BMJ Open

CTN 328: immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV): protocol for an observational cohort study

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To cite: Costiniuk CT, Singer J, Langlois M-A, *et al*. CTN 328: immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV): protocol for an observational cohort study. *BMJ Open* 2021;**11**:e054208. doi:10.1136/bmjopen-2021-054208

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-054208).

Received 05 June 2021
Accepted 25 November 2021

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ABSTRACT

Introduction Most existing vaccines require higher or additional doses or adjuvants to provide similar protection for people living with HIV (PLWH) compared with HIV-uninfected individuals. Additional research is necessary to inform COVID-19 vaccine use in PLWH.

Methods and analysis This multicentred observational Canadian cohort study will enrol 400 PLWH aged ≥16 years from Montreal, Ottawa, Toronto and Vancouver. Subpopulations of PLWH of interest will include individuals: (1) >55 years of age; (2) with CD4 counts <350 cells/mm^3; (3) with multimorbidity (≥2 comorbidities) and (4) 'stable' or 'reference' PLWH (CD4 T cells >350 cells/mm^3, suppressed viral load for ≥6 months and ≤1 comorbidity). Data for 1000 HIV-negative controls will be obtained via a parallel cohort study (Stop the Spread Ottawa), using similar time points and methods. Participants receiving ≥1 COVID-19 vaccine will attend five visits: prevaccination; 1 month following the first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose. The primary end point will be the percentage of PLWH with COVID-19-specific antibodies at 6 months following the second vaccine dose. Humoral and cell-mediated immune responses, and the interplay between T cell phenotypes and inflammatory markers, will be described. Regression techniques will be used to compare COVID-19-specific immune responses to determine whether there are differences between the 'unstable' PLWH group (CD4 <350 cells/mm3), the stable PLWH cohort and the HIV-negative controls, adjusting for factors believed to be associated with immune response. Unadjusted analyses will reveal whether there are differences in driving factors associated with group membership.

Ethics and dissemination Research ethics boards at all participating institutions have granted ethics approval for this study. Written informed consent will be obtained from all study participants prior to enrolment. The findings will inform the design of future COVID-19 clinical trials, dosing strategies aimed to improve immune responses and guideline development for PLWH.

Strengths and limitations of this study

- The largest and most comprehensive immunogenicity study in people living with HIV in Canada receiving COVID-19 vaccination.
- Emphasis on recruiting participants frequently excluded from pharmaceutical company-sponsored trials and those most likely to have poor outcomes following COVID-19 infection (including individuals of older age, immune non-responders and persons with multimorbidity).
- Assays used will enable differentiation between individuals with immunity from natural COVID-19 infection versus vaccine-induced immunity, in addition to detection of immunity towards key variants of concern.
- Involvement of community members from study conception to protocol development and study implementation.
- Limitations include relatively late study start, recruitment restricted to major urban centres and variations in timing between vaccine doses among participants.

Trial registration number NCT04894448.

BACKGROUND

In Canada today, an estimated 67 000 people are living with HIV (PLWH), 30% of whom are immune non-responders,[1] defined as achieving undetectable HIV viral levels without robust CD4 T cell count recovery (<350 cells/mm^3). Even with fully suppressed viral load on antiretroviral therapy (ART), chronic HIV infection is characterised by a low-grade elevation in pro-inflammatory and procoagulant biomarkers linked with higher mortality.[2–4]

Poor immunogenicity to common vaccines, including influenza,[5] pneumococcal,[6 7] meningococcal[6 7] and hepatitis A[8–10] and B vaccines,[11–13] is well-documented in PLWH with low CD4 T cell counts (<200 cells/mm^3) and unsuppressed viral loads.[14–16] PLWH face other intersecting vulnerabilities that increase their risk of SARS-CoV-2 acquisition and symptomatic/severe COVID-19; they commonly belong to low socioeconomic or racialized groups disproportionately affected by COVID-19 and have higher rates of risk factors for severe COVID-19 disease (eg, multiple chronic comorbidities).[17–19] Yet, this priority population has been understudied in COVID-19 vaccine clinical trials.[3 20] Most HIV-seropositive participants enrolled in COVID-19 vaccine trials had normal CD4 T cell counts (>500 cells/mm^3) and few comorbidities.[21 22] As such, the immunogenicity results may not represent the wide spectrum of PLWH who are followed in Canadian centres today.

