Journal of Virus Eradication 10 (2024) 100381

Contents lists available at ScienceDirect

Journal of Virus Eradication

journal homepage: www.sciencedirect.com/journal/journal-of-virus-eradication

Original research

Impact of interrupting antiretroviral therapy started during primary HIV-1 infection on plasma neurofilament light chain protein, a marker of neuronal injury: The SPARTAC trial

Jasmini Alagaratnam [a,b,*], Wolfgang Stöhr [c], Elizabeth Hamlyn [d], Kholoud Porter [e], Jamie Toombs [f], Amanda Heslegrave [f], Henrik Zetterberg [f,g,h,i], Magnus Gisslén [j,k], Jonathan Underwood [l], Mauro Schechter [m], Pontiano Kaleebu [n], Giuseppe Tambussi [o], Sabine Kinloch [p], Jose M. Miro [q,r], Anthony D. Kelleher [s], Abdel Babiker [c], John Frater [t,u], Alan Winston [a,b], Sarah Fidler [a,b], on behalf of The SPARTAC Trial Investigators

[a] Department of Infectious Disease, Faculty of Medicine, Imperial College London, London, United Kingdom
[b] Genitourinary Medicine/ HIV Department, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom
[c] Medical Research Council Clinical Trials Unit at University College London, London, United Kingdom
[d] Caldecot Centre, Kings College Hospital NHS Foundation Trust, London, United Kingdom
[e] Institute for Global Health, University College London, London, United Kingdom
[f] UK Dementia Research Institute at University College London, London, United Kingdom
[g] Department of Neurodegenerative Disease, UCL Institute of Neurology, University College London, London, United Kingdom
[h] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden
[i] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[j] Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[k] Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden
[l] Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom
[m] Projeto Praça Onze, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[n] Medical Research Council/Uganda Virus Research Institute, Entebbe, Uganda
[o] San Raffaele Scientific Institute, Milan, Italy
[p] Department of Infection and Immunity, Royal Free Hospital, Pond Street, London, United Kingdom
[q] Infectious Diseases Service, Hospital Clinic – IDIBAPS. University of Barcelona, Barcelona, Spain
[r] CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain
[s] Kirby Institute, UNSW Sydney, New South Wales, Australia
[t] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom
[u] National Institute of Health Research Biomedical Research Centre, Oxford, United Kingdom

ARTICLE INFO

Keywords:
HIV-1
Neurofilaments
Anti-retroviral agents
Viral rebound
Primary HIV infection

ABSTRACT

Objective: Antiretroviral therapy (ART)-conferred suppression of HIV replication limits neuronal injury and inflammation. ART interruption tests efficacy in HIV cure trials and viral rebound after ART interruption may induce neuronal injury. We investigated the impact of protocol-defined ART interruption, commenced during primary HIV-1 infection (PHI) on a biomarker of neuro-axonal injury (neurofilament light protein (NfL)), and its associations with inflammation (D-dimer and interleukin-6 (IL-6)) and HIV-1 reservoir size (total HIV-1 DNA).
Design: Retrospective study measuring plasma NfL in 83 participants enrolled in SPARTAC randomised to receive 48-weeks ART initiated during PHI, followed by ART interruption.
Methods: NfL (Simoa immunoassay, Quanterix™) was measured before ART, after 48 weeks on ART, and 12 weeks after stopping ART. Plasma D-dimer and IL-6, and total HIV-1 DNA in peripheral $CD4^+$ T-cells results were available in a subset of participants. Longitudinal NfL changes were assessed using mixed models, and associations with clinical and laboratory parameters using linear regression.
Results: NfL decreased following 48-weeks ART (geometric mean 6.9 to 5.8 pg/mL, $p = 0.006$) with no further significant change up to 12-weeks post-stopping ART despite viral rebound in the majority of participants

* Corresponding author. Chelsea & Westminster Hospital, 369 Fulham Road, London, SW10 9NH, United Kingdom.
E-mail address: j.alagaratnam@nhs.net (J. Alagaratnam).

https://doi.org/10.1016/j.jve.2024.100381
Received 26 March 2024; Received in revised form 11 June 2024; Accepted 12 June 2024
Available online 13 June 2024
2055-6640/© 2024 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(median 1.7 to 3.9 plasma HIV-1 RNA log_{10} copies/mL). Higher baseline NfL was independently associated with higher plasma HIV-1 RNA ($p = 0.020$) and older age ($p = 0.002$). While NfL was positively associated with D-dimer ($n = 48$; $p = 0.002$), there was no significant association with IL-6 ($n = 48$) or total HIV-1 DNA ($n = 51$). *Conclusions:* Using plasma NfL as a surrogate marker, a decrease in neuro-axonal injury was observed in a cohort of participants following ART initiation during PHI, with no evidence of neuro-axonal injury rebound following ART interruption for up to 12 weeks, despite viral rebound in the majority of participants.

