Application: BCC3

Melanie Murray - melanie.murray@cw.bc.ca
CTN+ Letter of Support Requests & Portfolio Study Submissions

Summary

ID: 0000000011
Status: In Review
Last submitted: Sep 5 2025 07:31 PM (PDT)
Labels: Fall 2025 Competition

Full Study Submission Form

Completed - Sep 5 2025

Full Study Submission Form



Please read the CTN+ Portfolio Study Submission and Review Process Guidance document prior to completing your submission.

If you have received a CTN+ Letter of Support and secured funding for your project, please use the form below to submit your study for full CTN+ support. Your application will be reviewed by the CTN+ review committee panel before receiving final adjudication at the next CTN+ Steering Committee meeting. In the case of incomplete information, you may be contacted by a CTN+ administrator with a request to provide additional details or documents.

If you have any questions or concerns about this process, please send an email to submissions@hivnet.ubc.ca.

Principal Applicant

First and Last Name

Melanie Murray

Email Address

melanie.murray@cw.bc.ca

Institutional Affiliation

University of British Columbia

Is the Principal Applicant a CTN+ Researcher?

If you have not yet submitted the **CTN+ Researcher Application form**, please fill out the form via this link before submitting the Full Submission form.

Yes

Submitter

Leave blank if this form is being submitted by the Principal Applicant.

First and Last Name

Melanie Murray

Email Address

melanie.murray@cw.bc.ca

Please upload the CTN+ Letter of Support you received for this project (if applicable).

CTNLetterBCC32025.pdf

Filename: CTNLetterBCC32025.pdf.pdf **Size:** 237.0 kB

Submission Details

Type of Submission

Final Submission

Regional Team Affiliation

Responses Selected:

BC & Yukon Region

Think Tank Affiliation

Responses Selected:

Treatment & Management Think Tank

Study Details

Study Name

(Long and short versions)

BCC3

Study Hypothesis

(In one sentence)

Hormone dysregulation will drive an older phenotypical age, along with greater inflammation and mitochondrial dysfunction among women living with HIV.

Lay Language Description

(300 words maximum)

Aging is a complex process characterized by several biological changes that occur and accumulate throughout life such as inflammation, mitochondrial dysfunction and immune senescence. People accumulate these changes at different speeds, so their biological age (or phenotypic age) is different from their chronological age. Females generally live longer than males, with fewer aging-related illnesses early in life. However, after menopause, an aging experience unique to females, they accumulate age-related illnesses at a similar rate to males. While reasons are not fully understood, they may relate to reduction in sex hormones.
Sex hormones play a crucial role in keeping our bodies functioning normally and when balanced the immune system performs well, and our cells age normally. However, abnormal hormone levels can lead to increased illness. For hormones to function normally, we need: enough hormone and functioning receptors for cells to respond.
Women living with HIV experience earlier loss of sex hormones, increased inflammation, mitochondrial dysfunction, and earlier immune senescence. Despite effective antiretroviral therapy extending the lives of people living with HIV, women living with HIV in Canada still experience reduced healthspan and lifespan than both men living with and women without HIV. A link between low sex hormones, and biological changes as a pathway to aging is emerging, though the effect of hormone receptors is not well understood.
The British Columbia CARMA-CHIWOS Collaboration (BCC3) is a study of 700 women living with and without HIV that employs a comprehensive approach to studying healthy aging. We will use HIV as a model of accelerated aging to study sex hormone/hormone receptor alterations in women, and how these relate to mitochondrial health, inflammation, immune senescence and phenotypic aging. This research will inform potential ways to promote healthy aging and support women-centered and informed decision-making in clinical care.

Number of Participants

(Total N, and N per Arm if applicable)

700

Please list the participating sites.

(Use 1 row per Site. Leave any unused rows blank.)

	Investigator (total)	Institution	Target Enrolment
Site 1	Melanie Murray	Oak Tree Clinic, UBC	550
Site 2	Melanie Murray (site contact: Jennifer Hoy)	Central Interior Native Health Society, Prince George	60
Site 3	Melanie Murray (site contact, Penny Bradford)	Vancouver Island, PWA Society, Victoria	60
Site 4	Melanie Murray	Interior Health	30
Site 5			
Site 6			
Site 7			
Site 8			
Site 9			

Background Information and Study Rational

(500 words maximum)

Background:

Aging is a complex process characterized by several biological changes or hallmarks that occur and accumulate throughout life. The rate at which these changes occur foretells their aging phenotype, or the rate of biologic aging compared to chronologic aging. Many of the hallmarks of aging are useful for assessing and understanding aging in a research setting, among them mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and chronic inflammation. People accumulate these changes at different rates, meaning their biological age (or phenotypic age) is different from their chronological age. Females generally live longer than males, with fewer aging-related illnesses early in life. However, after menopause, an aging experience unique to females, they accumulate age-related illnesses at a similar rate to males. While the reasons are not fully understood, they may, in part, be due to a reduction in sex hormones (e.g. estradiol), occurring during the menopause transition.

We know that states of low estradiol, such as early menopause (before age 45) or prolonged amenorrhea (no menstruation for >12 months), are experienced more frequently by women living with HIV than women without HIV. Women living with HIV have higher inflammation levels, exacerbated by menopause, and increased mitochondrial dysfunction. Furthermore, despite effective antiretroviral therapy extending the lives of people living with HIV, women living with HIV in Canada experience reduced healthspan and lifespan compared to both men living with and women without HIV. As such, HIV could be considered a model of accelerated aging.

A link between low sex hormones (such as with menopause and amenorrhea), inflammation, and mitochondrial dysfunction as a pathway to aging is emerging. We propose that the increased inflammation and mitochondrial dysfunction seen in women living with HIV is driven at least in part by hormone dysregulation and that this balance may play a significant role in the health disparities, accelerated cellular aging, and increased mortality rates in this population, and may ultimately represent an additional hallmark of aging.

Hypothesis: We hypothesize that the greater inflammation and mitochondrial dysfunction observed in women living with HIV compared with women not living with HIV is driven by hormone dysregulation. This imbalance may play a substantial role in accelerated cellular aging, health disparities, and lower health/life span in women living with HIV, and may ultimately represent an additional hallmark of aging for all women.

Study Hypothesis and Design

(1000 words maximum)

This section must include:

1. the hypothesis;
2. the study design (methodology and statistical analysis, including the rationale for the sample size calculation);
3. the study population (including participant inclusion/exclusion criteria);
4. the specific study intervention details;
5. expected outcomes; and
6. expected results and implications for the field.

RESEARCH DESIGN:
We will leverage the British Columbia CARMA-CHIWOS Collaboration (BCC3) study to investigate the hypothesis that hormone dysregulation will drive an older phenotypical age, along with greater inflammation and mitochondrial dysfunction. This could ultimately identify hormone dysregulation as an additional hallmark of aging.

AIMS 1 and 2:
For Visit #1, the BCC3 cohort has already enrolled 650 of a targeted 700 women (350 women living with HIV and 350 without HIV, age 16 and older and able to consent in English. Those who are pregnant or breastfeeding are invited to return at a later time). Biospecimens collected in Visit #1 will be used to assess bioavailable hormones and hormone-binding globulins by ELISA, systemic hormone receptor expression on T cell, NK cell, B cell, and monocytes and immune cell phenotyping (activation, exhaustion, and senescence) by flow cytometry, and an array of biomarkers, including soluble markers of inflammation (cytokines/chemokines) and mitochondrial health by flow cytometry for viability, mitochondrial (mt) mass, mt ROS, and mt intermembrane potential and mtDNA content by monochrome multiplex qPCR.
Lifetime E2 exposure will be calculated for participants similarly to published literature as reproductive lifespan (age and menopause or current age - age of menarche + years hormonal therapy + years pregnant - years of amenorrhea - years postpartum or lactating. This equation can be calculated using commonly assessed clinical parameters (menopause confirmation) and interview questions that can be used by clinicians without access to research laboratories.

Phenotypic age will be determined using Levine's PhenoAge expressed in years, which includes clinical blood parameters, albumin, alkaline phosphatase, creatinine, glucose, C-reactive protein, percent lymphocytes, mean cell volume, red cell distribution width, and white blood cell count. PhenoAgeAccel represents phenotypic age after accounting for chronological age and will be calculated as described by Li et. al.

AIM 3:
Three hundred (300) midlife participants aged 40-60 at Visit #1 will return for a second visit (Visit #2) as women in that age range are going through the period of greatest hormonal change (menopause transition), correlating with rapid changes in cardiometabolic and other health risks that may include accelerated phenotypic aging. Visit #2 will include collection of clinical, anthropometric, sociodemographic, psychosocial and structural data, with linked biospecimens and clinical lab tests in a manner similar to Visit #1.

Lifetime E2 exposure, bioavailable E2 levels and ERα/ERβ, PhenoAgeAccel, mtDNA content, mitochondrial dysfunction, inflammation,

and markers of immune cell senescence and exhaustion will be calculated as described in Aim 1 and 2 above. Weight gain between visits (Aim 3c) will be assessed , and their change between study visits calculated as value at Visit #2 – value at Visit #1 divided by time between visits.

POWER CALCULATIONS:
We have no preliminary data to calculate sample size/power for PhenoAgeAccel and hormones, but with >250 each of premenopausal and menopausal, and >600 females total, we have >80% power, with $\alpha=0.05$ to detect even a small effect size of as little as ±0.3 in PhenoAgeAccel for all hypotheses while controlling for relevant covariates and confounders. For all analyses, the significance level is $\alpha=0.05$, and we will correct for multiple comparisons using Holm-Bonferroni as appropriate.

DATA ANALYSIS:
Data will be log-transformed if not normally distributed. All below analyses will adjust for age, HIV status, ethnicity, body mass index (BMI), opioid and other substance use, smoking, food insecurity, and other covariates and confounders, as appropriate. Age will be included as an effect modifier as needed, should PhenoAgeAccel display differences between age groups and living with HIV. Linear or quantile regression will be used to determine associations between PhenoAge Accel and each of our outcomes of interest. Separate analyses will be done of E2 and progesterone in premenopausal and estrone in menopausal participants and exploratory analyses will examine the relationships between ERα/ERβ ratio and each hormone level using Pearson's or Spearman's correlation as appropriate to inform the inclusion of an interaction term between hormones and receptors. We will assess differences in weight gain between: 1) WLWH using integrase inhibitors at Visit #2, 2) WLWH not using integrase inhibitors at Visit #2, and 3) women not living with HIV, using ANOVA or Kruskal-Wallis according to data distribution with Tukey or Dunn's correction, respectively. Linear or quantile regressions will assess associations between weight gain and our outcomes of interest.

IMPACT:
Using HIV infection as a model of accelerated aging, we will study sex hormone alterations in women, and how these relate to phenotypic aging and biological hallmarks of aging. This knowledge will enable women living with and without HIV to lend their voices to the research, and receive back potentially actionable findings to improve their lives. We anticipate it may also inform potential ways to promote healthy aging and support women-centered and informed decision-making in clinical care.

Please list any barriers/potential risks that may impede the study and include mitigation strategies.

(100 words maximum)

AIMS 1 & 2:
Specimens have already been collected from >90% of participants for these aims. All assays are optimized and ready to go, thus we anticipate no barriers to completing these aims.

AIM 3:
98% of previous participants agreed to study biobanking and to be contacted for future study participation which exceeds our required sample size for Aim 3. Again, assays are optimized and ready to go once samples have been collected.

Estimated start date of recruitment/study start date

12/2020

Estimated final date of participant follow-up (if applicable)

9/2030

Estimated date of final study/project report

9/2031

Additional Documents

Please upload the Notice of Decision or Award Letter from your primary funding source.

BCC3_SpringProject_NoticeDecision.pdf

Filename: BCC3_SpringProject_NoticeDecision.pdf.pdf **Size:** 125.4 kB

Please upload the Reviewer Comments document related to the decision of funding (if applicable).

BCC3_Review_Documents_SpringProject.pdf

Filename: BCC3_Review_Documents_SpringProject.pdf.pdf **Size:** 71.4 kB

Please upload your Research Proposal document.

BCC3_Spring_Project_Grant.pdf

Filename: BCC3_Spring_Project_Grant.pdf.pdf **Size:** 856.0 kB

Please upload your Budget document.

BCC3_Spring_CIHR_Budget.pdf

Filename: BCC3_Spring_CIHR_Budget.pdf.pdf **Size:** 58.3 kB

Funding Source & CTN+ Service/Support Request

Confirmed funding source/agency/company name

CIHR

Is the study funded through a CIHR Project Grant?

