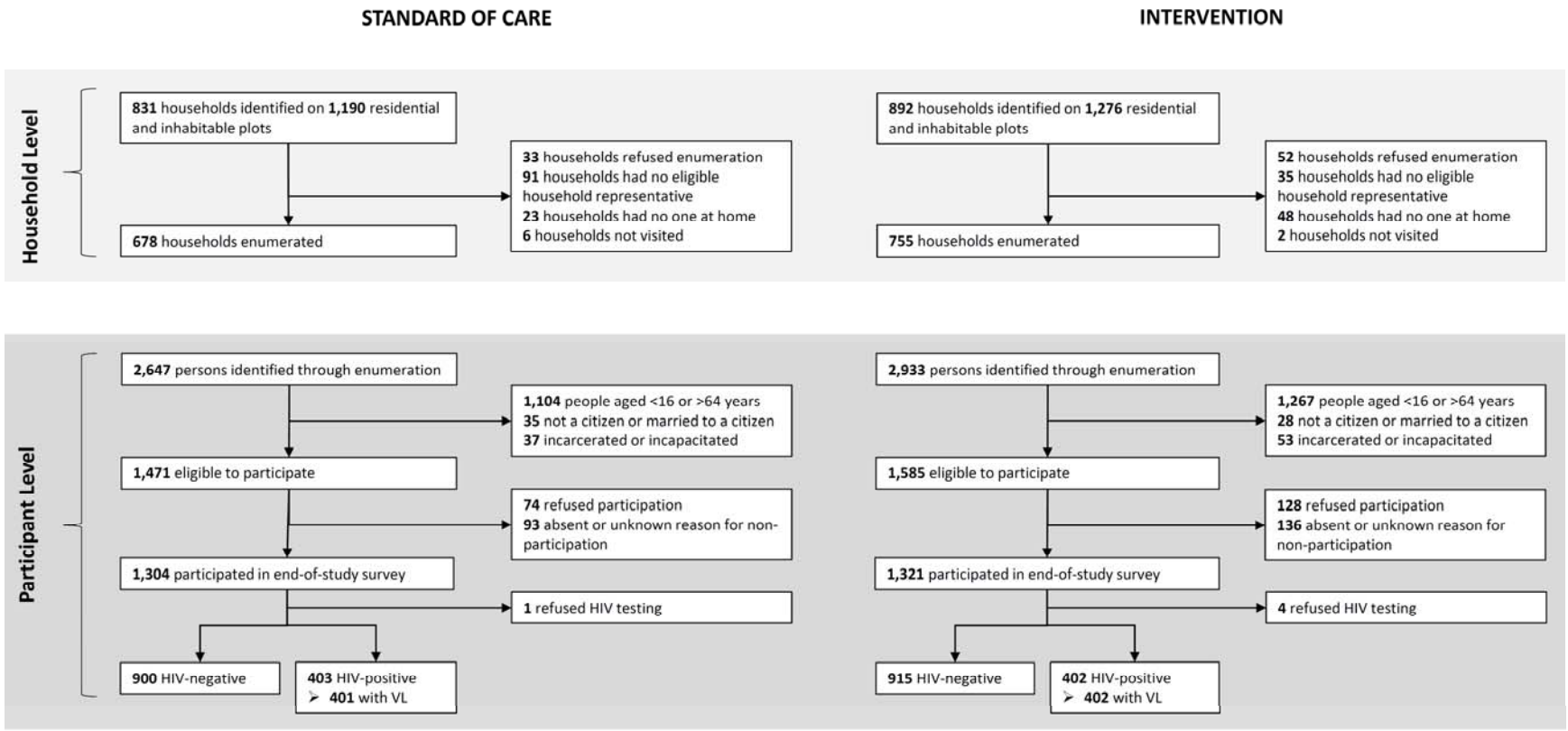
Population uptake of HIV testing, treatment, viral suppression, and male circumcision in Botswana: a cluster-randomized trial

Kathleen E. Wirth, ScD, Tendani Gaolathe, MD, Molly Pretorius Holme, MS, Mompati Mmalane, MD, FRCS, MSc, Etienne Kadima, MD, MPH, Unoda Chakalisa, MD, Kutlo Manyake, GN, Atang Mbikiwa, BA, Selebaleng V. Simon, BNS, Rona Letlhogile, BNS, Kutlwano Mukokomani, MBA, Erik van Widenfelt, BA, Sikhulile Moyo, PhD, Kara Bennett, MS, Jean Leidner, MS, Kathleen M. Powis, MD, Refeletswe Lebelonyane, MD, Mary Grace Alwano, MPH, Joseph Jarvis, MBBS, PhD, Scott Dryden-Peterson, MD, Coulson Kgathi, BCS, Janet Moore, PhD, Pam Bachanas, PhD, Elliot Raizes, MD, William Abrams, MA, Lisa Block, MS, Baraedi Sento, Vlad Novitsky, MD, PhD, Shenaaz El-Halabi, Tafireyi Marukutira, MD, Lisa A. Mills, MD, Connie Sexton, PhD, Sherri Pals, PhD, Roger Shapiro, MD, Rui Wang, PhD, Quanhong Lei, PhD, Victor DeGruttola, ScD, Joseph Makhema, MD, FRCP, M Essex, DVM, PhD Shahin Lockman, MD, Eric J. Tchetgen Tchetgen, PhD

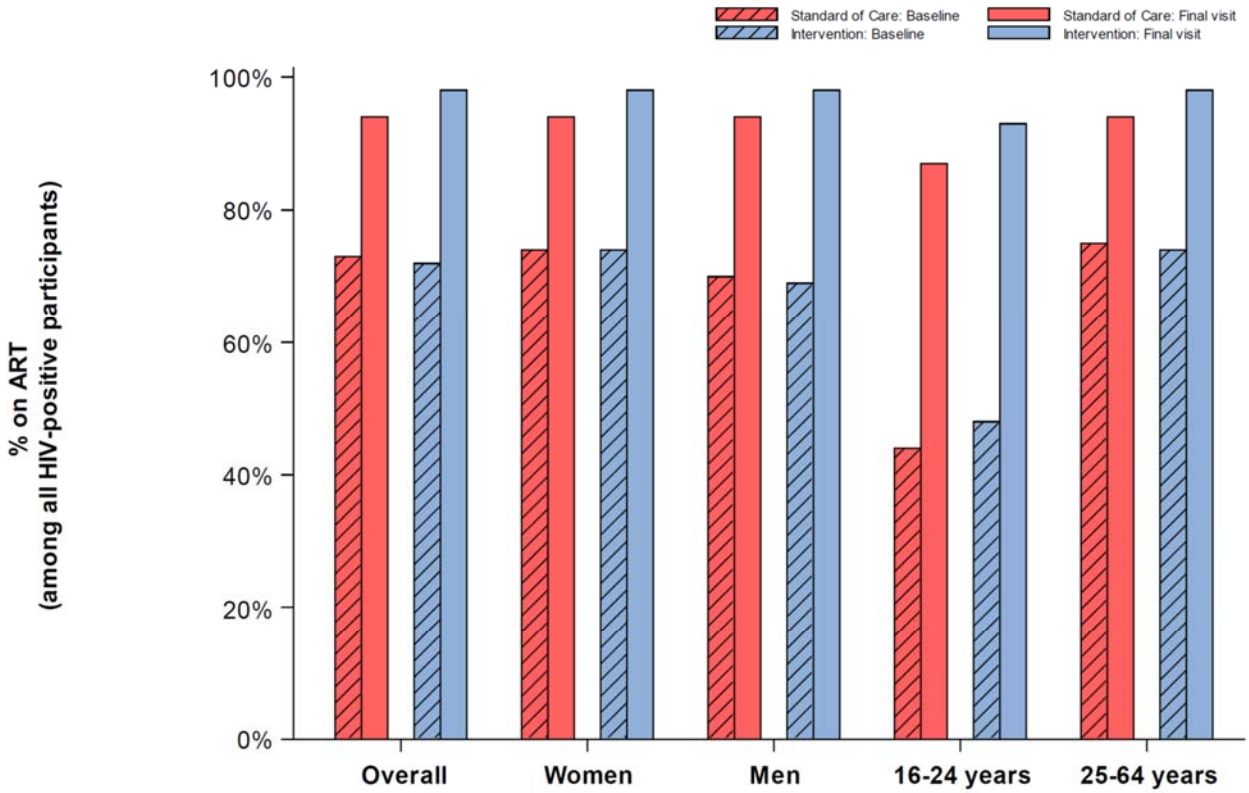
Supplementary Appendix

Figure S1. Recruitment, eligibility, and enrollment of participants in the 20% longitudinal cohort in six communities (3 matched pairs) in the Ya Tsie study at the household and participant levels according to randomization arm



Coverage at baseline and end of study among participants enrolled in the 20% longitudinal cohort from standard-of-care and intervention communities for the Ya Tsie study, overall and according to sex and age:

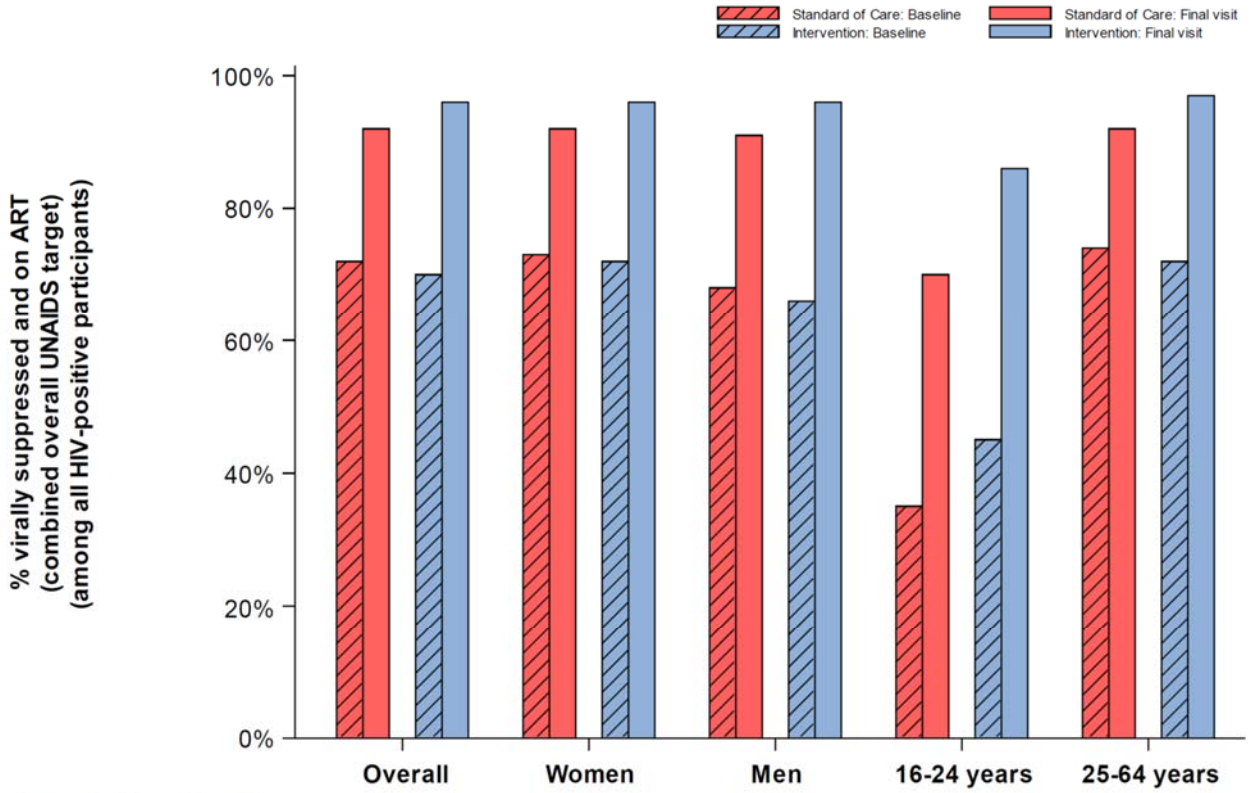
Figure S2.A. Longitudinal cohort: % on ART (among all HIV-positive participants)



	Overall	Women	Men	16-24 years	25-64 years
Standard of Care: Baseline	73% (1,295/1,771)	74% (971/1,306)	70% (324/465)	44% (48/110)	75% (1,247/1,661)
Standard of Care: Final visit	94% (1,479/1,576)	94% (1,109/1,182)	94% (370/394)	87% (45/52)	94% (1,434/1,524)
Intervention: Baseline	72% (1,322/1,825)	74% (979/1,329)	69% (343/496)	48% (54/112)	74% (1,268/1,713)
Intervention: Final visit	98% (1,530/1,564)	98% (1,141/1,167)	98% (389/397)	93% (42/45)	98% (1,488/1,519)

Coverage at baseline and end of study among participants enrolled in the 20% longitudinal cohort from standard-of-care and intervention communities for the Ya Tsie study, overall and according to sex and age:

