PBMCs, which could be due to the GALT harboring a larger population of CD4 T cells than peripheral blood. We cannot eliminate the presence of this mutation in other tissues of this PLWH, as we were unsuccessful to amplify RT in AT and LN. Even though, this G-to-A DRM is associated with resistance to ABC/3TC (current treatment of the patient), however, recent studies have shown that the presence of the sole M184V/I as an NRTI DRM in virologically suppressed PLWH is not associated with virological failure under 3TC/ABC/DTG or even 3TC/DTG (49,50). In a recent study, we demonstrated that the DRMs archived in the blood of three virologically suppressed PLWH are primarily present in defective genomes PLWH (51). Therefore, we cannot exclude that this mutation is present in defective genomes as some defects such as large deletion and MSD/Psi (Major Splicing Donor site and Psi packaging signal) defects are not detectable with the present study's sequencing technique. Globally all the

DRMs conferring high resistance to the DTG are probably harbored by defective proviruses.

Moreover, the fact that DRMs had no impact on the response to treatment and that they were archived in various tissues and not always in LNs that have the highest transcription level could also indicate that these DRMs may have been archived before ART in some reservoir cells and may not be the result of selection in compartment where ART could diffuse less efficiently.

This study has some limitations. For ethical considerations, the number of cells taken from each compartment was restricted. Therefore, we were unable to isolate specifically HIV-1 target cells for analyses. For the same reason, some virological analyses were not performed on all samples for all participants. For instance, intra-tissue proviral diversity was not analyzed in adipose tissue especially due to the low levels of infection in this compartment. In addition, as intact proviral DNA in circulating blood is very low, it is hard to show correlations between this parameter and transcriptional activity, and we cannot eliminate the fact that there could be an underlying viral transcription generated from intact proviral DNA. Furthermore, it is difficult to definitively affirm the absence of residual replication based on the analysis of HIV-1 DNA and residual replication at a single time point, even if the data on these different parameters are concordant. A longitudinal analysis of proviral diversity and the potential selection of DRMs are further needed to address this question (4).

Conclusion:

In conclusion, our results provide extensive insight into HIV-1 persistence in tissues of PLWH on effective ART containing Dolutegravir in multiple anatomical sites. We observed a wide range of HIV-1 DNA and transcription levels across tissues. Despite ongoing HIV-1 transcription under DTG, no major DRMs to DTG on intact proviruses were selected in any of the studied compartments. This indicates that HIV-1 persistence in deep tissues and the residual transcription under a therapeutic combination containing DTG is not associated with the risk of therapeutic failure.

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Competing interests:

VAF has received institutional grants from ViiV Healthcare and honoraria and travel grants from ViiV Healthcare and Gilead Sciences for participation in educational meetings and conferences. AC has received institutional grants from ViiV Healthcare and travel grants from Gilead Sciences. OR has received consulting fees and payment/honoraria for lectures from Gilead, MSD, Pfizer, and ViiV Healthcare. J. G. reports honoraria for consulting from Gilead Sciences, ViiV Healthcare, Bavarian Nordic and GSK. All other authors declare no competing interests.

Data availability:

The quantification data that support the findings of this study and the source data presented in figures can be found in Supplementary Data 1.

Sequenced fastq files are accessible on European Nucleotide Archive with the following accession number: **PRJEB104864 via https://www.ebi.ac.uk/ena/browser/view/PRJEB104864?show=reads.**

Author contributions

VAF, AC, LM and ABT conceptualized and designed the protocol. AC obtained funding. VAF supervised virological analyses. GM, AC and VAF designed experiments. GM, AM, EG carried out experiments. LA participated to data generation. VAF, GM, AM, KDS, ACha and FL analyzed data. LM supervised data monitoring. SO, and FC participated to the project administrative management. BL, JG, OR and JPV included participants. GM and VAF interpreted and analysed results and wrote the original manuscript. All authors critically reviewed the manuscript and contributed to the final version.