1. Introduction

Antiretroviral treatment (ART) has improved survival for people with HIV,[1] however, upon ART cessation, plasma viral load rebound occurs in most people.[2] The source of rebounding virus originates from latently infected cells, the 'reservoir'.[3] Whilst SMART[4] identified that interrupting ART with a $CD4^+$ count re-initiation criteria was unsafe, recent HIV-1 eradication research suggests that with monitoring, analytical treatment interruption (ATI) may be safe, whilst being the best way of testing efficacy and identifying remission.[5]

The main HIV reservoir resides in resting memory $CD4^+$ T-cells, but evidence suggests that the central nervous system (CNS)[6–12] is another important site. A concern with HIV-1 eradication strategies and ATI is that viral rebound in the CNS could precipitate immuno-activation and neuro-inflammation, leading to neuronal injury.[8] Evidence demonstrates that within 4 weeks of stopping ART initiated during primary HIV-1 infection (PHI), plasma biomarkers of inflammation (IL-6 and D-dimer) which decreased during ART, return to pre-ART levels[13] and that IL-6 and D-dimer mediate neuroinflammation and may cause neuro-axonal injury.[14–16] Early ART initiation is associated with lower HIV-1 DNA (a measure of HIV-1 reservoir),[17–19] and when stopping ART, is associated with a delayed time to virus rebound.[20,21]

Reported CNS adverse events during HIV-1 eradication studies including ATI are rare.[22–26] However, in many modern studies the period off ART is carefully monitored, with ART reinitiated at early viral rebound.[27] Nonetheless, it remains imperative to ensure CNS safety. Cerebrospinal fluid (CSF) neurofilament light protein (NfL) is a validated, sensitive and dynamic biomarker of CNS neuro-axonal injury[28–30] with elevated concentrations reported in neurological disorders, including across the spectrum of HIV infection.[31–33] The neurofilament complex form a major structural component of myelinated axons and sustain the structural and functional integrity of axons.[29] Neurofilaments make up around 85% of the cytoskeleton proteins and contain four main subunits with different molecular weights: NfL (68 kDa), neurofilament medium (150 kDa), neurofilament heavy (190–210 kDa) and α-internexin (66 kDa), of which NfL is the most abundant and most soluble.[28] In conditions involving cortical neuronal injury, neurofilament proteins can be used as a biomarker of neuro-axonal injury. Following an injury, neurofilament proteins from the damaged neuro-axonal units are released proportional to the severity of injury into interstitial fluid and enters the CSF, where they can then be measured.[33] However, restricted by accessibility, frequent CSF NfL measurement is difficult. A novel Simoa assay which can reliably measure blood NfL (usually 50–100 times lower than CSF NfL) has been developed,[34] thus removing the barriers faced by CSF sampling. Preliminary data demonstrates that blood NfL correlates moderately to strongly with CSF NfL[34–38] across a variety of neurological disorders, including HIV disease[34,39–42] and a recent meta-analysis demonstrated moderate correlations between CSF and blood NfL, especially when blood NfL was measured using Simoa or electrochemiluminescence assays, further strengthening the evidence for blood NfL as a reliable surrogate marker for CSF NfL.[43]

The primary aim of our study was to determine whether stopping ART was associated with increased neuro-axonal injury. Secondary aims were to investigate associations between neuro-axonal injury, inflammation and HIV-1 reservoir size.

2. Materials and methods

2.1. Participants

Short Pulse Anti-Retroviral Therapy at Seroconversion (SPARTAC)[44] was a multicentre, randomised controlled trial comparing 12 weeks ART or 48 weeks ART, with deferred ART (standard of care at the time), amongst participants diagnosed within six months of HIV-1 seroconversion. HIV-1 viral load and $CD4^+$ count measurement was 12-weekly until $CD4^+$ count <350 cells/mm^3, reflecting international treatment guidelines at the time.[45] Stored plasma samples from participants allocated to the 48-week ART arm at baseline (before ART), week 48 (after 48-weeks ART) and week 60 (12-weeks after stopping ART) were assessed. Plasma samples from participants were aliquoted and stored at −80 °C in the Kings College London Infectious Diseases Biobank, before shipment to the UK Dementia Research Institute, University College London for analysis. All participants gave written informed consent for future use of their stored samples; the trial was approved by research ethics committees in each country.[44] Further detail on the SPARTAC study including participant characteristics has previously been described.[44]

2.2. Laboratory analyses

Samples from weeks 0, 48 and 60 were analysed at the UK Dementia Research Institute, University College London, UK using the NF-light assay on the HD-X Simoa instrument (Quanterix™, USA).[46]

