Dual antiretroviral therapies are effective and safe regimens in the central nervous system of neurologically symptomatic people living with HIV

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Dual Antiretroviral Therapies are Effective and Safe Regimens in the Central Nervous System of Neurologically Symptomatic People Living with HIV

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Abstract:

Objective: Aim of this study was to compare cerebrospinal fluid (CSF) virological control, biomarkers and neurocognition of neurologically symptomatic patients on dual antiretroviral therapies (DT) versus 2NRTIs-based three-drug regimens (TT).

Design: Retrospective monocentric cross-sectional study.

Methods: We analysed data from people living with HIV (PLWH) undergoing lumbar puncture for clinical/research reasons with plasma HIV-RNA <200 cp/mL and neurological/neurocognitive symptoms without significant contributing comorbidities. We measured CSF HIV-RNA, inflammation, blood-brain barrier integrity, neuronal damage and astrocytosis biomarkers (5 biomarkers by ELISA and 5 indices by immunoturbidimetry) and recorded the neurocognitive performance (14 tests). CSF escape was defined as any case of CSF HIV-RNA 0.5 Log10 higher than viremia or any case of detectable CSF HIV-RNA coupled with undetectable viremia.

Results: 78 patients on TT and 19 on DT were included. Overall, 75.3% male, median age 51 years (46-58), current CD4 count 545 cells/mmc (349-735), time on current regimens 18 months (8-29), but length of plasma suppression 32 months (14-94). The two groups did not differ in terms of HIV-associated neurological diagnoses, demographic and viro-immunological features. Undetectable CSF HIV-RNA (73.7% in DT vs 78.2% in TT, p.67) and CSF escape (21.1% in DT vs. 19.2% in TT, p.86) did not differ. No difference was observed in depression, anxiety, neurocognition (in 63 participants) nor in any tested biomarker.

Conclusions: In PLWH with neurological/neurocognitive symptoms, peripherally effective DT can show CSF viro-suppression, inflammation, neuronal and astrocyte integrity and neurocognition comparable to TT.

Keywords: Dual antiretroviral therapy; HIV; Cerebrospinal fluid; Neurocognitive disorders; Viral escape; Biomarkers; Viral suppression.

Manuscript:

In the context of life-long combination antiretroviral therapy (cART) for people living with HIV (PLWH), long-term drug toxicities, adherence, costs and drug-drug interactions have prompted to look for treatment simplification strategies. Recently, specific two-antiretroviral-based regimens (DT) proved to be non-inferior compared to three-drugs therapies (TT) in terms of viro-immunological control [1]. Lacking experimental data, concerns regarding immune-activation, resistance-associated mutations (RAMs) selection and viral escape in anatomical reservoirs, such as lymph-nodes and central nervous system (CNS) have been raised [1–4]. HIV replication may persist in tissues of treated patients presenting undetectable viremia and relates to local lower drug exposure as compared to plasma levels [4–7]. This raises the question whether switching to DT can further increase the risk of virological escapes and impact persistent replicating viral reservoir, leading in turn to higher levels of immune-activation and inflammation-related comorbidities, including HIV-associated neurocognitive disorders (HAND). In this regard, clinical reports alerted about the risk of cerebrospinal fluid (CSF) viral escape (CVE) during DT, but this message could have been distorted by publication bias and by the DT used, which were not always supported by trials results [8,9]. Indeed, providing good adherence, CSF viral suppression seems to persist after switching to DT [10]. Among aviremic patients both AtLaS-M (Atazanavir and Lamivudine for treatment Simplification-Multicentric study) and SALT (Simplification to Atazanavir/Ritonavir + Lamivudine as Maintenance Therapy) trials have demonstrated that, comparing patients switching to DT to those remaining in TT, neurocognitive function is stable and similar up to 144 weeks [11,12]. Data from the neurocognitive sub-study of the NEAT001/ANRS143 trial (European

