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High concordance in plasma and CSF HIV-1 drug resistance mutations despite high cases of CSF viral escape in individuals with HIV-associated cryptococcal meningitis in Botswana

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Objectives: We compared the patterns of HIV-1 drug resistance mutations between the CSF and plasma of individuals with HIV-associated cryptococcal meningitis.

Methods: This is a cross-sectional study of archived CSF and plasma samples collected from ART-exposed participants recruited in the Phase 3 AmBisome Therapy Induction Optimisation randomized controlled trial (ISRCTN72509687) conducted in Botswana between 2018 and 2021. HIV-1 RT and protease genes were genotyped using next-generation sequencing and HIV-1 drug resistance mutations were compared between the CSF and plasma compartments stratified by thresholds of ≥20% and <20%.

Results: Overall, 66.7% (16/24) of participants had at least one HIV-1 drug resistance mutation in the CSF and/or plasma. A total of 15/22 (68.2%) participants had HIV-1 drug resistance mutations at ≥20% threshold in the plasma and of those, 11 (73.3%) had been on ART longer than 6 months. HIV-1 drug resistance mutations were highly concordant between the CSF and plasma at ≥20% threshold despite a substantial number of individuals experiencing CSF viral escape and with only 54.5% with CSF WBC count ≥20 cells/mm^3. Minority HIV-1 drug resistance mutations were detected in 20.8% (5/24) of participants. There were no mutations in the CSF that were not detected in the plasma.

Conclusions: There was high concordance in HIV-1 drug resistance mutations in the CSF and plasma, suggesting intercompartmental mixing and possibly a lack of compartmentalization. Some individuals harboured minority HIV-1 drug resistance mutations, demonstrating the need to employ more sensitive genotyping methods such as next-generation sequencing for the detection of low-abundance mutations.

Introduction

During the early course of infection, HIV-1 may enter the CNS by the migration of infected CD4+ T cells across the blood–brain barrier.[1] Once in the CNS, the virus can infect and adapt to cells that express low densities of CD4 (macrophages and microglial) leading to the evolution of compartmentalized HIV-1 variants in the CNS.[2] Compartmentalization may also arise due to limited penetration of some antiretroviral drugs into the CNS through the blood–brain barrier and ART interruptions.[3,4] The accumulated HIV-1 drug resistance mutations may persist for longer periods in the CNS, leading to poor response to ART in the future.[1,5]

Despite the widespread access to ART, poor ART adherence and late presentation to care remain the key challenges to ending HIV-associated opportunistic infections such as cryptococcal meningitis.[6] While people with cryptococcal meningitis can present with HIV-1 drug resistance mutations, compartmentalization of these mutations between the CSF and plasma requires further investigation. This study compares HIV-1 drug resistance mutations derived from the CSF and plasma of unsuppressed,

mostly ART-exposed individuals with HIV-associated cryptococcal meningitis in Botswana.

Methods

Study population

This was a cross-sectional study of archived CSF and plasma samples from participants enrolled in the Botswana site as part of the Phase 3 AmBisome Therapy Induction Optimisation randomized controlled trial conducted between 2018 and 2021. Briefly, the trial compared induction therapy of a single, high dose of liposomal amphotericin B given alongside 14 days of flucytosine and fluconazole with the WHO-recommended first-line regimen, which was 1 week of amphotericin B deoxycholate with flucytosine followed by 1 week of fluconazole. Eighty-four participants were recruited in the Botswana site, with 46 being ART-naive and 38 ART-exposed participants; however, we utilized samples from ART-exposed participants only. From the 38 ART-exposed participants, 28 and 36 participants had available CSF and plasma samples, respectively, collected on Day 1 of the trial, and 27 had CSF/plasma paired samples. The study was approved by the University of Botswana Institutional Review Board and the Health Research and Development Council at the Botswana Ministry of Health (HPDME 13/18/1) and all participants explicitly consented to future use of their samples.