Plasma D-dimer and IL-6 were measured previously in a subset of participants enrolled in Brazil, Australia, Italy and the United Kingdom at baseline, week 48 and week 60.[13] Total HIV-1 DNA from $CD4^+$ T-cells enriched from peripheral blood mononuclear cells was measured previously in participants with clade B virus at baseline and week 48.[20]

2.3. Data analysis

Statistical analyses were performed using Stata 17.0. P-values <0.05 were considered statistically significant. NfL was considered high if $>$ 10 pg/mL in participants aged <51 years, and if >15 pg/mL in participants 51–61 years.[34,47] The lower limit of quantification for HIV-1 RNA was <50 copies/mL, except in Africa, where it was <400 copies/mL which was the lower limit of detection using routine assays in Africa at the time of study. Longitudinal changes in NfL, D-dimer, IL-6 and total HIV-1 DNA were analysed using mixed models. Comparisons of high NfL between time-points was done using the exact McNemar test. Associations between baseline NfL with age, sex (sex assigned at birth), $CD4^+$ T-cell counts, $CD4^+/CD8^+$ ratio, plasma HIV-1 RNA, duration between seroconversion and randomisation, weight, creatinine clearance (Cockcroft-Gault formula), and in subgroups with D-dimer and IL-6, and total HIV-1 DNA data were analysed using linear regression. Correlations between NfL and laboratory parameters were assessed using Pearson's correlation. Missing values for baseline NfL ($n = 4$) and weight ($n = 5$) were imputed using multiple imputations by chained equations under the missing at random assumption, including all factors listed above in the imputation model and creating 20 imputed datasets. Predictors of change in NfL from baseline were analysed, adjusted for baseline NfL. A two-sample T-test of equal variance was performed to investigate whether NfL differed significantly in those with detectable

versus undetectable plasma HIV-1 RNA at all timepoints.

3. Results

Of 123 participants randomised to receive 48 weeks ART, 83 had stored plasma available from at least two timepoints and were included. Participant demographics are described in Table 1. Baseline characteristics of the participants included in this analysis were similar to the cohort of 123 participants allocated to 48 weeks ART.[44]

3.1. Longitudinal NfL

NfL geometric mean decreased from 6.9 (baseline) to 5.8 pg/mL (after 48 weeks ART), p = 0.006; (Table 2). There were no changes in NfL between weeks 48 and 60 despite plasma viral rebound in most during this time period (p = 0.70; Table 2). The proportion with high NfL was 17.7 % (14/79) at baseline, and 11.6 % (8/69), and 9.9 % (8/81) at weeks 48 and 60 (p = 0.31), respectively. 5/8 participants with high plasma NfL at week 48 also had so at week 60 (p = 1.0).

3.2. Factors associated with baseline NfL

In multivariable regression analysis, higher baseline NfL was independently associated with older age (0.13 [95% confidence interval (CI) 0.05, 0.21], $\log_{10}$ NfL per 10 years increase in age higher, p = 0.002) and baseline HIV-1 RNA (0.08 [95% CI 0.01, 0.14] $\log_{10}$ NfL per 1 $\log_{10}$ rise in plasma HIV-1 RNA higher, p = 0.020). No significant associations were seen between baseline NfL and sex (p = 0.56), baseline $CD4^+$ T-cell count (p = 0.28), creatinine clearance (p = 0.46) or weight (p = 0.88). Lower $CD4^+/CD8^+$ ratio and shorter time between seroconversion and randomisation were associated with higher NfL at baseline in univariable models, however, this was not significant when including HIV-1 RNA in the model (p = 0.23 and p = 0.10, respectively). None of the above factors were associated with change in NfL from baseline to week 48.

Table 1
Baseline characteristics of participants in the 48-week ART arm with plasma NfL measured at any time point (n = 83).

	All participants n = 83	Male n = 50	Female n = 33
Age, years	34 (27, 41)	36 (31, 46)	27 (22, 37)
Time from seroconversion to randomisation, weeks	13 (9, 15)	11 (7, 13)	14 (12, 17)
Weight, kg	73 (65, 82)	75 (68, 83)	65 (54, 79)
Creatinine clearance, mL/min	107 (97, 130)	113 (98, 128)	103 (81, 139)
Virus subtype			
- B	44 (53.0)	43 (86.0)	1 (3.0)
- C	26 (31.3)	1 (2.0)	25 (75.8)
- Other	13 (15.7)	6 (12.0)	7 (21.2)
Region			
- Europe[a]	42 (50.6)	39 (78.0)	3 (9.1)
- Africa[b]	30 (36.1)	0 (0)	30 (90.9)
- Australia & Brazil	11 (13.2)	11 (22.0)	0 (0)
Clinical manifestations of symptomatic HIV seroconversion illness	51 (61.5)	42 (84.0)	9 (27.3)
ART regimen initiated			
– 2 NRTI and bPI	75 (90.4)	42 (84.0)	33 (100)
– 2 NRTI and EFV	7 (8.4)	7 (14)	0 (0)
– 1 NRTI and bPI and T20	1 (1.2)	1 (2)	0 (0)

Values are median (IQR) or total (%).
ART = antiretroviral treatment, NfL = neurofilament light chain protein, NRTI = nucleoside reverse-transcriptase inhibitors, bPI = ritonavir-boosted protease inhibitor, EFV = efavirenz, T20 = enfuvirtide.
[a] Italy, Spain and United Kingdom.
[b] South Africa and Uganda.