For the AstraZeneca/Oxford COVID-19 vaccine trial (ChAdOx1, n=160 PLWH) inclusion criteria specified younger age (<55 years old) and high CD4 T cell count (>350 cells/mm^3) while excluding medical comorbidities (eg, heart, kidney, liver, respiratory diseases, etc).[21] The data obtained from PLWH were not included in the primary publication.[21] The Moderna trial included HIV-positive participants (n=176 PLWH) with CD4 T cell count ≥350 cells/mm^3 and an undetectable HIV viral load within the past year.[23] COVID-19 infection developed in 11 PLWH who received placebo but none who received the Moderna vaccine. The Johnson & Johnson trial (n=1218 PLWH) included participants with 'stable/well-controlled HIV infection' (defined as CD4 T cell counts ≥300 cells/µL within 6 months prior to screening and documented HIV viral load <50 copies/mL within 6 months prior to screening) but excluded participants with ongoing and progressive comorbidities associated with HIV infection.[24] COVID-19 infection developed in two vaccinated PLWH and four PLWH given placebo. The Novavax trial, conducted in South Africa, excluded PLWH with chronic cardiovascular disease (CVD), gastrointestinal disease, liver disease, renal disease, endocrine disorder and neurological illness, as well as participants with very high body mass index (≥40 kg/m^2).[22] As reported by Shinde *et al*, efficacy of the NVX-CoV2373 COVID-19 vaccine against the B.1.351 variant was examined in 1857 individuals in South Africa, of whom 30% (500 individuals) had HIV infection.[25] The vaccine efficacy estimate in baseline seronegative HIV-negative participants was 52.2% (95% CI −24.8 to 81.7). During the first 60 days of follow-up, the incidence of COVID-19 in HIV-negative placebo participants (5.3% (95% CI 4.3 to 6.6) was comparable to the incidence in PLWH placebo participants with HIV (5.2% (95% CI 3.6 to 7.2)).[26] Among HIV-negative participants, there were four and two cases of symptomatic COVID-19 among NVX-CoV2373 and placebo recipients, respectively (n<109 in each group).[26] No cases were observed in the baseline HIV-positive population (n<33 in each group).[26] Among 94% of participants without HIV, vaccine efficacy was 60.1%. The study was not powered to detect efficacy in the small population of PLWH.[26]

While several other trials included PLWH, they excluded their data from primary publication.[20 21 27] In a recent report by Ruddy *et al*, which examined COVID-19 antibody response in 12 PLWH with a median of 21 days (IQR 17–27) following the first dose of mRNA vaccine (50% Moderna and 50% Pfizer), antibodies were detected in all participants, although lower levels were observed in persons with lower CD4 T cell counts.[26] In this study, all 12 individuals were male, 8% were non-white, all had been on ART ≥6 months and 92% had an undetectable HIV viral load. Two individuals had a CD4 T cell count below 200 cell/mm^3.[26]

We lack robust data on vaccine immunogenicity and immune response durability in subpopulations of PLWH. No evidence is available on the durability of immunogenicity in PLWH beyond 3 months following vaccination. Since the hallmark of HIV infection is a reduced number and function of CD4 T cells, and cell-mediated immunity has emerged as a critical aspect of the COVID-19 immune response,[28–30] it is critical to characterise cellular immune and cytotoxic T cell responses to COVID-19 vaccination.[31–33]

Given that inflammatory markers may influence immune cell activation status and shift cell profiles towards either T helper (Th)1 or Th2 responses, impacting vaccine-elicited immune response,[34] understanding the interplay between immune activation and dysfunction is also important. To address this need, we are establishing a pan-Canadian prospective cohort of PLWH receiving COVID-19 vaccines to assess humoral and cellular immunogenicity and to describe the inflammatory milieu in this context. Safety and tolerability of COVID-19 vaccines in this cohort of PLWH will also be captured. Of note, COVID-19 vaccines currently approved for use in Canada include those manufactured by Pfizer, Moderna, AstraZeneca and Janssen (Johnson & Johnson) (https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/drugs-vaccines-treatments/authorization/list-drugs.html). Since the beginning of the vaccine rollout, Pfizer and Moderna vaccines were administered most often as they were the first to gain approval by Health Canada. Although approved, due to concerns associated with cerebral venous sinus thrombosis and vaccine-induced immune thrombotic thrombocytopenia, use of the AstraZeneca COVID-19 vaccine has been restricted in some provinces.

Study objectives

Primary objective

To evaluate the immunogenicity of COVID-19 vaccination in PLWH, by specific immunoglobulin G antibody ELISA, at 6 months following second vaccine dose.

Secondary objectives

1. To assess neutralisation capacity of COVID-19-specific IgG at 6 months following second vaccine dose.
2. To assess the durability of COVID-19-specific IgG response in PLWH at 12 months following vaccination.
3. To examine changes in the proportion and activation phenotype of CD4 T cells, CD8 T cells, B cells, natural

killer cells and monocytes, including gene expression and cytokine production, pre-COVID-19 and post-COVID-19 vaccination at 6 months following second vaccine dose.
4. To determine safety and tolerability of COVID-19 vaccines in PLWH, based on local or systemic adverse events following first or second injections.

