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No evidence of neuronal damage as measured by neurofilament light chain in a HIV cure study utilising a kick-and-kill approach

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ABSTRACT

Objective: HIV-remission strategies including kick-and-kill could induce viral transcription and immune-activation in the central nervous system, potentially causing neuronal injury. We investigated the impact of kick-and-kill on plasma neurofilament light (NfL), a marker of neuro-axonal injury, in RIVER trial participants commencing antiretroviral treatment (ART) during primary infection and randomly allocated to ART-alone or kick-and-kill (ART + vaccination + vorinostat (ART + V + V)).
Design: Sub-study measuring serial plasma NfL concentrations.
Methods: Plasma NfL (using Simoa digital immunoassay), plasma HIV-1 RNA (using single-copy assay) and total HIV-1 DNA (using quantitative polymerase chain reaction in peripheral $CD4^+$ T-cells) were measured at randomisation (following ≥22 weeks ART), week 12 (on final intervention day in ART + V + V) and week 18 post-randomisation. HIV-specific T-cells were quantified by intracellular cytokine staining at randomisation and week 12. Differences in plasma NfL longitudinally and by study arm were analysed using mixed models and Student's t-test. Associations with plasma NfL were assessed using linear regression and rank statistics.
Results: At randomisation, 58 male participants had median age 32 years and $CD4^+$ count 696 cells/μL. No significant difference in plasma NfL was seen longitudinally and by study arm, with median plasma NfL (pg/mL) in ART-only vs ART + V + V: 7.4 vs 6.4, p = 0.16 (randomisation), 8.0 vs 6.9, p = 0.22 (week 12) and 7.1 vs 6.8,

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p = 0.74 (week 18). Plasma NfL did not significantly correlate with plasma HIV-1 RNA and total HIV-1 DNA concentration in peripheral $CD4^+$ T-cells at any timepoint. While higher HIV-specific T-cell responses were seen at week 12 in ART + V + V, there were no significant correlations with plasma NfL. In multivariate analysis, higher plasma NfL was associated with older age, higher $CD8^+$ count and lower body mass index.
Conclusions: Despite evidence of vaccine-induced HIV-specific T-cell responses, we observed no evidence of increased neuro-axonal injury using plasma NfL as a biomarker up to 18 weeks following kick-and-kill, compared with ART-only.

1. Introduction

While antiretroviral treatment (ART) has improved survival of people with HIV (PWH),[1,2] ART alone is not a cure.[3,4] Upon stopping ART, plasma viral load rebounds within eight weeks.[5] The source of rebounding virus is cells latently infected with HIV, termed the reservoir,[3,6–8] formed when HIV genetic material integrates into the genome of the infected cell.[7] Reservoirs are established soon after HIV acquisition,[9] remain stable despite ART,[7,10,11] do not express viral antigens and thus, evade immune system detection.[12] The main HIV-1 latent reservoir is thought to be located in resting $CD4^+$ T-cells in circulation and lymphoid tissue[7,13] but additional reservoir compartments may include the lungs, genital tract and central nervous system (CNS).[6,13–17]

Several HIV-1 remission approaches are being trialled, such as kick-and-kill,[18,19] whereby latently infected cells are stimulated, leading to viral transcription and expression of viral antigens[7,13] which are recognised by the immune system and eradicated, to reduce HIV reservoir size.[20]

As ART for PWH is safe, well-tolerated and has improved life expectancy, any innovative cure strategy must be at least as safe as modern ART and hypothetical risks associated with HIV cure strategies need to be considered. While latency reversing agents (LRAs) aim to force HIV transcription in latently infected cells, they have broad epigenetic effects and can cause transcription of non-HIV genes and reactivate other integrated resting viruses.[21] The LRAs and/or associated viral protein expression following latency reversal may induce inflammation and immune-activation[22]; this is particularly risky in the CNS as this could cause neuro-toxicity and injury.[23–25]

To date, reported significant CNS adverse effects following kick-and-kill are rare,[26,27] but data are limited. In a trial in simian immunodeficiency virus-infected macaques that received ART and two LRAs, one macaque experienced significant viral rebound (higher in the cerebrospinal fluid (CSF) compared to plasma), increased CNS immune-activation and neuronal injury biomarkers as well as neurological symptoms necessitating euthanasia.[28]