AIDS Treatment Network 001/French National Agency for AIDS Research 143) on viremic patients starting first-line DRV/r+RAL versus DRV/r+2NRTIs are awaited. Limitations of these studies were variably the short-term follow-up [10], the restricted neurocognitive battery adopted [12] or the significant loss of patients undergoing the neurocognitive evaluation during follow-up [11]. Since HIV-related neuropathogenesis may not relate immediately and solely to uncontrolled viral replication within CNS and may arise after long periods of low-level tissue replication, other tools such as CSF biomarkers may be more accurate in detecting alterations at pre-symptomatic stages not yet evident at the neurocognitive assessment. On the other hand, archived RAMs and virus evolution in reservoirs during cART coupled with specific pharmacokinetic considerations in brain tissues may indicate that a surprising proportion of PLWH on TT is actually on mono/dual functional therapies [7,13–15], undermining the preconceived superiority of TT over well-tailored DT. Therefore, considering the unsolved concerns and the gaps between the ideal world of clinical trials and the reality of clinical settings, we have retrospectively analysed data of our Neuro-AIDS cohort consisting of more than 500 HIV-positive neurologically/neurocognitively symptomatic adult patients undergoing lumbar puncture (LP) for clinical and/or research reasons. Specifically, we have compared the virological control within CSF, several biomarkers of CNS functions and the neurocognition of patients presenting suppressed or low-level viremia (LLV) and no other neurological confounding on DT *vs* TT based on 2NRTIs plus a third drug. Our primary objective was to assess whether DT hold higher risk of CSF virological failure in such a clinical setting of high-risk adult patients. Our secondary aim was to evaluate whether DT associate with a heavier burden of CSF inflammation, CNS injury and/or poorer neurocognition.

Methods

Study design and patients

We performed a retrospective cross-sectional study nested in ongoing prospective studies on HIV-related neurological/neurocognitive disorders (Prospective study on predictors of neurocognitive decline in HIV-positive patients PRODIN, Study of nasal brushing collected olfactory mucosa samples in the diagnosis of human encephalopathies SOLFAMU, and Maraviroc-based treatment switch in HIV-positive patients with HAND: consequences of reducing antiretroviral-associated neurotoxicity MARANDX study, all approved by the local Ethics Committee). Data collected from PLWH undergoing LP for clinical and/or research reasons at our centre (Infectious Diseases Clinic, Amedeo di Savoia hospital, Torino) from 2010 to February 2019 were retrospectively analysed. Inclusion criteria were as follows: age ≥18 years; being on any type of DT or 2NRTIs-based TT; being stably on the same cART regimen since at least 3 months before LP; plasma HIV-RNA<200 cp/mL since at least 6 continuous months before LP. Exclusion criteria were as follows: active/previous CNS infective, neoplastic, traumatic, vascular, inflammatory or neurodegenerative disorders; disclosure of substance or alcohol abuse within the last year from LP; clinically relevant scores at the Beck Depression Inventory-II (≥30) or at the Hamilton Anxiety Rating Scale (≥25).

Neurocognitive evaluation

The neurocognitive battery consisted of: Trail Making Test A for processing speed/reaction time, Trail Making Test B and Stroop Colour test for executive functioning, Digit Span forward, Digit Span backward and Digit Symbol for attention/working memory, Corsi test and Disyllabic Words Serial Repetition test for

visuo-spatial and verbal short-term memory, Free and Cued Selective Reminding, Story Recall and Rey-Osterrieth Complex Figure Delayed Recall tests for verbal long-term memory and learning, Phonemic Verbal Fluency for language skills, Grooved Pegboard for Dominant/Non-dominant hand test for motor skills and Rey-Osterrieth Complex Figure Copy test for visuo-construction ability. Raw scores were converted to age-, sex- and education-adjusted normative t-scores in accordance with published manuals. HAND diagnosis was performed according to Frascati's criteria [16]. Daily functioning impairment was assessed by the Instrumental Activities of Daily Living.