Exploratory objectives

1. To determine if subpopulations of PLWH respond differently to COVID-19 vaccination. Subpopulations of interest include (1) PLWH >55 years of age; (2) immune non-responders (ART treated, and fully suppressed HIV RNA (<40 copies/mL), but CD4 T cell counts below 350 cells/mm^3 and CD4/CD8 T cell ratio <0.75); (3) PLWH with multimorbidity (two or more chronic diseases) and (4) PLWH 'reference' participants (with CD4 T cells >350 cells/mm^3, suppressed viral load for at least 6 months and have at most one comorbidity) (*note: groups will not be mutually exclusive but will likely have overlapping characteristics*).
2. To investigate if current COVID-19 vaccines elicit IgG that cross-recognise key COVID-19 variants of concern (VoC), and if this differs in PLWH compared with individuals without HIV.
3. To compare virus-specific T cell responses generated by COVID-19 vaccines in PLWH and compare results with HIV-negative populations.

METHODS AND ANALYSIS

Study design

This study is a multicentre prospective observational cohort study. Approximately 400 PLWH aged ≥16 years will be recruited from 4 sites in 3 Canadian provinces including (1) McGill University Health Centre (Montreal), (2) The Ottawa Hospital, (3) The University Health Network (Toronto) and (4) St. Paul's Hospital (Vancouver). These sites were selected since these siteshave four of the largest HIV clinics in Canada and established research infrastructures to support the recruitment, enrolment and follow-up of a high volume of diverse study participants. These sites also have strong track records for rapid enrolment of participants in CIHR Canadian HIV Trials Network (CTN) studies. Sites provide HIV care for many clients who are visible minorities and who have multiple comorbidities. These sites will recruit a study population representative of PLWH most likely to be impacted by detrimental COVID-19-related outcomes.[19] Data for HIV-negative individuals will be obtained from the Stop the Spread Ottawa (SSO) cohort. Since we will not perform additional analyses on the samples of the SSO cohort, we can include all the participants in the SSO study and matching of HIV-negative and HIV-positive participants will not be required.

Determination of which vaccine is administered at any point in time and to which individuals is dictated by Canada's provincial governments, with input from the National Advisory Committee on Immunization (NACI), and is not influenced by study investigators or staff. We will include participants irrespective of the specific type of COVID-19 vaccine. Furthermore, the duration of the interval between first and second doses time from when the vaccine was administered will not influence eligibility, since Canada has decided to extend dose intervals for all two dose vaccines to 4 months. However, the duration of interval between vaccine doses will be included as an outcome variable.

Methods: participants, intervention and comparator and outcomes

Inclusion criteria

(1) Age ≥16 years; (2) HIV-positive for HIV group, immunocompetent and generally in good health for the HIV-negative group and (3) receiving ≥1 dose of COVID-19 vaccine. Persons are still eligible to participate if they have already received one or two vaccine doses.

Exclusion criteria

(1) Receipt of any blood product or immunoglobulin preparation within 1 month of vaccine administration and until study completion; (2) signs/symptoms of active COVID-19 at the time of enrolment; (3) for the HIV-negative group: immunocompromised state or on immunosuppressant medications. Prior receipt of other vaccines <12 months or past COVID-19 infection is not an exclusion criteria but will be recorded. Detectable HIV viral load on ART is not exclusionary.

The following groups of PLWH will be prioritised for study enrolment:

1. *Older age* (55 years and above). Older age is associated with immunosenescence and results in lower vaccine efficacy.[35–37] We have selected 55 as the specific age cut-off since PLWH tend to develop comorbidities at an earlier age.
2. *Immune non-responders* (CD4 T cell count <350 cells/mm^3, CD4/CD8 <0.75 with undetectable viral load for 1+ year). Immune non-responders may be at risk of more adverse COVID-19-related outcomes than HIV immune responders.[38–41]
3. *Multimorbidity* (defined as having ≥2 comorbidities). Comorbidities may include CVD, co-infection, hypertension, dyslipidaemia, diabetes, chronic obstructive pulmonary disease and obesity among PLWH[2 42–45] and factors that contribute to worse outcomes with COVID-19.[46]
4. *HIV-positive 'stable' or 'reference'* group. These persons will have undetectable HIV viral load for >6 months, CD4 T cell counts >350 cells/mm^3 and a maximum of one comorbidity. To capture the full spectrum of individuals in the HIV-negative group, we will include this HIV-positive 'stable' group so that we can determine whether there are particular characteristics within PLWH which impact on immune response. In comparing the stable and unstable groups, we will be able to determine whether participants with low CD4

Table 1 Visits and procedure schedule

Visit number	1* (screen)	Vaccine	2	Vaccine	3†	4†	5†
Week number	–12 to 0 weeks	0	4 weeks		3 mo after dose 2	6 mo after dose 2	12 mo after dose 2
Window	–3 mo						
Inclusion/Exclusion	X		X				
Informed consent	X						
Medical history	X						
Blood draw: immunology‡	X		X		X	X	X
Blood draw: CD4/viral load§	X		X		X	X	X
Vaccination¶		X		X			
Participant diary**		X	X	X	X		
CITF†† questionnaire	X		X		X	X	X
Adverse events‡‡			X		X		
Concomitant meds	X		X		X	X	X