Careful monitoring of the CNS during kick-and-kill remains crucial but is challenging; routine brain biopsies are not practical, and neuroimaging is costly. CSF neurofilament light protein (NfL) is a validated, sensitive and dynamic biomarker of CNS neuro-axonal injury[29–31] and is a sensitive neuronal biomarker across the spectrum of HIV infection.[32–34] Neurofilaments are a heteropolymer family of neuronal intermediate filaments with a role in supporting the structural and functional integrity of axons.[29,35,36] Neurofilament proteins form the key structural components of axons, and the expression of these proteins is particularly high in large myelinated axons where they influence conduction speed.[37] Neurofilaments encompass about 85% of the cytoskeleton proteins and comprise four main subunits with different molecular weights: neurofilament light (68 kDa), neurofilament medium (150 kDa), neurofilament heavy (190–210 kDa) and α-internexin (66 kDa), of which neurofilament light chain protein (NfL) is the most abundant and most soluble.[29] In situations involving cortical neuronal injury, neurofilament proteins can be used as a biomarker of axonal injury. Following an insult, neurofilament proteins from the damaged neuro-axonal units are released proportional to the severity of damage into interstitial fluid and enters the cerebrospinal fluid, where they can then be measured.[34]

However, the invasive nature of CSF collection precludes frequent CSF NfL measurement. A novel Simoa assay which can reliably measure blood NfL (usually 50–100 times lower than CSF NfL) has recently been developed,[38] thus removing the barriers faced by CSF sampling and allowing more frequent measurements given that blood samples are easier to obtain. Preliminary data suggests that both plasma and serum NfL correlate moderately to strongly with CSF NfL across a variety of neurological disorders, including HIV disease.[38–42] A recent meta-analysis demonstrated moderate correlations between CSF and blood NfL, especially when blood NfL was measured using Simoa or electrochemiluminescence assays.[43]

RIVER[44] is the first open-label, randomised kick-and-kill trial assessing ART-alone versus ART plus HIV-1 prime/boost T-cell vaccination (ChAdV63. HIVconsv and MVA. HIVconsv) (ART + V + V) plus the LRA vorinostat (a histone deacetylase inhibitor) in individuals who initiated ART during primary HIV-1 infection.[45] Whilst the RIVER trial found a three-fold increase in histone acetylation following vorinostat dosing, and induction of robust HIV-specific T-cell responses, there was no significant benefit of this kick-and-kill approach compared with ART-alone on measures of peripheral blood[44] and gut[46] HIV-1 reservoir size. In order to investigate whether this kick-and-kill strategy caused neuro-axonal injury, we measured serial plasma NfL in participants enrolled into the RIVER trial[44] and assessed demographic and clinical factors associated with plasma NFL.

2. Material and methods

2.1. Participants and recruitment procedures into the RIVER trial

Participants aged between 18 and 60 years with recent acquisition of HIV infection and ART initiation within one month of confirmed HIV diagnosis were randomly assigned 1:1 to receive either ART-alone (control) or ART and vaccination with ChAdV63.HIVconsv prime and MVA.HIVconsv boost given eight weeks apart, followed by vorinostat taken orally in 10 doses of 400 mg every 3 days for 28 days (ART + V + V). Participants were enrolled between December 2015 and November 2017 at six clinical sites in the UK. Participants with viral co-infections (hepatitis B, hepatitis C and human T-cell lymphotropic virus (HTLV)) and concurrent malignancy or opportunistic infections were excluded. Recent HIV infection was defined as: positive HIV-1 serology within 12 weeks of negative HIV-1 serology or point-of-care test, positive p24 antigen and a negative HIV-1 serology test, negative HIV-1 antibody test with detectable HIV RNA or proviral DNA, Public Health England recency HIV antibody avidity assay reported as incident (<16 weeks prior to enrolment), weakly reactive or equivocal fourth-generation HIV antibody-antigen test, or equivocal or reactive HIV antibody test with less than 4 bands on a Western Blot analysis.[47]

2.2. Study visits

After enrolment, all participants were recommended to initiate a four-drug ART regimen, including raltegravir. Randomisation to continue ART-alone or to receive ART plus kick-and-kill took place after participants had been on cART for at least 22 weeks. After randomisation, all participants were followed up for 18 weeks. Study procedures included assessment of adverse events, adherence to medication and

anthropometric measurements.