Yes

If the study is *not* funded through a CIHR Project Grant, please list the type of grant or the type of agency/company support (e.g. Investigator-initiated grant in aid from a pharmaceutical company)

(If you responded "Yes" to the previous question, please leave this blank.)

(No response)

Please indicate which in-kind services you request from the CTN+ if your submission is approved.

You can find a full list of services on the resource page.

Responses Selected:

Basic Knowledge Mobilization services (plain language summary, recruitment materials, social media support)

Please indicate which of the following service support estimates you requested in your CTN+ Letter of Support to conduct your study.

You can find a full list of services on the resource page.

Responses Selected:

Methodology

Statistical Analysis

Advanced Knowledge Mobilization support

Does your study include a Participant Informed Consent Form?

Yes (please attach the document via the upload function below)

Upload your Participant Informed Consent Form (if applicable)

BCC3 Main Consent_06Sep2023.v10.docx

Filename: BCC3 Main Consent_06Sep2023.v10.docx.docx **Size:** 641.4 kB

Have you created a Community Engagement Plan?

Yes (please attach the document via the upload function below)

Upload your Community Engagement Plan (if applicable)

CBRdocument_BCC3_09_2025.docx

Filename: CBRdocument_BCC3_09_2025.docx.docx **Size:** 16.3 kB

Have you created an Indigenous Engagement Plan?

Yes (please attach the document via the upload function below)

Upload your Indigenous Engagement Plan (if applicable)

Indigenous Consultation_BCC3.pdf

Filename: Indigenous Consultation_BCC3.pdf.pdf **Size:** 58.3 kB

Do you have a Knowledge Mobilization Plan for your study?

Yes (please attach the document via the upload function below)

Upload your Knowledge Mobilization Plan (if applicable)

Sharing Study Results - Community Engagement Plan - CTN.pdf

Filename: Sharing Study Results - Community Engagement Plan - CTN.pdf.pdf **Size:** 242.8 kB

Budget Request

Funding Opportunity

Project Grant: Project Grant (2025-03-05)

Applicant

Last Name	First Name	Institution
Murray	Melanie	University of British Columbia

Period of support requested: **5 Year(s) 0 Month(s)**

Research Staff $417 000

Description

Lab Technician, Dr. Cote's lab (Izabella Gadawska): Will do all hormone assays, database maintenance, and assistance with Mitochondrial assays, MesoScale, Flow Cytometry assays for the grant (baseline and second visit assays).
(Dr. Kowatsch [PostDoctoral Fellow] has a physical disability where she is very limited in her ability to use her hands. The lab technician will help Dr. Kowatsch with assays.)
Salary ($89,250 with 3% annual increase) x 0.3 FTE for years 1-4 = $111,000
BCC3 CRA Coordinator: Will manage and train Community Research Associates (CRA), manage social media, webpage and other knowledge mobilization/translation activities. Salary ($78,450 with 3% annual increase) x 0.2 FTE years 1-2, 0.1 FTE year 3, 0.05 FTE years 4-5 = $49,000
BCC3 Research Coordinator: Contact, recruitment and part 1 of study visits for Visit #2 participants (includes anthropometric data, specimen collection and survey). Management of RedCap (clinical data entry platform) and participant contact for follow knowledge mobilization should they have indicated their wish to be contacted during enrolment. Salary ($78,450 with 3% annual increase) x 0.8 FTE for years 1-2, and 0.6 FTE for year 3 = $178,000
Community Liaison/Leadership Advocate/Administrative Assistant: Will be remunerated at a rate of $27/hr for training, working group meetings and KTE endeavours. This includes training, working groups and CAP meetings, along with monthly team meetings. This is estimated to be 400 hours/year at $27/hr (with annual $1/hr increase) x 5 years excluding holiday closures = $59,000
Analyst/Statistician: will consult on all statistical analysis. $5,000 each for years 2-5 = $20,000.

Trainees $350 000

Description

Postdoctoral fellow: Will optimize all Flow Cytometry assays and assist with running the Mitochondrial assays, MesoScale and Flow Cytometry assays. Statistical analysis for all 3 aims, supervision of students, leveraging cohort for additional studies and manuscript writing, coordination and running of knowledge mobilization events throughout the study along with attending regular CRA meetings.
Salary ($70,000) x 1.0 FTE for 5 years = $350,000.

Budget Request

Consumables $277 000

Description

Blood collection: Paying either BC Women's Hospital Lab or Life Labs for Visit #2 blood collection. $45/collection x 300 = $13,500.
Clinical labs for Phenotypic Age calculation: Visit #2 labs including complete blood count, renal profile, alkaline phosphatase, albumin, glucose. $50/participant x 300 participants as part of Visit #2 = $15,000.
Extractions: Extraction of peripheral blood mononuclear cells and plasma from non-clinical portion of the blood sample. A technician employed by the WHRI will do this work; this will pay for supplies, processing and aliquotting of samples. $45/sample x 300 Visit #2 participants = $13,500.
Cell culture: reagents required for cell thawing and tissue culture for assays, $16,000
Flow Cytometry Extracellular Antibodies (assuming 25% titration): antibody purchasing for extracellular cell phenotyping and identification, ~$3.25/antibody x 20 antibodies = $65/participant (n=700 Visit #1, 300 second Visit #2) assuming 25% titration of manufacturer's volume is achieved = $65,000.
Flow Cytometry Intracellular Antibodies: antibody purchasing for hormone receptor characterization, $4.20/hormone receptor x 5 receptors = $20/participant (n=700 Visit #1, 300 Visit #2) = $42,000
Mitochondrial dysfunction, and mitochondrial DNA content: $13/participant (n=700 Visit #1 for mitochondrial dysfunction only, 300 Visit #2 for both mitochondrial dysfunction and mitochondrial DNA content) = $13,000.
Mesoscale for markers of inflammation: $70/participant x 1000 (700 Visit #1, 300 Visit #2). spread over 7 single plex assays and 2 multiplex assays (n=700) = $70,000.
CRP assessment, Visit #2: $13.30/participant x 300 = $4000.
Hormone Assays: Each assay is $2.85 per participant, n=700 for CBG Visit #1 = $2,000
Sample collection and processing supplies for assay optimization: processing of local control blood for assay optimization and internal controls to be run throughout the study = $8,000.
Liquid nitrogen: Purchasing of liquid nitrogen to store peripheral blood mononuclear cells (PBMCs) for use in all Flow cytometry assays, $1,000/year = $5,000.
Plastics: Purchasing of plastics for sample processing such as 96 well plates, flow cytometry tubes, cryotubes for sample storage etc., $2,000/year = $10,000.

Non-Consumables $79 000

Description

FlowJo (software) license: Purchasing of software license required for analysis of data generated using flow cytometry, $1500/year for 4 years = $6000
Flow Core service contract: Service contract for use of flow cytometers (machines) for the acquisition of data via flow cytometry, $50/month for quality control + $40/hour = $6,000
MesoScale service contract: Service contract for the use of the machine required for the acquisition of Mesoscale markers of inflammation, $7000/year x 4 years = $28,000.
Minus 80 freezer replacement: purchase of the replacement -80 freezer due to loss of the old freezer in the past year, freezer is required for storage of plasma samples required for markers of inflammation and hormone assays, $24,000.
BCC3 website license: $3000/year for the licensing and maintenance of a study website = $15,000.

Knowledge Translation $33 000

Description

Publication fees and printing: 5 Journal articles @ $5000/article for Open Access publishing $25,000.
KT meetings, KT activities: $1000 in year 4, and $2000 in year 5. Includes room rentals, participant remuneration, audio-visual equipment rental, and catering during events. Total=$3,000. For additional KT events we will leverage funding from local sources such as the UBC Cues Funding, Health Research BC Reach grants etc.
Travel: Travel for Postdoctoral fellow to 2 national ($2000) and 1 international ($3000) conference during the course of the grant. Total=$7000.

Budget Request

Other $94 000

Description

Participant Honorarium: Study Visit #2 consist of 2 parts including the clinical survey where blood samples are acquired, and participants undergo anthropometric measures (~90 minutes, $70), followed by a community survey conducted by a Community Research Associate (CRA) collecting data on psychosocial and structural factors (~60-90 minutes, $50). Total: $120/participant for study Visit #2 x 300 participants = $36,000.
Knowledge User (KU) time remuneration: KUs who do not have a work position where research is a paid part of their mandate will be remunerated at a rate of $100/half day for working group meetings, CAB meetings and KM endeavours. We Anticipate requiring remuneration for ~8 individuals while others will be supported through their various organizations. $1000/year for years 1-3, $2000/year for years 4-5 = $7,000
Community Research Associate remuneration: Remuneration for time spent for outreach to previous study participants to invite them back for a second study visit, time liaising with study participants to arrange the community part of their Visit #2 study visit, and time to conduct the survey with participants.
$120/participant for study Visit #2 x 300 participants = $36,000.
Indigenous Elder honorarium: Remuneration for time spent working with the study, opening KT events, and consulting on OCAP principles with study data. Includes a small retainer for optional participant consultation with the Elder if they are distressed during either survey (this happens rarely). $2,000/year for 5 years = $10,000.
CRA training: $1,000/year for 5 years for CRA training and remuneration, this includes time spent, printing materials, and room bookings for in-person training = $5,000.

Total $1 250 000

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

17 July/Juillet 2025

Notice of Decision / Avis de décision

Project Grant Program / Programme de subventions Projet

Nominated Principal Applicant / Candidate principale désignée ou Candidat principal désigné :	Murray, Melanie Caroline M
Principal Applicants / Candidats principaux :	
Co-Applicants / Cocandidats :	Cai, R; Cardinal, CL; Côté, H; Elwood, C; King, EM; Kowatsch, M; Loutfy, MR; Magagula, PT; Povshedna, T; Sierocinski, T; Tognazzini, SW
Institution Paid / Établissement payé :	University of British Columbia
Title / Titre :	Hormones and Markers of Aging in Women.
Application Number / Numéro de la demande :	543325

Competition information / Information du concours

Competition / Concours :	202503PJT
Applications in Competition / Demandes dans le concours :	2814
Applications approved / Demandes approuvées au total :	435
• **Approved without equalization / Approuvées sans rééquilibrage :**	345
• **Approved through equalization / Approuvées en vertu du rééquilibrage :**	90

Decision on application / Décision sur la demande

Decision / Décision :	Approved / Approuvée	
Total Funding Amount / Montant total du financement :	$956,250	
Term / Durée :	5 years/ans	0 months/mois

Peer Review Committee Recommendation / Recommandation du comité d'évaluation par les pairs

Committee Name / Nom du comité :	Biological and Clinical Aspects of Aging (BCA) / Aspects biologiques et cliniques du vieillissement (BCA)
Applications reviewed / Demandes évaluées :	51
Applications approved / Demandes approuvées :	8

Your percent rank zone / Votre intervalle de rangs centiles :

100%-90%	89.99%-75%	74.99%-50%	49.99%-25%	24.99%-0%

Final rating / Cote finale :	4.27 (Discussed / Demande ayant fait l'objet d'une discussion)
Is your application a large grant? / Est-ce que votre demande est une grande subvention?	No

Additional Funding Opportunities/ Autres possibilités de financement

Name / Nom	Decision / Décision	Total Funding Amount / Montant total du financement	Competition / Concours	Application Number / Numéro de la demande
Project Grant - Priority Announcement: Aging / Subvention Projet - Annonce de priorités: Vieillissement	Not Approved / Non approuvée	$0	202503PJ2	545337
Project Grant - Priority Announcement: Sex and Gender in Health Research - Understanding sex and gender mechanisms and processes / Subvention Projet - Annonce de priorité : Recherche en santé sexe et genre - Comprendre les mécanismes et processus liés au sexe et au genre	Not eligible-Withdrawn / Non admissible-Retirée	$0	202503PX2	546273
Project Grant - Priority Announcement: HIV/AIDS and STBBI / Subvention Projet - Annonce de priorité - VIH/sida et ITSS	Not Approved / Non approuvée	$0	202503PJH	544974

Additional information / Autres Informations

This document is for information only. Official payment is stated on the CIHR Authorization for Funding.
Document à titre d'information seulement. Le paiement officiel est indiqué sur l'autorisation de financement des IRSC.