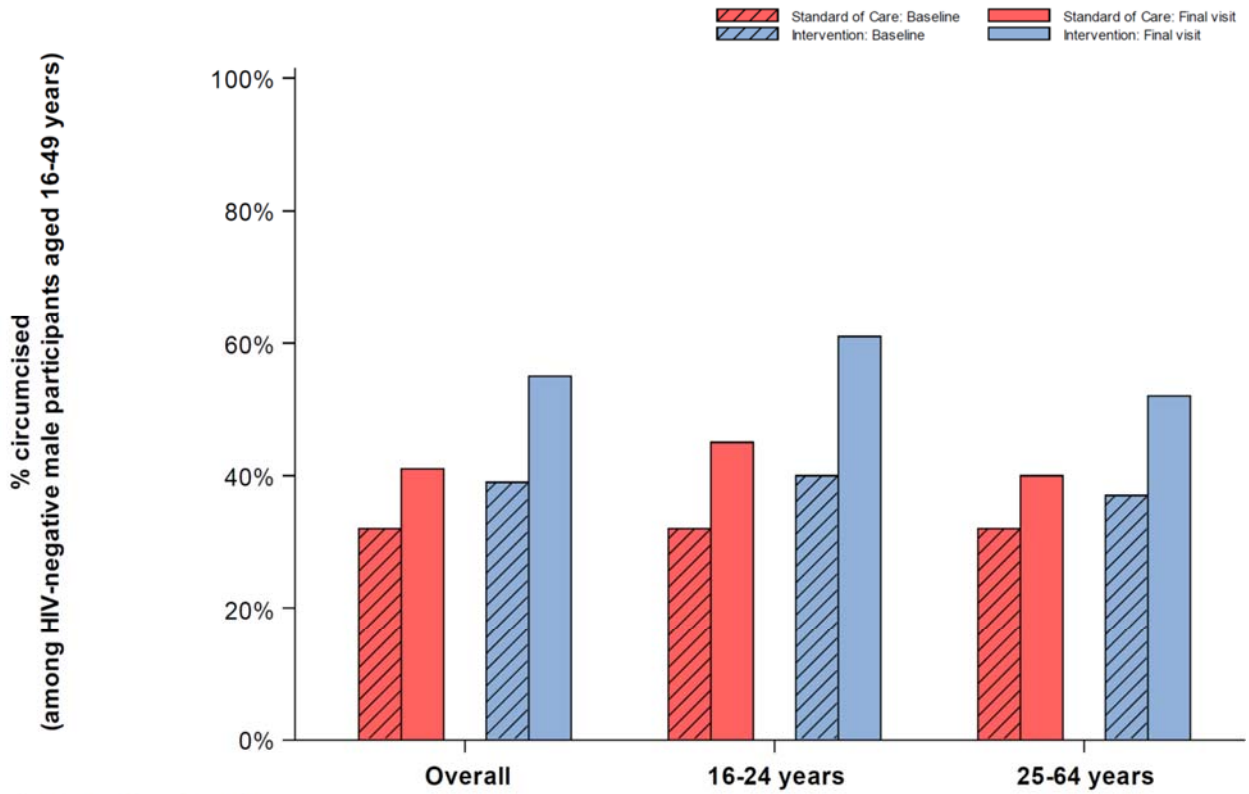
Figure S2.B. Longitudinal cohort: % virally suppressed, on ART (among all HIV-positive participants)



	Overall	Women	Men	16-24 years	25-64 years
Standard of Care: Baseline	72% (1,168/1,631)	73% (876/1,199)	68% (292/432)	35% (35/101)	74% (1,133/1,530)
Standard of Care: Final visit	92% (1,311/1,432)	92% (984/1,072)	91% (327/360)	70% (33/47)	92% (1,278/1,385)
Intervention: Baseline	70% (1,168/1,665)	72% (874/1,218)	66% (294/447)	45% (47/104)	72% (1,121/1,561)
Intervention: Final visit	96% (1,357/1,409)	96% (1,021/1,059)	96% (336/350)	86% (36/42)	97% (1,321/1,367)

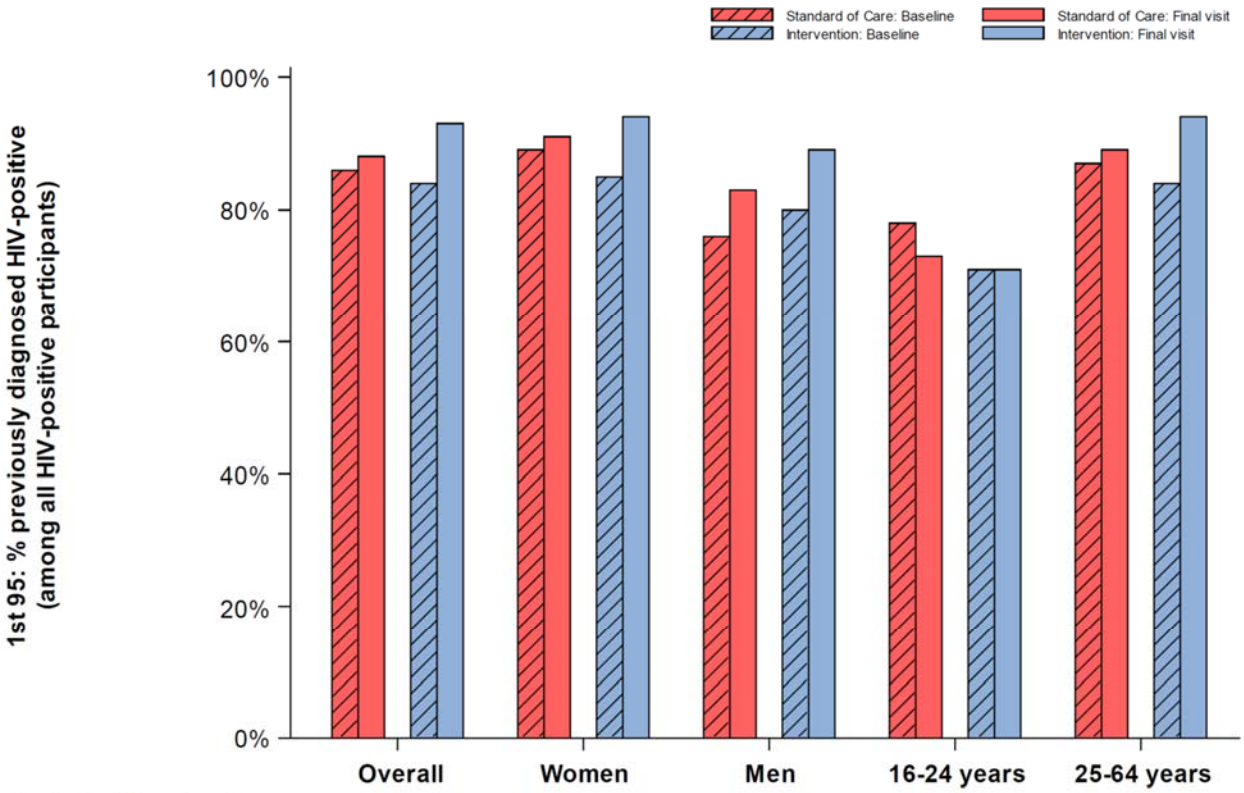
Coverage at baseline and end of study among participants enrolled in the 20% longitudinal cohort from standard-of-care and intervention communities for the Ya Tsie study, overall and according to sex and age:

Figure S2.C. Longitudinal cohort: % circumcised (among HIV-negative male participants aged 16–49 years)



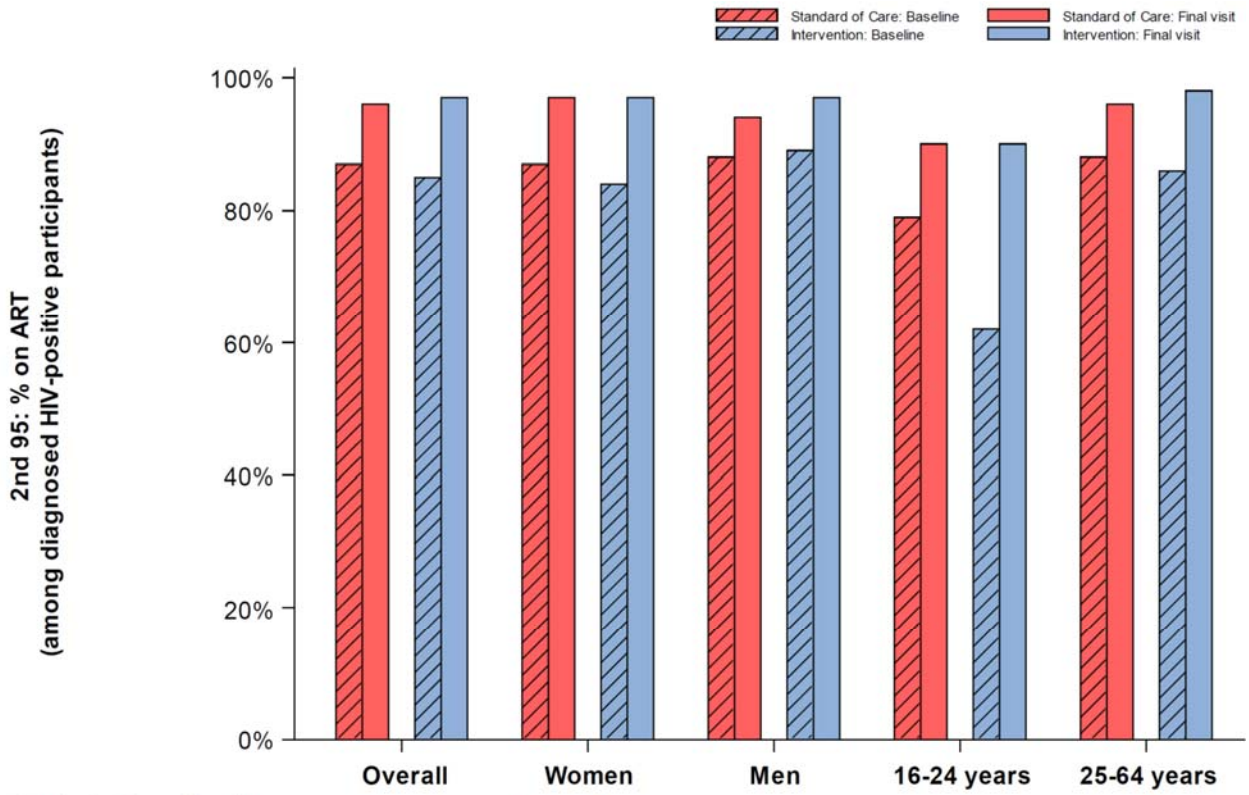
	Overall	16-24 years	25-64 years
Standard of Care: Baseline	32% (492/1,548)	32% (227/708)	32% (265/840)
Standard of Care: Final visit	41% (560/1,351)	45% (219/492)	40% (341/859)
Intervention: Baseline	39% (596/1,545)	40% (273/680)	37% (323/865)
Intervention: Final visit	55% (716/1,311)	61% (262/432)	52% (454/879)

Figure S3.A. End-of-study survey: 1st 95: % diagnosed (among all HIV-positive participants)



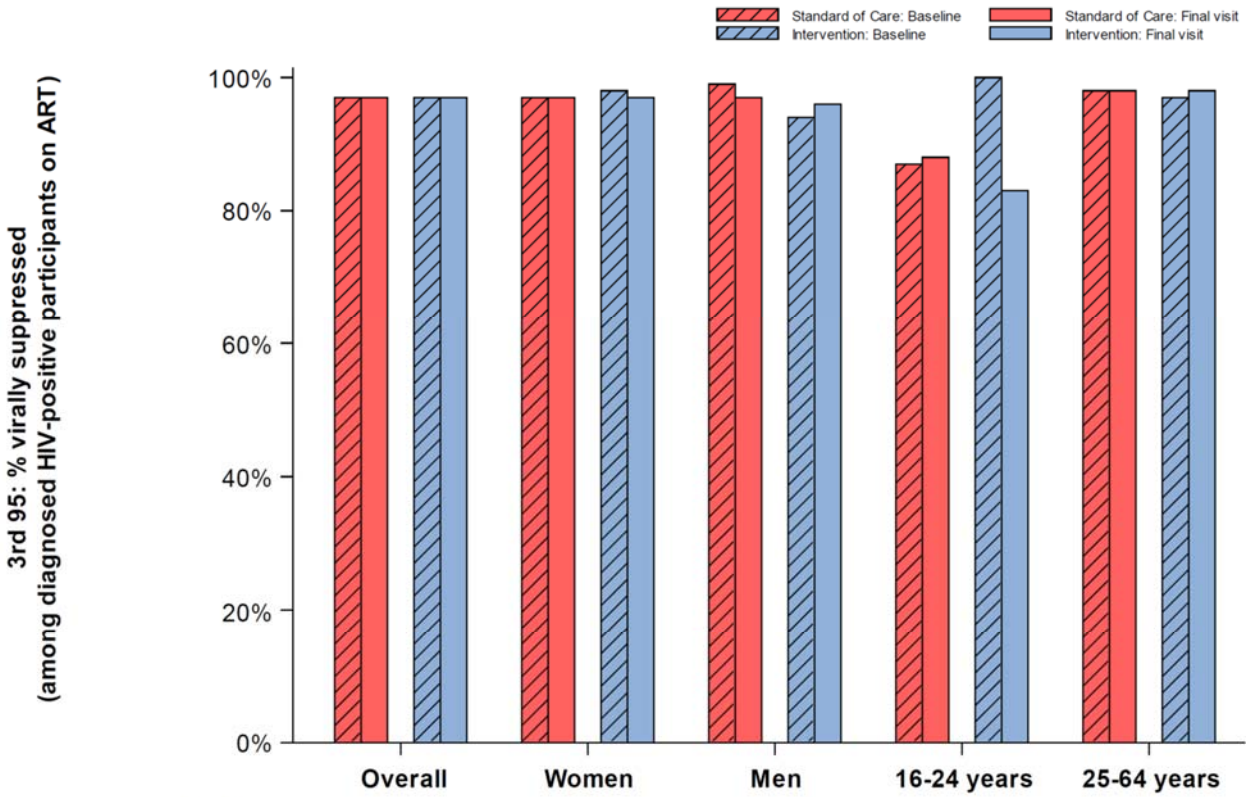
	Overall	Women	Men	16-24 years	25-64 years
Standard of Care: Baseline	86% (347/403)	89% (269/301)	76% (78/102)	78% (18/23)	87% (329/380)
Standard of Care: Final visit	88% (1,184/1,338)	91% (870/961)	83% (314/377)	73% (60/82)	89% (1,124/1,256)
Intervention: Baseline	84% (336/402)	85% (249/293)	80% (87/109)	71% (12/17)	84% (324/385)
Intervention: Final visit	93% (1,254/1,353)	94% (944/1,003)	89% (310/350)	71% (47/66)	94% (1,207/1,287)