For participants who develop COVID-19 symptoms 14+ days following vaccination. Participants will be asked to complete the COVID-19 Symptom Survey (online supplemental materials) and to go for a COVID-19 test at their nearest test centre and notify the study staff of their test result. If positive for COVID-19, the study staff will mail the participant six saliva collection kits by courier in order to collect information on SARS-CoV-2 variants.
Participants who have already received one vaccine dose: these individuals may be enrolled in the study at any duration of time post first dose as long as the baseline blood drawn is before the second booster. Visits 1 and 2 will be combined.
Participants who have already received two vaccine doses: these individuals must be enrolled in the study within 3 months of their second dose. Visits 1 and 3 will be combined and visit 2 will not be required. Participants will follow-up at visits 4 and 5.
*Screening assessment may be performed same day as vaccination but will be completed prior to vaccination.
†Visits 3, 4, 5 will be conducted at 3, 6, 12 months after dose 1, respectively, for COVID-19 vaccines administered as a one-dose schedule.
‡Immunoglobulin levels, flow cytometry and cytokine secretion (immunogenicity measures); blood will be collected at each visit. For participants who have already received a vaccine dose prior to study enrolment, 'baseline' immunoglobulin levels (ie, prevaccination) may not be available.
§Blood work such as CD4 and viral load can be collected as part of standard of care.
¶Participants will receive the COVID-19 vaccine outside of the study per standard of care as part of their provincial immunisation programme.
**Participants will be given a printed diary after vaccination during which they will record their vaccine reactions, oral temperature and any febrile respiratory tract symptoms as well as general changes to health and medications. The diary will be evaluated up to 30 days following each injection.
††The full CITF questionnaire will be completed at visit 1 and the modified CITF questionnaire will be completed at subsequent visits.
‡‡Adverse events will only be collected at 7 and 30 days postvaccination and for participants who receive a vaccine while currently enrolled in the study (ie, adverse events will not be collected retrospectively).
CITF, COVID-19 Immunity Task Force; mo, month.

counts (<350 cells/mm^3) differ in their response to vaccine from those with normal CD4 T cell counts while controlling for other characteristics. We aim to enrol HIV-negative and HIV-positive 'stable' individuals with overlapping characteristics (ie, some should have multiple comorbidities) so that the groups are comparable.

General methodology and participant timeline

Participants will attend 5 visits over 12 months: prevaccination; 1 month following first vaccine dose; and at 3, 6 and 12 months following the second vaccine dose (table 1). Each visit will last between 20 and 60 min.

Primary end point

Percentage of PLWH with COVID-19-specific antibodies at 6 months following second vaccine dose.

Secondary end points

Percentage of individuals with (1) COVID-19 neutralisation capacity at 6 months following second vaccine dose; (2) COVID-19-specific antibodies at 12 months following second vaccine dose and (3) proportion and activation status of CD4 T cells, CD8 T cells, B cells, natural killer cells and monocytes, prevaccination and 6 months post second vaccine dose.

Exploratory end points

COVID-19-specific antibodies at 6 months following second vaccine dose, stratified by subpopulations. Critically, we will also assess the ability of vaccine-elicited antibodies to cross-recognise SARS-CoV-2 S-protein variants, including N501Y and/or E484K, using in-house assays.

Sample size

Our primary outcome is the proportion of individuals in each group who mount a satisfactory immune response, although the best marker of what constitutes a satisfactory immune response is unclear at the moment and the science is rapidly evolving. We initially defined a successful immune response as a fourfold relative rise in IgG production at 6 months.[47] We anticipated that 90% of HIV-negative individuals would mount an adequate IgG response at this timepoint,[27 34] vs 70% of PLWH.[12 48] If 20% of the sample were to have the characteristic of interest

or predictor variable (eg, 20% with multimorbidities), enrolling 200 PLWH and 50 HIV-negative participants would provide sufficient statistical power (>80%) to detect a 20% difference in outcomes between groups whatever the exact proportions. This sample size was determined using the UCSF sample size calculator (https://data.ucsf.edu/research/sample-size) which uses the typical normal distribution assumption with the continuity correction as an approximation to the binomial distribution. In addition, we calculated that we would have >80% power to detect a 20% difference in outcome between those with suppressed CD4 count or unsuppressed viral load and the HIV-negative group. Previous studies of temporal differences in humoral and cellular responses to COVID-19 have shown differences between individuals when sample sizes included 100 participants or fewer.[49–53] We would also have >80% power to detect a 20% difference in outcome between the higher risk PLWH (ie, with CD4 counts <200 cells/mm^3 and/or unsuppressed HIV viral load) and the HIV-negative group. Such recruitment targets would also provide a sufficient buffer to account for potential drop-outs of 5%–10%. However, to increase our ability to detect differences in our primary outcome between the four subpopulations of interest (individuals of older age; immune non-responders; persons with multimorbidity and an HIV-positive 'stable' or 'reference' group), we plan to recruit 400 PLWH and use data from the *entire* cohort of HIV-negative individuals in the SSO study (approximately 1000 individuals) to increase power. Inevitably, the higher risk groups will be overlapping, so we will recruit a minimum of 20% of the 400 PLWH per category. By using the entire cohort of HIV-negative individuals in the SSO study, we avoid the need to match PLWH and HIV-negative participants.