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Adjudication Criteria/Critères de sélection

Initial Score/Cote Initiale: 4.0

Top/Bottom Selection/Groupe supérieur/inférieur

◯ **Top/Groupe supérieur**
◉ **Bottom/Groupe inférieur**

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Summary of Application/Résumé de la demande:

Dr. Murray is a Clinical Associate Professor in the Division of Infectious Diseases, Department of Medicine at the University of British Columbia. Her work focuses on the intersections of hormones, aging, and HIV. She is a Co-Leader of the British Columbia CARMA-CHIWOS Collaboration (BCC3; hivhearme.ca), a community- and clinic-based cohort study dedicated to improving healthy aging among women living with HIV. Dr. Murray is also a principal applicant in the CIHR-funded National Women's Health Research Initiative – Pan-Canadian Women's Health Coalition Hubs. She holds multiple research grants, including several CIHR Project Grants and several others from national and provincial agencies, reflecting a strong and sustained funding profile. There is no overlap between this grant and current funding. Her publication record includes numerous contributions—primarily as a co-author—to peer-reviewed journals focused on women's health and HIV. The application involves several co-applicants including long term collaborator Dr Helene Coté (UBC) who was the first to describe mtDNA changes in patients with HIV under treatment publishing a seminal paper in NEJM 2002.
Previous research by this team has shown that women living with HIV (WLWH) experience early menopause (before age 45), lower markers of ovarian reserve and prolonged amenorrhea (no menstruation for over 12 months) more frequently than women without HIV. WLWH also exhibit elevated levels of inflammation and increased mitochondrial dysfunction, both of which are further exacerbated by menopause. The investigators propose using HIV as a model of accelerated aging to show that hormone dysregulation is a key driver of early menopause and contributes to accelerated cellular aging.
To test this hypothesis, the study will leverage data from the British Columbia CARMA-CHIWOS Collaboration (BCC3) and is structured around three specific aims:
•Aim 1: Examine the relationship between hormone levels and phenotypic age acceleration. They will compare their cohort of females living with HIV with matched controls with respect to several socio-structural and demographic characteristics. Here, they will assess hormone levels (E2, progesterone, testosterone) and their receptors.
•Aim 2: Investigate the association between hormone dysregulation and molecular markers of cellular aging.
•Aim 3: Assess longitudinal changes in hormone levels and aging biomarkers among midlife women.

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Strengths and Weaknesses/Forces et faiblesses:

Strengths

1-The Project, we leverage the expertise, experience, and infrastructure of the CIHR-funded BCC3, a holistic cell-to-society community-based study of healthy aging in women. Specimens have been already collected and data from participant Visit #1 is available.

Weaknesses

1-Authors plan in aim 2 to use blood to measure several aging biomarkers. Blood is a mixture of cells with different proliferation rates, telomere length and mitochondrial content. Stress and lifestyle ma also impact these biomarkers. It will be hard to conclude anything about aging from these measurements. Blood is also constantly renewed from stem cells in the bone marrow and may not accurately represent aging occurring in key tissues.

2-Clonal hematopoiesis could have been proposed as an additional biomarker of stem cells problems originated in the blood marrow (see for example: doi: 10.1016/j.xcrm.2024.101835 and DOI: 10.1172/jci.insight.174783).

3-The proposal is largely descriptive. By the end of the grant, it is likely that we will have additional data reinforcing the notion that women living with HIV experience accelerated aging—something that is already well established. However, the proposal does not offer novel or mechanistic insights into why this accelerated aging occurs, nor does it identify potential points of intervention to improve outcomes for these women.

Overall, this grant builds on a well-organized and well-funded effort to characterize aging in women living with HIV. It offers a valuable opportunity to deepen our understanding of this population. The proposal could be further strengthened by incorporating complementary approaches aimed at uncovering mechanistic insights and identifying potential therapeutic strategies.

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Budget Recommendation/Recommandation budgétaire:

no concerns

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Please indicate your appraisal of the integration of sex as a biological variable as a strength, weakness, or not applicable to the proposal./Prière de sélectionner une option pour donner votre évaluation de l'intégration du sexe comme variable biologique en tant que point fort ou point faible de la proposition, ou en tant qu'élément non applicable à la proposition.

- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Please indicate your appraisal of the integration of gender as a socio-cultural determinant of health as a strength, weakness, or not applicable to the proposal./Prière de sélectionner une option pour donner votre évaluation de l'intégration du genre comme déterminant socioculturel de la santé en tant que point fort ou point faible de la proposition, ou en tant qu'élément non applicable à la proposition.

- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Review Type / Type d'évaluation:	Reviewer 1 / Évaluateur 1
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Sex and/or Gender Considerations/Notions de sexe et/ou de genre:

well addressed

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Adjudication Criteria/Critères de sélection

Initial Score/Cote Initiale: 4.5

Top/Bottom Selection/Groupe supérieur/inférieur

- (●) **Top/Groupe supérieur**
- ( ) **Bottom/Groupe inférieur**

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Summary of Application/Résumé de la demande:

Background: Aging is a complex process characterized by several biological changes or hallmarks that occur and accumulate throughout life, among them mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and chronic inflammation. People accumulate these changes at different rates, meaning their biological age (or phenotypic age) is different from their chronological age. A link between low sex hormones (such as with menopause and amenorrhea), inflammation, and mitochondrial dysfunction as a pathway to aging is emerging. We propose the increased inflammation and mitochondrial dysfunction seen in WLWH is driven at least in part by hormone dysregulation and that this balance may play a significant role in the health disparities, accelerated cellular aging, and increased mortality rates in this population; ultimately representing an additional hallmark of aging.

Research Plan: The candidates will leverage the British Columbia CARMA-CHIWOS Collaboration (BCC3) study to investigate the hypothesis that hormone dysregulation will drive an older phenotypical age, along with greater inflammation and mitochondrial dysfunction. This could ultimately identify hormone dysregulation as an additional hallmark of aging. The BCC3 cohort has already enrolled 640 of a targeted 700 women (350 WLWH and 350 without HIV) for Visit #1 study visits, providing a robust platform for this research.

Biospecimens collected in visits will be used to assess bioavailable hormones, hormone-binding globulins, hormone receptor expression, immune cell phenotyping, and an array of biomarkers, including markers of inflammation and mitochondrial health. For Aim 3, participants aged 40-60 at Visit #1 will be asked to return for Visit #2.

Specific Aims: In the BCC3 cohort of women living with and without HIV we will assess: Aim 1: Hormones and phenotypic age acceleration. Aim 2: Hormone dysregulation and markers of cellular aging. Aim 3: Longitudinal changes in hormones and aging markers among midlife women.

Impact: Using HIV infection as a model of accelerated aging, the candidates will study sex hormone alterations in women, and how these relate to phenotypic aging and biological hallmarks of aging. The candidate anticipate it may also inform potential ways to promote healthy aging and support women-centered and informed decision-making in clinical care.

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Strengths and Weaknesses/Forces et faiblesses:

Strengths

Strong research team working together since the beginning of the building of the cohort.

Excellent productivity.

State-of-the art technology proposed

Important research questions biologically and societally.

Use of PhenoAge as a measure of biological age in the context is innovative

Weaknesses

The determination of the ER on immune cells date back to 1970s and therefore it is not new. These measurements were performed in numerous pathologies and for many purposes.

The lack of CD28 may be associated with senescence but it is not a marker of senescence.

The receptor number on cells is not informative until the receptor signaling is not investigated concomitantly.

There are very few preliminary data to support the proposal. The preliminary data intuitive if you have amenorrhea have also less E2.

The HIV may be an accelerated aging but this is a defined disease so it has its own hallmarks on the whole organism which may resemble those occurring in certain aging trajectories (but not in all) also as a result of other chronic inflammatory diseases which appear well before the so-called aging. Therefore, HIV should not be considered only in this perspective but also as a disease which impact per se the hormonal status and may be the target of treatment.

In case of aging, due to our present knowledge, the HRT has no indication.

The studies do not give answer why even if the hormones diminish in older women the females live longer and better than men, therefore other factors should be investigated. In semi-supercentenarians the only biological process which was related to longevity was the inflammation. Therefore inflammation/anti-inflammation should be considered as an adaptation and not only always pathological.

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Budget Recommendation/Recommandation budgétaire:

No concerns, adequate.

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

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- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Please indicate your appraisal of the integration of gender as a socio-cultural determinant of health as a strength, weakness, or not applicable to the proposal./Prière de sélectionner une option pour donner votre évaluation de l'intégration du genre comme déterminant socioculturel de la santé en tant que point fort ou point faible de la proposition, ou en tant qu'élément non applicable à la proposition.

- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Review Type / Type d'évaluation:	Reviewer 2 / Évaluateur 2
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Sex and/or Gender Considerations/Notions de sexe et/ou de genre:

Sex and gender well considered.

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Adjudication Criteria/Critères de sélection

Initial Score/Cote Initiale: 4.3

Top/Bottom Selection/Groupe supérieur/inférieur

- (●) **Top/Groupe supérieur**
- ( ) **Bottom/Groupe inférieur**

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Summary of Application/Résumé de la demande:

HIV is associated with accelerated aging, as well as with decreased sex hormones in women. This proposal seeks to understand the role of sex hormones in aging women, and to the accelerated aging shown by women with HIV. This study is an extension of an existing study to collect longitudinal data in middle aged women with and without HIV. The proposal comprises three aims. In Aim 1, the team will investigate the contribution of sex hormones and receptors to phenotypic age, based on epigenetic markers of age. In Aim 2, the relationship between hormones and other markers of aging will be investigated, including mitochondrial dysfunction, inflammation and senescence. In Aim 3, changes in hormones will be linked to the same biomarkers as in Aims 1 and 2.

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Strengths and Weaknesses/Forces et faiblesses:

Strengths

This is a well-written and compelling proposal, which seeks to investigate a novel and important questions related to female aging in a comprehensive way.
The study harnesses an existing and highly valuable cohort, which will be extended with this proposal, adding to its original value by adding longitudinal data.
The team is well suited to this proposal given its existing involvement with the proposed cohort.
The indigenous component is a strength of the proposal, relying on an existing relationship with the local indigenous community.
The methods are carefully designed and comprehensive. Especially, the measurement of both total hormone and bioavailable hormone is a strength.

Aspects that could be improved

The timing of the blood test for regularly menstruating women and menopause women is clear, but how timing is determined for individuals in the more irregular perimenopausal period (but not amenorrhea) is not clear. I assume it will be random, but it would be helpful to have this explicitly stated.

In the proposal, it is mentioned that multiple comparison correction will be applied, but what type of correction is unclear.

The use of highly exploratory univariate analyses in the proposal, while understandable given the novel nature of the proposal, seems like a missed opportunity. Greater stratification of outcomes as well as the use of multivariate analyses would help establish the combined effects of some of the factors investigated.

For Aim 3, a mixed model design may better capture the intra- and inter-individual differences in the longitudinal data.

The role and importance of integrase inhibitors could be described more.

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Budget Recommendation/Recommandation budgétaire:

N/A

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

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- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Please indicate your appraisal of the integration of gender as a socio-cultural determinant of health as a strength, weakness, or not applicable to the proposal./Prière de sélectionner une option pour donner votre évaluation de l'intégration du genre comme déterminant socioculturel de la santé en tant que point fort ou point faible de la proposition, ou en tant qu'élément non applicable à la proposition.

- (●) **Strength/Point fort**
- ( ) **Weakness/Point faible**
- ( ) **Not applicable/Non applicable**

Review Type / Type d'évaluation:	Reviewer 3 / Évaluateur 3
Name of Applicant / Nom du chercheur:	Murray, Melanie
Application No. / Numéro de demande:	543325
Agency / Agence:	CIHR/IRSC
Competition / Concours:	Project Grant/Subvention Projet
Committee / Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title / Titre:	Hormones and Markers of Aging in Women.

Sex and/or Gender Considerations/Notions de sexe et/ou de genre:

Sex and gender is appropriate

Review Type/Type d'évaluation:	SO Notes /Notes de l'agent scientifique
Name of Applicant/Nom du chercheur:	Murray, Melanie Caroline M.
Application No./Numéro de demande:	543325
Agency/Agence:	CIHR/IRSC
Competition/Concours:	2025-03-05 Project Grant/Subvention Projet
Committee/Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title/Titre:	Hormones and Markers of Aging in Women.

Assessment/Évaluation:

The proposal focuses on healthy aging in women using a cohort of women living with HIV experiencing early menopause before age of 45 modelling accelerated aging.

Strengths (including SGBA considerations):

- The cohort is established and samples/data for this project are available.
- The indigenous component of the cohort is strong.
- Measuring bioavailable hormones is valuable.