Figure S3.B. End-of-study survey: 2nd 95: % on ART (among diagnosed HIV-positive participants)



	Overall	Women	Men	16-24 years	25-64 years
Standard of Care: Baseline	87% (308/353)	87% (238/273)	88% (70/80)	79% (15/19)	88% (293/334)
Standard of Care: Final visit	96% (1,150/1,196)	97% (849/877)	94% (301/319)	90% (57/63)	96% (1,093/1,133)
Intervention: Baseline	85% (289/340)	84% (211/252)	89% (78/88)	62% (8/13)	86% (281/327)
Intervention: Final visit	97% (1,228/1,262)	97% (922/948)	97% (306/314)	90% (44/49)	98% (1,184/1,213)

Figure S3.C. End-of-study survey: 3rd 95: % virally suppressed (among diagnosed HIV-positive participants on ART)



	Overall	Women	Men	16-24 years	25-64 years
Standard of Care: Baseline	97% (299/308)	97% (230/238)	99% (69/70)	87% (13/15)	98% (286/293)
Standard of Care: Final visit	97% (1,102/1,136)	97% (816/840)	97% (286/296)	88% (49/56)	98% (1,053/1,080)
Intervention: Baseline	97% (280/289)	98% (207/211)	94% (73/78)	100% (8/8)	97% (272/281)
Intervention: Final visit	97% (1,166/1,202)	97% (878/902)	96% (288/300)	83% (34/41)	98% (1,132/1,161)

Table S1. End-of-study survey: HIV-tested (prior 12 months) or prior knowledge of HIV-positive status, unadjusted prevalence ratios (PR) and 95% confidence intervals (CI) (complete model details)

			PR (95% CI)
Matched pair	5		(ref)
	7		1·18 (0·93, 1·48)
	13		1·18 (1·02, 1·36)
Time	Baseline		(ref)
	Final visit		1·15 (1·10, 1·20)
Matched pair by time interaction	Pair 5	Baseline	(ref)
	Pair 7	Final visit	0·79 (0·71, 0·88)
	Pair 13	Final visit	0·61 (0·56, 0·66)
Randomized arm	Standard of care		(ref)
	Intervention		0·82 (0·69, 0·98)
Randomized arm by time interaction	Standard of care	Baseline	(ref)
	Intervention	Final visit	1·29 (1·17, 1·43)

Table S2. End-of-study survey: Prior knowledge of HIV-positive status, unadjusted prevalence ratios (PR) and 95% confidence intervals (CI) (complete model details)

			PR (95% CI)
Matched pair	5		
	7		1·02 (0·97, 1·08)
	13		1·10 (1·06, 1·14)
Time	Baseline		
	Final visit		1·03 (0·95, 1·11)
Matched pair by time interaction	Pair 5	Baseline	
	Pair 7	Final visit	1·04 (0·96, 1·13)
	Pair 13	Final visit	0·95 (0·88, 1·03)
Randomized arm	Standard of care		
	Intervention		0·97 (0·95, 1·00)
Randomized arm by time interaction	Standard of care	Baseline	
	Intervention	Final visit	1·08 (1·04, 1·13)

Table S3. End-of-study survey: Documented current use of ART, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

			PR (95% CI)
Matched pair	5		(ref)
	7		1·04 (1·03, 1·05)
	13		1·19 (1·18, 1·21)
Time	Baseline		(ref)
	Final visit		1·16 (1·09, 1·23)
Matched pair by time interaction	Pair 5	Baseline	(ref)
	Pair 7	Final visit	1·04 (0·99, 1·11)
	Pair 13	Final visit	0·89 (0·83, 0·95)
Randomized arm	Standard of care		(ref)
	Intervention		0·94 (0·93, 0·95)
Randomized arm by time interaction	Standard of care	Baseline	(ref)
	Intervention	Final visit	1·12 (1·07, 1·17)

Table S4. End-of-study survey: HIV-1 RNA ≤ 400 copies/mL, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

			PR (95% CI)
Matched pair	5		(ref)
	7		1·04 (1·02, 1·05)
	13		1·21 (1·19, 1·22)
Time	Baseline		(ref)
	Final visit		1·16 (1·12, 1·20)
Matched pair by time interaction	Pair 5	Baseline	(ref)
	Pair 7	Final visit	1·06 (1·02, 1·09)
	Pair 13	Final visit	0·88 (0·84, 0·92)
Randomized arm	Standard of care		(ref)
	Intervention		0·94 (0·93, 0·94)
Randomized arm by time interaction	Standard of care	Baseline	(ref)
	Intervention	Final visit	1·13 (1·09, 1·17)

Table S5. End-of-study survey: Self-report of circumcision, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

			PR (95% CI)
Matched pair	5		(ref)
	7		1·13 (1·05, 1·23)
	13		0·93 (0·86, 1·01)
Time	Year 1		(ref)
	Final visit		1·22 (1·16, 1·28)
Matched pair by time interaction	Pair 5	Year 1	(ref)
	Pair 7	Final visit	1·02 (0·93, 1·11)
	Pair 13	Final visit	0·73 (0·68, 0·78)
Randomized arm	Standard of care		(ref)
	Intervention		0·92 (0·86, 0·99)
Randomized arm by time interaction	Standard of care	Year 1	(ref)
	Intervention	Final visit	1·26 (1·17, 1·35)

Table S6. Longitudinal cohort: Documented current use of ART, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

Covariate			RR (95% CI)
Matched pair	Pair 5		Ref.
	Pair 1		0·93 (0·79, 1·10)
	Pair 2		0·94 (0·82, 1·08)
	Pair 3		0·91 (0·80, 1·03)
	Pair 4		0·92 (0·80, 1·06)
	Pair 6		1·08 (0·98, 1·20)
	Pair 7		1·04 (0·93, 1·16)
	Pair 8		1·12 (1·01, 1·25)
	Pair 9		0·92 (0·81, 1·05)
	Pair 10		1·17 (1·05, 1·31)
	Pair 11		1·08 (0·96, 1·21)
	Pair 12		1·15 (1·03, 1·27)
	Pair 13		1·19 (1·08, 1·32)
	Pair 14		1·16 (1·04, 1·28)
	Pair 15		1·11 (1·00, 1·23)
Time	Baseline		Ref.
	Final visit		1·35 (1·25, 1·46)
Matched pair by time interaction	Pair 5	Final visit	Ref.
	Pair 1	Final visit	1·04 (0·89, 1·22)
	Pair 2	Final visit	0·99 (0·87, 1·12)
	Pair 3	Final visit	1·07 (0·95, 1·21)
	Pair 4	Final visit	1·07 (0·94, 1·22)
	Pair 6	Final visit	0·95 (0·86, 1·05)
	Pair 7	Final visit	0·98 (0·88, 1·10)
	Pair 8	Final visit	0·90 (0·82, 1·00)
	Pair 9	Final visit	1·08 (0·95, 1·22)
	Pair 10	Final visit	0·88 (0·79, 0·98)
	Pair 11	Final visit	0·95 (0·85, 1·06)
	Pair 12	Final visit	0·88 (0·80, 0·97)
	Pair 13	Final visit	0·85 (0·78, 0·94)
	Pair 14	Final visit	0·89 (0·81, 0·99)
	Pair 15	Final visit	0·91 (0·83, 1·01)
Randomized arm	Standard of care	Baseline	Ref.
	Intervention	Baseline	1·00 (0·96, 1·04)
Randomized arm by time interaction	Standard of care	Final visit	Ref.
	Intervention	Final visit	1·05 (1·01, 1·09)

Table S7. Longitudinal cohort: HIV-1 RNA ≤ 400 copies/mL, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

Covariate			RR (95% CI)
Matched pair	Pair 5		Ref.
	Pair 3		0.90 (0.79, 1.03)
	Pair 4		0.91 (0.79, 1.05)
	Pair 6		1.08 (0.97, 1.20)
	Pair 7		1.04 (0.92, 1.17)
	Pair 8		1.13 (1.01, 1.26)
	Pair 9		0.90 (0.78, 1.04)
	Pair 10		1.18 (1.04, 1.33)
	Pair 11		1.05 (0.93, 1.19)
	Pair 12		1.14 (1.03, 1.28)
	Pair 13		1.21 (1.09, 1.34)
	Pair 14		1.15 (1.03, 1.28)
	Pair 15		1.12 (1.00, 1.25)
Time	Baseline		Ref.
	Final visit		1.34 (1.23, 1.46)
Matched pair by time interaction	Pair 5	Final visit	Ref.
	Pair 3	Final visit	1.09 (0.95, 1.24)
	Pair 4	Final visit	1.10 (0.96, 1.27)
	Pair 6	Final visit	0.96 (0.86, 1.06)
	Pair 7	Final visit	1.00 (0.89, 1.13)
	Pair 8	Final visit	0.90 (0.81, 1.01)
	Pair 9	Final visit	1.06 (0.92, 1.22)
	Pair 10	Final visit	0.89 (0.79, 1.01)
	Pair 11	Final visit	0.98 (0.86, 1.10)
	Pair 12	Final visit	0.89 (0.80, 0.99)
	Pair 13	Final visit	0.85 (0.76, 0.94)
	Pair 14	Final visit	0.90 (0.81, 1.00)
	Pair 15	Final visit	0.91 (0.81, 1.01)
Randomized arm	Standard of care	Baseline	Ref.
	Intervention	Baseline	0.99 (0.95, 1.03)
Randomized arm by time interaction	Standard of care	Final visit	Ref.
	Intervention	Final visit	1.07 (1.02, 1.11)

Table S8. Longitudinal cohort: Self-report of circumcision, unadjusted prevalence ratios (PR) and 95% confidence interval (CI) (complete model details)

Covariate			RR (95% CI)
Matched pair	Pair 5		Ref.
	Pair 1		0·91 (0·64, 1·29)
	Pair 2		1·21 (0·95, 1·55)
	Pair 3		1·03 (0·79, 1·34)
	Pair 4		1·26 (0·99, 1·62)
	Pair 6		1·20 (0·92, 1·55)
	Pair 7		1·21 (0·90, 1·63)
	Pair 8		1·07 (0·81, 1·42)
	Pair 9		1·11 (0·86, 1·44)
	Pair 10		1·07 (0·75, 1·51)
	Pair 11		1·12 (0·84, 1·51)
	Pair 12		1·35 (1·05, 1·74)
	Pair 13		0·95 (0·73, 1·25)
	Pair 14		1·41 (1·08, 1·84)
	Pair 15		1·29 (1·01, 1·65)
Time	Baseline		Ref.
	Final visit		1·29 (1·13, 1·48)
Matched pair by time interaction	Pair 5	Final visit	Ref.
	Pair 1	Final visit	1·06 (0·81, 1·40)
	Pair 2	Final visit	1·00 (0·85, 1·19)
	Pair 3	Final visit	1·04 (0·87, 1·24)
	Pair 4	Final visit	1·08 (0·91, 1·29)
	Pair 6	Final visit	1·09 (0·90, 1·32)
	Pair 7	Final visit	1·25 (0·99, 1·57)
	Pair 8	Final visit	1·13 (0·93, 1·37)
	Pair 9	Final visit	1·00 (0·83, 1·19)
	Pair 10	Final visit	0·95 (0·77, 1·18)
	Pair 11	Final visit	0·90 (0·75, 1·07)
	Pair 12	Final visit	0·96 (0·81, 1·14)
	Pair 13	Final visit	0·99 (0·83, 1·18)
	Pair 14	Final visit	0·90 (0·76, 1·06)
	Pair 15	Final visit	0·92 (0·79, 1·08)
Randomized arm	Standard of care	Baseline	Ref.
	Intervention	Baseline	1·22 (1·11, 1·34)
Randomized arm by time interaction	Standard of care	Final visit	Ref.
	Intervention	Final visit	1·07 (1·00, 1·14)

Data Sharing Statement: A complete de-identified data set from the BCPP household cohort will be made publicly available approximately one year after the completion of the primary analyses. The location of this information has not yet been confirmed. Please contact the corresponding author for further details.

The Botswana Combination Prevention Project (BCPP) Evaluation Protocol (Protocol #1)

Research Design and Impact Evaluation

Version 7.0

January 16, 2019

Collaborating Institutions:
Botswana–Harvard AIDS Institute Partnership (BHP)
Botswana Ministry of Health (MOH)
Harvard T.H. Chan School of Public Health
U.S. Centers for Disease Control and Prevention (CDC)

Funded by: The United States President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC)

i. PRINCIPAL INVESTIGATORS

Myron Essex, DVM, PhD
Lasker Professor of Health Sciences
Harvard T.H. Chan School of Public Health
FXB 402, 651 Huntington Avenue
Boston, MA 02115-6017
Email: messex@hsph.harvard.edu
Phone: +1-617-432-0975
Fax: +1-617-739-8348

Joseph Makhema, MBChB, FRCP
Chief Executive Officer
Botswana Harvard AIDS Institute
Private Bag BO320
Gaborone, Botswana
Email: jmakhema@bhp.org.bw
Phone: +267-390-2671
Fax: +267-390-1284

Shahin Lockman, MD, MS
Associate Professor of Medicine; Associate Professor in the Department of Immunology and Infectious Diseases
Harvard T.H. Chan School of Public Health
FXB 406a, 651 Huntington Avenue
Boston, MA 02115
Email: shahin.lockman@gmail.com
Phone: +1-617-771-8780
Fax: +1-617-739-8348

Eric Tchetgen Tchetgen, PhD
Professor of Biostatistics and Epidemiologic Methods
Departments of Biostatistics and Epidemiology
Kresge, Room 822
677 Huntington Avenue
Boston, MA 02115
Email: etchetge@hsph.harvard.edu
Phone: 617-432-5970
Fax: 617-432-1884

Molly Pretorius Holme, MS
Senior Research Manager
Department of Immunology and Infectious Diseases
Harvard T.H. Chan School of Public Health

FXB 406a, 651 Huntington Avenue
Boston, MA 02115
Email: mpretori@hsph.harvard.edu
Phone: +1-617-432-4377
Fax: +1-617-739-8348

ii. ROLES OF INSTITUTIONS AND PRINCIPAL INVESTIGATORS

- **Institutions**: Close coordination between MOH, Harvard Chan School, CDC, and BHP is needed to ensure seamless implementation of research and program activities. However, MOH and CDC will lead, and ultimately be responsible for, program implementation and monitoring and evaluation, while Harvard Chan School/BHP will lead, and ultimately be responsible for, the evaluation of HIV incidence and viral genetic linkage analyses.