Recruitment

Participants will be informed about the study through a recruitment flyer during routine physician visits to their HIV clinic and via established recruitment strategies through our community partners and the CTN via webpages, email and social media platforms. Individuals followed for routine HIV care at clinics other than the four enrolment sites are eligible to participate if they can come to the enrolment site for study visits. Participants will be compensated US$40 per study visit to help offset the time commitment and parking fees. We will make a concerted effort, through the use of recruitment quotas, to ensure the HIV-negative and HIV-positive 'stable' groups have overlapping characteristics (eg, age >55 years, CD4 count <350 cells/mm^3, comorbidities) so that the groups will be comparable.

Data collection

Medical history and HIV history will be gathered from both patient interviews and clinic chart reviews following written informed consent. Information extracted will include comorbidities, year of HIV diagnosis, CD4 T cell nadir (if known) in addition to tobacco smoking and cannabis use history. Medications will be recorded in addition to the ART regimen. History of COVID-19 infection and date of confirmatory test will be recorded.

Sample collection

At each visit, blood will be collected to isolate serum, plasma and peripheral blood mononuclear cells (PBMCs).

Humoral immunity (SARS-CoV-2 binding antibodies)

We will evaluate levels of immunoglobulins M and A (IgM, IgA) and IgG targeting the SARS-CoV-2 Spike (S) protein receptor-binding domain (RBD) and nucleocapsid protein using a high-throughput automated ELISA co-developed and validated by Dr Marc-Andre Langlois,[54] thereby distinguishing vaccine-induced (S only) from infection-induced (S and nucleocapsid (N)) responses. We will also evaluate samples for IgM and IgG antibody cross-recognition of RBD derived from VoCs, including those harbouring *N501Y* and/or *E484K* (eg, Alpha and Beta strains, respectively) using a commercial multiplex ELISA (V-Plex, Meso Scale Discovery). This assay is updated regularly by the manufacturer to accommodate emerging spike variants. We will test plasma samples for their capacity to block viral entry using a well-established neutralisation assay based on retroviruses pseudotyped with the SARS-CoV-2 S protein.[49 51]

Cellular immunity

Flow cytometry will be performed to enumerate CD4 T cells (including helper and regulatory subsets), CD8 T cells and other T cell subsets (including naïve, central memory, transitional memory, effector memory and terminally differentiated cells), B cells (including naïve, memory and antibody-secreting B cells), natural killer cells and monocytes (classical, inflammatory and non-classical). We will also evaluate markers of cellular immune activation, senescence and exhaustion. Following high-resolution human leucocyte antigen class I/II typing, we will examine COVID-19-specific T cell responses using an activation-induced marker (AIM) assay. Briefly, PBMCs will be stimulated overnight with pools of SARS-CoV-2 S peptides. Activated CD4 and CD8 T cells will be quantified by flow cytometry-based expression of CD137, OX40 and/or CD69. Gene expression will be assessed by single-cell RNA sequencing of PBMC as described previously.[55] T and B cell epitope specificity will be confirmed using virus-derived antigens (peptide/HLA or RBD dextramers, respectively). Plasma levels of inflammatory markers including interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-4 IL-6, IL-8, IL-10, IL-13, IL-17, transforming growth factor-β, IFN-γ-induced protein-10 (IP-10), IL-12p70 and tumour necrosis factor-α will be measured using multiplex Luminex assays, D-dimer, C reactive protein and markers of microbial translocation lipopolysaccharide, beta-d-glucan (ßdG) and soluble CD14 will be evaluated by ELISA.[56]

Exploratory safety and tolerability of COVID-19 vaccines in PLWH

In the prospective cohort, we will explore vaccine safety and efficacy to inform subsequent studies. Reactogenicity: *symptoms diary*: participants will be asked to document specific local and systemic reactions in a diary for 1 week and 1 month following each injection, as was done in Pfizer-BioNTech phase III studies.[47] We will report the proportion of participants developing local (redness, pain or swelling at the injection site) or systemic effects (fatigue, headache, muscle pain, fever, joint pain, diarrhoea) within 7–30 days following each vaccine dose with 95% CIs (online supplemental information).

COVID-19 questionnaires

We will administer the COVID-19 System Questionnaire to participants who develop a flu-like illness to confirm the illness, along with PCR-based tests for COVID-19. Participants will complete the COVID-19 Immunity Task Force (CITF) Standardised Core Survey Data Elements questionnaire prior to vaccination and a modified CITF questionnaire (minus the demographic information) at follow-up visits (online supplemental information).