Weaknesses (including SGBA considerations):

- Main: It was agreed that the proposal is largely descriptive; Suggested experiments do not aim to reveal mechanism or points of intervention. Stratifying the outcomes with more rigorous statistics would have helped.
- Blood cell biomarkers are suggested to measure biological age, while blood cells may not be suitable; clonal hematopoiesis will be better.
- Estrogen receptors on immune cells are not a novel observation.
- CD28 is not a marker for senescence.
- More preliminary data would strengthened the proposal.
- Hormone replacement therapy is not discussed.
- The gender component could be mentioned in the main proposal.

Budget:

Review Type/Type d'évaluation:	SO Notes /Notes de l'agent scientifique
Name of Applicant/Nom du chercheur:	Murray, Melanie Caroline M.
Application No./Numéro de demande:	543325
Agency/Agence:	CIHR/IRSC
Competition/Concours:	2025-03-05 Project Grant/Subvention Projet
Committee/Comité:	Biological and Clinical Aspects of Aging/Aspects biologiques et cliniques du vieillissement
Title/Titre:	Hormones and Markers of Aging in Women.

Assessment/Évaluation:

The budget is accepted as is.

Note: The final rating of the application, provided in the Notice of Recommendation (NOR) and Notice of decision (NOD), is the average rating of the peer review committee members following the discussion of the application during the committee meeting, and therefore may differ from the ratings provided by the assigned reviewers in their respective reviews.

Remarque : La cote définitive de la demande, qui apparaît dans l'avis de recommandation et l'avis de décision, représente la moyenne des cotes accordées par les membres du comité d'évaluation par les pairs après avoir débattu de la demande à la réunion du comité. Elle peut donc différer de celle donnée par les évaluateurs dans leur évaluation respective.

CIHR Pan-Canadian Network for HIV and STBBI Clinical Trials Research
Réseau pancanadien pour des essais cliniques sur le VIH et autres ITSS

February 12, 2025

Canadian Institutes of Health Research (CIHR)
CIHR Project Grants
C160 Elgin Street, 9th Floor
Ottawa, ON K1A 0W9

RE: Letter of support for Dr. Melanie Murray's application to the Project Grant competition entitled *"Hormones and Markers of Aging in Women."*

Dear Review Committee Members,

On behalf of the CIHR Pan-Canadian Network for HIV/AIDS and Sexually Transmitted and Blood-Borne Infections Clinical Trials Research (CTN+), I am pleased to confirm our support for the proposed Project Grant entitled "*Hormones and Markers of Aging in Women.*" The assembled team is comprised of well-established STBBI experts and community partners with an excellent track record of collaboration and productivity. This project to address an important research gap in aiming to better understand the underlying mechanisms of premature aging and other comorbidities seen in people with HIV, with particular attention on how, as well as factors related to sex and gender such as endogenous and exogenous hormones and socio-structural factors such as stigma, sexism, and discrimination, affect markers aging and health/mortality risk.

Originally established in 1990 as a cornerstone of the federal AIDS Strategy, the CTN has broadened its focus to include all sexually transmitted and blood-borne infections (STBBIs), and is now known as CTN+. Under the leadership of National Director Dr. Marina Klein at the Research Institute of the McGill University Health Centre (RI-MUHC) in Montréal, the network undertakes clinical trials to find new ways to prevent, treat and manage HIV and other STBBIs, prioritizing a community-based approach to ensure relevant solutions for Canada's most at-risk populations. The CTN+ also invests in developing the next generation of Canadian researchers, to increase the country's capacity to conduct clinical trials and improve the health and wellbeing of people living with and affected by STBBIs. CTN+ is a Canada-wide collaborative and inclusive partnership of researchers, people living with HIV and/or STBBIs and their caregivers, governments, health advocates and the pharmaceutical and biotechnology industry. Since the CTN's inception, the Network has trained over 100 postdoctoral fellows, enrolled over 24,000 study participants and conducted more than 330 clinical studies. With our research excellence, community engagement and robust infrastructure, we pursue an integrated approach across the continuum of prevention, testing, initiation of care and treatment, and ongoing care and support.

The current proposal focuses on HIV as a model of accelerated aging, particularly in women. Dr. Murray will leverage the British Columbia CARMA–CHIWOS Collaboration (BCC3; CTN 335) study investigate how hormone disruptions among women living with and without HIV intersect with factors such as chronic/latent viral infections, and inflammation to drive aging processes. Our Network has provided support for this study in the form of project management, statistical planning and analysis, and communications and knowledge translation. We are pleased to provide continued support the extension of this project in-kind (approximately 10,000 CAD/year) by providing time during our regular CTN+ meetings to discuss study design and findings, including during the sessions led by our Treatment and Management Think Tank, provide methodologic input and additional community engagement opportunities, and identify and plan knowledge translation strategies. Through our Community Leadership Team, Regional Teams, and Community-Centered Knowledge Mobilization Hub, we can further support the participation and engagement of community partners and other stakeholders, as well as translate and disseminate new knowledge gained from the study.

Premature aging, inflammation, and other comorbidities in people with chronic viral infections, including HIV, are important community priorities and expanding our understanding of these processes and improving their clinical management is a key priority of our network. Dr. Murray and several of the co-applicants are key leaders in our network, engaging as outstanding investigators and collaborators in CTN+ activities. We are enthusiastic about this proposed work and the CTN+ strongly supports the activities described in this grant, and urges CIHR to fund this proposal. Please do not hesitate to contact us to clarify any of the above points.

Kind regards,



Marina Klein, MD, MSc, FRCP(C)
Professor of Medicine, McGill University Health Centre
Research Director, Division of Infectious Diseases and Chronic Viral Illness Service
National Director, CIHR Canadian HIV/AIDS & STBBI Trials Network (CTN+)

E-mail: Marina.Klein@mcgill.ca or info@ctnplus.ca

CONCEPT (SIGNIFICANCE & IMPACT)

OVERVIEW

Aging is a complex process characterized by several biologic changes, or hallmarks, that accumulate as one navigates life[1,2]. The rate at which these changes occur foretells their aging phenotype, or the rate of biologic aging compared to chronologic aging. Worldwide, females live on average 5 years longer than do males[3], and in their younger years, are less likely to experience aging-related illness[4]. Female-specific aging is not fully understood, because many studies historically excluded females due to concerns they may become pregnant, or that fluctuating hormones could affect study results.

Females have a unique experience of aging—***menopause***—after which they start to accumulate age-related comorbidities at a similar rate to males[4]. The aging process of females may be contingent upon the sex hormones that change so significantly during the transition to menopause. In this context, it is crucial that we understand the role played by estradiol, the main circulating estrogen pre-menopause, and other sex and stress hormones in women's biological aging and illness. To date, we know that:

- In the general population, both menopause (>1y after final menstruation) and prolonged amenorrhea (>1 year without menstruation) are associated with decreases in the sex hormones estradiol and progesterone[5], and low estradiol levels are associated with a decrease in estrogen receptors on cells[6]. Menopause is associated with increased inflammation levels in humans [7,8], alterations in mitochondrial function, biogenesis and dynamics in mice[9], and progression of age-associated diseases affecting the brain, renal, hepatic, bone and cardiovascular systems, as well as glucose/lipid metabolism[10-15].
- HIV as a model of accelerated aging: We know that HIV seropositive status reduces both health span[16,17] and lifespan[17-19], even after considering psychosocial and structural factors. For women living with HIV (WLWH; for this proposal, referring to cis women), life expectancy is 5-10 years shorter[18,20,21] than that of same-age women without HIV; WLWH lose 3-4 more years of life expectancy than do males with HIV[22]. While the reasons for these disparities are not clear, we do know that WLWH are more likely to have a history of prolonged amenorrhea[23-28], experience more primary ovarian insufficiency[29], and may reach menopause earlier[29,30] than women without HIV. A low sex hormone state earlier in life may therefore confer earlier and higher risk for many comorbidities (hereafter referred to as ***concurrent conditions,*** in line with community recommendations) in WLWH compared to women without HIV, in addition to the risk associated with HIV-related inflammation[31].

We propose that for females, aging rate relates in part to alterations in sex hormones that normally protect women's health during the 30-45 years of menstruating life. Specifically, we *hypothesize* that hormonal dysregulation drives changes in several of the hallmarks of aging and affects phenotypic age.

In this Project, we address this hypothesis by leveraging the ***British Columbia CARMA-CHIWOS Collaboration (BCC3).*** BCC3 combines two decade-long, prospective cohorts of women living with and without HIV living in BC: (i) CARMA (Children and Women: AntiRetroviral therapy and Markers of Aging) and (ii) CHIWOS (Canadian HIV Women's Sexual and Reproductive Health Study) into a holistic society-to-cell framework[32]. BCC3 is a community-based study responding to calls from the community to study aging in WLWH and understand existing gaps in health and lifespan. It offers a model of accelerated aging for WLWH that provides a unique opportunity to answer aging questions for all women. We will leverage our BCC3 study infrastructure, already-collected data and bio-specimens, along with data from a new study visit, to enable a fulsome assessment of hormonal status and investigate associations with phenotypic age and hallmarks of cellular aging. Specifically, we will compare key measures in WLWH and HIV-negative women to examine aims covering 3 topics:

Aim 1: Hormones and phenotypic age acceleration.
Aim 2: Hormone dysregulation and markers of cellular aging.
Aim 3: Longitudinal changes in hormones and aging markers among midlife women.

This work is crucial to understanding how clinical care can be adapted or modified to encompass female reproductive health, and to inform guidelines for comprehensive women-centered care.

BACKGROUND

Hallmarks of aging: In 2013, Lopez-Otin et. al. put forth the hallmarks of biologic aging[1] including "genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and intercellular communication". This was updated in 2023 to include "macroautophagy, chronic inflammation, and dysbiosis"[2]. Many of these hallmarks are useful to assess and understand aging in a research setting[33], but their use beyond research is limited by lack of standardization, cost, and access to clinical testing.

Phenotypic age: Levine's *PhenoAge* is a measure of biological age validated in the NHANES IV[34] cohort that may be calculated using commonly available clinical lab tests. It is "highly correlated to all-cause and cause-specific mortality independent of chronological age, demographic characteristics and disease history, and is robust to stratifications by age, race/ethnicity, education, disease count, and health behaviours"[34]. In contrast to aging "clocks," which require expensive and specialized DNA methylation data, PhenoAge may serve as a readily calculable marker of aging that could be completed in the clinic setting, and which could guide clinical care.

Hormones and women's health: Sex hormones, including estrogens, progesterone, and testosterone, are key modulators of human health that can influence inflammation, and aging processes in females. Females produce three major endogenous estrogens, namely estriol, estradiol (E2), and estrone[35]. Estriol is produced by the placenta during pregnancy[36]. In premenopausal females, E2, the most potent and primary circulating estrogen, is produced mainly in the ovaries, along with progesterone and testosterone[37], and all three are lower in premenopausal females with prolonged amenorrhea[38]. During menopause, the main estrogen shifts from E2 to estrone, the latter primarily made in adipose and muscle tissues[39], and E2 and progesterone levels decline[40]. Testosterone is produced by ovaries, adrenal glands, and adipose tissue[41,42], and declines with age independent of menopause[43,44].

Sex hormones and health: Beyond their role in reproductive health, sex hormones are important to the health of several organ systems and to metabolic regulation[45]. E2 is a potent antioxidant in the liver, slowing hepatic fibrosis progression by suppressing reactive oxygen species (ROS)[46]. With the onset of menopause, low E2 levels are correlated with a higher risk of insulin resistance, type 2 diabetes mellitus, dyslipidemia, central adiposity, metabolic syndrome, cardiovascular disease, and non-alcoholic fatty liver disease[47]. Altered E2 and/or testosterone levels can also affect body weight, skin quality, perimenopausal symptoms, as well as sexual drive, mood, and mental health[48]. Sufficient E2 but lower progesterone levels, as seen with subclinical ovulatory disturbances within regular cycles, are related to stressors and implicated in osteoporosis, liver fibrosis and hypertension[36,49-52]. Ovary removal (oophorectomy) before age 45-50y decreases E2 (along with progesterone and testosterone) and is associated with higher cardiovascular and all-cause mortality[53,54]. The protective effects of estrogens last beyond the reproductive years. A low estrone level is associated with impaired endothelial function and greater all-cause mortality in menopausal women[55,56].

Binding globulins: When measuring hormone levels, it is important to consider sex hormone-binding globulin (SHGB), which binds E2 and testosterone[57], and corticosteroid-binding globulin (CBG), which binds progesterone and cortisol[58]. When hormones are bound to SHBG or CBG, they are biologically unavailable to tissues[59,60]. SHBG levels rise with age and are higher in people living with HIV[61], decreasing levels of bioavailable hormone (E2, testosterone). *BCC3 data* (**Fig 1a, b)** confirms similar total E2 levels, but lower levels of bioavailable E2, in WLWH compared with controls.