- **Principal Investigators**:

  Myron Essex: Harvard Principal Investigator (PI) responsible for preparation of the study protocol, design and evaluation of research, monitoring of research progress, creation and monitoring of research budgets, and preparation of research progress reports; responsible for design of initial proposal, including virological analyses, and supervision of Harvard and BHP investigators.

  Joseph Makhema: BHP Co-Principal Investigator (Co-PI) responsible for preparation of study protocol, preparation of progress reports, and overseeing field research staff in Botswana.

  Shahin Lockman: Harvard Co-Principal Investigator (Co-PI) responsible for preparation of the study protocol, and monitoring of research progress / revisions of research protocol and procedures as needed; and assisting with monitoring/revision of research budgets and preparation and review of research progress reports.

  Eric Tchetgen Tchetgen: Harvard Co-Principal Investigator (Co-PI) responsible for monitoring of research progress, facilitation of internal and external study monitoring, and preparation of research progress reports; responsible for overseeing statistical design and analyses as well as their interpretation.

  Molly Pretorius Holme: Harvard Co-Principal Investigator (Co-PI) responsible for preparation of the study protocol, and monitoring of research progress / revisions of research protocol and procedures as needed; and assisting with monitoring/revision of research budgets and preparation and review of research progress reports.

iii. INVESTIGATOR LISTS BY INSTITUTION

Botswana Ministry of Health (MOH), Gaborone, Botswana (Alphabetical Order)

Name	Role	Contact Email
Marina Anderson, MD, MPH	Surveillance	manderson@gov.bw
Shenaaz El-Halabi (DPS), MPH	In-country oversight	sel-halabi@gov.bw
Ernest B. Fetogang, PhD	M&E	efetogang@gov.bw
Refeletswe Lebelonyane, MD	In-country oversight	rlebelonyane@gov.bw
Madisa Mine, PhD	Laboratory	mmine@bhp.org.bw

Harvard T.H. Chan School of Public Health, Boston, MA, USA (Alphabetical Order)

Name	Role	Contact Email
Ingrid Bassett, MD	Cost-effectiveness	ibassett@partners.org
Kara Bennett, MS	Statistical Consultant	karabennett@earthlink.net
Victor DeGruttola, DSc	Senior Statistical Scientist	degrut@hsph.harvard.edu
Scott Dryden-Peterson, MD	Treatment and PMTCT	scott_peterson@post.harvard.edu
Kenneth Freedberg, MD	Cost-effectiveness	kfreedberg@partners.org
Vladimir Novitsky, MD, PhD	Lab	vnovi@hsph.harvard.edu
Kathleen Powis, MD, MPH	Research Operations, Treatment and PMTCT	kpowis@partners.org
Roger Shapiro, MD, MPH	Treatment and PMTCT	rshapiro999@gmail.com
Rochelle Walensky, MD	Cost-effectiveness	rwalensky@partners.org
Rui Wang, PhD	Statistics	rwang8@partners.org
Kathleen Wirth, ScD	Statistics	kwirth@hsph.harvard.edu

Botswana–Harvard AIDS Institute Partnership (BHP), Gaborone, Botswana (Alphabetical Order)

Name	Role	Contact Email
Tendani Gaolathe, MD	Treatment and PMTCT, Field research operations	tgaolathe@bhp.org.bw
Simani Gaseitsiwe, PhD	Lab	gaseitsiwe@bhp.org.bw
Poloko Kebaabetswe, PhD, MPH	Qualitative research consultant	polokokebaabetswe@yahoo.com
Mompati Mmalane, MD, FRCS-Ed, MSc	BHP oversight and research operations	mmmalane@bhp.org.bw
Mosepele Mosepele, MBBS, MSc	Clinical outcomes	mmosepele@bhp.org.bw
Sikhulile Moyo, MSc, MPH, PhD	Lab	smoyo@bhp.org.bw
Rosemary Musonda, MSc, PhD	Lab	rmusonda@bhp.org. bw
Erik van Widenfelt, BA	Research data systems	ewidenfelt@bhp.org.bw

Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, and Gaborone, Botswana (Alphabetical Order)

Name	Role	Contact Email
Pam Bachanas, PhD, MS	HTC	dtt6@cdc.gov
Ebi Bile, BSc, MA	In-country lab oversight	efb6@bw.cdc.gov
Arielle Lasry, PhD	HTC Costing	ftn9@cdc.gov
Tafireyi Marukutira, MD	Clinical	xwt5@cdc.gov
Lisa A. Mills, MD	Co-Investigator	lmills1@cdc.gov
Janet Moore, PhD	Co-investigator	jym3@cdc.gov
Sherri Pals, PhD	Statistics	sfv3@cdc.gov
Elliot Raizes, MD	Clinical Consultant	gwq0@cdc.gov
Chunfu Yang, PhD	Laboratory	cxy0@cdc.gov

iv. TABLE OF CONTENTS

Cross-Protocol Appendix Number	Title
C2	Table A: Study Communities and Matching Criteria for Community Pairs Table B: Back-up Communities if Selected 30 Communities Decline, are Unavailable or Deemed Unsuitable for Study Inclusion Figure A: Map of Study Communities in Three Regions Figure B: Map of Study Communities in the Southern Region Figure C: Map of Study Communities in the Central Region Figure D: Map of Study Communities in the Northern Region
C3	Table of Study Population Sizes
C4	Data Safety Monitoring Plan

Protocol 1 Appendix	Title
LOA#1	Letter of Amendment #1 (December 13, 2013) to Protocol 1 v1.0 dated September 24, 2013
1-1	Summary of Protocol Changes

v. LIST OF TABLES AND FIGURES

vi. ACRONYMS AND ABBREVIATIONS

AE	adverse event
AHS	annual household surveys (follow-up visits of the BHS and HIC)
ANC	antenatal clinic (or care)
ART	antiretroviral treatment (or therapy)
ARV	antiretroviral
BCPP	Botswana Combination Prevention Project
BHHRL	Botswana–Harvard HIV Reference Laboratory
BHP	Botswana–Harvard AIDS Institute Partnership
BHS	baseline household survey
CCC	closed clinical cohort
CDC	Centers for Disease Control and Prevention
CEPAC	Cost-Effectiveness of Preventing AIDS Complications
CI	confidence interval
CP	combination prevention
CRT	community-randomized trial
DMC	Data Management Center
EDC	electronic data capture (system used by BHP)
EC	enhanced care
ELISA	enzyme-linked immunosorbent assay
ESS	end-of-study survey
ETC	expanded treatment cohort
FDE	full disk encryption
FGD	focus group discussion
FRR	false recent rate
FTE	full-time equivalent
GCLP	Good Clinical and Laboratory Practice
HIV-1 RNA	HIV-1 RNA load in plasma, viral load
HIC	HIV Incidence Cohort
HPLC	high performance liquid chromatography
HSPH	Harvard School of Public Health
HTC	HIV testing and counseling
IAS	International AIDS Society
IDI	individual in-depth interviews
IDR	immunodominant region
IRB	institutional review board
IPMS	integrated patient management system
ISPOR	International Society for Pharmacoeconomics and Outcomes Research
LTC	linkage to care
LTCM	Linkage-to-care Manager
LTFU	loss/lost to follow-up

MC	male circumcision
M&E	monitoring and evaluation
MoH	Botswana Ministry of Health
MTCT	mother-to-child (HIV) transmission
NNRTI	non-nucleoside reverse transcriptase inhibitor
OHRP	Office for Human Research Protections
OI	opportunistic infection
Option B+	indefinite continuation of ART for women who start ART for PMTCT
PEPFAR	President's Emergency Plan for AIDS Relief
PI	protease inhibitor
PII	personally identifiable information
PIMS	Patient Information Management System
PMTCT	prevention of mother-to-child (HIV) transmission
POB	pregnancy/breastfeeding
POC	point-of-care
PSA	probabilistic sensitivity analysis
QA/QC	quality assurance/quality control
QALY	quality adjusted life-year
QOL	quality of life
RDS	respondent driven sampling
SAE	serious adverse event
SD	standard deviation
SMC	Safe Male Circumcision (Botswana government initiative)
SOC	standard of care
SOP	standard operating procedures
SRH	sexual and reproductive health
study arm	a group of 15 communities that is randomized to receive either enhanced care or combination prevention
TB	tuberculosis
TDR	transmitted drug resistance
UNAIDS	Joint United Nations Programme on HIV/AIDS
UTT	universal test and treat
VPN	virtual private network
VDC	Village Development Committee
WHO	World Health Organization
YLS	Year of Life Saved

vii. BOTSWANA COMBINATION PREVENTION PROJECT SCHEMA

INTRODUCTION

Background on Combination Prevention

In recent years, several new HIV prevention interventions have proven to be highly effective at reducing transmission of HIV and have offered hope for containing and possibly reversing epidemics in sub-Saharan Africa. Despite considerable success in scaling up these interventions in many sub-Saharan African countries, adult HIV incidence remains high in many regions in Southern Africa. This may in part be attributed to the insufficient scale and quality of HIV prevention interventions. Modeling studies suggest that investment in rapid, coordinated scale-up of a combination of evidence-based HIV prevention interventions, including HIV testing and counseling (HTC), male circumcision (MC), antiretroviral therapy (ART) and prevention of mother-to-child transmission (PMTCT), can significantly reduce population-level HIV incidence and may be cost-effective or even cost-saving in the long term. Rigorous evidence-based outcomes to support these projections of population-level impact are needed if national and international bodies are to justify further investment in combination prevention strategies.

Study Hypothesis

The BCPP study was designed to test the hypothesis that implementing a combination prevention package (described below) will impact the HIV/AIDS epidemic by significantly reducing population-level, cumulative HIV incidence in a defined geographic area over a period of 3 years and will be cost-effective. With the changes introduced in version 5.0 of this protocol, HIV incidence will be measured over a shorter period, approximately 2.5 years.

Proposed Combination Prevention Package

The proposed combination prevention package for the BCPP includes:

- Rapid scale-up of HTC services, with a target of ensuring >90% of HIV-infected adults aged 16-64 have documentation of their HIV-infected status.
- Rapid scale-up of universal ART for all HIV-infected adults, with a target of ensuring that >93% of adults diagnosed with HIV infection are receiving ART.
- Rapid scale-up of retention in care and adherence interventions, with a target of ensuring that >95% of HIV-infected adults receiving ART are virally suppressed with HIV-1 RNA <400 copies/ml.
- Rapid scale-up of linkage to male circumcision services, with a target of ensuring >60% of HIV-negative men (aged 16–49) are circumcised.
- Rapid strengthening of PMTCT services, with a target of ensuring >90% of women initiated on indefinite ART (Option B+) during pregnancy remain in care and on treatment at 12 months post-delivery.

Study Arms

Thirty communities (described in ***Appendix C2***) in three geographic areas considered representative of Botswana have been proposed for inclusion in the community randomized trial (CRT): (1) around Gaborone in the south east, (2) around Francistown in the north east, and (3) in the region around Mahalapye, Serowe, and Palapye in the central eastern region. Communities will be randomized to either the Combination Prevention arm or the Enhanced Care arm. Combination Prevention

communities will receive the proposed combination prevention package described above. Enhanced Care Communities will receive guidance and technical support for quality management and data systems at all laboratories and local clinics at which individuals receive HIV care and treatment (and participants in the survey occurring in ~20% of households in the Enhanced Care communities may benefit from study provision of home-based HIV counseling and testing and, for HIV-infected residents, point of care CD4 count and HIV-1 RNA testing).

High Viral Load Treatment and Universal Test and Treat as Combination Prevention Package Components

Data show that higher levels of HIV-1 RNA (viral load) are independently associated with both higher rates of HIV transmission and more rapid HIV clinical disease progression and CD4 decline. This study therefore originally included the use of a modified "treatment as prevention" approach that targeted not only those adults who need ART for their own health, but also those adults who are most likely to perpetuate HIV transmission within the community (those with viral load ≥10,000 copies/ml). At the time of designing the BCPP in 2012 there was strong experimental evidence supporting initiation of ART at CD4≤350 cells/μL, or WHO Stage III/IV, but conclusive experimental evidence supporting patient benefit of ART initiation at higher CD4 counts, especially CD4 >500 cells/μL was lacking.

The 2013 WHO guidelines recommended initiation of ART in all HIV-infected adults with CD4≤500 cells/μL regardless of clinical stage. In an attempt to provide programmatic data for an anticipated change in CD4 threshold for ART initiation in the future in Botswana and to enhance the power of the study to answer its primary objective, the threshold for ART initiation in the Combination Prevention arm was modified in early 2015 (from CD4≤350 to CD4≤500 regardless of viral load, in addition to offering expanded ART to persons with CD4>500 and viral load ≥10,000).