2.3. Participants included in this sub-analysis

Participants enrolled into RIVER who had given written consent to be included in future sub-studies were included. Ethics committee approval was obtained from all participating sites (14/SC/1372) and the trial was conducted in accordance with the principles of the Declaration of Helsinki. Stored plasma samples taken at three timepoints were retrieved and analysed for NfL: at randomisation (following ≥22 weeks of ART), week 12 (before receiving the tenth and final vorinostat dose and 4 weeks after completing the vaccination course in the ART + V + V arm) and week 18[44] (Fig. 1).

2.4. Laboratory parameters

We measured plasma NfL using the commercially available NF-light assay on a HD-X Simoa instrument (Quanterix, Billerica, MA, USA).[48] Samples were analysed in duplicate, diluted 1:4 and the lower limit of quantification of the assay was 0.174 pg/mL, as determined by the manufacturer. Analyses were performed by the same technician using a single batch of reagents and the intra- and inter-assay coefficients of variations were below 6% and 15%, respectively.

The following parameters were measured previously in the main study[44]: total HIV-1 DNA (in $CD4^+$ T-cells isolated from cryopreserved peripheral blood mononuclear cells (PBMC) according to previously described methods[49]) and plasma HIV-1 RNA (by single-copy assay) at randomisation, week 12 and week 18. T-cells specific to the HIVconsv-vaccine were quantified by intracellular cytokine staining, as described previously,[50] at randomisation and week 12.

2.5. Statistical analysis

Plasma NfL was $\log_{10}$-transformed to reduce data skewness, where appropriate. Differences in plasma NfL between study arms at each timepoint were analysed using Student's t-test, and longitudinal changes using linear mixed models. Correlations between plasma NfL and HIV-1 RNA, total HIV-1 DNA and polyfunctional $CD4^+$ and $CD8^+$ T-cell responses were analysed using Spearman's rank statistics. Using linear regression, we analysed the association between plasma NfL and the following factors at baseline: patient's age at randomisation, ethnicity, body mass index, duration since primary HIV infection diagnosis, $CD4^+$ T-cell count, $CD8^+$ T-cell count, estimated glomerular filtration rate (calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula)[51] and total HIV-1 DNA. *P*-values <0.05 were considered statistically significant throughout. Statistical analyses were performed using Stata 15.1 (StataCorp LLC, Texas, USA).

3. Results

In total, 58/60 participants enrolled into RIVER gave written consent to be included in future sub-studies and were included in this analysis.

Table 1
Baseline characteristics of participants from RIVER trial included in this analysis.

	All participants (n = 58)	ART-only (n = 29)	ART + V + V (n = 29)
Age, years	32 (28, 40)	31 (30, 38)	34 (28, 44)
Male	58 (100%)	29 (100%)	29 (100%)
Ethnicity			
White	40 (69%)	15 (52%)	25 (86%)
Black African/Caribbean	4 (7%)	4 (14%)	0
Other	14 (24%)	10 (34%)	4 (14%)
Route of HIV acquisition			
Sex between men	53 (91%)	25 (86%)	28 (97%)
Heterosexual intercourse	2 (3)	1 (3%)	1 (3%)
Sex between men and IDU	2 (3)	1 (3%)	0
Unknown	1 (2)	2 (7%)	0
Time from primary HIV infection diagnosis to ART start, weeks	2 (1, 3)	2 (0, 3)	2 (1, 3)
Time from primary HIV infection diagnosis to randomisation, weeks	28.1 (27.0, 37.1)	28.0 (26.6, 41.0)	28.1 (27.1, 34.4)
Body mass index, kg/m^2	24 (22, 27)	24 (22, 26)	24 (22, 27)
$CD4^+$ T-cell count, cells/μL	696 (566, 785)	675 (561, 790)	704 (579, 740)
$CD8^+$ T-cell count, cells/μL	660 (452, 828)	670 (461, 946)	642 (438, 735)
CD4/CD8 ratio	1.08 (0.86, 1.42)	1.09 (0.77, 1.26)	1.08 (0.92, 1.46)
Plasma HIV-1 RNA, copies/mL			
<50	57 (98%)	28 (97%)	29 (100%)
50 to <200	1 (2%)	1 (3%)	0
Serum creatinine	78 (71, 86)	83 (74, 89)	75 (71, 82)
Estimated glomerular filtration rate, mL/min/$1.73m^2$	110 (99, 117)	107 (97, 117)	111 (104, 117)