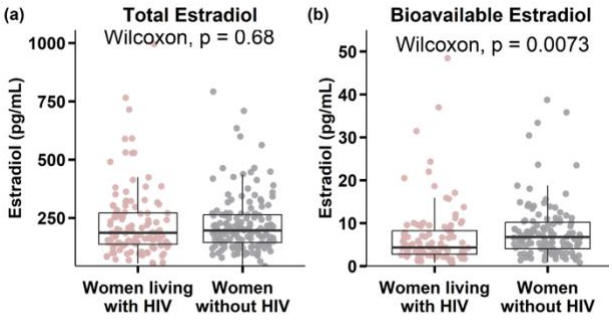


Figure 1. a) Total Estradiol, and b) Bioavailable Estradiol

Hormone receptors: Sensitivity at the hormone receptor level is a key factor in determining an

individual's response to a given hormone[35,43,62] . Tissues involved in reproduction express receptors for hormones such as estrogens, progesterone and testosterone. Recent studies show that immune cells including T cells, B cells, natural killer (NK) cells, and monocytes also express hormone receptors, and that hormone binding to these receptors results in modulation of immune responses[63-66]. For instance, estrogens bind three types of estrogen receptors (ER), the intracellular receptors ERα, ERβ, and the extracellular G-protein coupled ER (GRER). Testosterone binds the androgen receptor (AR), while progesterone binds both the progesterone receptor (PR) and glucocorticoid receptor (GR). These receptors are all expressed on lymphocytes and other immune cells (collectively, peripheral blood mononuclear cells or PBMCs). In PBMCs, ERα and ERβ have been implicated in inflammatory and anti-inflammatory responses, respectively[63-65], such that a *higher ratio of ERα to ERβ results in a more pro-inflammatory state.* PR and GR suppress the immune response in females[67,68], while AR has been shown to suppress the immune response in males, and transgender individuals[68]. ERs, particularly ERβ also modulate mitochondrial function[64]. In adipocytes, loss of ERα is associated with reduced mitochondrial DNA (mtDNA) content in cultured cells[69] and weight gain in mice[70].

Hormones and cellular aging: At the cellular level, declining mitochondrial function is associated with cellular senescence and development of age-related diseases[71]. E2 appears to protect mitochondria by decreasing formation of reactive oxygen species (ROS)[72], regulating mitochondrial DNA (mtDNA) content, and inducing antioxidant responses[73], preserving mitochondrial function in cardiac, skeletal muscle and brain tissues[64]. Estrogens protect telomeres (the caps at the end of chromosomes that shorten with age) against oxidative DNA damage, while stimulating telomerase activity[74,75]. This finding may partially explain the longer leukocyte telomere length and lifespan observed in females vs males. In pre- and post-menopausal females, shorter telomeres are seen with low estrogen (estrone, E2) levels[76].

HIV as a model of accelerated aging: Antiretroviral therapy successfully extends the lives of people living with HIV. *So why is it that WLWH have reduced healthspan[16,17] and lifespan[17-19] compared to women not living with HIV*, and lose more years of life than do men living with HIV[77]? WLWH experience an increased prevalence of concurrent conditions, such as cardiovascular, liver, and kidney diseases, osteoporosis and malignancies[16,19,78-83]. Inflammation is well established as a key driver in the onset and progression of these conditions[84,85]. REPRIEVE, a large randomized controlled trial with 7769 participants across 12 countries, 32% of whom were female, revealed that WLWH have higher levels of inflammation compared to male counterparts[86]; and that this disparity is greater after menopause[86,87]. HIV and some antiretrovirals also cause mitochondrial toxicity, affecting blood mtDNA copy number or content (a marker of mitochondrial health)[88-91].

Compared to females without HIV, WLWH are more likely to experience prolonged amenorrhea (no menses for ≥1 year before menopause, not due to pregnancy, lactation or contraception) [27]. Both amenorrhea and menopause are associated with reduced sex hormone levels[38,40], higher inflammation[87], and an increased prevalence of concurrent conditions[49,87,92]. The synthesis of sex hormones is mitochondria-dependent[92,93] and so adverse mitochondrial effects of HIV and/or its treatment may represent underlying mechanism(s) of hormonal dysregulation in WLWH.

Given this link between amenorrhea, menopause, heightened inflammation, and mitochondrial dysfunction, *we propose that the greater inflammation and mitochondrial dysfunction observed in WLWH compared with women not living with HIV is driven by hormone dysregulation.* This imbalance may play a substantial role in accelerated cellular aging, health disparities, and lower health/life span in WLWH, and may ultimately represent an additional hallmark of aging for all women.

FEASIBILITY

RESEARCH PLAN: APPROACH & METHODS

Leveraging BCC3: In this Project, we leverage the expertise, experience, and infrastructure of the CIHR-funded BCC3, a holistic cell-to-society community-based study of healthy aging in women[94]

that was launched in 2021. BCC3 comprises CARMA and CHIWOS cohorts, and has so far recruited 640/700 women (cis, trans) living with and without HIV, aged ≥16 years, who can consent in English, attend an in-person visit, and are not currently pregnant or breast/chest-feeding for Visit #1 of the study. Women ***not*** living with HIV are recruited to be well-matched to WLWH with respect to several socio-structural and demographic characteristics. To address our project aims, we will use already-collected specimens and data from participant Visit #1 and will invite midlife females (i.e. baseline aged 40-60 years) to return for Visit #2, at 3-5 years after their baseline (Visit #1) study visit.

Approach to research: The BCC3 cohort embraces the principles of antiracism, equity, diversity, and inclusion, and values the lived experience of WLWH. We follow principles of GIPA (Greater involvement of People Living with HIV/AIDS)[95] and MEWA (Meaningful engagement of Women living with HIV/AIDS)[96]. The BCC3 Community Advisory Board (CAB) is inclusive of persons representing the broad lived experience of WLWH in BC, Indigenous Elders, knowledge holders, community leaders, and HIV/AIDS service organisations. WLWH (CAB members, study members, and community research associates) were involved in development of the study aims, the design and piloting of our surveys, preparation of operations manuals and data collection tools, data collection, and have led knowledge mobilization (KM) throughout the study.

Other study considerations: While this proposal discusses "women" in the context of cisgender females, we recognize that hormones and aging in transgender and non-binary individuals and men are also important and require study. Although trans women do not experience menopause, and our current study lacks power to analyse hormone levels in this group, we are committed to inclusivity. We have enrolled trans women and non-binary femme individuals in our study and will complete all baseline (Visit #1) measures above and present their data descriptively. We are building relationships with the trans community and have applied for funding to complete aims informed by the trans community in a separate application. We also have access to a parallel cohort of men living with and without HIV and intend to explore the interplay of hormones and aging in men in a future application.

Study visits, specimen collection, database & biobank

Study visits: Each study visit for BCC3 study participants includes a clinical survey, height, weight, waist circumference, blood pressure, and biospecimen (blood, urine, hair) collection, followed by a community survey that is administered in person or virtually by one of four BCC3 Community Research Associates (WLWH with research training). Our clinical survey collects data on clinical and reproductive history, sociodemographic factors, medications (including antiretrovirals), concurrent illnesses and substance use, while the community survey collects data on psychological, socio-structural, and behavioural determinants of health, mental health, and sexual health. Our consent includes survey data and collection of biospecimens, with an option for biobanking (separate consent, *98% of 640 participants have consented*). All participants undergo testing of renal function, urine albumin-creatinine ratio, albumin, liver enzymes, platelet count, lipid and glucose metabolism. The residual blood sample is processed and sent to the Côté lab (UBC) for all research assays. *We have enrolled 299/350 WLWH and 341/350 women without HIV for Visit #1.*

Menstrual/menopause status and hormone testing: At each study visit, regularly menstruating females are asked for date of their last menstrual period to establish day of menstrual cycle. Females with normal menstrual cycles in the 3 months prior to the study visit are considered premenopausal, and they provide timed blood samples to measure E2 levels (early follicular phase, cycle days 2-5 post menses start[97]), and progesterone (mid-luteal phase, cycle day 21-23 after menstruation, or 7 days before next flow[98]) collected during their current or next cycle. If women report no menses for ≥1 year and have an FSH level ≥25 mIU/mL[23], they are considered menopausal. Those with current amenorrhea not meeting criteria for menopause have random E2, progesterone, prolactin, LH, androstenedione, and dehydroepiandrosterone sulphate (DHEAS) levels tested to explore reasons for amenorrhea. Testosterone is measured on all participants. We know hormone levels are pulsatile and

vary from cycle to cycle but chose to do a single timed hormone measurement because one value has been shown to be similar to levels averaged over time[99], and this is feasible with our population.
Database, biobank and statistical support: Blood is processed at BC Women's Hospital, then shipped and stored in the Côté lab at UBC, where CARMA and BCC3 specimens have been housed since study inception. Data are stored in our BCC3 Research Electronic Data Capture (REDCap)[100] database, managed by BCC3 staff, and hosted in the Clinical Research Support Unit at BC Children's Hospital Research Institute. This allows highly secure real-time data entry, access, and facilitates timely analyses. Co-I **Sierocinski** or a **CTN+** Statistician will advise on all below statistical analyses.

AIM 1: To examine the associations between phenotypic age acceleration and a) bioavailable/free hormone levels, b) immune cell hormone receptor expression, and c) lifetime E2.

Hypotheses: Greater baseline phenotypic age acceleration (PhenoAgeAccel)[34,101] will be associated with:

1a. Lower levels of bioavailable/free hormone: E2 and estrone (primary outcomes), progesterone and/or testosterone (secondary outcomes).
1b. Altered PBMC receptor levels: ERα/ERβ ratio (primary outcome); ERα, ERβ, ARs, PR, and/or GRs (secondary outcomes).
1c. Lower estimated lifetime E2 exposure.

Rationale: In general, menopause and amenorrhea are associated with reduced sex hormone levels (E2, progesterone, testosterone)[38], and increased inflammation[7,8,102], both of which are associated with accelerated progression of age-associated diseases[28,103-108]. Through their interaction with ER on immune cells, estrogens affect innate and adaptive immune responses[109]; thus, low levels of estrogens may affect inflammation and subsequent aging. It is less well-known how testosterone or progesterone may affect this pathway. To gain a holistic view of hormone levels and their relation to inflammation and aging, we must assess both bioavailable hormone levels and hormone receptors. As hormone levels vary across the lifespan of an individual, estimating lifetime hormone exposure may provide additional information for estimating their effect on biological aging. Finally, the literature describes several aging "clocks" that may be used to assess accelerated aging. Levine's PhenoAge[34] (described above) provides a clinically actionable and available measure that correlates well with all cause and disease specific mortality. There are few data regarding the relationship of hormone dysregulation and PhenoAge, or with PhenoAgeAccel (i.e. phenotypic age after accounting for chronological age) described by Li *et. al* [110,111].

Preliminary data: BCC3 participants with current amenorrhea (random hormone levels) have lower total E2 and progesterone levels than do those with regular menstrual cycles (timed hormone levels) (**Fig 2**). However, WLWH have lower bioavailable E2 (**Fig 1b**), and testosterone compared to controls. Total progesterone is lower in WLWH vs controls (**Fig 3**); we have yet to assay CBG to assess effect on bioavailable progesterone.

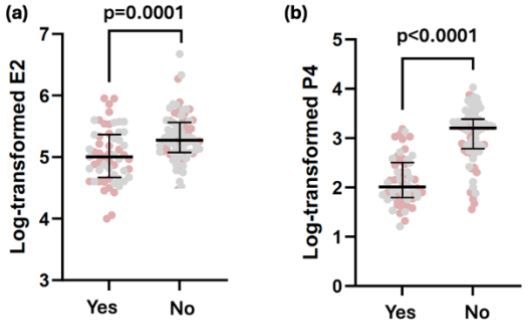


Figure 2. Hormone levels in women with (yes) and without (no) amenorrhea. (a) E2 (estradiol), (b) P4 (progesterone).

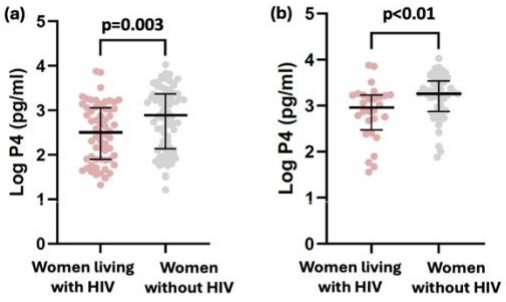


Figure 3. Progesterone (P4) levels in (a) All specimens, (b) Timed specimens only.