Cerebrospinal fluid analysis

Quantitative determination of intrathecal synthesis and blood-brain barrier (BBB) damage markers (CSF-serum albumin ratio CSAR, IgG index, intrathecal synthesis, Tourtelotte and Tibbling indices) were measured by immunoturbidimetric methods (AU5800, Beckman Coulter, Brea, CA, USA) and calculated by Reibergram, as previously described [17]. Reference values: CSAR <6.5 up to 40 years and <8.0 in patients aged above; IgG index <0.7; intrathecal synthesis 0%. CSF total tau (t-tau), phosphorylated tau (p-tau), β-amyloid1-42 (Aβ1-42), neopterin and S100β were measured by immunoenzymatic methods (Fujirebio diagnostics, Malvern, USA; R&D Systems Europe, Ltd. Abingdon, UK; DRG Diagnostics, Marburg, Germany; Diametra Srl, Spello, Italy). Reference values: t-tau <300 pg/mL in patients aged 21–50, <450 pg/mL in patients aged 51–70 and <500 pg/mL in older; p-tau <61 pg/mL; Aβ1–42 >500 pg/mL; neopterin <1.5 ng/mL; S100β <380 pg/mL. HIV-RNA was quantified by the Roche Amplicor assay v2.0 (Hoffman-La Roche, Basel, Switzerland) with a lower limit of quantification of 20 copies/mL. CNS penetration-effectiveness score (CPE) was derived from Letendre et al and updated with approximation on similarities and

preliminary data for newer drugs [18]. CVE was defined as any case of CSF HIV-RNA 0.5 Log_{10} higher than plasma HIV-RNA or any case of detectable CSF HIV-RNA coupled with undetectable plasma HIV-RNA [19]. Any plasma HIV-RNA determination between 20 and 200 cp/ml was defined as LLV.

Statistical analysis

Data were analysed using standard non-parametric statistical methods considering the variables distribution (Mann–Whitney, Chi-squared and Fisher exact tests). Continuous variables were described as medians (interquartile ranges) and discrete variables were described as absolute number (percentage). The significance threshold to reject the null hypothesis was set at 5% (α=0.05, two-tailed predictions). Bonferroni correction was applied when appropriate. Data analysis was performed using SPSS software for Windows (version 25.0. IBM Corp).

Results

Population

97 patients met our inclusion criteria and were analysed. 19 patients were on DT and 78 on TT. DT were as follows: 12 INI+boosted PI (63.2%), 3 INI+nNRTI (15.8%), 2 boosted PI+nNRTI (10.5%) and 2 boosted PI+NRTI (10.5%). 1 started DT as naïve, while the others (94.7%) switched from other regimens. Before starting DT, 10 patients (52.6%) showed a plasma HIV-RNA<20 cp/mL, being the median plasma HIV-RNA among unsuppressed patients 33 cp/mL (22-690). The median time on DT was 20 months (12-60), while the time spent on previous regimen was 27 months (16-44). Demographic and clinical data of the 19 patients on DT are reported in details in Supplementary fig.1 http://links.lww.com/QAD/B773. Among TT, 24 patients (30.8%)

were on 2NRTIs+boosted PI and 27 (34.6%) on 2NRTIs+INI and 2NRTIs+nNRTI both. They were on the current TT since 16 months (7-27) and spent 23 months (15-41) on previous regimens (79.5% coming from another TT, 19.2% starting the current TT as naïve and 1 from DRV/r+RAL).

Indications for LP were as follows (the same patient may present more than one): neurological signs or symptoms (DT: 12, 63.2%; TT: 50, 64.1%), neurocognitive complaints (DT: 8, 42.1%; TT: 36, 46.2%), brain MRI abnormalities (DT: 14, 73.7%; TT: 44, 56.4%), other clinical reasons (DT: 1 syphilis and 1 follow-up in previous rebound HIV encephalitis, 10.5%; TT: 2 Non Hodgkin lymphoma prophylaxis, 2 syphilis, 5.1%) and research purposes (DT: 5, 26.3%; TT: 18, 23.1%). No difference was observed between DT *vs* TT at the comparison for LP indications.

Similarly, after the diagnostic work-up, no significant difference was observed in terms of prevalence of diagnosed clinical conditions. Specifically (multiple diagnoses per individual were recorded): HAND (for patients who underwent the neurocognitive assessment, see below; DT: 10/11, 90.9%; TT: 38/52, 73.1%), CVE (DT: 4, 21.1%; TT: 15, 19.2%), isolated brain MRI abnormalities (DT: 4, 21.1%; TT: 18, 23.1%) and alternative diagnoses excluded (DT: 3, 15.8%; TT: 14, 17.9%). A definitive diagnosis among patients complaining of neurocognitive or neurological signs or symptoms was reached in similar percentage (9/13, 69.2% in DT vs 34/51, 66.7% in TT; p.86).