Shortly thereafter, in July 2015, the WHO announced that it would revise ART guidelines to recommend universal treatment (regardless of CD4 count or HIV disease severity). The WHO's plans were informed by results from two clinical ART trials. One, the START trial, reported that early drug treatment of people with HIV cut their risk of serious illness or death by 57% (Lundgren, Babiker et al. 2015). Another, the TEMPRANO study, released findings that early ART significantly decreased morbidity overall and when restricted to patients with baseline CD4>500 cells/μL (Danel, Moh et al. 2015). WHO published an early release guideline on when to start ART and on pre-exposure prophylaxis for HIV in September 2015 (World Health Organization 2015). The BCPP study team, with the support of the DSMB and funder, revised the intervention treatment initiation criteria to take these results and WHO guidelines into account. Universal test and treat (UTT) is therefore included in the combination prevention package starting with version 4.0 of the protocols as of June 2016.

Also as of June 2016, with the launch of its "Treat All" campaign, Botswana treatment guidelines allow initiation of ART for all HIV-infected citizens regardless of CD4 count or WHO stage. Enhanced Care community residents are eligible to receive ART according to these updated Botswana national guidelines. Residents in the Combination Prevention communities are continuing to receive additional expanded treatment as prevention.

Through additional modeling, the study team adjusted effect size and power calculations to reflect a move to universal ART in both the Combination Prevention and Enhanced Care arms, using a range of roll-out timelines. This revised modeling also used estimates of coverage (by HTC, linkage to care, ART,

and MC) that are consistent with what had been observed in BCPP thus far, rather than using the higher coverage "targets". Model-based estimates suggest that the impact of a change in treatment policy on the power to detect a difference in cumulative incidence (the primary objective of BCPP) may still yield at least 60% power, but the impact depends on the exact nature and timing of the actual implementation of changes in policy as well as other prevailing conditions. The retention of this level of power even with Enhanced Care communities moving to expanded access to ART is at least in part attributable to the fact that BCPP is implementing multiple HIV prevention interventions (including higher HTC coverage, linkage to HIV care/treatment, adherence support, etc.). With version 5.0 of the protocol, we are dropping the third annual household survey and instead performing the second annual household survey approximately 6 months later than originally planned. This will result in a total average follow-up of the BHS/HIC participants of approximately 30 months rather than 36 months. This change is being made for budgetary reasons, and also because the randomized arms are becoming more similar over time with regard to HIV treatment (particularly with the move to UTT in both arms in June 2016). Shortening the follow-up period from the originally planned 36 months to an average of 30 months with version 5.0 of the protocol reduces estimated power for the first primary objective to approximately 63%.

Indefinite ART following PMTCT (Option B+)

Women completing ART for PMTCT will be offered indefinite ART continuation (regardless of pre-treatment CD4, viral load or clinical stage). Option B+ was part of the original combination prevention package at the launch of this study in 2013. Upon the Botswana government's adoption of Option B+ as national policy (April 2015), Option B+ is offered to qualifying women in Enhanced Care communities as well as Combination Prevention communities per national guidelines.

Relationship of BCPP Study Protocols

The Botswana Combination Prevention Project and related procedures were initially described in three separate, but inter-related, protocols: an *Evaluation Protocol (#1), Closed Clinical Cohort Protocol (#2)*, and *Intervention Protocol (#3)*.

In 2015, the *Closed Clinical Cohort Protocol* (#2) was terminated due to concerns about low study power, evolving ART guidelines, and feasibility.

BCPP STUDY OBJECTIVES

Primary objectives covered by the BCPP study protocols include:

1. To determine whether implementation of a combination prevention (CP) package can significantly reduce population-level, cumulative HIV incidence in 16-64 year old residents in Botswana over a period of approximately 30 months. This evaluation is a primary objective of the *Evaluation Protocol*. Implementation of the combination prevention package is described in detail in the *Intervention Protocol.*
2. To estimate population-level uptake of HIV testing, ART, VL suppression, male circumcision, and PMTCT services and compare service uptake between Enhanced Care communities and Combination Prevention communities at baseline and at study end. This is a primary objective of the *Evaluation Protocol*.
3. To estimate the cost per additional infection averted in Combination Prevention communities compared with Enhanced Care communities. This is a primary objective of the *Evaluation Protocol*.

4. To implement the combination prevention intervention package in Combination Prevention communities and describe uptake of these interventions in Combination Prevention communities (expanded HIV Testing and Counseling, strengthened Male Circumcision, expanded HIV Care and Treatment, and strengthened PMTCT services). This is the primary objective of the *Intervention Protocol*.

STUDY DESIGN

The over-arching design is a pair-matched CRT that will be completed over the course of approximately 5 years. A randomized design is selected to provide experimental evidence of combination prevention package impact, and because population-level HIV incidence is the outcome of interest, we propose a community (rather than individual) randomized trial. A detailed description of the combination prevention package and how these interventions go beyond standard of care (SOC) are described in detail in the *Intervention Protocol*. In brief, the interventions consist of the following four components:

1) Expand HTC and strengthen linkage-to-care services
2) Strengthened and expanded HIV care and treatment services
3) Expand male circumcision (MC) services
4) Strengthened PMTCT services, specifically identification, linkage, and treatment of HIV-positive pregnant women

STUDY METHODS

The study communities will be matched into pairs based on community characteristics thought to be associated with the outcome of interest (adult HIV incidence). Pair-matching is done to improve balance between study arms and statistical efficiency.

The study methods are divided into five components that are described in detail in the BCPP protocols and summarized here.

1) Baseline Household Survey **(BHS)** followed by Annual Household Survey **(AHS)*** Visits

Following matching, within each community, a baseline household survey (BHS) of approximately 20% of randomly selected households will be implemented to (1) establish an HIV incidence cohort (HIC) consisting of all eligible consenting HIV-negative household members aged 16-64 years, and (2) estimate baseline and annual coverage of the combination prevention package. The BHS and subsequent annual household survey (AHS) visits are described in the *Evaluation Protocol.* Individuals who enroll in BHS will be followed with AHS visits, regardless of whether they are assigned to the HIC (see below) to assess uptake in both study arms of HTC, ART including viral suppression, MC and PMTCT services and describe major outcomes.

*Revisions in Version 5.0 of the protocol extended the interval between the first annual household follow-up visit at T1 and the second/final household visit at T2 to approximately 18 months, rather than 12. "AHS" will still be used to refer to follow-up visits to the 20% households.

2) HIV Incidence cohort **(HIC)**

The HIC, enrolled during the BHS, will be followed to assess cumulative HIV incidence over approximately 30 months, which is the primary study outcome of interest. HIV genotypes of incident infections will be analyzed to allow description of which incident infections in the HIC are linked to HIV-

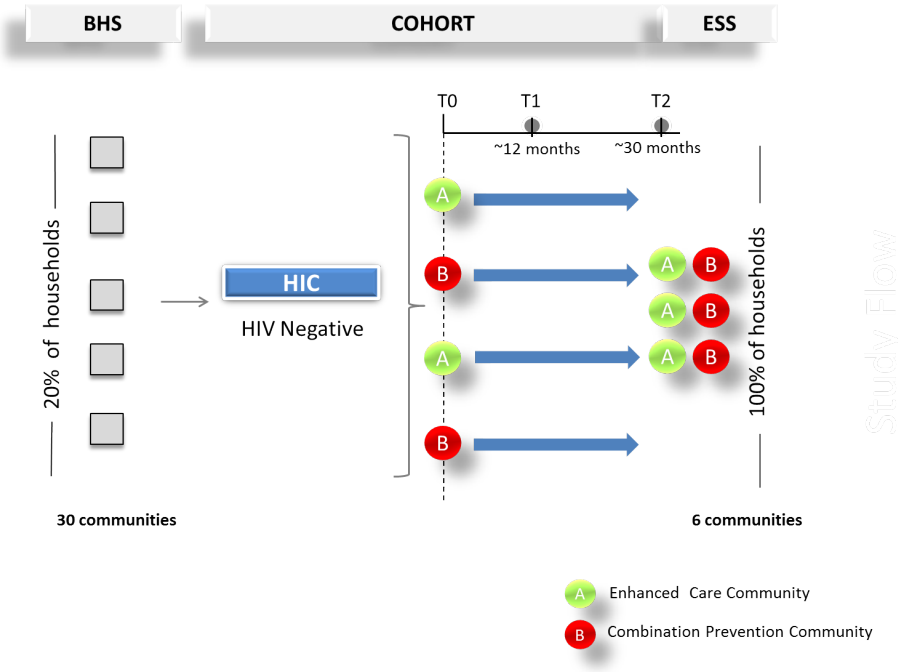
infected persons residing in the same study arm. Comparison of linked HIV incidence rates between arms may inform analysis of intervention effectiveness. The HIC is described in the *Evaluation Protocol.*

3) Programmatic Interventions **(HTC, Universal ART, Strengthened PMTCT and MC Services)**
Matched community pairs will be randomized 1:1 to Enhanced Care or Combination Prevention arms (15 communities per arm). Combination Prevention communities will receive the combination prevention intervention package, which includes strengthened HIV testing and counseling, universal ART, and strengthened male circumcision services. To reach high coverage levels of HTC, campaigns will be implemented after BHS and continue with ongoing services in the Combination Prevention arm. Universal ART with linkage to care and adherence support will be offered to eligible HIV-infected persons referred through the study from BHS/AHS or HTC services and routine testing, as well as to existing patients already enrolled in care at the Combination Prevention community clinics. Services for pregnant women will be strengthened for third trimester retesting of women with a previous HIV-negative test; linkage to HIV treatment for all HIV-infected pregnant and post-partum women; and retention on ART post-delivery. To reach high coverage levels of male circumcision in HIV-uninfected adult men, MC campaigns with strengthened linkage to MC services will be conducted in Combination Prevention communities. Programmatic intervention activities are described in the *Intervention Protocol*.

4) End of study survey **(ESS)**
An end of study survey (ESS) targeting 100% of households in three community pairs (3 Combination Prevention communities and 3 Enhanced Care communities) will be used to estimate and compare Combination Prevention package uptake between arms. The ESS is described in the *Evaluation Protocol.*

5) Laboratory Research Activities **(Viral Genotype Analysis and Point-of-Care Viral Load Validation)**
Laboratory research activities will be used to map genotypes from HIV-infected individuals identified during the BHS, HTC, and ESS and from seroconverted individuals in the HIC. All HIV-infected adults will be asked for consent to allow viral genotype analysis. Genotyping will help to inform viral genotype maps of Enhanced Care community and Combination Prevention community arms. These viral genotype analyses will be used to assess whether the proportion of new infections that are linked to circulating HIV-1 variants in the same arm, declines over time in the Combination Prevention arm. Procedures related to this genotyping are described in the *Evaluation Protocol.* The accuracy and feasibility of point-of-care viral load testing will also be assessed in comparison to standard viral load platforms.

FIGURE S1: Overview of Surveys and HIV Incidence Cohort in the Evaluation Protocol



Equipoise

In individually randomized trials involving human participants, equipoise is the ethical requirement that, at the start of a trial, there be a state of honest, professional disagreement in the community of expert practitioners as to the preferred treatment. Beneficence, the moral obligation not to harm needlessly, and when possible, to promote the welfare of research subjects, is a key ethical principle underpinning the concept of equipoise.

In this study, investigators believe there is equipoise, since impact of combination HIV prevention programs on population-level adult HIV incidence has not yet been rigorously demonstrated, and professional disagreement about the effectiveness of combination prevention and the need for a thorough evaluation of its impact has been published in the scientific literature. Therefore, based on current scientific evidence for and against the combination prevention package, randomizing a community to either Enhanced Care or Combination Prevention arms does not breach the trust relationship between the state and the community.

Combination prevention will be compared to an enhanced standard of care in the Enhanced Care communities. The Enhanced Care communities will benefit in several ways from participation in the project. First, residents in the 20% households participating in the BHS and related activities (outlined in the Evaluation Protocol) will be offered the following procedures, which may confer direct health benefits: home-based HIV counseling and testing; point-of-care CD4 testing for HIV-infected, treatment-naive residents; HIV-1 RNA testing for HIV-infected residents (HIV-1 RNA sample drawn in the household); and referral to local HIV care and treatment clinics for HIV-infected residents who are not on treatment and/or not actively engaged in care. Second, the BCPP will also provide guidance and technical support for quality management and data systems at all laboratories and local clinics at which individuals receive HIV care and treatment, including those in the Enhanced Care communities. The MOH will receive technical assistance to support improved implementation and utilization of

clinical data systems (primarily PIMS2 and IPMS) in all 30 study communities. It is likely that resulting improvements in medical data systems and in quality management will help improve the general quality of care and treatment of all community residents attending these clinics.

To maintain the trust relationship between the study team and the community during study conduct, analyses are proposed for interim DSMB review to ensure that the DSMB can recommend early stopping of the trial if efficacy is demonstrated early. If efficacy is demonstrated, scale-up of the combination prevention package in Enhanced Care communities would be encouraged by investigators, although implementation would be based on funding availability and guidelines from the MOH.

BCPP STUDY COORDINATION AND GOVERNANCE

Implementation of the BCPP protocols will be coordinated using BCPP standard operating procedures and manuals, integrated across protocols where possible. Roles and responsibilities for program implementers and study investigators are defined in each of the protocols and will be described in detail in the BCPP Study Manual. While some overlap exists due to the design of the different protocols, Harvard Chan School/BHP will have primary responsibility for the *Evaluation Protocol* and CDC/MOH will have primary responsibility for the *Intervention Protocol*. During its abbreviated implementation the CDC/MOH had primary responsibility for the *CCC Protocol*. Due to the interlocking nature of the BCPP protocols, a joint project data management team, chaired by representatives from MOH, Harvard Chan School, BHP, and CDC, will be formed to build and manage the research data collection systems/overall research database for the BCPP.

viii. EVALUATION PROTOCOL SYNOPSIS

The *Evaluation Protocol* of the BCPP describes the rationale for and procedures related to evaluation of the primary endpoint (HIV incidence), as well as some key related secondary endpoints. This protocol therefore focuses on the Baseline Household Survey; the HIV Incidence Cohort; research-related blood sample collection; and an End of Study Survey.