Values are median (interquartile range) or total (%). Abbreviations: IDU = injecting drug use, ART = antiretroviral treatment, ART + V + V = antiretroviral treatment and vaccination and vorinostat.

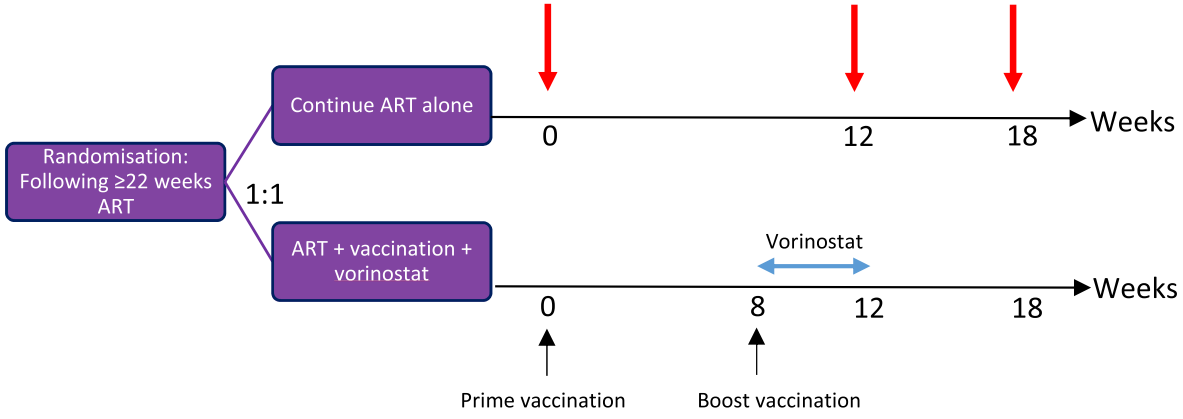

Fig. 1. RIVER trial schema.
RIVER trial schema illustrating the 1:1 randomisation into the two study arms. The red arrows signify the time points when plasma samples were analysed for NfL, in both study arms. The black arrows signify the time points for the prime and boost vaccinations, respectively. The blue arrow signifies the 28 day vorinostat dosing period.
Abbreviations: NfL = neurofilament light chain protein; ART = antiretroviral treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

All 58 participants were male, 69% of white ethnicity and 91% acquired HIV via sex between men (Table 1). At randomisation, median age was 32 years, duration since PHI diagnosis was 28 weeks, $CD4^+$ count was 696 cells/μL and all had plasma HIV-1 RNA <200 copies/mL (Table 1). Participant characteristics at randomisation were well-balanced by study arm.

3.1. Plasma NfL longitudinally and by study arm

Geometric mean plasma NfL was 6.8 pg/mL at randomisation, 7.4 pg/mL at week 12, and 6.9 pg/mL at week 18, without significant difference between the time points (p = 0.12) (Table 2). Plasma NfL in the ART-only and ART + V + V arms was similar at each timepoint (Table 2 and Fig. 2), and the two arms did not differ in change in plasma NfL from randomisation (p = 0.43 for interaction study arm x timepoint).

3.2. Correlations with plasma NfL

No significant correlations were seen between plasma NfL and HIV-1 RNA, total HIV-1 DNA, polyfunctional $CD8^+$ T-cell or $CD4^+$ T-cell responses, in the overall cohort at any of the timepoints (Table 2) or in the separate study arms at weeks 12 and 18 (where applicable) ($p > 0.05$ for all, results not shown).