No significant differences in terms of demographic and viro-immunological features were observed, as shown in Tab.1. DT showed a trend for a higher proportion of patients with a history of LLV and for a smaller proportion presenting plasma HIV-RNA<20 cp/mL at LP (Tab.1). Furthermore, the groups did not differ in the duration of continuous virological suppression, while the length of time from HIV diagnosis was

longer for DT but the difference did not reach the significance threshold and none was diagnosed during primary HIV infection (Tab.1).

Cerebrospinal fluid virological suppression

As shown in Tab.1, despite the expected lower RAMs-unadjusted CPE score in DT, the amount of CSF HIV-RNA did not differ between the groups: 14 patients on DT (73.7%) presented a CSF HIV-RNA below the detection limit and similarly it was in TT (78.2%; Tab.1). Among patients with detectable CSF virus, CSF HIV-RNA was 120 (45-182) and 54 cp/mL (43-77) in DT and TT, respectively (p.1.0; Tab.1). Lastly, the prevalence of CVE was similar among the groups: 4 cases in DT and 15 among TT (21.1% vs 19.2%; p.86).

Neurocognition

63 patients (64.9%) underwent the neurocognitive assessment: 11 on DT and 52 on TT. As shown in Tab.2, the groups did not score differently at the depression and anxiety questionnaires. HAND prevalence did not differ between DT and TT (90.9% vs 73.1%; p.21), as well as the distribution of HAND grades (Tab.2). Comparing the groups, there was no difference in raw or adjusted scores in any neurocognitive tests (data not shown).

Cerebrospinal fluid biomarkers

Comparing patients on DT vs TT, there was no difference at any tested CSF biomarkers of intrathecal humoral response (intrathecal synthesis, IgG index, Tourtelotte and Tibbling indices), inflammation and monocyte/macrophages activation (cells, proteins, neopterin), BBB integrity (CSAR), astrocytosis (S100β), neuronal injury (tau, p-tau) and amyloid metabolism (Aβ1-42), as shown in Tab.2-Fig.1. Since CSAR and CSF tau

have different age-adjusted cut-offs of normality, we also assessed the proportion of patients with altered CSAR and CSF tau. No significant difference was registered between the groups: altered CSF tau levels in 5.9% vs 2.9% of patients on DT and TT, respectively (p.55); impaired BBB integrity in 5.6% vs 21.9% of patients on DT and TT, respectively (p.11).

Discussion

In our cohort of adult PLWH with suppressed or LLV undergoing LP due to neurological or neurocognitive issues or neurologically-oriented research purposes, DT showed similar efficacy in suppressing viral replication within CSF compared to standard TT.

The study subjects represent a clinical group of patients characterized by a high risk of presenting CSF alterations and/or an abundant compartmentalized CNS reservoir, as confirmed by the significantly higher prevalence of observed CVE and HAND than those recently reported [19–22]. Given this, patients on DT did not show greater prevalence of CVE than TT and the amount of quantifiable CSF virus was also similar. Since we were not able to calculate for every patient a genotypic susceptibility-adjusted CPE score, which seems to better relate to neurocognitive performance and CVE risk [23,24], we cannot rule out that the observed equivalence in CSF viro-suppression could be partially affected by discrepancies in RAMs between the groups. Among patients with available data (37 TT; 11 DT), those on TT presented a lower prevalence of cumulative genotype testing positive to RAMs compared to DT (reverse transcriptase and protease 37.8% and 31.4% vs 72.7% and 45.4%). Although the available data for TT may not be representative for the whole group, we can infer that our patients on TT

should have a similar or lower RAMs prevalence than those on DT, reliably reducing the risk of a biased non-rejection of the null hypothesis.