Primary Objectives of the Evaluation Protocol:

1. To determine whether implementation of a combination prevention (CP) package can significantly reduce population-level, cumulative HIV incidence in 16-64 year old residents in Botswana over a period of approximately 30 months.

2. To estimate population-level uptake of HIV testing, ART, VL suppression, male circumcision, and PMTCT services and compare service uptake between Enhanced Care and Combination Prevention communities at baseline and at study end.

3. To estimate the cost per additional HIV infection averted in combination prevention compared with Enhanced Care communities.

Study Design:
The study is a pair-matched community-randomized trial.

Study Population:
Thirty community clusters will be selected from three geographic regions in Botswana. Prior to randomization, community pairs will be matched on community characteristics thought to be associated with the outcome of interest (HIV incidence). The average total population size of each of the targeted communities is 6,027, giving a total population across all 30 communities of about 180,800, of whom about 105,000 are adults aged 16-64.

Study Procedures:
Matched community pairs will be randomized 1:1 to Enhanced Care or Combination Prevention arms (15 communities per arm). Randomization will occur prior to initiation of study activities (including the BHS).

Within each community, a baseline household survey (BHS) of approximately 20% of randomly selected households will be implemented to (1) establish an HIV incidence cohort (HIC) consisting of eligible consenting HIV-negative household members aged 16-64 years, and (2) estimate baseline coverage of the CP package.

Combination Prevention communities will receive the Combination Prevention intervention package immediately after completion of the BHS. All study communities, including Enhanced Care communities will receive guidance and technical support for quality management and data systems at all laboratories and local clinics at which individuals receive HIV care and treatment.

The HIC will be followed during AHS visits to assess cumulative HIV incidence over approximately 30 months, which is the primary study outcome of interest. Study participants who are not eligible for the

HIC will also be followed in the AHS, to assess, in both study arms, uptake of HTC, ART, MC and PMTCT in each arm and to describe major health outcomes (such as death).

To meet the second primary objective, an end-of-study survey (ESS) targeting 100% of households in selected pairs of communities will be used to estimate and compare CP package uptake between arms.

To understand HIV transmission patterns in Combination Prevention and Enhanced Care communities, HIV-1 genotyping will be performed. Because sexual mixing outside of a study arm will likely be an important source of contamination, circulating HIV-1 variants in Combination Prevention and Enhanced Care communities will be genotyped, and genotyping data will be translated into HIV transmission network(s). The genotyping data will be used to estimate the spatial location (community) of HIV infection for identified newly infected individuals. Specifically, the HIV genotyping data will estimate a potential link for each incident case in the study, and distinguish between *linked* (new incident infections between members of the same study arm), and *unlinked* (new incident infections transmitted from outside the study arm). The HIV-1 genotyping in Enhanced Care and Combination Prevention arms will be performed through sample collection from consenting HIV-infected adults in the BHS and newly infected individuals in the 20% households taking part in the household surveys throughout the study; from HIV-infected adults newly-identified through the HTC campaigns in the Combination Prevention communities; from all HIV-infected adults accessing HIV care and treatment at local clinics in the Combination Prevention communities; and from participants in the ESS in both the Enhanced Care and Combination Prevention arms. The HIV genotyping data will also be used to assess whether the proportion of new infections that are linked to circulating HIV-1 variants in the same arm declines over time at a different rate from those infections that are not linked to circulating variants from the same arm, in the Combination Prevention communities.

Duration:
Study activities will occur over a period of approximately 50 months. The BHS will be completed in approximately 24 months. Each incidence cohort will be followed for an average of approximately 30 months.

Sample Size:
With approximately 300 HIV-negative individuals enrolled in the HIC in each of 30 communities, the estimated size of the HIC will be 4,500 per study arm or 9,000 in total. Original model simulations estimate that over a 3-year study follow-up period of the HIC, rapid scale-up of interventions will result in cumulative adult HIV incidence of 0.024 in the Combination Prevention arm compared with 0.041 in the Enhanced Care arm (40% reduction). Assuming a coefficient of variation (k) of 0.26, and alpha of 0.05, an average community HIC size of 300, with 15 communities per arm, in a pair-matched design, should allow 86% power to detect the estimated difference in cumulative adult HIV incidence between study arms, by the end of three years of HIC follow-up. Sample size was determined based on these original power estimates. To update estimates of study power, a simulation study was undertaken using a range of updated input parameters. These were based on observed BCPP data from the first several community pairs and on speculation regarding roll-out of UTT. As expected, these demonstrate some reduction in power compared to that described in the previous versions of the protocol, but power exceeded 60% in the tables displayed in Section 5.1.1.

1. INTRODUCTION AND BACKGROUND

1.1. Epidemic in Botswana

Thirty years into the global HIV pandemic, HIV prevalence and incidence remain high in sub-Saharan Africa. Although this region represents only 12% of the world's population, it accounts for approximately 67% of the world's 34 million HIV-infected persons, and annually, about 70% of the world's 2.7 million new infections occur in this region (UNAIDS 2010). Despite Botswana's substantial progress in providing HIV treatment and prevention of mother-to-child transmission services (PMTCT) to its citizens, an estimated 25% of its adult residents are HIV-infected, the second highest adult HIV prevalence in the world; and about 14,000 persons in Botswana are newly infected with HIV annually, the third highest national HIV incidence rate globally (UNAIDS 2010).

1.2. Combination Prevention

In recent years, several new HIV prevention interventions have proven to be highly effective at reducing transmission of HIV and have offered hope for containing and possibly reversing epidemics in sub-Saharan Africa. For HIV-negative men, male circumcision has been shown to significantly reduce HIV acquisition from HIV-infected female partners. Three randomized controlled trials of circumcision, involving more than 10,000 men in sub-Saharan Africa, demonstrated a 60% reduction in HIV acquisition risk (Auvert, Taljaard et al. 2005; Bailey, Moses et al. 2007; Gray, Kigozi et al. 2007). Additionally, antiretroviral treatment (ART) of HIV-infected persons has been shown to reduce viral load and consequently the risk of HIV transmission to uninfected partners in sero-discordant couples by as much as 96% (Cohen, Chen et al. 2011). Similarly, the use of antiretrovirals to prevent transmission of HIV from infected mothers to their infants during pregnancy, birth, and breastfeeding can reduce transmission to about 1% (Shapiro, Hughes et al. 2010).

Despite considerable success in scaling up these interventions in many sub-Saharan African countries, especially those supported by the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), adult HIV incidence has remained high (>1%) in many regions in Southern Africa (Botswana, Swaziland, Lesotho, Kwa-Zulu Natal in South Africa, and Southern Mozambique). Multiple factors may account for high HIV incidence in this part of the world. Firstly, viral characteristics of HIV-1 subtype C, endemic in Southern Africa, may make it more infectious than other viral subtypes (Novitsky, Wang et al. 2010; Novitsky, Ndung'u et al. 2011). For example, recent evidence suggests that about one third of persons infected with HIV-1 subtype C, maintain very high viral loads (VL>50,000 copies/ml) for more than 6 months after sero-conversion.

Secondly, the scale and quality of the HIV prevention interventions (HTC, ART, MC and PMTCT) may have been insufficient to reduce HIV incidence among adults in Southern Africa to levels observed in other parts of the world. For example, the main mechanism by which scale-up of HTC and ART reduces HIV transmission risk in the community, is by reducing viral load in resident HIV-infected persons, ideally to undetectable levels (VL<400 copies/ml). Studies have suggested that every 0.5 log reduction in an individual's HIV-1 viral load is equivalent to a 40% reduction in individual HIV transmission risk (Modjarrad, Chamot et al. 2008), and once the viral load is fully suppressed (VL <400 copies/ml) individual HIV transmission risk is virtually zero (Attia, Egger et al. 2009). Despite considerable scale-up

of access to HTC and ART, poor retention of HIV-infected persons in the HIV care and treatment cascade from diagnosis to ART follow-up (Rosen and Fox 2011), has certainly mitigated the ability of HTC and HIV care and treatment programs in Southern Africa to reduce the prevalence of HIV-infected persons with detectable viral loads (VL>400 copies/ml). In a meta-analysis of pre-ART retention in Africa, Rosen et al. reported that only 59% of newly diagnosed adults are successfully linked to HIV care and treatment services, only 46% are retained from initial registration and staging at the facility to ART-eligibility, and only 68% are retained from ART eligibility to ART initiation (Rosen and Fox 2011). Of those adults who initiate ART, ~30% are no longer on therapy by 3 years of follow-up, with most of those no longer in care considered lost to follow-up (Fox and Rosen 2010). Therefore, even if 50% of HIV-infected adults in Botswana know their HIV status (Central Statistics Office 2009), a considerably lower proportion are expected to have a suppressed viral load (Gardner, McLees et al. 2011). The remaining HIV-infected adults with unsuppressed viral load are still infectious and spreading virus in the community. Additionally, achieving high coverage of male circumcision among HIV-negative males has also been challenging with most countries, including Botswana, unable to meet ambitious 80% coverage targets to date (Curran, Njeuhmeli et al. 2011).

In summary, despite having the tools to end the HIV epidemic (Fauci and Folkers 2012), insufficient scale, quality, and coordination of prevention interventions has significantly limited southern Africa's ability to sufficiently curb the HIV epidemic. This has led public health experts to call for a "combination prevention" strategy that involves implementing multiple prevention interventions with known efficacy in a geographic area at a scale, quality, and intensity to impact the epidemic (Merson, Padian et al. 2008). Although most experts agree that synchronized implementation of a combination of prevention interventions is needed, there remains considerable debate about what components should be included in the essential combination prevention package (Merson, Padian et al. 2008; Halperin 2009). However, four evidence-based interventions including HTC, ART, MC and PMTCT followed by indefinite ART are recommended for scale-up by almost all prevention experts and will form the cornerstone of the combination prevention package proposed as part of this project (see Section 1.3 below).

1.3. The Proposed Combination Prevention (CP) Package

Based on experimental evidence for prevention interventions discussed above, the proposed combination prevention (CP) package to be delivered as part of the BCPP *Intervention Protocol* will include accelerated scale-up and expansion of HTC, active linkage to HIV care and treatment according to local eligibility criteria, expanded ART among all HIV-infected residents regardless of WHO Stage or CD4 count; strengthened support for retention in care and ART adherence, and strengthened active linkage to expanded MC and PMTCT services. Under versions 1.0 and 2.0 of this protocol, the combination prevention package included expanded ART for individuals with CD4>350 cells/µL plus HIV-1 RNA ≥10,000 copies/mL, or pregnancy/breastfeeding (Option B+). Under version 3.0 of this protocol, the combination package included expanded ART for individuals with WHO Stage I/II and CD4 counts 351–500 cells/µL or with WHO Stage I/II and CD4 count>500 cells/µL and high HIV-1 RNA levels (≥10,000 copies/mL). Option B+ was offered beginning with version 3.0 in both Combination Prevention and Enhanced Care communities with the adoption of this policy by the Botswana government in April 2015. Beginning with version 4.0 of this protocol, universal test and treat was offered in the Combination Prevention arm. Enhanced Care communities have received the Botswana standard of care in effect as it changes over time (see the Schema and Section 5.1.1 for discussion of

the standard of care and services in Enhanced Care communities). Version 5.0 of the Evaluation Protocol takes into account that universal ART is available to residents of both Enhanced Care and Combination Prevention Communities following the Botswana government's adoption of "Treat All" in June 2016.

In 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) proposed new targets directed at ending the AIDS epidemic (UNAIDS 2014). Namely, that by 2020, 90% of all people living with HIV will know their HIV status; 90% of all people with diagnosed HIV infection will receive sustained combination antiretroviral therapy (ART); and 90% of all people receiving ART will have viral suppression The targets of the study combination prevention package meet, and in some cases exceed, those for the UNAIDS 90-90-90.

To reach stated scale-up targets (see BCPP Schema) several service uptake initiatives will need to be implemented, including (1) community mobilization to encourage HTC and MC uptake, (2) strengthening linkage to ART and/or PMTCT services for newly diagnosed HIV-infected adults, (3) strengthening of linkage to MC services for HIV negative males identified at HTC services, (4) expansion of HTC, ART, PMTCT and MC services through recruitment of additional health care personnel, and where necessary, creation of new infrastructure (e.g. new male circumcision surgical units), and (5) monitoring and evaluation of prevention programs to ensure stated targets are reached.

Adults aged 16-64 will be targeted for service uptake because recent, nationally representative surveys of HIV prevalence and incidence (Central Statistics Office 2009) suggest that HIV incidence remains high (>1%) throughout this age group. Modeling exercises (presented in Section 5) suggest that meeting scale-up targets by the end of two years of program implementation, and maintaining treatment coverage thereafter, could result in community HIV incidence reductions of approximately 40% by the end of 2.5 years of implementation.

The *Intervention Protocol* describes the details of implementing the CP package in the study communities.

2. STUDY OBJECTIVES

2.1. Primary Study Hypothesis

Implementing the CP package will impact the HIV/AIDS epidemic by reducing the incidence of HIV infection in a defined geographic area within approximately 2.5 years and will be cost-effective.

2.2. Primary Objectives

1) To determine whether implementation of a combination prevention (CP) package can significantly reduce population-level, cumulative HIV incidence in 16-64 year old residents in Botswana over a period of approximately 30 months.

2) To estimate population-level uptake of HIV testing, ART, VL suppression, male circumcision, and PMTCT services and compare service uptake between Enhanced Care and Combination Prevention communities at baseline and at study end. The service uptake indicators will include:
   - The proportion of 16-64 year-old community residents who report knowing that they are HIV-infected, or report testing HIV-negative in the preceding 12 months.
   - The proportion of 16-49 year-old HIV-negative men, resident in the study communities who are circumcised.
   - The proportion of HIV-infected 16-64 year-old adult community residents who know they are HIV-positive and are receiving ART.
   - The proportion of HIV-infected 16-64 year-old community residents who know they are HIV-positive, are receiving ART, and have HIV-1 RNA ≤400 copies/mL.
   - The proportion of eligible, HIV-infected pregnant women, resident in study communities, who remained in care and on treatment 12 months post-delivery after initiating ART for Option B+.