Table 2
Plasma NfL concentration over time, and correlation with plasma HIV RNA, total HIV DNA and polyfunctional $CD4^+$ and $CD8^+$ HIVconsv-specific T-cell responses.

		Randomisation (following ≥22 weeks ART)	Week 12 (on final day of intervention in ART + V + V)	Week 18
Plasma NfL, pg/mL	Overall[a]	6.9 (6.2, 7.6)	7.4 (6.5, 8.3)	6.9 (6.3, 7.7)
	ART-only[a]	7.4 (6.5–8.4)	8.0 (6.6–9.7)	7.1 (6.2–8.0)
	ART + V + V[a]	6.4 (5.4–7.6)	6.9 (5.8–8.1)	6.8 (5.7–8.1)
	p-value[b]	0.16	0.22	0.74
Plasma HIV-1 RNA, copies/mL	Overall[c]	14 (3, 25)	5 (1, 12)	6 (1, 17)
Correlation with plasma NfL[d]	Rho	0.16	0.14	0.25
	p-value	0.35	0.34	0.07
Total HIV-1 DNA, copies/10^6 peripheral CD4 cells	Overall[c]	1581 (569, 2939)	1177 (527, 2399)	1501 (554, 2741)
Correlation with plasma NfL[d]	Rho	0.12	0.14	0.01
	p-value	0.37	0.32	0.93
% $CD154^+$ IFN-g^+ $CD4^+$ cells[b]	Overall[c]	0.009 (0.000, 0.023)	0.031 (0.005, 0.112)	n/a
Correlation with plasma NfL[d]	Rho	0.01	−0.19	
	p-value	0.96	0.21	
% $CD107a^+$ IFN-g^+ $CD8^+$ cells[b]	Overall[c]	0.074 (0.008, 0.289)	0.125 (0.033, 0.291)	n/a
Correlation with plasma NfL[d]	Rho	0.31	0.16	
	p-value	0.03	0.26	

n/a: not assessed at this timepoint.
Abbreviations: NfL = neurofilament light chain protein, ART = antiretroviral treatment, ART + V + V = antiretroviral treatment and vaccination and vorinostat.
[a] Geometric mean (95% confidence interval).
[b] *p*-values relate to differences between ART-only and ART + V + V arms.
[c] Overall median (interquartile range) of both study arms combined.
[d] Correlation with plasma NfL.

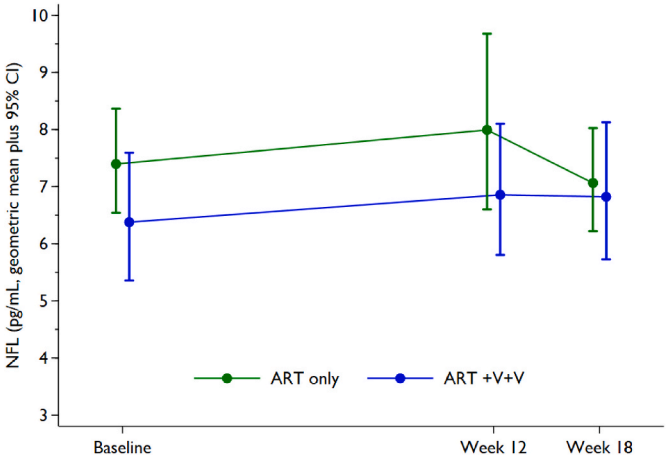

Fig. 2. Longitudinal changes in plasma NfL by study arm.
Line graph demonstrating longitudinal changes in plasma NfL by study arm, ART-only versus ART+V+V.
Abbreviations: NfL = neurofilament light protein, ART = antiretroviral treatment, V+V = vaccination and vorinostat, CI = confidence interval, Baseline = point of randomisation.