Laboratory investigations

HIV-1 viral load and CSF viral escape results, which were previously reported, were used in this analysis.[7] HIV-1 RNA was extracted from 140 μL of plasma and cell-free CSF using the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany). Complementary DNA strand from protease and RT of HIV-1 *pol* was amplified as previously described.[8] The purified amplicons were used for next-generation sequencing on Illumina MiSeq (Illumina K.K., Tokyo, Japan). The sequence reads obtained were analysed using PASeq (https://paseq.org/) and HyDRA Web (http://hydra.canada.ca).

HIV-1 drug resistance mutations were considered high frequency if detected at ≥20% allele frequency, while low-frequency mutations were defined as those occurring between 5% and 20% allele frequency. HIV-1 drug resistance mutations detected were reported using the Stanford HIV drug resistance database (http://hivdb.stanford.edu). The CSF/plasma consensus sequences obtained were included in the maximum-likelihood phylogeny inferred in IQ-TREE 2 under the General Time Reversible model of nucleotide substitution and a total of 1000 bootstrap replicates.[9] Sequences were subtyped using the REGA subtyping tool version 3.[10]

Results

Participant characteristics

Baseline demographics and clinical characteristics of the 24 ART-exposed participants with successful genotyping results are shown in Table S1, available as Supplementary data at *JAC* Online. The median age was 39.5 years (IQR: 34.0–44.8 years) and two-thirds of the participants were male. Most participants (41.7%) were on tenofovir, lamivudine and efavirenz. The median duration on ART was 62.5 months (IQR: 1.5–100 months). The median CD4+ T cell count at enrolment was 30 cells/mm^3 (IQR: 10.8–60.0 cells/mm^3) while median (IQR) CSF and plasma HIV-1 viral load were 4.4 (3.6–5.2) $\log_{10}$ copies/mL and 4.8 (3.9–5.3) $\log_{10}$ copies/mL, respectively.