Considering also other acknowledged risk factors for CVE [25,26], DT and TT did not differ in terms of CD4 nadir and duration of cART, while the former were disadvantaged by a trend to significance of more subjects with a LLV history and of longer duration of the infection. Nevertheless, both the groups showed an equivalent long-lasting plasma viral suppression, whereby, although these mild unfavourable differences against DT in terms of CVE risk, CVE prevalence in DT and in TT overlapped. In line with this, also the neurocognitive performance and the alterations in all the measured CSF biomarkers were similar between the groups. In contrast to clinical trials reporting neurocognitive data, our sample was larger [11] or analysed through an extensively wider battery [12] assessing 9 neurocognitive domains. As for trials aviremic patients [11,12], compared to patients on TT, those on DT did not present a higher prevalence of HAND nor reduced performances in specific tasks that may be expected considering recent data on intertwined associations between drug classes/molecules, neurocognitive functions and differential penetration in brain areas [27,28]. This observation has to be interpreted knowing that HAND development timing is not clear and that our neurocognitively tested patients were on DT since a median time of almost 4 years but with a large variability within the sample (minimum 4 – maximum 124 months).

Concerning the time of the development of CNS complications, neuropathogenesis may not relate immediately and solely to uncontrolled viral replication within CNS and may arise after long periods of low-level CSF viremia or even optimal suppression [29,30]. In fact, we have analysed CSF cell-free HIV-RNA only, but both cell-associated HIV-

DNA and HIV-RNA have been detected with a significant higher prevalence compared to CSF cell-free HIV-RNA among on cART patients and the presence of the former has been associated with worse neurocognitive outcomes regardless of age and nadir CD4 count prior to the patient's initiation of cART [31]. If available, the assessment of HIV-DNA and RNA within CSF cells may reveal differences between DT and TT that the simple cell-free RNA cannot, especially in cohorts of patients with high prevalence of undetectable CSF HIV-RNA, such as ours.

The absence of any difference in CSF biomarkers of intrathecal humoral response, inflammation, monocyte/macrophages activation, BBB integrity, astrocytosis, neuronal injury and amyloid plaques deposition, already variably associated with viral replication, CVE and with the presence and severity of HAND [26,32–35], once again confirms the equality in safety and effectiveness of DT and TT in our clinical setting.

Interestingly, some of the pathways linked to the CSF biomarkers used here have also been associated to cART-related neurotoxicity [36–38]. The lack of any difference in CSF biomarkers in an era where one of the main driver to DT simplification is the pro-active prevention of cART-related toxicity could be disappointing. This conclusion has to be confirmed by larger samples, longer observational periods, alternative biomarkers and analyses and should consider possible legacy effects of previous regimens and treatment holidays as well as the ARVs molecules included/excluded from DT. For instance, we found that patients on TT have BBB alterations four times more commonly than those on DT. Despite the difference was not statistically significant, the finding requires further studies since evidence exists on BBB injury caused by some ARVs, primarily EFV [37,39], which is not included in any current DT.

Lastly, from a clinical point of view, neurological and neurocognitive issues of patients on DT were similar to those registered among TT, as well as for the results of the diagnostic work-up. Furthermore, among patients complaining of signs or symptoms, a final conclusive diagnosis was reached in identical proportions. Despite preliminary, this observation may also suggest that CNS complications and challenges in clinical management may not be affected by being on a DT instead of a TT.

The subsequent follow-up of the patients allowed us to exclude those with neurological conditions unclear at LP but already likely present biasing CSF analysis. Thus, despite retrospective, clinical confounders were limited.

Only 1 included DT can be considered as a modern DT (DTG-based) and, overall, only 7 (36.8%) are currently recommended by guidelines (DTG+RPV; RAL+boosted DRV). Therefore, our DT sample is mostly represented by PI-based or old unconventional non-PI-based DT, presenting possible representativeness issues that require a confirmation in larger samples of modern DT. Considering our sample size and inclusion criteria and the fact that the majority of patients on DT switched from TT, further studies on larger samples of neurologically and neurocognitively asymptomatic PLWH including naïve starting on DT are also warranted to assess our findings in different clinical settings.

Several reasons can explain the observed equality between DT and TT in terms of CNS efficacy and safety. Firstly, it is the result of balancing direct and indirect viral neurotoxicity with cART-related direct and indirect neurotoxicity. Secondly, taking into account ARVs differential and molecule-specific penetration across BBB, their diffusion and concentration through the several brain areas and cells and their differential intracellular inhibitory potential within the cells types (macrophages/glial cells versus neurons versus astrocytes), as well as possible archived RAMs and the

established CNS reservoir with its dynamics of decay and evolution under cART [7,13–15,27,40], the residual activity of some TT (actually functional dual therapies) may overlap the one of well-patient-tailored DT, but with an addition of toxicity. Lastly, an effective long-lasting and continuous virological suppression in blood, characterising both the groups, may be enough to restore an efficient immune system and this, in turn, may be sufficient for an adequate CNS functioning [12,41].