3) To estimate the cost per additional HIV infection averted in Combination Prevention compared with Enhanced Care communities.

2.3. Evaluating the Impact of Combination Prevention: Design and Rationale to Address Primary Objectives

Although modeling studies suggest that implementation of the stated CP Package can significantly reduce HIV incidence and may be cost-effective, rigorous evidence to support these projections of population-level impact is needed if national and international bodies are to justify further investment in combination prevention strategies (Lancet 2010; Padian, McCoy et al. 2011). For funders to be confident in the effectiveness of the proposed CP package, this evaluation would need to: (1) demonstrate a measureable reduction in adult HIV incidence and (2) be able to attribute any observed incidence reduction to scale-up of the CP package. The first two primary study objectives for the BCPP *Evaluation Protocol* are designed to meet these needs.

Current global economic realities have resulted in level or declining availability of funding for HIV/AIDS programs (Stover, Korenromp et al. 2011). Therefore, the proposed third primary objective is to evaluate the cost-effectiveness of CP package implementation in Botswana.

A randomized design is proposed to provide experimental evidence of CP package impact, and because population-level HIV incidence is the outcome of interest, we propose a community (rather than individual) randomized trial (CRT).

In summary, 30 study communities, considered representative of the rural and peri-urban populations of Botswana, will be selected and matched into pairs based on community characteristics thought to be associated with HIV incidence, which is the outcome of interest (see section 4.2 and Appendix C2 for matching criteria). Pair-matching is done to improve balance between study arms and statistical efficiency (Hayes and Moulton 2009). Communities within pairs will be randomized to Combination Prevention or Enhanced Care arms. Following community matching and randomization, a baseline 20% household survey (BHS) will be implemented to recruit an HIV incidence cohort (HIC) of eligible, consenting, HIV-negative adults aged 16-64. Through activities described in the BCPP *Intervention Protocol*, the CP package will be scaled up to target levels within two years post-randomization in

Combination Prevention communities with treatment coverage levels maintained thereafter. The HIC will be followed at approximately 12 months (T1) and 30 months (T2) after enrollment to measure cumulative HIV incidence. A small number of communities may receive a third follow-up at 36 months (T3) as stipulated by protocol versions 1.0-4.0, depending upon the timing of approval and roll-out of version 5.0 of the *Evaluation Protocol*. In addition, a small number of communities will have had their second follow-up visit at T2 according to the original study design and schedule. All data collected from these study visits under previous versions of the protocol and the former study schedule will contribute to the final analyses. Cumulative HIV incidence will be compared between Enhanced Care and Combination Prevention arms to assess CP package impact. Households that participated in the BHS will be visited (as part of the HIC follow-up activities). Residents of these households who are not participating in the HIC will also be asked to complete a survey at each follow-up visit to help provide CP package coverage estimates during the course of the study. The overall evaluation study design is illustrated in Figure S1 in the BCPP Schema (section vii), and the rationale for the sample sizes is provided in Section 5.

We propose a parallel group CRT design rather than a step-wedge design primarily because a step-wedge design is considered infeasible. A step-wedge design would entail enrollment of multiple HIV incidence cohorts sampled, recruited and followed within the short time period of any one "step" and this would be expensive and beyond the realm of feasibility. In addition, a parallel group CRT is generally regarded as providing stronger experimental evidence of impact compared with a step-wedge design because any pre-existing trends in HIV incidence will be controlled for at the design stage, rather than modeled in an adjusted analysis as would need to occur in a step-wedge trial (Hussey and Hughes 2007; Hayes and Moulton 2009).

A pre- versus post-study design would not generate evidence of similar strength to that of a parallel group CRT (Habicht, Victora et al. 1999; Hayes and Moulton 2009). Previous pair-matched CRTs to assess impact of complex program interventions on adult HIV incidence have been successfully implemented in Africa, and this lends support to the feasibility of our proposed design (Hayes, Mosha et al. 1995).

We propose directly measured HIV incidence as the primary outcome of interest, because fewer assumptions are required for direct HIV incidence measures, and this is still regarded as the gold standard technique for estimating incidence (Kim, Hallett et al. 2011).

We propose that the HIV incidence cohort (HIC) remain open such that HIV-negative adults aged 16-64 who migrate into HIC households, or adolescents who age into eligibility during study conduct, or other participants who become eligible during study conduct, be offered the opportunity to enroll in the HIC at T1. An open, rather than a closed cohort, has been selected because (1) this should help to keep the incidence cohorts representative of their respective communities, (2) this has some sample size advantages, and (3) analysis techniques to manage open rather than closed HIV incidence measures are available and not inferior. The advantages of an open, rather than a closed, cohort should outweigh the disadvantages, which includes possible intervention contamination of Enhanced Care communities (see Section 2.5.1).

To meet the second primary objective, CP package coverage in the community will be estimated at baseline and annually in the 20% households, and through an end of study survey (ESS) that targets all

households in 6 of the 30 study communities. Targeting 100% of households in 6 communities in the ESS allows for obtaining more precise estimates of CP package coverage (see Section 5 for precision estimates), and permits comparison of estimated coverage in the 20% households with that in the rest of the community.

To meet the third primary objective, costing data will be collected prospectively, and used in combination with impact estimates derived from the CRT. The cost-effectiveness analysis and methods for collecting costing data are described in Section 7.

2.4. Secondary Objectives

1) To estimate the extent to which incident HIV infections in Combination Prevention and in Enhanced Care communities arise from HIV strains circulating within communities randomized to the same study arm as the incident case or from strains circulating outside those communities (sexual network mixing) by: (1) genotyping the circulating HIV-1 variants in Enhanced Care and Combination Prevention arms, and (2) estimating the proportions of new HIV infections that can and cannot be phylogenetically linked to HIV-infected persons in the same study arm.

2) To estimate the efficacy of the Combination Prevention package on reducing the rate of new infections with HIV strains circulating within communities in the same study arm.

3) In the intervention arm only, to estimate over time, the proportion of incident HIV-1-infections that can be linked to HIV-infected adults of the same study arm.

4) During the end of study survey (ESS) to compare Combination Prevention (CP) package uptake within 20% households to CP package uptake in the broader communities they represent.

5) To obtain secondary cross-sectional estimates of community HIV incidence at baseline and study end in Enhanced Care and Combination Prevention communities through use of HIV-incidence assays.

6) At baseline, during study conduct, and at study end, to estimate the proportion of recent HIV infections in Combination Prevention and Enhanced Care communities with evidence of transmitted HIV drug resistance.

7) To estimate the proportion of HIV-infected adults with undetectable viral load (VL<400 copies/ml) and to estimate the community viral load at baseline, during study conduct, and study end in Combination Prevention and Enhanced Care communities.

8) To estimate the association between HIV-1 viral load and HIV transmissions using viral linkage data.

9) To project HIV infections and deaths (attributable to AIDS) averted in the combination prevention and Enhanced Care communities beyond the time frame of the trial.

10) To estimate the cost of provision of intervention services.

11) To estimate the cost-effectiveness of combination prevention (taking into account both infections averted and clinical costs) using the two arms from the trial as well as an additional model-based strategy.

12) To compare CD4 and viral load trajectories over time, among HIV-infected ART-naive persons who reside in Combination Prevention vs. Enhanced Care communities.

13) To assess the accuracy and feasibility of point-of-care viral load testing in comparison to standard viral load platforms.

14) To understand (through qualitative interviews) barriers to and facilitators of linkage to care and ART initiation among BHS participants.

15) To use BCPP data in analyses of the rates of and risk factors for major AIDS and non-AIDS illnesses/injuries/mortality among persons living with and without HIV in Botswana.

2.5. Design and Rationale to Address Secondary Objectives

2.5.1. Contamination

There are several sources of potential contamination in the proposed trial. This contamination can affect interpretation of results concerning the efficacy of the ART-related interventions in reducing HIV transmission in this trial, and is hence addressed in secondary objectives #1-2 (sexual network contamination) and secondary objective #4 (intervention contamination).

Sexual network contamination will almost certainly occur; available evidence from a recently conducted community survey in Mochudi (a district in southern Botswana) showed at least 30% of surveyed adults reported sexual partnerships with adults outside the community (unpublished data). Therefore, during trial conduct, sexual network contamination may occur in four ways:

- Enhanced Care HIC members may become HIV-infected through relationships with: (1) non-study community members, or (2) Combination Prevention community members
- Combination Prevention HIC members may become HIV-infected through relationships with: (1) non-study community members, or (2) Enhanced Care community members

Because Botswana's population is congregated largely in the eastern part, which constitutes a relatively small geographic area, and because the population is widely mobile anyway (Central Statistics Office 2009), attempting to separate study communities by large "buffer zones" or geographic boundaries (e.g. mountainous regions) is not considered a feasible option for trying to reduce sexual network contamination. Instead, investigators propose HIV-1 genotyping to enumerate circulating HIV-1 variants in Combination Prevention and Enhanced Care communities. Clustering of HIV-1 sequences implies that hosts are connected by a chain of viral transmission. Clustered HIV-1 sequences represent viral variants that circulate and spread in the communities where the analyzed blood specimens originate. The planned genotyping will help assess which new incident infections in the HICs were transmitted from members of the same study arm (phylogenetically linked infections), and which were transmitted from outside the study arm (unlinked infections). Similar methodology

has been successfully used in HIV prevention trials involving sero-discordant couples (Cohen, Chen et al. 2011). More details related to the proposed analyses are described in Sections 8 and 10.

A high density of sampling is necessary to increase the accuracy of viral linkage analysis. To assess patterns of HIV-1 transmission, blood samples for HIV-1 genotyping will be collected from:

- All consenting HIV-infected individuals identified in the BHS upon enrollment, and at AHS visits and during the ESS in participating Combination Prevention and Enhanced Care communities.
- All consenting newly identified HIV-infected adults identified in the HIC in both Combination Prevention and Enhanced Care communities, at T1 and T2.
- All consenting HIV-infected adults who present to HIV care/treatment clinics after being identified in the first HTC campaigns in Combination Prevention communities.
- All consenting HIV-infected adults receiving HIV care and/or treatment at local clinics in the Combination Prevention communities (including those already in care/on ART at the start of the study).
- All consenting *newly diagnosed* HIV-infected adults in the second HTC campaign (**HTC2**) and thereafter in the Combination Prevention communities who present to HIV care/treatment clinics.

The importance of genotyping in assessing and possibly controlling for sexual network contamination is captured in the first two secondary objectives. To meet the first secondary objective, we will assess the proportions of *linked* vs. *unlinked* incident cases in the Combination Prevention and Enhanced Care communities that are identified within the 20% of households participating in surveys, over the course of the study. This will allow us to estimate the extent of sexual network contamination of the HIV incidence cohorts.

To meet secondary objective #2, we will evaluate genotypes from the same groups as in secondary objective #1 (i.e., HIV-infected residents of the 20% of households participating in surveys over the course of the study, in both arms). This analysis, however, will include only phylogenetically *linked* incident cases. This analysis will therefore control for sexual network contamination by including only linked incident cases in the analysis of CP package impact.

The additional purpose of HIV-1 genotyping is captured in the 3rd secondary objective, which targets all HIV-infected residents in Combination Prevention communities. In secondary objective #3, we will estimate how the proportion of linked incident cases (in Combination Prevention communities only) changes over time. If the CP package is effective in reducing population-level HIV incidence, there should be a reduction in the proportion of recent infections that can be phylogenetically linked to HIV-infected persons in Combination Prevention communities.

Intervention contamination will occur mainly in three ways: (1) through Enhanced Care community members accessing interventions in Combination Prevention communities, (2) through Combination Prevention community members accessing Enhanced Care outside the Combination Prevention communities, and (3) through study procedures (such as HIV counseling and testing) related to the BHS and HIC (which may affect the Enhanced Care communities, in particular).

Several approaches (detailed in the *Intervention Protocol*) will be undertaken in an attempt to decrease intervention contamination between Enhanced Care and Combination Prevention communities. However, to assess the extent and nature of the intervention contamination that will inevitably occur, CP package uptake will be compared between Enhanced Care and Combination Prevention communities during the end of study survey (ESS), as described in primary objective #2.

With regard to contamination related to the BHS and HIC, BHS and HIC study procedures include: (1) HTC and point-of-care CD4 and referral of HIV-infected persons to HIV care and treatment services, and (2) encouragement of annual HTC and risk reduction measures for HIV-negative adults, combined with counseling about MC benefits for HIV-negative males. These procedures could introduce an unintentional intervention in the Enhanced Care communities (in the BHS and HIC participants). These procedures could also make the HIC less representative of the broader community of residents, even in the Combination Prevention communities. Unfortunately, this type of contamination is unavoidable as the study cannot be conducted without implementing these study procedures in all communities. To assess possible intervention contamination introduced by study procedures in the BHS and HIC, investigators propose the 4th secondary objective.

Another aspect of study design may result in HIV incidence measured in the HIC not being representative of broader study communities (in addition to the intervention contamination described above): despite being an open cohort, observed incidence in the HIC may be different than incidence in the communities that it represents, because behavior of HIC enrollees may change simply because they are part of a study (the Hawthorne effect). Laboratory-based methods for estimation of HIV incidence are important tools for monitoring of HIV/AIDS epidemics and are attractive due to their simplicity, ease of use, low cost and application to single cross-sectional specimens collected during routine surveys. For accuracy of testing and reliable scale-up, cross-sectional HIV incidence estimates need to be compared with the longitudinal HIV incidence data and properly validated. Use of incidence assays will categorize HIV infections observed at baseline (during the BHS and first HTC campaign), and newly diagnosed and incident HIV-infections during study conduct (during HTC activities thereafter), during follow-up of the 20% households including the HIC, and at study end (during the ESS) as either prevalent or recent (new infection in the preceding 3-12 months depending on the algorithm of incidence testing). For cross-sectional estimates of HIV incidence, the cross-sectional assay-based data will be compared and validated by the longitudinal data on HIV incidence. In sum, the 5th secondary objective will assess whether cumulative HIV incidence observed in the HIC is representative of community incidence.