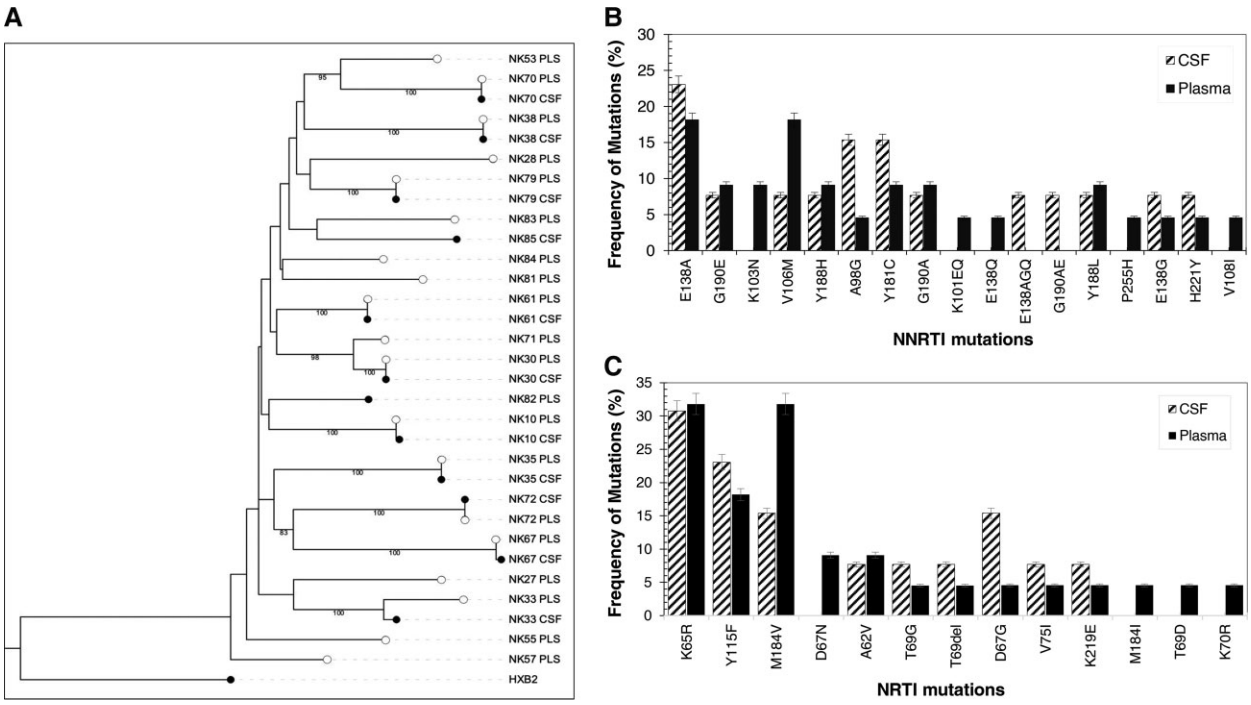

Figure 1 (a) Maximum-likelihood phylogenetic tree of 35 *pol* sequences and reference genome (HXB2). The sequences were isolated from plasma (open circles, PLS) and CSF (closed circles, CSF). Bootstrap values above 90 are indicated, with paired sequences having a bootstrap value of 100. (b) Frequency of NNRTI-associated mutations in participants' CSF and plasma samples (both paired and unpaired). (c) Frequency of NRTI-associated mutations in participants' CSF and plasma samples (both paired and unpaired).

Table 1. HIV drug resistance mutations in the CSF and plasma at ≥20% and <20% threshold in people with HIV-associated cryptococcal meningitis in Botswana

			HIV-1 viral load (log_{10} copies/mL)				HIV-1 drug resistance mutations (≥20% frequency)		HIV-1 drug resistance mutations (<20% frequency)	
ID	ART regimen	Months (days) on ART	CSF	Plasma	CSF WBC (cells/mm^3)	CSF viral escape	CSF	Plasma	CSF	Plasma
1	TDF/3TC/EFV	74 (2259)	165 508	390 036	2	No	None	None	None	None
2	TDF/3TC/EFV	8 (260)	414	79 899	2	No	N/A	K65R, Y115F, E138A, G190E	N/A	None
3	TDF/3TC/EFV	66 (2027)	N/A	9534	390	N/A	N/A	M184V, D67N, K103N, V106M, E138A	N/A	A62V, K65R
4	TDF/3TC/EFV	64 (1964)	322 572	422 256	455	No	None	None	None	None
5	TDF/3TC/DTG	0 (7)	4730	1143	62	Yes	None	Y188H	None	None
6	TDF//3TC/EFV	34 (1063)	362 670	49 275	10	Yes	K65R, Y115F, T69G, A98G, Y181C, Y188H, G190A	L90M, K65R, Y115F, T69G, A98G, Y181C, Y188H, G190A	L90M, T69del	T69del, K101EQ
7	TDF/3TC/LPV/r	112 (3420)	1 129 270	119 019	130	Yes	None	None	None	None
8	ZDV/3TC/NVP	139 (4231)	N/A	102 408	7	N/A	N/A	E138Q	N/A	None
9	TDF/3TC/DTG	0 (16)	670	120	8	Yes	None	N/A	K65R, Y115F, A98G, E138A, G190A, Y188H, L90M, D67G, E138AGQ, G190AE, Y181C	N/A
10	TDF/3TC/EFV	0 (10)	6356	1566	80	Yes	N/A	M184V, Y188L	N/A	K65R, Y115F, V106M
11	Unknown	41 (1250)	43354	258 912	2	No	E138A	E138A	None	None
12	ZDV/3TC/EFV	160 (4875)	9432	66 285	2	No	N/A	M184V, K103N, P225H	N/A	None
13	TDF/3TC/DTG	0 (5)	N/A	40 470	208	N/A	N/A	None	N/A	None
14	DTG/other	67 (2061)	50 812	66 576	10	No	M184V	M184V	None	None
15	TDF/3TC/EFV	72 (2213)	26 088	184 959	2	No	A62V, K65R, D67G, V75I, K219E, E138G, G190E, H221Y	A62V, K65R, D67G, V75I, K219E, E138G, G190E, H221Y	None	None
16	TDF/3TC/DTG	2 (77)	38 864	3393	85	Yes	E138A	E138A	None	None
17	ABC/3TC/DTG	3 (107)	N/A	2130	13	N/A	N/A	M184I, A98G	N/A	None
18	TDF/3TC/EFV	109 (3338)	13 200 792	2 019 123	145	Yes	K65R, Y115F, M184V, V106M, Y188L	K65R, Y115F, M184V, V106M, Y188L	None	None
19	TDF/3TC/DTG	0 (14)	2142	24 492	5	No	None	None	None	None
20	TDF/3TC/DTG	97 (2955)	4324	135 471	2	No	N/A	K65R, M184V	N/A	None
21	TDF/3TC/EFV	138 (4202)	N/A	586 368	2	N/A	N/A	None	N/A	None
22	TDF/3TC/EFV	131 (3990)	N/A	43 704	5	N/A	N/A	D67N, T69D, K70R, M184V, K219E, V106M, Y181C, G190A	N/A	V108I
23	unknown	61 (1861)	N/A	269 532	5	N/A	N/A	None	N/A	None
24	TDF/3TC/DTG	0 (6)	1142	3219	2	N/A	None	N/A	None	N/A