In conclusion, our retrospective analysis on a small but highly characterized sample suggests that, among symptomatic patients with peripheral virological control, DT can be as effective and safe as TT within the CNS compartment. Further studies on larger samples, different clinical conditions and with long-term prospective design are warranted to confirm that the removal of a drug over three of a standard regimen might not make the difference within CNS in patients with durable virological suppression in blood.

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data; Daniela Vai and Simone Domini performed the neurocognitive evaluation; Cristiana Atzori, Marco Nigra and Lorenzo Mighetto performed the laboratory analyses; all the authors helped in interpreting the results and revising the manuscript.

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Figure:

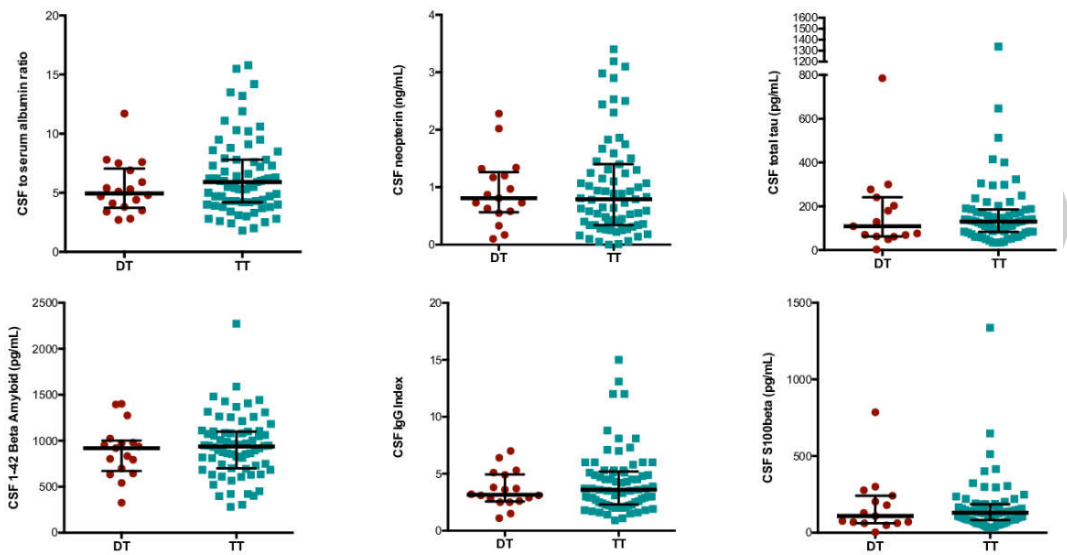

Figure 1. Comparison of representative cerebrospinal fluid biomarkers in patients on dual versus 2NRTI-based three-drug regimens

The median CSF concentrations of CSF-serum albumin ratio, neopterin and total tau protein (top left, middle and right corner, respectively) did not significantly differ between DT and TT, as well as the median CSF concentrations of Amyloid β 1-42 fragment, IgG index and S100β protein (lower left, middle and right corner, respectively). Data were analysed by Mann-Whitney test. Legend: DT Dual therapies; TT 2NRTI-based three-drug therapies; CSF Cerebrospinal fluid; IgG Immunoglobulin G.

Tables:

Table 1. Demographic and viro-immunological characteristics of individuals on dual regimens versus 2NRTIs-based three-drug regimens