3.3. Factors associated with plasma NfL at randomisation

In multivariate regression analysis, higher plasma NfL at randomisation was independently associated with older age (0.10 (95% CI 0.06, 0.16) $\log_{10}$ NfL per 10 years older, p < 0.001), higher $CD8^+$ T-cell count (0.02 (95% CI 0.00, 0.03) $\log_{10}$ NfL per 100 cells/mm^3 higher, p = 0.03), and lower body mass index (−0.02 (95% CI -0.03, −0.01) $\log_{10}$ NfL per 1 kg/m^2 higher, p < 0.001) (Table 3).

4. Discussion

Despite evidence of vaccine-induced HIV-specific T-cell responses and histone deacetylation,[44] we observed no evidence of increased neuro-axonal injury using plasma NfL as a surrogate biomarker, up to 18 weeks following this kick-and-kill strategy compared with ART-only in participants enrolled into the RIVER trial who initiated ART during primary HIV-1 infection.

Our results are in keeping with published literature demonstrating no evidence of CNS adverse effects when assessed using CSF biomarkers following panobinostat, a histone deacetylase inhibitor.[27] Our results also reflect the published positive association between plasma NfL and age.[38] While age-related reference ranges for CSF NfL are defined,[33] similar reference ranges for plasma NfL are yet to be ascertained. The finding that higher $CD8^+$ cell count is independently associated with increased plasma NfL is novel and may indicate that higher $CD8^+$ T-cell counts (linked with ongoing immune activation and poorer immune reconstitution) may be associated with ongoing inflammation, which can lead to neuronal injury. The finding that lower body mass index is associated with higher plasma NfL has been observed previously,[52] perhaps indicative of a lower volume of distribution and hence higher plasma NfL concentration.

Strengths of our study include the random allocation of participants in the RIVER trial to the control and kick-and-kill arms, which enables us to assess the impact of kick-and-kill without risk of bias through confounding by indication. Several explanations for the apparent lack of CNS signal need to be taken into account; if viral transcription leads to neuro-axonal injury, then the lack of plasma viral transcription following this kick-and-kill strategy may explain the absence of impact on plasma NfL. While increased peripheral histone acetylation was observed following vorinostat dosing,[44] vorinostat concentration and

Table 3
Multivariable linear regression model to identify factors associated with baseline plasma NfL (log_{10}).

	Univariable analysis		Multivariable analysis	
Variable	Parameter estimate[a](95% confidence interval)	*p*-value	Parameter estimate[a](95% confidence interval)	*p*-value
Age (per 10 years older)	0.11 (0.06, 0.15)	<0.001	0.11 (0.06, 0.16)	<0.001
Body mass index (per kg/m^2)	−0.02 (−0.03, −0.01)	0.002	−0.02 (−0.03, −0.01)	<0.001
White ethnicity	−0.02 (−0.12, 0.08)	0.68	0.00 (−0.08, 0.09)	0.96
Duration since PHI diagnosis (per 5 weeks increase)	0.01 (−0.01, 0.03)	0.19	0.00 (−0.01, 0.02)	0.62
eGFR (per 10 μmol/L/min increase)	−0.05 (−0.08, −0.02)	0.001	−0.01 (−0.04, 0.02)	0.52
CD4$^+$ T-cell count (per 100 cells/μL increase)	−0.01 (−0.04, 0.01)	0.31	−0.00 (−0.03, 0.02)	0.75
CD8$^+$ T-cell count (per 100 cells/μL increase)	0.01 (−0.01, 0.02)	0.58	0.02 (0.00, 0.03)	0.03
Total HIV DNA (per 1 log_{10} copy/10^6 peripheral CD4$^+$ T-cells increase)	0.05 (−0.05, 0.14)	0.31	−0.02 (−0.10, 0.06)	0.62

Abbreviations: PHI = primary HIV diagnosis; eGFR = estimated glomerular filtration rate.
[a] Parameter estimates reflect the associated impact of each independent variable in the model on baseline plasma NfL.

histone acetylation in the brain was not measured, and we are unable to confirm whether vorinostat crossed the blood-brain barrier and affected the CNS in terms of neuronal toxicity, viral transcription and/or neuronal injury; preliminary studies suggest that the current form of vorinostat poorly crosses the blood-brain barrier. Nonetheless, the extent to which this finding can be translated to more potent LRAs is unknown. The lack of change in plasma NfL concentrations following the induction of potent HIV-specific CD4$^+$ and CD8$^+$ T-cell responses after vaccination suggests that this approach is safe from a neuro-axonal perspective.