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Table 1: Participants clinical and biological characteristics

Subject ID	Current * treatment	Nadir CD4 (mm^3)	CD4 level (inclusion) (mm3)	Zenith Viral load (log cp/ml)	Time on ** ART (days)	Duration of HIV-1 *** RNA's undetectability (months)
ID1	DTG/ABC/3TC	60	304	4.8	535	59
ID2	DTG/3TC	436	990	4.6	2267	73
ID3	DTG/ABC/3TC	420	702	6.0	2377	72
ID4	DTG/3TC	601	801	3.5	1557	49
ID5	DTG/TDF/FTC	596	1258	5.4	693	13
ID6	DTG/ABC/3TC	496	777	5.3	2055	54
ID7	DTG/ABC/3TC	30	259	4.7	2556	82
ID8	DTG/ABC/3TC	NA	432	NA	1590	48
ID9	DTG/TDF/FTC	24	451	4.8	1288	40
ID10	DTG/ABC/3TC	705	1097	3.9	2103	69
ID11	DTG/ABC/3TC	480	861	4.2	1341	30
ID12	DTG/ABC/3TC	669	1442	3.2	2650	25
ID13	DTG/ABC/3TC	50	743	7.0	2238	68
ID14	DTG/ABC/3TC	360	953	4.8	1727	52
ID15	DTG/ABC/3TC	320	676	5.4	1590	51
ID16	DTG/ABC/3TC	377	687	4.3	2427	87
ID17	DTG/3TC	17	322	5.0	1270	39
ID18	DTG/3TC	626	1000	4.9	2049	63
ID19	DTG/3TC	810	1200	3.3	1407	35
ID20	DTG/3TC	477	878	5.5	953	25

*DTG, Dolutegravir; TDF, Ténofovir disopoxil fumarate; FTC, Emtricitabine; ABC, Abacavir; 3TC, Lamivudine.
**Time on ART: time since the first antiretroviral therapy
***Duration of HIV-1 RNA's undetectability: time with continuous undetectable viral load

Figure 1. Levels of HIV-1 persistence markers in different anatomical compartments
Scatter dot plot sorted by sampled compartment and colored by participants with the y axis showing (a) the level of Total HIV-1 DNA in log copies of Total HIV-1 DNA/10^6 cells (b) the level of HIV-1 ca-RNA in log copies of HIV-1 RNA/μg of total RNA. Bars represent median levels. Paired-wise comparisons are plotted on Figure 1a and 1b (two-tailed Wilcoxon matched-pairs signed rank test). * p value <0.05 ** p value <0.01 ***p value <0.001. Unplotted comparisons were statistically not significant (p values detailed in supplementary table 4).

Figure 2. Correlation analysis between persistence markers in different compartments and participants biological and clinical parameters
Heat maps showing Spearman's rank correlation coefficient (r) between a) Total HIV-1 DNA levels in different compartments; b) HIV-1 ca-RNA levels in different compartments; c) Total HIV-1 DNA and HIV-1 ca-RNA in each compartment, d) Virological parameters in each compartment (Total HIV-1 DNA, HIV-1 ca-RNA, HIV-1 proviral diversity) and clinical and biological parameters, e) Intact and defective proviral DNA

and Total and HIV-1 ca-RNA. The r-coefficient is color coded. * two-tailed p value <0.05 ** p value <0.01 ***p value <0.001. (p values are detailed in supplementary tables 5 and 6).

Figure 3. Drug resistance mutations to INSTIs and to NRTIs archived in anatomical sites
Histogram showcasing by participant and by anatomical compartment, the frequency of archived drug resistance mutations. IN: Integrase, RT: reverse transcriptase.

Figure 4. HIV-1 quasispecies diversity in different anatomical compartments.

Scatter dot plot sorted by sampled compartment and colored by participants with the y axis showing the pairwise genetic distance between V3-V4 ENV sequences. Bars represent median levels of diversity.

ED Summary:
Mchantaf et al. explore HIV-1 persistence in five anatomical compartments in people living with HIV on successful Dolutegravir therapy. They find the highest levels of infection and viral transcription in lymph nodes, and that persistence is not associated with the selection of drug resistance mutations to Dolutegravir.