Table 2
Clinical parameter trends over the study period.

	Week 0: Before starting ART	Week 48: After 48 weeks of ART	Week 60: 12 weeks after stopping ART
Plasma NfL, pg/mL[a]	N = 79 6.92 (5.97–8.01)	N = 69 5.77 (4.94–6.74)	N = 81 5.75 (5.08–6.52)
Plasma HIV-1 RNA, $\log_{10}$ copies/mL	N = 79 4.59 (4.03–5.18)	N = 68 1.70 (1.70–2.60)	N = 81 3.78 (2.82–4.51)
$CD4^+$ T-cell count, cells/μL	N = 79 608 (465–760)	N = 68 794 (597–995)	N = 80 714 (479–867)
CD4: CD8 T-cell ratio	N = 79 0.53 (0.38–0.82)	N = 68 0.98 (0.73–1.32)	N = 80 0.70 (0.47–1.00)
Subgroup analysis			
D-dimer, mg/L[a]	N = 44 0.36 (0.29–0.44)	N = 32 0.28 (0.24–0.33)	N = 45 0.31 (0.25–0.38)
IL-6, pg/mL[a]	N = 44 1.38 (1.08–1.78)	N = 34 1.42 (1.08–1.88)	N = 45 1.48 (1.22–1.78)
Total HIV DNA, $\log_{10}$ copies/10^6 $CD4^+$ T-cells	N = 45 3.79 (3.47–3.97)	N = 51 3.26 (3.09–3.44)	n/a

Values are median (IQR) unless stated otherwise.
n/a: not assessed at this timepoint.
[a] Values are geometric mean (95 % confidence interval).

HIV-1 RNA was <400 copies/mL in 7.6 % (6/79), 83.8 % (57/68), 24.7 % (20/81) of all participants at baseline, week 48, and week 60, respectively. Whereas participants with HIV-1 RNA ≥400 copies/mL had significantly higher NfL at baseline than participants with HIV-1 RNA <400 copies/mL (geometric mean 7.2 versus 4.0 pg/mL; p = 0.028), there was no significant difference at week 48 (5.2 versus 5.9 pg/mL; p = 0.57), or week 60 (6.0 versus 5.1 pg/mL; p = 0.29), respectively.

3.3. D-dimer, IL-6 and total HIV-1 DNA per $CD4^+$ T-cell analysis

D-dimer and IL-6 results were available in 48/83 (Table 2). D-dimer decreased significantly from baseline to week 48 (from geometric mean 0.36 to 0.28 mg/L, p = 0.017), with no further change to week 60 (p = 0.46). D-dimer significantly correlated with NfL at baseline (r = 0.66, p < 0.001), week 48 (r = 0.45, p = 0.010) and week 60 (r = 0.53, p < 0.001). Baseline D-dimer was also associated with baseline NfL (0.53 [95% CI 0.20, 0.85] $\log_{10}$ NfL per 1 $\log_{10}$ rise in D-dimer, p = 0.002) when included into a multivariable model with age and baseline HIV-1 RNA as independent factors, whereas there was no association between NfL and HIV-1 RNA (p = 0.91). In contrast, there was no change in IL-6 between baseline, week 48 and week 60 (Table 2), and there was no significant association between NfL and IL-6 at any timepoint. Total HIV-1 DNA results were available in 51/83 participants (Table 2); total HIV-1 DNA decreased significantly between baseline and week 48 (from geometric mean 5689 to 1730 copies/10^6 $CD4^+$ T-cells; p < 0.001, Table 2). However, there was no significant association between NfL and total HIV-1 DNA at baseline (r = 0.13, p = 0.40) or week 48 (r = 0.24, p = 0.13), or when analysing the two variables as change from baseline (r = 0.33, p = 0.064).

4. Discussion and conclusion

From this large, international cohort of individuals randomly allocated to interrupt ART initiated during PHI, we observed that despite evidence of neuro-axonal injury (using plasma NfL) during untreated PHI, we saw no evidence of recurrence in neuro-axonal injury up to 12 weeks post-ART interruption. Our results are in keeping with the study

in individuals with treated chronic HIV-1 disease, where following ATI, no evidence of increased neuronal injury was demonstrated at the first point of plasma HIV-1 RNA >1000 copies/mL.[48]

Data suggests that during very early HIV-1 infection, neuronal injury is often delayed compared to viral and inflammatory changes[49]; no detectable rise in CSF NfL was seen in participants with hyperacute HIV-1 infection[50] whereas CSF NfL was elevated in half of individuals several months after acquiring HIV-1.[51] A study of eight individuals on suppressive ART initiated during chronic infection who interrupted ART, demonstrated that while none developed neurological symptoms, three experienced significant rises in CSF NfL.[52] Taken together, evidence suggests that when closely monitored, short periods of ATIs are safe from a neurological perspective.