Methods: <u>Hormone measurements:</u> Visit #1 hormone levels have been completed for >600 participants to date. We measured total hormone levels in women not using exogenous hormones: plasma E2 and progesterone in pre-menopausal females (timed if regular menstrual cycles, untimed if amenorrheic), estrone in menopausal females; and SHBG and testosterone in all women. We will measure CBG in our participants to determine free progesterone levels. We

elected to use immunoassays for hormone measures which are reliable and practical for generating real time data and widely used in our hospital. For each hormone, SHBG, and now CBG, we chose/will choose an ELISA kit with evidence of low cross-reactivity and high performance against mass spectroscopy. Kits from single vendors and internal controls are used to monitor inter-lot variability for each hormone. Hormones are assayed from plasma after solvent extraction to eliminate interfering lipids and proteins, allowing assaying of larger volumes if levels are low, and for reliable results[112-114].

Bioavailable/free hormone levels: Bioavailable and free hormone levels will be calculated using the equations proposed by Mazer *et al*[115]; i) Bioavailable hormone = Total hormone / [1+ K_{SHBG} * SHBG)] and ii) Free hormone = Total hormone / [1 + (K_{SHBG} * SHBG) + (K_{alb} * Albumin)]. In these formulae, hormone, binding globulins, and albumin will be expressed in moles and K_{SHBG} and K_{alb} represent the binding constants for SHBG and albumin to each hormone[116]. CBG will be substituted for SHBG for progesterone in the equation. Albumin, SHBG and hormone levels have been assayed for Visit #1.

Hormone receptor expression: As hormones cannot function without receptor expression, we will assess intracellular expression of ERα and ERβ, total AR, PR and GR expression using flow cytometry as part of an immune cell characterization panel similar to one used routinely in the Côté lab, which has been adapted to include the hormone receptors. This panel will allow for the extracellular characterization of several live PBMC subsets (**Table 1**). Precision count beads[117] will be used to calculate absolute cell number to control for variations in CD4 T cell number between women with and without HIV.

Table 1: Immune Cell Characterization Panel

Cell Subset	Identity Markers
Memory CD4/CD8 T Cells	CD45RA, CCR7
T regulatory cells	CD25, CD127, FoxP3
B cells, plasma cells	CD19, HLA-DR, CD38
Natural Killer (NK) cells	CD16, CD56
Monocytes	CD14, CD16, HLA-DR
Cell Phenotyping	**Markers Used**
Activation	HLA-DR, CD69, CD38
Senescence	CD28, CD57
Exhaustion	PD-1, TIGIT
Hormone receptors	ERα and ERβ, PR, AR, GR

Our *primary outcome* (ERα/ERβ ratio) will be assessed on each immune cell subset. ERα/Erβ ratio will be calculated as described by Maselli[63]. There are few data on AR, PR and GR expression in immune cells, so these will be assessed in parallel to allow for exploratory analysis. To measure co-expression of immune phenotyping markers, we (co-I Kowatsch) will use multi-dimensional reduction (Uniform Manifold Approximation and Projection; UMAP)[118] and clustering (X-Shift)[119] with a standardised analysis pipeline.[120] This will allow a more comprehensive understanding of the relationships between immune status and hormone receptor expression and limit comparisons to phenotypes present.

Table 2: Lifetime E2 Exposure

Variable	Method of Calculating
Years of reproductive life	(Age of enrollment or menopause@) – (Age of menarche)
Years of endogenous E2	(Yrs reproductive life) – (Yrs postpartum and/or lactating) – (Yrs without menses*)
Actual years of E2 exposure	(Yrs endogenous E2) + (Yrs of menopausal hormone therapy)
Lifetime E2 exposure	$\frac{\text{Actual years of E2 exposure}}{\text{Eligible years of E2 exposure}}$

@Age at enrollment if not menopausal, age at menopause if is; Years postpartum=6 weeks/pregnancy; Years without menses = years without menses not due to pregnancy, lactating, or polycystic ovarian syndrome. E2=estradiol; Yrs=years.

Lifetime estradiol exposure will be calculated for all participants in similar fashion to Park *et al.*[121] **(Table 2)** but employing a ratio to standardise for the broad range of participant ages. Lifetime E2 exposure will be expressed as a ratio ranging from 0 to 1.

Phenotypic age will be determined using Levine's Pheno-Age[34] expressed in years (**Table 3**). Phenotypic age acceleration (PhenoAgeAccel) represents phenotypic age after accounting for chronological age and will be calculated as described by Li *et. al*[110,111].

Table 3: Elements of PhenoAge

Item	Units	Organ System
Albumin	g/L	Liver
Alkaline Phosphatase	U/L	Liver
Creatinine	μmol/L	Kidney
Glucose	mmol/L	Metabolic
C reactive Protein (log)	mg/dL	Inflammation
Lymphocytes	%	Immune
Mean cell volume	fL	Immune
Red cell distribution width	%	Immune
White blood cell count	1000 cells/μL	Immune
Age	years	-

Power calculations: We have no preliminary data to calculate sample size/power for PhenoAgeAccel and hormones, receptors or lifetime E2. However, with >250 females in each of the premenopausal (E2) and menopausal (estrone) groups, and >600 for hypothesis 1c, we have >80% power, with α=0.05 to detect even a small

effect size[122] of as little as ±0.3 in phenotypic age for all hypotheses while controlling for relevant covariates and confounders[123].

Data analysis: We will report descriptive statistics for the whole cohort and by menopausal status. For all analyses, significance level is α=0.05, and we will correct for multiple comparisons as appropriate. Data will be log-transformed if not normally distributed. All below analyses will adjust for age, HIV status, ethnicity, body mass index (BMI), opioid and other substance use, smoking, food insecurity, and other covariates and confounders, as appropriate. Age will be included as an effect modifier as needed should phenotypic age display differences between age groups and living with HIV. Hypothesis 1a: We will determine if greater PhenoAgeAccel is associated with lower free/bioavailable hormone levels (E2, estrone as primary outcomes; progesterone and testosterone as secondary outcomes), using linear or quantile regression as appropriate. Separate analyses will be done of E2 and progesterone in premenopausal and estrone in menopausal participants. Women using hormonal contraceptives (oral, transdermal or Depo-Provera) or menopausal hormone therapy in the past 3 months, or those with contraceptive implants will be included in other aims but are excluded from these analyses. We will retain data from women with copper or hormone eluting intrauterine devices as data suggest little effect on circulating hormone levels[124]. Hypothesis 1b: We will use linear or quantile regression modelling as appropriate to determine if PhenoAgeAccel is independently associated with ERα/ERβ ratio (primary outcome), or altered levels of ERα, Erβ, GR, AR or PR hormone receptors (secondary outcomes) on PBMCs. Exploratory analyses will examine the relationships between ERα/ERβ ratio and each hormone level using Pearson's or Spearman's correlation as appropriate to inform the inclusion of an interaction term between hormones and receptors. Hypothesis 1c: We will use linear regression to determine whether PhenoAgeAccel is associated with estimated lifetime E2 exposure.

AIM 2: To examine the associations between hormone exposure and markers of aging.

Hypotheses:

2a. At Visit #1, lower estrogen levels and/or lower lifetime E2 exposure will be associated with i) altered immune cell mtDNA content, ii) mitochondrial dysfunction, iii) chronic inflammation, and iv) immune cell senescence and/or exhaustion.

2b. These effects will be greater among WLWH than in HIV-negative women.

Rationale: Both amenorrhea and menopause are associated with lower sex hormone levels[38,40], increased prevalence of concurrent conditions[49,87], and higher inflammation[86,87], which may in turn lead to immune cell senescence[125]. In females, low E2 and estrone levels are associated with shorter telomere length[76]. In the CARMA cohort, WLWH are more likely to have hormonal dysregulation[126,127], non-HIV concurrent conditions[82], and lower blood mtDNA content[88] than control women without HIV. For WLWH, amenorrhea may, in part, be related to antiretroviral effect on mitochondria resulting in reduced sex hormone production[107,108]. Given these links, we propose that hormone dysregulation will be associated with these markers of aging.

Preliminary data: Among BCC3 participants, hormone levels are lower (**Fig 1b, 3)**, and amenorrhea more frequent among WLWH versus controls**.**

Methods: mtDNA content: Total DNA will be extracted from whole blood using the QIAamp DNA Mini kit. We will quantify mtDNA content using the monochrome multiplex qPCR assay developed by the Côté lab[128], using a LightCycler 480. mtDNA will be defined as a ratio of the number of mitochondrial DNA copies/nuclear DNA copies[129].

Mitochondrial dysfunction: Live frozen PBMC will be thawed quickly, stained for viability and mitochondrial (mt) intermembrane potential, mt mass, and mt ROS, using markers routinely used to assess mitochondrial dysfunction, and a standardized assay in the Côté lab[130]. This will be conducted on previously described cell subsets (see **Table 1).** T regulatory cells will be excluded from this analysis due to permeabilization requirement which is incompatible with mitochondrial dyes used. Data will be acquired using flow cytometry on a Cytek Aurora spectral cytometer at the UBC core flow

facility. Unstained and single stained cells will be used to assess proper gating and fluorescent background as per Cytek instructions. Mitochondrial dysfunction will be calculated as ***mt intermembrane potential/mt mass ratio*** from the median fluorescence intensity of each measure, which will estimate the intermembrane potential while controlling for the mitochondria present[131].

Plasma markers of inflammation will be measured using a custom Mesoscale V-Plex panel that includes CRP, IFNγ, TNFα, IL-6, IL-10, IL-1β, and sCD163, chosen as they are known to be altered by both HIV and age[1,87,132-134]. Assays will be standardized using manufacturers' quality controls and three internal controls (plasma from 3 participants) to assess inter-plate variability. To allow for inclusion of plasma markers of inflammation in multivariable analysis we will calculate *inflammatory index* as described by others[135,136], which generates a single variable representative of a participant's inflammatory status and is a more rounded approach[135,136]. Briefly, cytokines will be assigned a score based on their percentile in the entire cohort (>75th, ≥50th, <50th). The resulting scores per cytokine will be summed to generate a continuous variable representing the number of elevated inflammatory cytokines per participant. This method quantifies inflammation in different participants where inflammation may be driven by different inflammatory cytokines.

Immune cell senescence and exhaustion will be assessed using the same flow cytometry panel as in Aim 1 (**Table 1**) for hormone receptor expression. Both senescent cells (CD28+CD57) and exhausted cells (PD-1+TIGIT+) will be expressed as proportion of cell subset (0-100%).

Other covariates: Study surveys will collect demographic data (gender, income [dichotomized at $20,000], education, food insecurity and housing etc), reproductive history, substance use (smoking, alcohol, opiates, stimulants), medications, and concurrent illnesses via self-report as described[94]. Height and weight are measured to obtain BMI.

Power calculations: For hypothesis 2a we will have ample power to detect even a clinically weak[122] regression coefficient of r=±0.3 (sample size required for 80% power, α=0.05, with up to 12 predictors is n=205)[137]. For hypothesis 2b, based on current BCC3 bioavailable E2 levels, given median for WLWH of 4.34 pg/mL and 6.87 pg/mL for controls with a population standard deviation of 8.78 pg/mL, and using a sample size calculator that employs this data[138], we require a total of n=230 individuals to detect a difference of 2.3 pg/mL between the groups with 80% power, α=0.05. We have already enrolled more than the required numbers of women in each group, even when only including women not using hormones. We lack preliminary lifetime E2 exposure data, but with 640 women enrolled, we have >80% power, with α=0.05 to detect an effect size as little as ±0.3 in lifetime E2[123].

Data analysis: Aim 2 data will be considered as continuous variables and descriptive statistics will be reported by HIV status. Hypothesis 2a & 2b: Linear or quantile regressions will be used as appropriate to assess associations between lifetime E2 exposure and bioavailable estrogen levels with the outcome variables (mtDNA content, mt intermembrane potential/mt mass ratio, inflammation index, immune cell senescence and/or exhaustion), and by living with HIV. Analysis will be adjusted for age, smoking, opioid use, and other potential covariates and confounders. Data will be log transformed if not normally distributed. Inflammation will be defined and assessed in two ways; i) cellular chronic activation (HLA-DR+CD38+), and ii) inflammatory index as described in Aim 1. We will adjust for multiple comparisons as appropriate.