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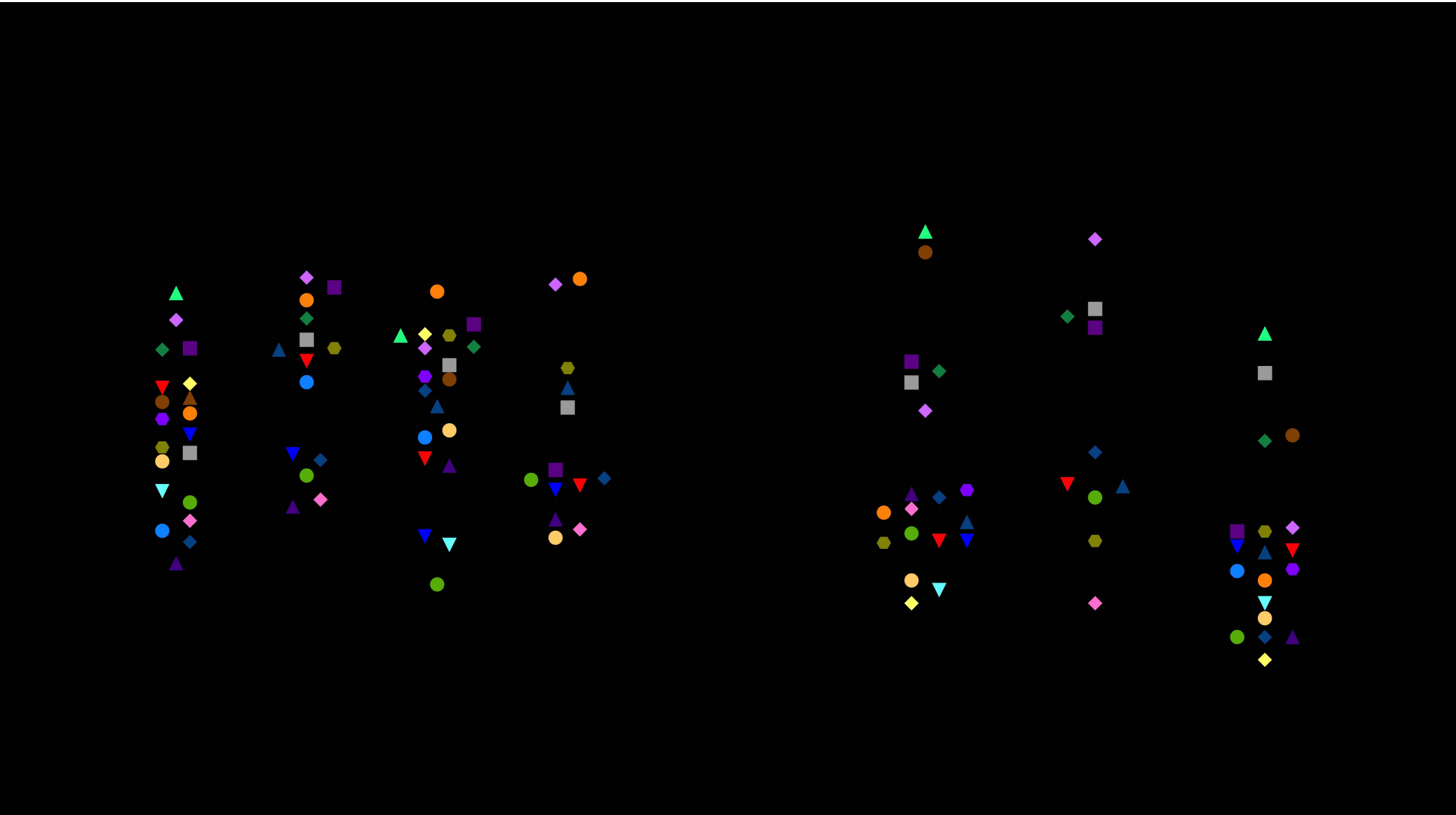