The upper limit of normal for plasma NfL is age-dependant[34,47]; in our study the proportion of participants with plasma NfL above the threshold considered normal remained similar and low across all timepoints. This may reflect that amongst these individuals, there is little neuronal injury due to the short duration of infection.

Our results are in keeping with published data demonstrating a positive association between NfL with age and plasma HIV-1 RNA.[34] While we saw a positive association between NfL and D-dimer (biomarker of pro-coagulation), we saw no evidence of an association with IL-6 (pro-inflammatory cytokine). Data from SPARTAC demonstrated that within 4 weeks of interrupting ART initiated during PHI, 78% had detectable plasma HIV-1 RNA ≥400 copies/mL,[53] plasma biomarkers of inflammation (IL-6 and D-dimer) which decreased during ART had returned to pre-ART levels[13] and plasma HIV-1 RNA strongly correlated with plasma D-dimer.[54] These findings suggest a biological explanation why viral transcription might lead to a pro-coagulation and pro-inflammatory milieu, resulting in neuro-axonal injury.

Strengths of our study include the protocol-indicated ART interruption, enabling us to assess the impact of ATI without risk of bias through confounding by indication. SPARTAC was an international study with 40 % female participants, thus our results are uniquely generalisable. Limitations include the relatively short follow-up period after stopping ART and not all participants had D-dimer, IL-6, and total HIV-1 DNA results available. Stronger correlations between CSF and blood NfL have been reported in conditions with higher CSF and blood NfL concentrations.[34–37] However, plasma NfL concentrations were generally low across all timepoints in this sub-study. Evidence suggests that the correlation between blood and CSF NfL is lower at lower NfL concentrations, thus the current assays may still be insufficiently sensitive to detect changes at these low concentrations, due to low signal-to-noise ratio. Furthermore, the lack of concurrent CSF NfL limits our knowledge about parallel trends in CSF NfL during this time period. Data on underlying comorbidities in the participants throughout the study period were not available to us, thus potential confounding factors which may have independently affected NfL concentrations including central and peripheral neurological conditions, could not be controlled or accounted for. Of note, the participants enrolled into the SPARTAC study were a relatively young cohort (see Table 1), and the prevalence of comorbidities in this population is expected to be generally low.

Our overall results are reassuring, but it is unclear whether they can be extrapolated to other populations, such as those undergoing HIV eradication strategies followed by ATI, with low nadir $CD4^+$ counts, chronic HIV infection or receiving more contemporary antiretroviral regimens.

When using plasma NfL as a surrogate marker, we observed a decrease in neuro-axonal injury in a cohort of participants following ART initiation during PHI, with no evidence of neuro-axonal injury rebound following ART interruption for up to 12 weeks. The ability to identify individuals undergoing ATI experiencing neuro-axonal injury with a blood test may be invaluable for ATI monitoring.

Sources of funding

This study is independent research funded by a grant awarded by the British HIV Association (BHIVA) Grant reference number: A1Algaratnam.

The SPARTAC trial was funded by the Wellcome Trust (grant reference number: 069598/Z/02/Z) and supported by GlaxoSmithKline plc and Abbott Laboratories. SPARTAC Controlled-Trials.com number: ISRCTN76742797 and EudraCT number: 2004-000446-20.

A Wellcome Trust Multi-User Equipment grant to HZ and AH funded the instrument used for the biomarker measurements.

Data statement

Data and materials may be made available upon reasonable request in writing to the corresponding author.