If participants develop COVID-19 symptoms 14 days+ after vaccination, we will also collect CITF system questionnaire (length of illness and symptomatology, which vaccine was administered, number of vaccine doses received) and saliva specimens to enable study of COVID-19 VoC (https://www.covid19immunitytaskforce.ca/covid-19-immunity-task-force-releases-standardized-core-survey-data-elements/).

Data management

The study sponsor, the CTN, will be responsible for national project management, database development, data management and data analysis. To facilitate data sharing, we will: (1) use standard encodings for CITF-defined Core Data Elements; (2) request immunogenicity study participants consent to data sharing as guided by the CITF, including collecting survey elements and saliva 14+ days after vaccination on symptomatic participants to determine whether the infecting strain is a COVID-19 VoC and (3) rapidly share interim data and all requested study metadata for cataloguing.

Confidentiality

All participant-related information, including Case Report Forms, laboratory specimens, evaluation forms and reports, will be kept strictly confidential. All records will be held in a secure, locked location and only accessible to research staff. Participants will be identified using a coded number specific to each participant. All computerised databases will identify participants by numeric codes only and will be password protected. On request, and in the presence of the investigator or his/her representative, participant data will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups and applicable regulatory agencies to verify clinical study procedures and/or data, as permissible by local regulations.

Statistical analyses

We will use regression techniques to compare COVID-19-specific immune responses applying data transformation where necessary to conform with distributional assumptions in order to determine whether there are differences between the 'unstable' PLWH group (CD4 <350 cells/mm3), the stable PLWH cohort and the HIV-negative controls, taking into account factors that are believed to be associated with immune response. We will also perform unadjusted analyses to determine whether there are differences which may or may not be driven by factors associated with group membership. We will report data from exploratory analyses with descriptive statistics and data for vaccines from different manufacturers separately and combined. We will stratify results by the number of doses received and the time interval between the two doses in case these factors drive response and differ between groups. We will stratify immunogenicity data by sex as females and males have differences in both vaccine-elicited immune responses[57] and adverse effects from vaccines.[58] Furthermore, we will stratify analyses by individuals who are naïve to COVID-19 versus those with pre-existing antibodies as a result of prior COVID-19 infection. This will be important since antibody responses (particularly after the first dose) will be much higher in convalescent individuals,[59 60] so it is not appropriate to include them with individuals who do not have pre-existing antibodies to COVID-19.

ETHICS AND DISSEMINATION

Ethics approval and consent to participate

Written informed consent will be obtained from all study participants. The study will be conducted in accordance with the Declaration of Helsinki. At the time of initial manuscript submission (June 2021), a very closely related protocol had been approved by the University of British Columbia/Providence Health Care Research Institute and Simon Fraser University Research Ethics Boards. The present protocol was later approved by the Research Institute of the McGill University Health Centre (second review), the Ottawa Hospital Research Ethics Board, the University of Toronto Research Ethics Board. Patient enrolment for this trial began June 2021. Both the protocol and informed forms were reviewed and approved by the CTN Community Advisory Committee.

Availability of data and materials

De-identified participant data will be stored on a secure password-protected RedCap database. Access to the database will be controlled by the CTN. Access to the final study database will be provided on written reasonable request to the corresponding author/principal investigator following publication and CTN and CITF approval.

We will standardise reagents and analysis strategies, where possible, working with the Immune Sciences Network and Testing Working Party recommendations to enable data sharing and avoid duplication in consultation with other CIHR and CITF-funded vaccine surveillance projects. We will also contribute results to SeroTracker, a knowledge hub that tracks and synthesises findings from SARS-CoV-2 serosurveillance efforts worldwide (https://www.covid19immunitytaskforce.ca/serotracker/). To inform COVID-19 immunisation guidelines and future interventions for PLWH in Canada and internationally, we are committed to sharing results with all stakeholders and will adhere to Wellcome's *Sharing research data and findings relevant to the COVID-19 outbreak* statement.[61] Data sharing agreements will be obtained between CTN sites to use data not sent to the CITF.

Serology results will be provided to individual study participants at the end of the study, along with a summary of study results and their implication in lay language. We prioritise meaningful community engagement and our Community Advisory Committee includes PLWH and representation from the Canadian Aboriginal AIDS Network. The Community Advisory Committee reviews protocols and informed consent forms and advises on community priorities. Our well-established relationship with CATIE, Canada's source for HIV information, will enhance KT. We will leverage our KT staff to share lay language updates via press releases, media coverage, website, e-newsletters and social media and undertake targeted KT activities to mobilise knowledge across our network and report results to senior policymakers via research summaries and policy briefs, community groups and participants via factsheets. Our team will publish manuscripts, contribute to guidelines and present to stakeholders. A first manuscript outlining the results of the primary objective will be submitted for publication within 6 months of participants completing the 6-month post second vaccine dose study visit. A second manuscript outlining the durability results will be submitted for publication within 6 months of participants completing the 12-month post second dose study visit. Data will also be shared with CTN members at the semi-.annual meetings and through conference abstracts.