AIM 3: To longitudinally compare changes in hormones and aging markers in midlife women.

Hypotheses:

3a. Greater change in PhenoAgeAccel between visits will be associated with: i) lower lifetime E2 exposure, ii) lower Visit #1 bioavailable E2, iii) higher Visit #1 ERα/ERβ ratio, iv) living with HIV (primary outcomes).

3b. Greater change in aging hallmarks (mtDNA content, mitochondrial dysfunction, chronic inflammation, immune cell senescence and/or exhaustion) between Visits #1 and #2 will be associated with i) lower lifetime E2 exposure, ii) lower Visit #1 bioavailable E2, iii) higher Visit #1

ERα/ERβ ratio, iv) living with HIV (secondary outcomes).

3c. At Visit #2, greater weight gain among WLWH using integrase inhibitors will correlate with i) Lower mtDNA content, ii) greater mitochondrial dysfunction, and/or iii) higher ERα/ERβ ratio.

Rationale: Midlife women (aged 40-60y) will be asked to return for a second study visit (Visit #2) as women in that age range are going through the period of greatest hormonal change (menopause transition)[139], and this correlates with rapid changes in cardiometabolic and other health risk[140]. Menopause accelerates phenotypic aging[141] and is associated with low sex hormone levels. WLWH may have earlier menopause[29,30,142]. As concurrent illness is also more prevalent in WLWH versus control women[82], we anticipate greater PhenoAgeAccel and markers of aging in WLWH vs controls.

Integrase inhibitors (e.g. dolutegravir, bictegravir) have been associated with weight gain in some individuals[143-145], with midlife and older women experiencing the greatest weight gain[146,147]. As dolutegravir has been associated with impaired mitochondrial health[130], weight gain with menopause[148], and altered ERα/ERβ ratio with lower E2[6], we contend lower mitochondrial health and/or ERα/ERβ ratio may relate to weight gain on integrase inhibitors.

Preliminary data: Pilot data from a sample of 200 BCC3 participants suggests PhenoAgeAccel differs between women living with and without HIV (see Aim 1 power calculation). Our team previously showed that dolutegravir (an HIV integrase inhibitor) reversibly alters mitochondrial health[130]. Based on current British Columbia prescribing practices, we anticipate many participants will have changed to an integrase-containing regimen since completing Visit #1.

Methods: 300 midlife participants (150 WLWH and 150 controls) aged 40-60 years at Visit #1 will be invited to return 3-5 years after Visit #1 for a Visit #2 which will include collection of clinical, anthropometric, sociodemographic, psychosocial and structural data, with linked biospecimens and clinical lab tests in a manner similar to Visit #1 described under "Study Visits" on Page 4 [94]. We have ethics approval to contact participants (98% agreed to be contacted). With our privileged access to participants, history of success enrolling large cohorts, active KM engagement from BCC3 participants, and links with key community groups, we expect no difficulty reaching our target.

Lifetime E2 exposure, bioavailable E2 levels and ERα/ERβ ratios will be calculated as described in Aim 1. PhenoAgeAccel (Aim 1), mtDNA content, mitochondrial dysfunction, inflammation, and markers of immune cell senescence and exhaustion (Aim 2), and weight gain (Aim 3c) will be assessed as above, and their change between study visits calculated as value at Visit #2 – value at Visit #1 divided by time between visits.

Power calculations: Hypothesis 3a: Pilot analysis of Visit #1 data for a random sample of 200 BCC3 participants, showed median PhenoAgeAccel of +1.1 years for WLWH; -1.8 years for controls (positive PhenoAgeAccel suggests older phenotypic age), and a population standard deviation of ±9.06. For repeated measures, assuming $\alpha=0.05$, and correlation between repetitions of 0.75, we have > 80% power to detect a 2-year difference in PhenoAgeAccel given our sample size of 300 women between 40-60 years at baseline[149]. This calculation includes an expected 10% attrition rate, based on our experience, in part due to BC's opioid crisis. Hypothesis 3c: Based on published weight gain values in midlife WLWH taking (mean±SD) +2.3 ±1.2 kg versus not taking integrase inhibitors -0.3±0.8 kg[146], if we conservatively assume a SD of ±3 kg for our study, we have 80% power, $\alpha=0.05$ to detect a difference in weight gain of 1 kg between the groups, with n=142 per group.

Data analysis: Descriptive statistics will summarize prevalence and distribution of variables. Hypothesis 3a, 3b: Linear or quantile regressions will assess whether changes in PhenoAgeAccel (3a) or change in aging hallmarks (3b) are associated with lifetime E2 exposure, lower Visit #1 bioavailable E2, higher baseline ERα/ERβ ratio, or living with HIV. Hypothesis 3c: We will assess differences in weight gain between: 1) WLWH using integrase inhibitors at Visit #2, 2) WLWH not using integrase inhibitors at Visit #2, and 3) women not living with HIV, using ANOVA or Kruskal-Wallis according to data distribution with Tukey or Dunn's correction, respectively. Linear or quantile regressions will

assess associations between weight gain and i) mtDNA content, ii) mitochondrial dysfunction, and iii) ERα /ERβ ratio with integrase use and weight gain as an interaction. All models will be adjusted for age, smoking, opioid use, time on integrase inhibitors, and other covariates and confounders.

TIMELINE (Table 4).

Table 4. Timeline

Aim	Research	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5
1,2,3	Ethics amendment					
3	Enroll Visit 2 participants (n=300)					
1,2,3	Hormone, binding globulin, measurements					
1,2,3	Calculation of lifetime E2 exposure					
1,2,3	Hormone receptor measurements					
2,3	Immune cell phenotyping					
2,3	Mitochondrial dysfunction					
2,3	Assessment of plasma inflammation					
1,2,3	Statistical Analysis					
All	Knowledge mobilization					

KNOWLEDGE MOBILIZATION

Our protocol/surveys/policies are publicly available on the BCC3 website[94]. A diverse and inclusive group of women representing Indigenous, African/Caribbean/ Black, youth, older women, and others have, and will continue to, lend lived experiences to this project. Following the CHIWOS research model, WLWH have informed research questions/survey formulation, administer all community surveys and routinely co-present findings[150]. We will strive to conduct research in a Good Way, with Indigenous Knowledge Holders, Community Research Associates, and BCC3 CAB members guiding KM strategy and content. In addition to classic KM (conference presentations/abstracts/peer-reviewed publications), we will communicate findings and receive feedback from women attending local community organisations, through Sharing Circles, retreats, and education sessions (we have held 14 in the last 3 years). We will hold events for care providers and share KM through the CIHR-funded Canadian Women-Centred HIV Care Hub (***Murray, King*** and ***Côté***), community partners (***ACPnet, WHRI, Ribbon Community***), the ***CIHR CTN+*** (who will also provide statistical support), our study website, and social media. Study clinicians will incorporate findings into clinical care guidelines, and patient care management.

EXPERTISE & ROLES

We have assembled an inclusive team and are uniquely positioned to undertake this project, leveraging the BCC3 cohort staff, structure, database, and biospecimens. We have access to all equipment and services required; we meet biweekly to ensure seamless project implementation. As NPA, I (***Murray***) will lead oversight, ethics, enrolment, data analysis and interpretation, and KM. ***King, Loutfy***, and ***Murray*** bring expertise in community-based research, clinical HIV care, and psycho-socio-structural contributors to health in WLWH. ***Elwood*** and ***Prior*** bring clinical expertise in hormones and menstrual cycles/menopause. ***Côté*** is a basic scientist HIV research focus; testing for hormones, receptors, mtDNA content and dysregulation, and inflammatory markers will be done in her lab. ***Murray*** and ***Kowatsch*** will lead Aims. Knowledge Holders ***Cardinal, Magagula, Tognazzini***, Community Research Associates (3 WLWH) and students (***Cai, Povshedna***) will ensure integrated KM. ***Sierocinski*** has big data analysis expertise and will guide statistical analysis along with **CTN+**.

IMPACT

The BCC3 cohort provides a unique opportunity, by employing HIV as a model of accelerated aging, to study how hormones influence the aging process in all women, and to investigate it comprehensively, integrating biomedical and socio-structural factors. Our work will inform future mechanistic, and therapeutic studies that examine the roles sex hormones play in healthy aging with an aim of improving the health and quality of life of women across the life course. Our findings may inform clinical trials of hormone therapy for women with prolonged premenopausal amenorrhea, or early onset menopause – especially those living with HIV. Greater knowledge of the role of modifiable and non-modifiable factors on hormonal imbalance will inform women and their care providers with respect to screening, counselling, and support of healthy aging, particularly as midlife women enter the menopause transition. Ultimately, this research will highlight the importance of hormones in the aging process and potentially identify hormones as an additional hallmark of aging.

Statement of Research Focus

Over the past 5 years I have worked closely with Dr. Helene Côté (a basic scientist at the University of British Columbia), and Dr. Angela Kaida (a social scientist at Simon Fraser University) to conceive of and build the British Columbia CARMA-CHIWOS Collaboration. BCC3 combines two, decade-long, prospective cohorts of women living with HIV (CARMA and CHIWOS) into a society-to-cell framework examining healthy aging among women living with and without HIV holistically from psycho-socio-structural, clinical and cellular vantages. As one of the three co-Principal Investigators on this study, my particular research focus is the role of hormones in healthy aging.

Progress/Productivity/Impact:

Thus far, my research has shown that women (refers to cis women in this context) living with HIV 1) are more likely to experience prolonged amenorrhea prior to menopause; 2) have lower markers of ovarian reserve than negative controls; and 3) experience more comorbidity (hereafter concurrent conditions as per community recommendations), earlier in life than same age women without HIV.

In the general population, female reproductive hormones estrogen and progesterone are anti-inflammatory, and protective to many organ systems, including the bone, brain, liver, kidney, and cardiovascular systems. Lower estrogen correlates with earlier onset of age-related disorders. In our study, women living with HIV who have a history of prolonged amenorrhea also have a higher risk of osteopenia and liver fibrosis than women living with HIV without this history. Notably, risk of these illnesses is greater for women with both HIV and amenorrhea, than controls with amenorrhea.

As socio-structural factors commonly intersect with hormone dysregulation and concurrent conditions, control women are recruited to be as well matched as possible to their peers living with HIV. These factors are routinely included in our analyses (we have endeavored to match our cohort by age, ethnicity, and British Columbia health authority, and recruit participants from similar locations/environments to reflect the population of women living with HIV in British Columbia). This will power us to draw conclusions about aging in women with similar socio-structural backgrounds.

To date, despite COVID-19 impacts, the study launched in March 2021. Thus far, we have recruited 640/700 participants (Visit #1), held fourteen knowledge mobilization events, presented 26 abstracts (posters/oral presentations), and published ten papers, with several more nearing submission.

Through past funding (below) and in preparation for this grant, we have done the following:

AIM 1: For enrolled participants, we assessed Visit #1 estradiol and progesterone on premenopausal women, estrone on menopausal women, and SHBG and testosterone in all women. Flow cytometry panels for this proposal have been optimized and hormone receptors (ERα, ERβ, PR, GR, and AR) have been characterized on lymphocytes from donor peripheral blood mononuclear cells (PBMCs). We have reviewed the literature to generate the equation that will be used for lifetime E2 exposure. Finally, we already have Visit #1 results for all parameters required for phenotypic age calculation for all enrolled participants. Furthermore, in a random sample of 200 participants, we have calculated baseline phenotypic age for use in power calculations, and while not currently significant, have observed that women living with HIV are on average 3 years older phenotypically than women not living with HIV.

AIM 2: The flow cytometry panel proposed for assessing mitochondrial dysfunction and the qPCR for mitochondrial DNA (mtDNA) content are standardized assays in the Côté lab. We have optimized the flow panel for phenotypic characterization (senescence, exhaustion, and proliferation) of all proposed cell subsets. In consultation with the literature, we have selected the inflammatory plasma markers and assessed their distribution in a random sample (distributed for age, ethnicity, and number of non-HIV viruses) to ensure detection using our chosen method (MesoScale). Finally, for **AIM 3**: We have ethics approval and 98% of participants have consented to be contacted for future visits. We know from experience to expect an attrition rate of ~10%, however, despite this we are still powered for our proposed analyses. Methods used in aim 3 have been optimized as described for aims 1 and 2.

Using HIV as a model of accelerated aging, we aim to address key knowledge gaps in women's health by exploring the relationship between hormones, phenotypic age, and cellular aging. We will

invite 300 midlife women to return for Visit #2 (~3-5 years later) to assess longitudinal changes in hormones and aging markers at a time in their lives when we are most likely to see change.