CRediT authorship contribution statement

Jasmini Alagaratnam: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wolfgang Stöhr:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Elizabeth Hamlyn:** Writing – review & editing, Formal analysis. **Kholoud Porter:** Writing – review & editing, Methodology, Formal analysis. **Jamie Toombs:** Writing – review & editing, Investigation. **Amanda Heslegrave:** Writing – review & editing, Investigation. **Henrik Zetterberg:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Magnus Gisslén:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **Jonathan Underwood:** Writing – review & editing. **Mauro Schechter:** Writing – review & editing, Investigation. **Pontiano Kaleebu:** Writing – review & editing, Investigation. **Giuseppe Tambussi:** Writing – review & editing, Investigation. **Sabine Kinloch:** Writing – review & editing, Investigation. **Jose M. Miro:** Writing – review & editing, Investigation. **Anthony D. Kelleher:** Writing – review & editing, Investigation. **Abdel Babiker:** Writing – review & editing. **John Frater:** Writing – review & editing, Investigation. **Alan Winston:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Sarah Fidler:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Jasmini Alagaratnam has received support to attend scientific conferences and received speaker's fees from MSD and Gilead Sciences and has received research grant funding from Gilead Sciences. Kholoud Porter has received funding from ViiV healthcare, Gilead Sciences and MSD. Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (outside submitted work). Henrik Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018–02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931 and #ALFGBG-717531)) and the UK Dementia Research Institute at UCL. Magnus Gisslen is supported by grants from the Swedish Research Council (#2021–05405 & #2021–06545), Swedish State Support for Clinical Research (#ALFGBG-965885 and #ALFGBG-717531), and by SciLifeLab from the Knut and Alice Wallenberg Foundation (#2020.0182 &

#2020.0241), research grants from Gilead Sciences and honoraria as speaker, DSMB committee member, and/or scientific advisor from Amgen, AstraZeneca, Biogen, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline/ViiV, Janssen-Cilag, MSD, Novocure, Novo Nordic, Pfizer and Sanofi. Jonathan Underwood has received honoraria for preparation of educational materials and has served on advisory boards for Gilead Sciences and Viiv Healthcare and is supported by the Medical Research Council [grant number MR/T023791/1]. Mauro Schechter has served on advisory Boards, received honoraria as speaker and received research grants from Gilead Sciences, Janssen, Merck, GSK/ViiV. Sabine Kinloch has received consultant honoraria from Janssen and Viiv. Jose M Miro has received consulting honoraria and/or research grants from AbbVie, Angelini, Contrafect, Cubist, Genentech, Gilead Sciences, Janssen, Lysovant, Medtronic, MSD, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. Jose M Miro received a personal 80:20 research grant from Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, during 2017–23. Anthony D Kelleher has received support for clinical research and diagnostic assays from GSK/Viiv and MSD, received speaker's fees from Gilead Sciences, received support of patents from Cytognos, CSL (all payments made to Institution). Alan Winston has received honoraria or research grants on behalf of Imperial College London or been a consultant or investigator in clinical trials sponsored by Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Roche and ViiV Healthcare. Sarah Fidler has received honoraria or research grants on behalf of Imperial College London or been a consultant or investigator in clinical trials sponsored by Gilead Sciences and ViiV Healthcare. For the remaining authors, none were declared.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank the following groups and individuals for their contributions (listed alphabetically):

Brighton and Sussex University Hospitals NHS Trust, Brighton, UK

- Martin Fisher (in memoriam)

Kirby Institute, Sydney, Australia

- David Cooper (in memoriam)

HIV Prevention Research Unit, South African Medical Research Council

- Gita Ramjee (in memoriam)

Imperial College London, London, UK

- Jonathan Weber
- Hanna Box

Imperial College National Institute of Health Research (NIHR) Biomedical Research Centre (BRC) for infrastructure support.