ABC, abacavir; 3TC, lamivudine; ARV, antiretroviral drug; ZDV, zidovudine; DTG, dolutegravir; EFV, efavirenz; LPV/r, ritonavir-boosted lopinavir; N/A, data not available; None, no major drug resistance mutations; TDF, tenofovir disoproxil fumarate.

HIV-1 drug resistance mutations in the CSF and plasma

Of the 24 participants with successful genotyping results, 11 were CSF/plasma pairs, 11 were unpaired plasma and 2 were unpaired CSF. Maximum-likelihood phylogenetic analysis revealed a 100% bootstrap value for all the pairs confirming patient-level viral sequence uniqueness [Figure 1(a)]. A total of 15/22 (68.2%) participants had HIV-1 drug resistance mutations at ≥20% threshold in the plasma, and of those, 11 (73.3%) had been on ART longer than 6 months (Table 1). NRTI- and NNRTI-associated mutations were found in 11/15 (73.3%) and 13/15 (86.7%) participants, respectively, while PI-associated mutations were detected in 1/15 (6.7%) participants. Overall, 5/24 (20.8%) participants harboured minority HIV-1 drug resistance mutations in the CSF or plasma. Out of 11 participants with paired CSF/plasma samples, 5 (45.5%) had CSF viral escape, while 7/11 (63.6%) had pleocytosis (CSF WBC count ≥10 cells/mm^3, as previously described).[1] There were 8/11 participants with paired samples harbouring HIV-1 drug resistance mutations in the CSF and/or plasma; of those, 5 (62.5%) experienced pleocytosis and 4 (50.0%) had CSF viral escape, while 4 (50.0%) were mutually inclusive of both variables. There were 2/11 (18.2%) participants with discordant HIV-1 drug resistance mutations; however, there were no mutations in CSF that were not present in plasma, but two participants had additional mutations in the plasma.

The most prevalent NNRTI-associated mutations in the plasma and CSF were V106M and Y181C, which cause high-level resistance to nevirapine and efavirenz, respectively [Figure 1(b)]. K65R and M184V were the most prevalent NRTI mutations in the plasma, both occurring in 31.8% (7/22) of participants, while K65R also predominated in the CSF, with a prevalence of 30.8% (4/13) [Figure 1(c)].