Patient and public involvement

The CTN Community Advisory Committee (CAC) was involved in the peer-review process of this study proposal and deemed that the research questions addressed were of very high priority to PLWH. The CAC's critiques of the initial proposal were taken into account in the revised proposal. Two members of the CAC (SM and EM) were involved in finalising the study design, inclusion/exclusion criteria, outcome measures and monitoring plans and are formal study investigators and coauthors. Community consultants will receive financial compensation to recognise their time commitment and expertise.

DISCUSSION

Herein, we present the protocol for an observational cohort study to evaluate COVID-19 vaccine-elicited immunogenicity in PLWH, with a priority of determining immunogenicity in PLWH who are of older age, immune non-responders and those with multimorbidity. These three groups were selected since they represent subpopulations most likely to experience poor outcomes following COVID-19 infection and have a weaker immune response to vaccination.

PLWH immune non-responders are at risk of more adverse COVID-19-related outcomes than HIV immune responders. In the study performed by Braunstein *et al*, PLWH with COVID-19 had a higher proportion of hospital admissions, intensive care unit (ICU) admission and death. Those who experienced these COVID-19-related outcomes had CD4 T cell counts below 500 cells/mm3.[3 38] Similarly, the study by Dandachi *et al* found that having CD4 T cell counts below 200 cells/mm^3 was associated with severe outcomes such as ICU admission, intubation and death.[39] Furthermore, a multicentre cohort study by Hoffman *et al* showed that CD4 T cell counts <350 cells/mm^3 were associated with severe COVID-19 (adjusted OR 2.85, 95% CI 1.26 to 6.44).[40] However, in a group of patients with inborn errors of immunity, Kinoshita *et al* demonstrated robust T cell activity and humoral immunity against COVID-19 structural proteins in some patients with antibody deficiency,[62] underscoring the heterogeneity and complexity of immune response.

A major challenge with planning this study is the unprecedented, rapidly changing nature of the COVID-19 pandemic and evolving scientific information. As a result, data on the optimal time points to assess immune responses post-COVID-19 vaccination administration are rapidly changing, resulting in multiple adjustments in our protocol plans. Within Canada, the vaccination schedule is determined by provincial vaccination programmes based on review of evidence, population risk factors and local infection rates, along with input from NACI. However, differences exist between provinces regarding to vaccine supply, eligibility criteria, type of vaccine administered and time period between vaccine doses. We are mitigating these challenges by holding monthly meetings with teams to discuss these issues over the previous month, troubleshoot and adjust recruitment priorities accordingly for the upcoming months. Ideally, and under non-pandemic circumstances, we would establish, a priori, methods for analysing data from single versus two-dose vaccines. However, in the current context with uncertainty of vaccine supply and distribution, such detailed plans are impractical and will depend on the methods of vaccination used in the participants enrolling in this study. This statement holds true for other variables we will likely encounter, such as different dosing intervals among persons receiving two-dose vaccines.

The importance of advanced age in impaired vaccine-induced immunogenicity is well-documented. Due to

a combination of disrupted post-transcriptional regulation, T cell receptor signalling and metabolic function, older individuals demonstrate reduced quantity and functionality of T cells.[63 64] When this balance is disrupted, T cells exhibit shorter-lived effector phenotypes rather than memory or follicular helper T cells and vaccine-induced antibodies are less protective than in younger persons.[63 64] As the elderly are considered a priority vaccination group, many of them were eligible for vaccination in early 2021 in Canada, before this current study began, meaning that baseline plasma, serum and PBMCs will not be available for participants in this subpopulation of interest. Another drawback with starting this study in May 2021 is that we may miss other important groups of PLWH, including Indigenous persons, who were prioritised as high-risk populations for immunisation and were eligible to receive COVID-19 vaccines at a younger age than the general population.

Another major challenge with the planning of this study was the need to ensure an adequate sample size to meet the primary objective in the group as a whole, but also in important subpopulations of PLWH. Enrolment of such a large number of individuals, with follow-up until 12 months following the second vaccine dose, is very resource-intensive and requires dedicated study participants. We will provide individualised antibody results to participants at study completion to increase study engagement and prevent drop-outs. Furthermore, due to the need to rapidly enrol participants to match the pace of the COVID-19 vaccine rollout, we decided to use CTN study sites with large clinic volumes and proven capacity to recruit. However, these clinics are based in urban centres and, therefore, may follow individuals who differ systematically from PLWH who live in rural areas. PLWH who live in cities may be of higher socioeconomic status and consist of more men who have sex with men. Therefore, each site will need to make a concerted effort to recruit sufficient participants with other profiles. For this reason, we will employ a flexible recruitment strategy, whereby sites that can recruit the required participants with characteristics of interest more easily than other sites can help to make up for lower recruitment flexibility at other sites. As with many studies, recruitment of women living with HIV may be challenging. Due to our connection with other studies within the CTN and the help of our community advisory board, we are encouraging clinics with predominantly female clients to ensure they inform their female patients living with HIV about this study.