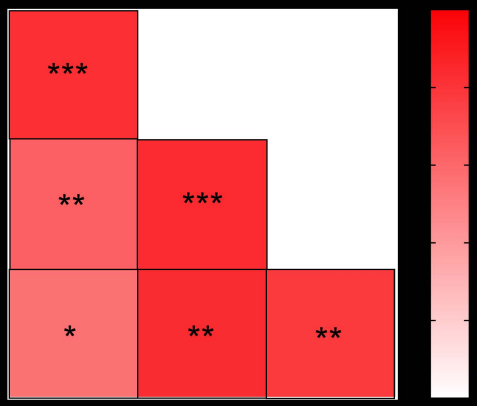

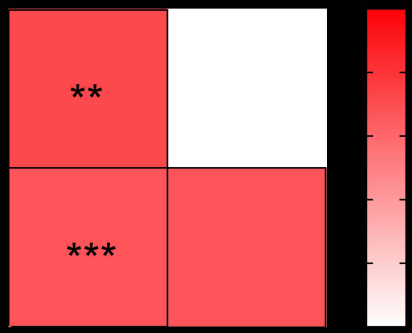

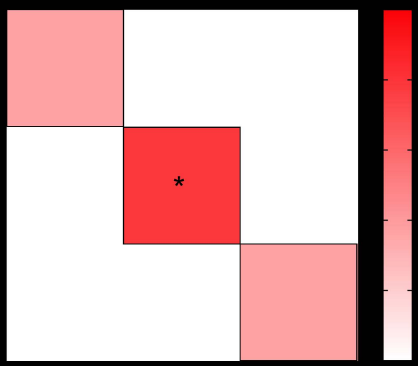

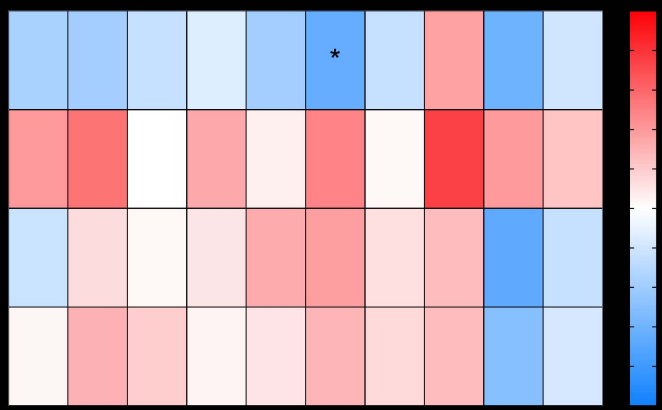

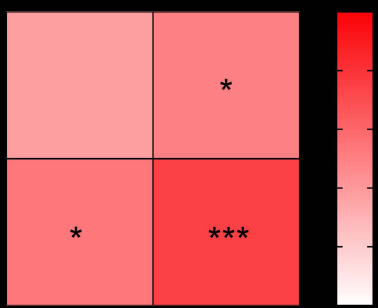

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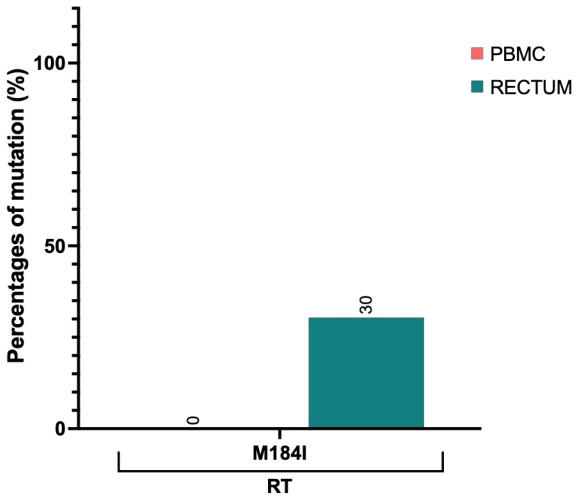