Parameter	DT (19)	TT (78)	*p*
Male, n	14 (73.7%)	59 (75.6%)	.86
Age, years	57 (51-60)	50 (45-56)	.11
Caucasian, n	18 (94.7%)	74 (94.9%)	.98
Risk factor, n			.59
MSM	7 (36.8%)	28 (35.9%)	
Heterosexuals	6 (31.6%)	17 (21.8%)	
Previous IDU	6 (31.6%)	33 (42.3%)	
HCV coinfection, n	7 (36.8%)	28 (35.9%)	.94
Time from HIV diagnosis, months	242 (177-316)	162 (67-227)	.092
Time on current cART, months	20 (12-60)	16 (7-27)	.57
Continuous virological suppression, months	59 (24-87)	31 (14-95)	.28
Current CD4 count, cell/mmc	626 (375-919)	541 (338-734)	.96
CD4/CD8 ratio	0.7 (0.4-1.0)	0.8 (0.5-1.2)	.71
CD4 count nadir, cell/mmc	99 (35-256)	190 (70-284)	.64
CD4 count nadir<200 cell/mmc, n	11 (57.9%)	41 (52.6%)	.68
Plasma HIV-RNA <20 cp/mL, n	15 (78.9%)	72 (92.3%)	.088
Plasma HIV-RNA, Log10 cp/mL°	2.0 (1.8-2.1)	1.5 (1.5-1.7)	.52
History of LLV, n	3 (15.8%)	3 (3.8%)	.054
CPE score	6 (5-6)	7 (6-8)	.020

CSF HIV-RNA<20 cp/mL, n	14 (73.7%)	61 (78.2%)	.67
CSF HIV-RNA, Log10 cp/mL*	2.1 (1.6-2.3)	1.7 (1.6-1.9)	1.0

°Among patients with detectable plasma HIV-RNA at lumbar puncture time. *Among patients with detectable CSF HIV-RNA. Mann–Whitney test and Chi-squared test were used for continuous and discrete variables, respectively. Legend: DT Dual therapies; TT 2NRTI-based three-drug therapies; MSM Males who have sex with other males; IDU Intravenous drug users; cART Combination antiretroviral therapy; LLV Low-level viremia; CPE Central Nervous System Penetration and Effectiveness; CSF Cerebrospinal fluid.

Table 2. Neurocognitive performance and cerebrospinal fluid biomarkers levels in patients on dual regimens versus 2 NRTIs-based three-drug regimens

Parameter	DT (11)	TT (52)	*p*
Neurocognition			
Education, years	8 (8-13)	8 (8-13)	.81
BDI-II score	4 (1-9)	6 (2-17)	.54
HARS score	3 (1-13)	3 (1-8)	.88
HAND, n			.12
Unimpaired	1 (9.1%)	14 (26.9%)	
ANI	7 (63.6%)	32 (61.5%)	
MND	3 (27.3%)	4 (7.7%)	
HAD	0	2 (3.8%)	
Parameter	**DT (19)**	**TT (78)**	***p***
Intrathecal Synthesis and CSF Humoral response			
Tourtelotte index	0.55 (0-7.55)	0 (0-8.9)	.75
Tibbling index	0.60 (0.48-0.92)	0.60 (0.40-0.80)	.91
IgG index	0.31 (0.26-0.49)	0.36 (0.23-0.52)	.60
Intrathecal synthesis, %	0 (0-31)	0 (0-0)	.61
CSF Inflammation and Immune-activation			
CSF cells, cell/ml	0 (0-2)	0 (0-0)	.29
CSF proteins, mg/ml	43 (35-55)	48 (38-60)	.51
CSF neopterin, ng/ml	0.77 (0.56-1.2)	0.79 (0.34-1.4)	.82
Blood-brain barrier integrity & cell-line specific CSF biomarkers			

CSF-Serum Albumin ratio	4.9 (3.7-7.1)	5.9 (4.2-7.8)	.29
CSF tau, pg/ml	135 (38-248)	153 (86-242)	.59
CSF phospho-tau, pg/ml	29 (22-44)	35 (28-48)	1.0
CSF Aβ 1-42, pg/ml	919 (671-1002)	938 (700-1100)	.96
CSF S100β, pg/ml	109 (62-241)	130 (83-186)	.60

Mann–Whitney test and Chi-squared test were used for continuous and discrete variables, respectively. Legend: DT Dual therapies; TT 2NRTI-based three-drug therapies; BDI-II Beck Depression Inventory II; HARS Hamilton Anxiety Rating scale; HAND HIV-Associated Neurocognitive Disorders; ANI Asymptomatic Neurocognitive Impairment; MND Mild Neurocognitive Disorders; HAD HIV-Associated Dementia; CSF Cerebrospinal fluid; IgG Immunoglobulin G; Aβ 1-42 Amyloid β 1-42 fragment.