Currently, the immune correlate of protection against COVID-19 is undefined. T cell and B cell responses are usually used as surrogates of protection.[65] We decided to use a fourfold rise from baseline in IgG production following second vaccine dose as the criteria for a successful vaccine response, as was indicated in the Pfizer study.[47] Data from the Pfizer study submitted to the Food and Drug Administration and published by Walsh *et al* report geometric mean titres that were compared with those from a human SARS-CoV-2 convalescent serum panel as a benchmark. Increased titres were expressed in logarithmic fold increases.[66] There are currently no national standards for presenting the serology data. Some groups prefer to report the raw signal values, whereas others normalise data as fold increases. Since individual baseline values will not be available for participants who have already received their first vaccine dose, one option is to use the cohort baseline data derived from non-vaccinated participants. Alternatively, we may opt to examine relative titres, or fold-changes, and therefore baseline data will not actually be required. Since science is continuously evolving, we will use the definition of a successful vaccine response which is most widely accepted at the time of publication.

The best methodology to match PLWH with the HIV-negative group remains unclear, and there is no optimal approach to match participants to controls. As discussed by Wong *et al*, the ideal comparison group would be individuals who are identical to HIV-negative adults in all aspects except HIV status.[67] As PLWH have distinct characteristics, traditional risk factors, lifestyle factors and socioeconomic factors compared with the general population, the general population may not be the ideal comparison group.[67] Differences include increased tobacco smoking,[68] substance use[69] and comorbidities[70] among PLWH compared with the general population. However, PLWH also undergo more screening for age-related comorbidities due to frequent contact with healthcare providers.[67] Since matching is challenging, we will avoid the need to match individuals entirely, by using a large existing dataset of over 1000 individuals without HIV infection. As previously mentioned, as long as we ensure there are 20% of individuals with the characteristics of interest in the HIV-negative and HIV-positive 'stable' cohorts, we can compare groups while adjusting for these characteristics via regression.

The findings from this study will provide valuable insight into COVID-19 vaccine-induced immunogenicity in important subpopulations of PLWH. HIV-positive persons of older age, with CD4 counts <350 cells/mm^3 and multimorbidity were not included in the early clinical trials, yet are most likely to suffer from poor outcomes if infected with COVID-19. These findings will inform clinical guidelines and recommendations for PLWH and, in turn, reduce COVID-19-induced morbidity and mortality.

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Acknowledgements The authors thank all of the CTN staff who were involved in the planning and preparation of this study, in addition to the CTN Scientific Review Committee and the Community Advisory Board, for their expedited review and helpful comments.CTC is supported by a Fonds de recherche du Québec-Santé (FRQS) Junior 2 career award. AB holds a Canada Research Chair Tier 2 Canada Research Chair (CRC) in sexually transmitted infection (STI) prevention. M-AL holds a CRC in Molecular Virology and Intrinsic Immunity. M-AJ holds a CRC, Tier 2, in human immunovirology. SW is supported by a Research Chair in HIV Clinical Care and Aging from the Ontario HIV Treatment Network. DT is supported by a Tier 2 CRC in HIV Prevention and STI Research. MB holds a Canada Research Chair, Tier 2, in viral pathogenesis and immunity. ZB holds a Scholar Award from the Michael Smith Foundation for Health Research. J-PR holds a Lowenstein Chair in Hematology.

Contributors Co-principal investigators of the study are CTC, CC and AHA. CTC and CC conceived the study, led the proposal and protocol development. CTC wrote the first draft of the manuscript. JS is the biostatistician who provided methodological expertise and performed sample size calculations. All other authors, including AB, JN, IK, SM, EM, MO, CK, DT, SW, MHa, MHu and JBA contributed to protocol development, study design and development of the proposal. CTC, M-AL, M-AJ, MO, MB and ZB designed the laboratory evaluations. M-AL will be responsible for studies on humoral immunity. M-AJ will be responsible for flow cytometric studies to define proportions of immune cells and their subsets, while CTC will be responsible for cytokine assessment. MB and ZB will be responsible for RNA profiling. M-AL and MB will perform the analysis of antibodies to COVID-19 variants. MO and MB will perform analyses of T cell responses to vaccine immunogens. Markers of gut barrier damage, microbial translocation and CMV IgG titres will be performed by J-PR. HLA typing will be performed as necessary for participants evaluated for T cell responses by MO, ZB and MB. JS will oversee data analysis between groups and subgroup analyses. All authors critically reviewed and approved the final manuscript.

Funding This project is being supported by funding from the Public Health Agency of Canada, through the Vaccine Surveillance Reference group and the COVID-19 Immunity Task Force (grant number: N/A) and the CIHR CTN (grant number: N/A).

Competing interests The authors declare that they have no competing interests

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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