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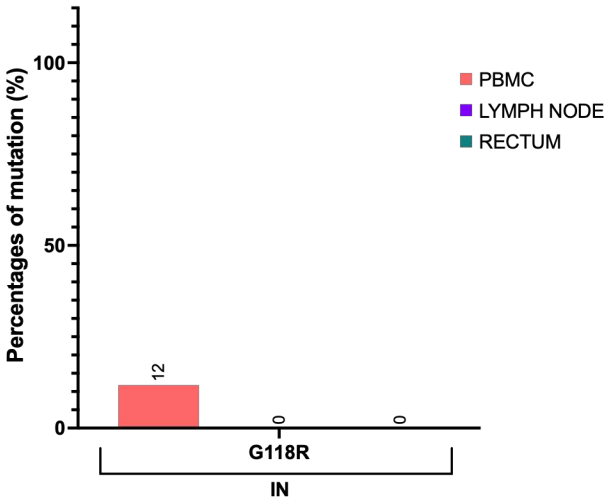

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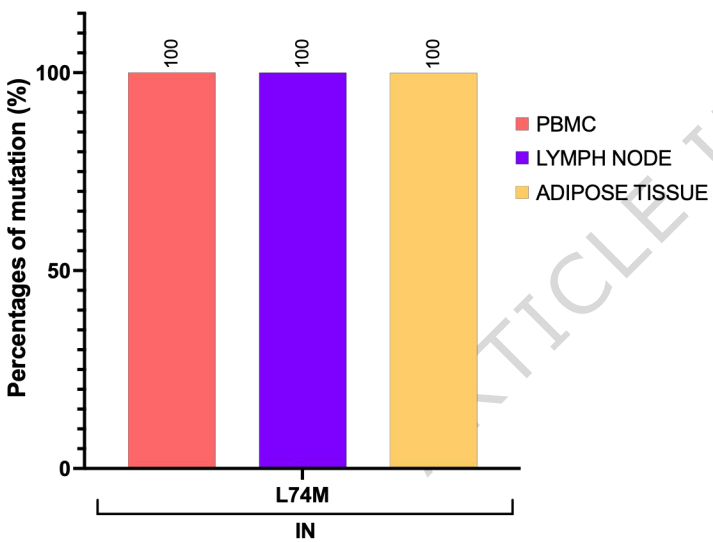

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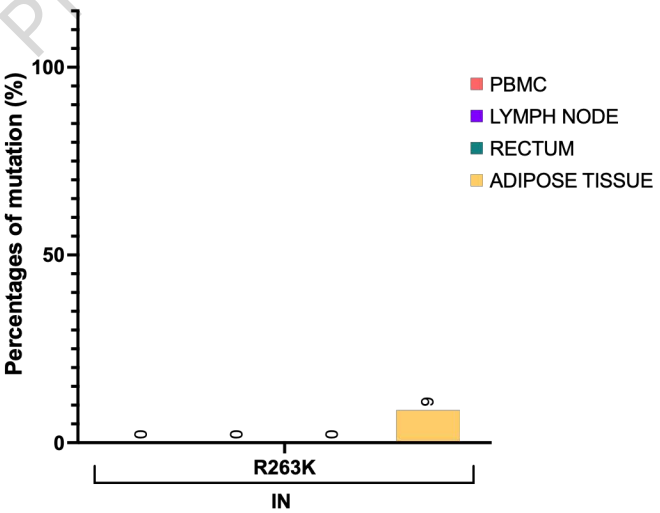

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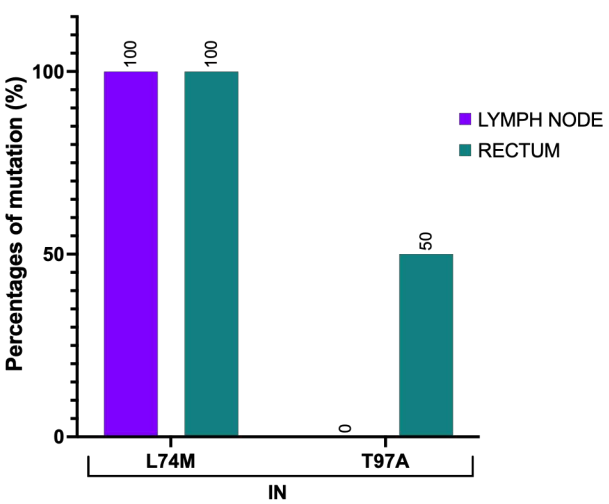

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