Discussion

We report a high prevalence (66.7%) of HIV-1 drug resistance mutations in ART-exposed individuals with HIV-associated cryptococcal meningitis, which may contribute to the transmission of these mutations. The most predominant mutations were M184V and K65R, indicating long-term exposure and poor adherence to the tenofovir plus lamivudine or emtricitabine regimen. Although these individuals can safely continue tenofovir, lamivudine and dolutegravir or switch to zidovudine, lamivudine and dolutegravir,[11] the long-term efficacy of these regimens in patients harbouring HIV-1 with these mutations requires further investigations. Our results suggest that both poor adherence to ART and the development of HIV-1 drug resistance mutations contribute to HIV disease progression and the development of opportunistic infections like cryptococcal meningitis.

Our study also revealed a high concordance in the CSF and plasma HIV-1 drug resistance mutations harboured by these individuals and there were no mutations in the CSF that were not detected in the plasma. Furthermore, approximately half of the participants with paired samples harbouring drug resistance mutations experienced pleocytosis and CSF viral escape. These results corroborate previous research findings demonstrating minimal HIV-1 compartmentalization and intercompartmental mixing of the viral populations.[12] This also provides additional evidence that the high influx of HIV-1-infected CD4+ T cells into the CNS accounts for the equilibration of these viruses in the two compartments and possibly a lack of independent evolution and replication of the virus in the CNS.[1,13] These findings may provide reassurance to ART prescribers in that, in this cohort, a switch in the patient's ART regimen based on the drug resistance profile from plasma would still be effective in the CSF, unlike in scenarios where there are additional mutations in the CSF. We further noted two participants with additional HIV-1 drug resistance mutations in the plasma, suggesting possible selective drug pressure in the plasma as opposed to the CSF.[14]

When comparing the HIV-1 drug resistance mutations in the CSF and plasma using next-generation sequencing we found 20.8% of the participants harbouring HIV-1 drug resistance minority variants. This shows that the use of next-generation sequencing is critical in detecting low-abundance mutations that may have an impact on treatment outcomes and potentially drive viral escape. Although conventional next-generation sequencing has limitations of decreasing accurate sampling of the original viral population and high error rates affecting the downstream analysis and interpretation, conventional next generation-sequencing is still widely used, even in studies with public health impact,[15] and it outperforms Sanger sequencing in detecting mutations at <20% threshold.[16] To mitigate these problems, we recommend the use of unique primer IDs, which will increase the sensitivity for the detection of minor variants.[17,18] Another limitation of this study is that in our sequencing approach, we did not include the HIV-1 integrase gene; however, studies show that the prevalence of pretreatment integrase inhibitor resistance is relatively low since they have been recently introduced.[19] Lastly, our study had a modest sample size utilizing all available samples but was conducted in the context of a well-designed clinical trial and high rates of virological suppression in Botswana. We, therefore, recommend further studies with larger sample sizes to profile and especially evaluate the impact of HIV-1 drug resistance mutations in the CSF.

In summary, the high prevalence of HIV-1 drug resistance mutations shows the need to enhance ART adherence and monitor HIV-1 viral load in individuals on ART to control the transmission of HIV-1 drug resistance mutations and the development of opportunistic infections. The presence of highly concordant HIV-1 drug resistance mutations between the CSF and plasma could indicate intercompartmental mixing and lack of HIV compartmentalization in the CNS in individuals with HIV-associated cryptococcal meningitis.

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Author contributions

N.K., R.M., I.K., D.S.L., M.M., T.S.H., J.N.J., S.M. and S.G conceived and designed the study; K.L. and T.B.L. collected and prepared the study samples; N.K. performed the laboratory experiments; N.K., W.T.C., S.M. and S.G. conducted formal analysis and prepared the original manuscript draft; I.K., S.G. and S.M. supervised the research; S.M., R.M. and S.G. acquired funding for the research; N.K., W.T.C., K.L., T.B.L., I.K., R.M., D.S.L., M.M., T.S.H., J.N.J., S.M. and S.G. participated in manuscript editing and approved the final version; all authors read and agreed to the published version of the manuscript.

Data availability

Data contained within the article and the relevant sequences are available on GenBank under accession numbers ON773625 to ON773655.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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