Infectious Diseases BioBank, King's College London

- Christine Mant

University of Oxford

- Helen Brown
- Nicola Robinson
- Matthew Pace

References

1. May MT, Gompels M, Delpech V, et al. Impact on life expectancy of HIV-1 positive individuals of CD4+ cell count and viral load response to antiretroviral therapy. *AIDS*. 2014;28:1193–1202.
2. Davey RT, Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci U S A*. 1999. https://doi.org/10.1073/pnas.96.26.15109. Published Online First.
3. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity*. 2012. https://doi.org/10.1016/j.immuni.2012.08.010.
4. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*. 2006. https://doi.org/10.1056/NEJMoa062360. Published Online First.
5. Julg B, Dee L, Ananworanich J, et al. Recommendations for analytical antiretroviral treatment interruptions in HIV research trials—report of a consensus meeting. In: *The Lancet HIV*. 2019. https://doi.org/10.1016/S2352-3018(19)30052-9.
6. Gray LR, Roche M, Flynn JK, Wesselingh SL, Gorry PR, Churchill MJ. Is the central nervous system a reservoir of HIV-1? *Curr Opin HIV AIDS*. 2014. https://doi.org/10.1097/COH.0000000000000108.
7. Churchill MJ, Cowley DJ, Wesselingh SL, Gorry PR, Gray LR. HIV-1 transcriptional regulation in the central nervous system and implications for HIV cure research. *J Neurovirol*. 2015. https://doi.org/10.1007/s13365-014-0271-5. Published Online First.
8. Hellmuth J, Valcour V, Spudich S. CNS reservoirs for HIV: implications for eradication. *J virus Erad*. 2015;1:67–71.
9. Joseph SB, Arrildt KT, Sturdevant CB, Swanstrom R. HIV-1 target cells in the CNS. *J Neurovirol*. 2015. https://doi.org/10.1007/s13365-014-0287-x. Published Online First.
10. Wong JK, Yukl SA. Tissue reservoirs of HIV. *Curr Opin HIV AIDS*. 2016;11:362–370.
11. Gianella S, Pond SLK, Oliveira MF, et al. Compartmentalized HIV rebound in the central nervous system after interruption of antiretroviral therapy. *Virus Evol*. 2016. https://doi.org/10.1093/ve/vew020. Published Online First.
12. Kincer LP, Joseph Sarah Beth Gilleece MM, Hauser BM, et al. Rebound HIV-1 in cerebrospinal fluid after antiviral therapy interruption is mainly clonally amplified R5 T cell-tropic virus. *Nat Microbiol*. 2023:260–271.
13. Hamlyn E, Stöhr W, Cooper DA, et al. The effect of short-course antiretroviral therapy initiated in primary HIV-1 infection on interleukin-6 and D-dimer levels. *AIDS*. 2015. https://doi.org/10.1097/QAD.0000000000000675. Published Online First.
14. Kempuraj D, Thangavel R, Natteru PA, et al. Neuroinflammation induces neurodegeneration. *J Neurol Neurosurg spine*. 2016.
15. Gveric D. Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. *Brain*. 2003. https://doi.org/10.1093/brain/awg167. Published Online First.
16. Blokhuis C, Peeters CFW, Cohen S, et al. Systemic and intrathecal immune activation in association with cerebral and cognitive outcomes in paediatric HIV. *Sci Rep*. 2019. https://doi.org/10.1038/s41598-019-44198-z. Published Online First.
17. Ananworanich J, Chomont N, Eller LA, et al. HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early ART. *EBioMedicine*. 2016;11:68–72.
18. Burbelo PD, Price RW, Hagberg L, et al. Anti-human immunodeficiency virus antibodies in the cerebrospinal fluid: evidence of early treatment impact on central nervous system reservoir? *J Infect Dis*. 2018. https://doi.org/10.1093/infdis/jix662. Published Online First.
19. Gisslén M, Hunt PW. Antiretroviral treatment of acute HIV infection normalizes levels of cerebrospinal fluid markers of central nervous system (CNS) inflammation: a consequence of a reduced CNS reservoir? *J Infect Dis*. 2019. https://doi.org/10.1093/infdis/jiz031.
20. Williams JP, Hurst J, Stöhr W, et al. HIV-1 DNA predicts disease progression and post-treatment virological control. *Elife*. 2014;3, e03821.
21. Assoumou L, Weiss L, Piketty C, et al. A low HIV-DNA level in peripheral blood mononuclear cells at antiretroviral treatment interruption predicts a higher probability of maintaining viral control. *AIDS*. 2015. https://doi.org/10.1097/QAD.0000000000000734. Published Online First.
22. Henrich TJ, Hanhauser E, Marty FM, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann Intern Med*. 2014. https://doi.org/10.7326/M14-1027. Published Online First.
23. Rasmussen TA, Tolstrup M, Moller HJ, et al. Activation of latent HIV by the histone deacetylase inhibitor panobinostat: a pilot study to assess effects on the CNS. *Open Forum Infect Dis*. 2015;2, ofv037.
24. Chan P, Ananworanich J. Perspective on potential impact of HIV central nervous system latency on eradication. *AIDS*. 2019. https://doi.org/10.1097/QAD.0000000000002264. Published Online First.
25. Hellmuth J, Muccini C, Colby DJ, et al. Central nervous system safety during brief analytic treatment interruption of antiretroviral therapy within four HIV remission trials: an observational study in acutely treated people living with HIV. *Clin Infect Dis*. 2020. https://doi.org/10.1093/cid/ciaa1344. Published Online First.
26. Hsu DC, Silsorn D, Inthawong D, et al. Impact of analytical treatment interruption on the central nervous system in a simian-HIV model. *AIDS*. 2019;33. https://doi.org/10.1097/QAD.0000000000002270.
27. Wen Y, Bar KJ, Li JZ. Lessons learned from HIV antiretroviral treatment interruption trials. *Curr Opin HIV AIDS*. 2018;13. https://doi.org/10.1097/COH.0000000000000484.
28. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018. https://doi.org/10.1038/s41582-018-0058-z.
29. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry*. 2019. https://doi.org/10.1136/jnnp-2018-320106.
30. Bridel C, Van Wieringen WN, Zetterberg H, et al. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. *JAMA Neurol*. 2019. https://doi.org/10.1001/jamaneurol.2019.1534. Published Online First.
31. Yilmaz A, Blennow K, Hagberg L, et al. Neurofilament light chain protein as a marker of neuronal injury: review of its use in HIV-1 infection and reference values for HIV-negative controls. *Expert Rev Mol Diagn*. 2017;17:761–770.