A note on trans and non-binary individuals and men: The BCC3 study is not currently powered to draw conclusions on hormone levels for trans-women, however, the study is trans and non-binary femme inclusive. Seven trans-women have completed the study so far. We will present data descriptively, generate hypotheses, and conduct a larger study investigating hormones and healthy aging in trans individuals. We have built relationships with the trans community to address some of their healthy aging research priorities (Team Grant - Embracing Diversity to Achieve Precision & Health Equity; Letter of Intent - Funded). In addition, through partnerships with Dr. King, and the BCC3 affiliated CARMA study, we have access to men living with and without HIV. We anticipate assessing the role of hormones in healthy aging for these groups in future applications.

Budget requested in relation to overall funding held currently or in the past:

There is no overlap of funding between this application and funds that are currently or have in the past been held. This application leverages the data collected through previous grants to delve deeper into relationships between hormones, inflammation and cellular aging in women.

Current and past funding for the BCC3 study includes the following:

1. ***"The BCC3 cohort: Cellular aging in women living with HIV." CIHR Project Grant. (04/2019-03/2024). NPA Helene Cote, Co-I Melanie Murray, $822,375.*** Funding established BCC3 cohort for 500 participants: Focus on chronic viral infections (Epstein-Barr virus, cytomegalovirus, hepatitis B, C, herpes viruses 1 and 2, cytomegalovirus, Kaposi's sarcoma virus) and cellular aging (telomere length, mitochondrial DNA content) among WLWH and well-matched control women.
2. ***"Hormones and Healthy Aging in Women Living with HIV: A Study By, With and For Community" CIHR CBR Grant (04/2020-03/2023). NPA Melanie Murray, $450,000.*** This funding supported the Visit #1 enrollment of 200 participants, supported us in improving community-based research practices in our cohort, and enabled measuring of total estradiol and progesterone levels of pre-menopausal participants (n=400), and estrone in menopausal participants (n=300).
3. ***"Role of Female Sex and Hormones on Aging in Women Living with HIV" CIHR Project Grant (04/2021-03/2026). NPA Melanie Murray, $382,500.*** This funding allowed the assessment of Visit #1 telomere length, mitochondrial DNA content, and chronic viral co-infections among additional 200 participants enrolled above (2), and measurement of hair cortisol levels for all 700 participants.
4. ***"The British Columbia CARMA-CHIWOS Collaboration (BCC3): the role of female sex hormones and chronic/latent co-infections on cellular aging and comorbidities in women living with HIV." CIHR HIV Clinical Trials Network Grant (12/2021-12/2026). NPA Melanie Murray, $119,583.*** This grant enabled our team to increase our knowledge mobilization activities, consult with an Elder on OCAP principles, conduct recruitment drives at sites distant from Vancouver (Victoria, Prince George, the interior of British Columbia), and increased remuneration for participants and community research associates (WLWH who have research training and conduct participant surveys).
5. ***"The Women-Centred HIV Care Hub: Mobilizing and Scaling-up the Women-Centred HIV Care Model across Canada." (07/2023-06/2027). PA Melanie Murray. $840,000.*** This grant is entirely for National Knowledge Mobilization of the Women Centered HIV Care Model. No overlap.
6. ***"What healthy aging means to me: a community-collaborative knowledge mobilization initiative with, by, and for, women living with HIV." (10/2024-04/2026). PA Melanie Murray. $20,000.*** This grant is entirely for knowledge mobilization to community partners in BC.

*For the current proposal, total estradiol, estrone, progesterone, SHBG, mtDNA content, and lab parameters for PhenoAge calculation from Visit #1 have been measured from the above funding. Requested funding will allow Visit #2 for 300 participants, testing of hormone receptors, mitochondrial dysfunction, cell senescence and inflammatory markers (both Visits), mtDNA content (Visit #2).

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BCC3 Study

Knowledge translation and exchange: communicating research study results to the BCC3 study participants

The purpose of this document is to outline the consultations done around the knowledge translation and exchange project embedded within the BCC3 study – communicating research study results to the BCC3 study participants. This project is in response to numerous inquiries by the women enrolled in the BCC3 study, who told us they are interested in receiving their personalized study results. This work will be in addition to the original BCC3 protocol; participation in this project will not impact status within the main BCC3 study.

BACKGROUND

As a community-based cohort study, BCC3 works with the community members of our study team (Community Research Associates), study participants, and other community stakeholders to improve the relationship between research and community in a meaningful way. During the informed consent process, many women now enrolled in the study expressed interest in receiving research study results back. While communicating the standard laboratory test results either to the participant and/or their healthcare provider (based on the preferences indicated during the consent process), is already a part of the BCC3 protocol, numerous research measures related to healthy aging are not communicated back individually to women. In response to this interest, we secured funding from the UBC Public Scholars Initiative (PSI) and Michael Smith Health Research BC to initiate consultations with the community and ultimately organize Knowledge Translation and Exchange (KTE) events for the BCC3 study participants who are interested in receiving individualized study results. We believe that these events will help "complete the circle" for the community-based aging research and empower women to take charge of their healthy aging journey and equip them with tools and knowledge to support it.

METHODS

The objectives of the consultations were to:

- To identify the key themes in healthy aging research that are of interest for women living and aging with HIV.
- To identify the list of BCC3 research results that are appropriate to communicate back to the participants and how to communicate them in a good way.
- To discuss and develop the framework of the KTE events (duration, number of participants, speakers, information booklet, venue, infographics/poster).
- To develop in-person and virtual options to all BCC3 participants regardless of geographical location can receive the KTE events.

The consultations (n=4) have been held on a virtual platform (zoom) and involved the BCC3 team members leading the project (Tetiana Povshedna, Monika Kowatsch), BCC3 PIs (Drs Helene Cote, Melanie Murray, Angela Kaida, Elizabeth King), BCC3 Peer Research Associates and other team

members who were interested. We invited some of the BCC3 Community Advisory Board partners and other community members to give us advice and feedback. A $28/h honorarium was provided. This part of the KTE project is not research and is not something we intend to publish.

The zoom account was used in accordance with the University of British Columbia (UBC) zoom security measures which include using a licensed version of Zoom (UBC or SFU licensed versions will be used), having a unique Zoom link for the meeting, and sharing that link only with participants. In addition, we used a virtual "Waiting Room" and required a password on the zoom call and allowed for identity confirmation while simultaneously allowing participants to remain anonymous in the zoom room.

The KTE pipeline was developed based on the consultations, and will be the subject of a future protocol amendment, which will be accompanied by an example of the information booklet that will be shared with interested BCC3 participants, poster/infographics, event plan, venue details, etc.

DATA ANALYSIS

To identify themes and priorities with regards to study results communication, the first wave of consultations have been performed and consultations were transcribed verbatim to be further used for the KTE pipeline development. A second wave of consultations is in progress, building on results of the first. A third wave of consultations is planned for 2029-30, this will depend upon receipt of future grants.

DATA ACCESS AND STORAGE

Our recorded transcripts are kept in password-protected files and only the project lead (Tetiana Povshedna) and BCC3 PIs will have access to these files. We will not collect any identifying information during the consultations and any information that could be used to identify an individual will not be included in transcriptions from these meetings. Once KTE pipeline is developed, the recorded transcripts will be destroyed.

KNOWLEDGE TRANSLATION & EXCHANGE

This is necessary for the development of the KTE pipeline that will allow for the effective and meaningful communication of individual BCC3 study results back to the participants. The details of the proposed KTE plan will be described in detail, pending the second wave of community consultations.

Statement of Indigenous experience - BC CARMA CHIWOS Collaboration Team

To ensure that our research contributes positively to the communities that we study, we have partnered with Indigenous leaders who have guided, and will continue to guide, our approaches to ensure that our team contributes to respectful and relevant health research that includes Indigenous people. Elder Sheila Nyman, a Syilx Metis woman from the Lower Similkameen in the Okanagan Valley and Clinical Social Worker, has been involved in the BCC3 since its inception and has guided our team to ensure that our work is respectful and culturally-safe. Sheila also provides counseling services to any member of the BCC3 study team, study participant, or community partner that is in need of support, particularly regarding trauma relating to racism and colonialism. Claudette Cardinal, a descendent of the Central Plains Cree of Alberta of the Maskwa (Bear) clan and Indigenous Community Researcher brings the lived and living experience of HIV to our team. Claudette has been involved in several aspects of the BCC3 study activities ranging from guiding study design, interpretation of findings, co-presenting at conferences, and leading knowledge translation activities. Both Sheila and Claudette will continue to be involved in the BCC3 study during the next steps of our research journey, and their guidance will be critical to our evolving data management plan. Furthermore, Indigenous voices are an important part of our community advisory board (CAB), and our CAB includes representation from individuals and Indigenous community service organizations from across the province (Carrier Sekani Family Services [Northern BC], Central Interior Native Health Society [Northern BC], and Kilala Lelum [Lower Mainland]). Together, these individuals and organizations represent a diversity of Indigenous voices that ensure that our research remains relevant, attentive to, and respectful of the communities that we study. In addition, several members of the BCC3 team have contributed to a recently published set of guiding principles for the conduct of meaningful research of Indigenous women living with HIV that will be implemented in our study approach (https://hivhearme.ca/chiwos-paw/; Nicholson et al. *Int Journal of Qual Methods* 2022; 21). These principles were developed through extensive consultation with Indigenous women living with HIV and will be used as a framework to guide our research. Aligned with these principles, we seek to create research that is action-oriented, strengths-based, and uses inclusive and honoring language. Our knowledge translation events will incorporate arts-based methods and build in time to connect with the lands and integrate ceremony, all approaches identified through this consultation. We will work with Indigenous partners to ensure that our work minimizes harms and maximizes benefits and that all knowledge is shared with communities who contributed to this work. Guided by these principles and Indigenous voices, we believe that we will be equipped to conduct culturally-safe and meaningful research with, by, and for Indigenous persons.

Research Project and Indigenous Peoples - BC CARMA CHIWOS Collaboration Team

Indigenous people are overrepresented among those living with HIV in Canada. This is particularly true of women where Indigenous women comprise more than 30% of women living with HIV in Canada. Therefore, to carry out ecologically valid and safe work, inclusion

of Indigenous people in our study is essential, and Indigenous women also represent approximately 30% of BCC3 participants, in each HIV group. Our overall goal is to conduct culturally sensitive and participatory research that is guided by Indigenous voices. To do so, we have developed a strategy that prioritizes Indigenous engagement throughout all stages of our work. We enact principles from Chapter 9 of TCPS-2 including community engagement, collaboration, diversity of representation, and integration of customs and codes into research practices. Below, we summarize the key aspects of our approach to ensure that our research addresses the rights of Indigenous Peoples:

i) *Centering our research on community priorities* - To ensure collective benefit, we have engaged with Indigenous women living with HIV to understand their needs, priorities, and perspectives. We have shaped our research questions, study design and knowledge translation plans around these priorities, and remain responsive to ongoing guidance from Indigenous voices to ensure that our research directly benefits their communities.

ii) *Integrating culturally-safe methods and Indigenous ways of knowing into research approaches* - Our research prioritizes the inclusion of women, particularly Indigenous women, in a culturally-safe manner. We do this by collaborating with Indigenous Elders to ensure study materials are respectful, by providing cultural sensitivity training to all team members, and by partnering with Indigenous leaders to offer real-time counseling, and integrating Indigenous customs into team activities and knowledge translation events.

iii) *Applying principles of ownership, control, access and possession (OCAP) to data use* We have consulted with local Indigenous allies (Nicholson, Cardinal, Nyman) to ensure that our current data use practices respect OCAP principles. Funds from the LOI will allow us to expand this consultation process and establish a detailed data management plan that integrates OCAP, FAIR and SHARE principles to develop an approach that balances data sharing with safeguarding Indigenous data sovereignty.

iv) *Building capacity in Indigenous Health Research* - Our team is committed to building capacity to allow for long-term engagement of Indigenous partners in research. We will continue our tradition of producing research that is co-built with community members, including our Indigenous partners. To extend capacity-building efforts to smaller communities in BC, we will recruit and train an Indigenous community research associate from rural BC, providing support for them to eventually assume a leadership role in research, thereby strengthening capacity in remote areas of the province.

In conclusion, our commitment to conducting ecologically valid and safe research necessitates the meaningful inclusion of Indigenous people, particularly women. Guided by Indigenous voices, community partnerships and principles of data sovereignty and TCPS-2, we hope to contribute to positive and sustainable research that addresses the unique needs of Indigenous communities.