Clinical parameters, such as patient-reported outcome measures of CNS function, cognitive function or cerebral imaging were not performed and may have provided further clinical insights. Published studies report various degrees of correlation between CSF and blood NfL, with stronger correlations seen in conditions with higher CSF and blood NfL concentrations. Throughout the study in both study arms, plasma NfL concentrations were generally low and similar in range to that seen in HIV-negative individuals (median (IQR) 9.3 (5.9–13.1) pg/mL and neuroasymptomatic individuals living with untreated HIV and with CD4$^+$ T-cell counts >350 cells/μL (median (IQR) 9.0 (6.5–14.3) pg/mL).[38] This may reflect the protective effect of early ART initiation during primary HIV infection and the potential lack of neuronal injury following this particular HIV remission strategy in this cohort of individuals. The lack of concurrent CSF NfL in this study restricts our knowledge about the corresponding CNS NfL trends. Whilst the RIVER study was powered for its primary endpoint, our sub-study may have been underpowered to detect changes in plasma NfL in this setting.

All participants enrolled into the RIVER trial initiated ART during primary HIV infection, when HIV reservoir size is presumed to be lowest[53]; furthermore there are data suggesting a relative delay in the establishment of the CNS reservoir, compared with the systemic reservoir.[54,55] Thus, the impact of kick-and-kill on markers of neuronal injury in participants who initiated ART during chronic HIV infection when HIV CNS reservoir size is larger, remains unknown. Whilst our results are reassuring, the majority of participants enrolled into the RIVER trial were young, white men who have sex with men, and it is unclear whether we can extrapolate our findings to other populations, such as women and those of non-white ethnicity.

In summary, significant neuronal injury was unlikely following the kick-and-kill strategy employed in the RIVER trial, supporting the CNS safety of this strategy. Continued monitoring for CNS adverse events remains an important aspect of HIV-remission research, especially when investigating other potentially more potent kick-and-kill strategies.

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Principal contributions made by each author

Jasmini Alagaratnam, Alan Winston and Sarah Fidler conceptualised the original idea for the study; Jasmini Alagaratnam, Jonathan Underwood, Henrik Zetterberg, Magnus Gisslen, Alan Winston and Sarah Fidler obtained the funding for the study; Jamie Toombs performed the plasma NfL laboratory work; Amanda Heslegrave and Henrik Zetterberg oversaw the plasma NfL laboratory work; Maryam Khan measured plasma HIV RNA by single-copy assay; Matthew Pace performed the total HIV DNA quantification work; John Frater oversaw the total HIV-1 DNA measurement and was the laboratory lead for the RIVER trial; Sarah Pett was the RIVER trial physician at the MRC CTU and responsible for recruitment and follow-up of participants at one of the trial sites; Mark Nelson, Amanda Clarke, Nneka Nwokolo, Margaret A Johnson, Julie Fox, Sabine Kinloch and Sarah Fidler were responsible for the recruitment and follow-up of participants; Tomas Hanke designed the vaccines used in the RIVER trial; Jakub Kopycinski performed the HIV-immunology assays; Lucy Dorrell oversaw the HIV immunology assay work; Sabine Kincloch provided research and clinical oversight for all vaccination visits; John Frater and Sarah Fidler were co-principal investigators of the RIVER trial; Sarah Fidler was the clinical chief investigator for the RIVER trial; Wolfgang Stöhr was the RIVER trial statistician and curated the RIVER trial results database; Wolfgang Stöhr, Jasmini Alagaratnam, Alan Winston and Sarah Fidler planned the data analysis; Wolfgang Stöhr performed the formal data analysis; Jasmini Alagaratnam wrote the first draft of the manuscript; all authors reviewed the draft manuscript for intellectual content and contributed to the final manuscript.

Data statement

Data from this analysis can be made available upon reasonable request to the corresponding author.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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