32. Peterson J, Gisslen M, Zetterberg H, et al. Cerebrospinal fluid (CSF) neuronal biomarkers across the spectrum of HIV infection: hierarchy of injury and detection. *PLoS One*. 2014. https://doi.org/10.1371/journal.pone.0116081. Published Online First.
33. Abdulle S, Mellgren Å, Brew BJ, et al. CSF neurofilament protein (NFL) - a marker of active HIV-related neurodegeneration. *J Neurol*. 2007;254:1026–1032.
34. Gisslén M, Price RW, Andreasson U, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. *EBioMedicine*. 2015. https://doi.org/10.1016/j.ebiom.2015.11.036.
35. Marques TM, Van Rumund A, Oeckl P, et al. Serum NFL discriminates Parkinson disease from atypical parkinsonisms. *Neurology*. 2019. https://doi.org/10.1212/WNL.0000000000007179. Published Online First.
36. Mattsson N, Andreasson U, Zetterberg H, et al. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA Neurol*. 2017. https://doi.org/10.1001/jamaneurol.2016.6117. Published Online First.
37. Novakova L, Zetterberg H, Sundström P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology*. 2017. https://doi.org/10.1212/WNL.0000000000004683. Published Online First.
38. Alagaratnam J, De Francesco D, Zetterberg H, et al. Correlation between cerebrospinal fluid and plasma neurofilament light protein in treated HIV infection: results from the COBRA study. *J Neurovirol*. 2022;28. https://doi.org/10.1007/s13365-021-01026-3.
39. Bergman J, Dring A, Zetterberg H, et al. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS. *Neurol - Neuroimmunol Neuroinflammation*. 2016. https://doi.org/10.1212/nxi.0000000000000271. Published Online First.
40. Novakova L, Zetterberg H, Sundström P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology*. 2017. https://doi.org/10.1212/WNL.0000000000004683. Published Online First.
41. Hansson O, Janelidze S, Hall S, et al. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder. *Neurology*. 2017. https://doi.org/10.1212/WNL.0000000000003680. Published Online First.
42. Meeter LH, Dopper EG, Jiskoot LC, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. *Ann Clin Transl Neurol*. 2016. https://doi.org/10.1002/acn3.325. Published Online First.
43. Alagaratnam J, von Widekind S, De Francesco D, et al. Correlation between CSF and blood neurofilament light chain protein: a systematic review and meta-analysis. *BMJ Neurol Open*. 2021;3. https://doi.org/10.1136/bmjno-2021-000143.
44. Fidler S, Porter K, Ewings F, et al. Short-course antiretroviral therapy in primary HIV infection. *N Engl J Med*. 2013. https://doi.org/10.1056/NEJMoa1110039. Published Online First.
45. World Health Organization. *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach*. 2010, 2010 Revision.
46. Sheet SD, Simoa TM. *NF-Light*. Advantage Kit Data Sheet; 2017:1–2.
47. Simrén J, Andreasson U, Gobom J, et al. Establishment of reference values for plasma neurofilament light based on healthy individuals aged 5-90 years. *Brain Commun*. 2022;4:4.
48. De Scheerder MA, Van Hecke C, Zetterberg H, et al. Evaluating predictive markers for viral rebound and safety assessment in blood and lumbar fluid during HIV-1 treatment interruption. *J Antimicrob Chemother*. 2020. https://doi.org/10.1093/jac/dkaa003. Published Online First.
49. Spudich S, Peterson J, Fuchs D, Price RW, Gisslen M. Potential for early antiretroviral therapy to reduce central nervous system HIV-1 persistence. *AIDS*. 2019. https://doi.org/10.1097/QAD.0000000000002326. Published Online First.
50. Peluso MJ, Valcour V, Ananworanich J, et al. Absence of cerebrospinal fluid signs of neuronal injury before and after immediate antiretroviral therapy in acute HIV infection. *J Infect Dis*. 2015;212:1759–1767.
51. Peluso MJ, Meyerhoff DJ, Price RW, et al. Cerebrospinal fluid and neuroimaging biomarker abnormalities suggest early neurological injury in a subset of individuals during primary HIV infection. *J Infect Dis*. 2013;207:1703–1712.
52. Gisslen M, Rosengren L, Hagberg L, Deeks SG, Price RW. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. *AIDS Res Ther*. 2005;2:6.
53. Hamlyn E, Ewings FM, Porter K, et al. Plasma HIV viral rebound following protocol-indicated cessation of ART commenced in primary and chronic HIV infection. *PLoS One*. 2012. https://doi.org/10.1371/journal.pone.0043754. Published Online First.
54. Hamlyn E, Fidler S, Stöhr W, et al. Interleukin-6 and D-dimer levels at seroconversion as predictors of HIV-1 disease progression. *AIDS*. 2014. https://doi.org/10.1097/QAD.0000000000000155. Published Online First.