2.5.2. Transmission Dynamics

Surveillance of transmitted HIV drug resistance (TDR) among recently infected individuals can inform treatment guidelines and provide feedback on the success of HIV-1 treatment and prevention programs. Increasing prevalence of transmitted drug-resistance mutations in the population may adversely impact the use of HIV treatment regimens. Therefore, surveillance of transmitted HIV drug resistance will address whether a rapid scale-up of ART in Combination Prevention communities is associated with the increasing prevalence of transmitted drug resistance (as compared with Enhanced Care communities). Secondary objective #6 addresses this concern.

As described earlier, the mechanism by which expanded access to ART should achieve a prevention effect is through lowering the proportion of HIV-infected adults in the intervention community with detectable viral loads (VL>400 copies/ml) (Montaner, Lima et al. 2010). To explore whether the treatment strategy in Combination Prevention communities achieved this aim, investigators propose to estimate the proportion of HIV-infected adults participating in the BHS, HIC and ESS with undetectable HIV-1 viral loads. This is captured in the 7th secondary objective.

Assessing the prevention efficacy of any single prevention intervention on its own will not be possible as part of this CRT as multiple prevention interventions will be implemented simultaneously. However, among linked recent HIV infections that are identified at baseline, during 20% household follow-up, during HTC in Combination Prevention communities, and ESS, assessing the proportion that are associated with HIV transmission from adults with high viral load (e.g. VL ≥10,000 copies/ml and other thresholds) could improve understanding of the proportion of incident infections in study communities that might be averted by the initially proposed treatment-as-prevention strategy and/or UTT. This is captured in the 8th secondary objective.

2.5.3. Cost and Cost-effectiveness

Assessing cost-effectiveness of the proposed CP package is crucial if potential funders and national governing bodies are to continue investment in the CP package after study completion.

The Cost-Effectiveness of Preventing AIDS Complications International (CEPAC-I) model will be used to assess expected outcomes from the proposed combination prevention programs in Botswana. The cost effectiveness analysis will evaluate three combination HIV prevention strategies: the two arms from the trial and an additional model-based strategy, the “optimized package”. The “optimized package” provides a model-based scenario based on the intervention arm, but will assess the case where linkage to routine HIV care, ART, and HIV screening are further optimized, pre-exposure prophylaxis is administered, and may incorporate additional prevention strategies.

Model-based cost-effectiveness analysis is critical to understanding the value of clinically effective prevention and therapeutic strategies. Model-based analyses have a number of attractive features, such as allowing one to: a) organize and synthesize trial data; b) handle uncertainty and forecast beyond a single trial via sensitivity and “what-if” analysis and c) extrapolate to longer time horizons, new geographic settings, and alternative target populations. With regard to HIV/AIDS, models have been used in multiple settings to evaluate the epidemiologic and policy implications of preventive interventions, to study the cost effectiveness of different treatments, to evaluate screening programs, to determine future resource needs, and to inform the design of clinical trials.

Secondary objectives (#9-11) pertain to cost and cost-effectiveness analyses. These will provide critical information to policy makers, following completion of the trial.

3. OVERVIEW OF STUDY METHODS

As described in the BCPP Schema (section vii), the over-arching design is a pair-matched community randomized trial (CRT) that will be completed over the course of approximately 5 years. To facilitate

description in this protocol the study methods are divided into six components. Harvard/BHP will have responsibility for 1, 2, 4, and 5 and CDC/MOH will have responsibility for 3.

1) The baseline household survey (BHS) (with subsequent annual household surveys, AHS)
2) Follow-up of the HIV incidence cohort (HIC) and BHS participants
3) Programmatic interventions in Combination Prevention communities
4) The End of Study Survey (ESS)
5) Laboratory Research Activities (Viral Genotype Analyses)

This protocol describes the BHS/AHS, HIC, and ESS, and the viral genotype analyses of the BCPP impact evaluation including the following: study population eligibility criteria (Section 4.4), sample size estimates and sampling methods (Section 5), study procedures for the BHS/AHS, HIC and ESS and Research Blood Draws (Section 6), cost effectiveness analysis (Section 7), laboratory procedures (Section 8), data management issues (Section 9), approaches to analysis (Section 10) and issues related to ethical interaction with human subjects (Section 11). Section 12 provides a suggested study safety and clinical monitoring plan, and Section 13 a description of study logistics and responsibilities, including a data dissemination plan.

4. STUDY POPULATIONS

4.1. Selection of Study Communities

Thirty communities (Appendix C2) in three geographic areas in Botswana: (1) around Gaborone in the south east, (2) around Francistown in the north east, and (3) in the region around Mahalapye, Serowe, and Palapye in the central eastern region, are proposed for inclusion in the CRT. The rationale for selecting 30 communities is provided in Section 5 (sample size calculations). The communities in Appendix C2 are purposively selected and proposed based on (1) desired size, and (2) feasibility.

- **Community size**
  Communities need to be large enough to meet sample size requirements yet not be so large that achieving ambitious CP package coverage targets is infeasible. Results of sample size calculations show that an average cluster size of about 300 HIV incidence cohort (HIC) enrollees per community is needed. To achieve this sample size, we are aiming to enroll approximately 500 potential HIC enrollees per community (300/0.85/0.70=500). The proportion of potential enrollees incorporates the expected impact of missing, refusal, capping the size of larger communities, and using back-up households.

  Because investigators wish to minimize intervention contamination that may be introduced by repeat HIV testing and counseling of the HIC in Enhanced Care communities, we propose to keep the community HIC size at no more than 20% of the entire study community. Therefore, the ideal average community size proposed by investigators is about 5,750 persons [500/(0.58*0.75*0.2) = 5,747] [Note: 0.58 is the proportion of the community considered age-eligible (Appendix C2), 0.75 is the proportion of adults HIV-negative, and 0.2 is the desired sampling proportion for the HIC]. Investigators therefore searched for communities of size 2,500—15,000, aiming for an average size of 5,750. Slightly less than 20% of households from the 30 study communities will take part in the BHS and HIC. Note that although this sample will likely be somewhat less than 20% of households,

we will refer to these as the "20% households" from this point forward, for ease of reference (please see Sections 5.1.3 and 5.1.4 for details regarding selection of the 20% households).

- **Feasibility**
  This project represents a significant logistical operation over the course of 5 years. To maintain feasibility, investigators propose that all communities need to:
  - Have a health center, from which HIV care and treatment, PMTCT, and HTC or MC services can be provided.
  - Be reachable by road, via a ≤8 hour drive from Gaborone or Francistown. (CDC and MOH have permanent, well-staffed offices in Gaborone and Francistown, while Harvard has a permanent office in Gaborone. This makes the study easier to implement and monitor for quality control purposes).
  - Have a clear geographic boundary such that it is reasonably easy to identify a household as either being part of, or outside of, the study community.

  These feasibility inclusion criteria resulted in the exclusion of urban areas from the study, and the inclusion of rural and peri-urban areas.

  The 30 study communities proposed meet the criteria described above. The average total population size of each of the targeted communities is 6,027. Therefore, we estimate a total population in study communities of about 180,800 people, with 58% (104,864) aged 16-64 (Appendix C3). HIV prevalence among residents aged 16-64 is estimated at 25%, resulting in an estimated 26,216 HIV-infected residents aged 16-64, or 13,108 per study arm.

4.2. Matching of Study Communities

Due to the small number of communities that can be enrolled in community randomized trials for cost and feasibility reasons, there exists a chance of imbalance of potential confounders between study arms. Therefore, in this study we considered restricted randomization, matching, and stratification as strategies to ensure balance between arms. We chose a pair-matched design because:

- This approach is more likely than stratification to achieve balance between study arms;
- Matching has the added advantage of improving statistical efficiency compared with restricted randomization, and;
- Chosen matching covariates (see below) are quite closely correlated with the outcome of interest (adult HIV incidence), making a matched design of similar or increased statistical efficiency compared with a stratified design, even though a matched design entails greater loss of degrees of freedom.

Possible disadvantages of a pair-matched design include:

- Loss of both communities in a pair if one community drops out.
- It is not possible to assess whether the intervention effect varies between matched pairs, whereas, in a stratified design, opportunity to assess effect modification across strata would be possible.

The following community matching criteria were chosen:

- **Community size**: Although community size in Botswana may not be associated with the outcome of interest (HIV incidence), achieving sample size balance between arms improves statistical efficiency. Appendix C2 provides a comparison of communities within proposed matched pairs. Overall, proposed community HIC sizes range from 230 to 1,105; however, following matching, the median difference between community HIC size within matched pairs is 61, and ranges from 1-313.

- **Baseline Access to Health Services including ART**: Access to ART is associated with a decrease in new HIV diagnoses (Montaner, Lima et al. 2010; Tanser, Bärnighausen et al. 2013). Although some communities within matched pairs have access to primary care clinics, and others have access to primary care hospitals, investigators believe that in the context of Botswana, this does not represent a significantly different level of access to health services.

- **Age structure**: Adult HIV prevalence and incidence are correlated with adult age (Central Statistics Office 2009). Age structures are very similar within the study community pairs. If we use the proportion of adults (16-64) who are at highest risk for HIV infection (25-34) as an indicator of age structure, the median difference in this proportion is 2% (range 0-7%). Note that residents up to age 64 are included in the study, due to relatively high HIV prevalence (15-25%) in persons aged 50-64 years in Botswana, as well as a spike in HIV incidence (5.5%) in 60-64-year old men, both noted in the 2008 Botswana AIDS Indicator Survey, or BAIS. *[Note: we do not have age structure data for Mandunyane or Mmadinare, but based on the age structure of the other 28 selected communities, do not expect any significant differences in age structure within the matched pairs].*

- **Geographic location** as measured by proximity to major urban centers (Hayes, Mosha et al. 1995). Geographic locations of communities within pairs, as estimated by average distance to the nearest large urban centers, are quite similar. For example, across 30 communities, the range of average distances to nearby urban centers ranges from 18-380 kilometers. However, following matching, the median difference in distance to an urban center between communities in a matched pair is 20 kilometers (range 3-150).

We do not have access to community-specific measures of HIV prevalence [e.g. HIV prevalence measured from antenatal care (ANC) clinics]. Previous successful pair-matched CRTs (Hayes, Mosha et al. 1995), where HIV incidence was the outcome of interest, have also addressed the problem of absent baseline HIV prevalence estimates by using geographic location and other proxies of HIV prevalence as matching criteria.

4.3. Definition of a Study Community Resident

The following definitions of study community residents will be used in the protocol:

- **Permanent study community resident:** An individual who reports spending on average ≥14 nights each month in that community over the preceding 12 months or an individual who recently moved into the community and reports spending on average ≥14 nights each month since arriving.

- **Part-time study community resident:** An individual who reports spending on average 3–13 nights each month in that community over the preceding 12 months or an individual who recently moved into the community and reports spending on average 3-13 nights each month since arriving.

- **Non-study community resident:** An individual who reports spending on average <3 nights each month in that community over the preceding 12 months.

- **Household member:** an individual who reports spending more nights (on average) in the household being sampled than in any other household in the same community over the preceding 12 months.

- **Botswana Citizen:** A Botswana citizen is anyone who can produce proof of Botswana citizenship. Usually this is the Omang number, which can be found on national ID cards, drivers licenses, Botswana passports, or an Omang application receipt. The Omang number is needed in Botswana to access routine government services. It is anticipated, based on a community survey in Mochudi, and the 2011 census (Central Statistics Office 2009), that less than 3% of residents will be non-citizens.

- **Spouse of Botswana Citizen:** A spouse of a Botswana citizen is a person who can produce evidence of being married to a Botswana citizen, usually a marriage certificate, and is therefore eligible to receive free medical services in Botswana.

- **Non-citizen:** A non-citizen is anyone who declares himself or herself as a non-citizen and/or cannot produce proof of Botswana citizenship.

4.4. Study Inclusion and Exclusion Criteria

4.4.1. Baseline 20% Household Survey (BHS)

The BHS will be conducted in the 20% households that are sampled from the participating study communities that have a total population of ≤6,000 residents; and approximately 273 households from the participating study communities that have a population over 6,000 residents (the latter is approximately 20% of residential and habitable plots for 6,000 residents). Eligible adults residing within these households will be asked to take part in the BHS. Eligible residents of the participating 20% households who did not take part in the BHS at T0--e.g. due to absence, etc.--and who are present during the subsequent AHS visits (prior to the final visit) will also be asked to complete the same procedures that are offered in the BHS. Following, we define inclusion/exclusion criteria (with regard to the BHS) for: (1) households, and (2) adults living within selected households.

- **Household** Eligibility Criteria for BHS**:**
  Inclusion criteria
  - The household should be located within the study community enumeration area map established for the 2011 national population census (Central Statistics Office 2009) or within established community boundaries where these maps are not available.

- **Household Representative** Eligibility Criteria for BHS:
  Inclusion criteria:
  - Aged 18 years or older
  - Household member
  - Willing to provide household information following administration of verbal consent script

- **Household Member** Eligibility Criteria for BHS:
  Inclusion criteria:
  - Aged 16-64 years
  - Permanent or part-time study community resident in any of the 30 study communities
  - Household member
  - Botswana citizen or spouse of a Botswana citizen
  - Able to provide informed consent if ≥18 years old, or able to provide assent to complement a guardian's permission, if a minor (aged 16 or 17)

4.4.2. HIV Incidence Cohort (HIC)

The HIV Incidence Cohort (HIC) will be enrolled from the 20% households participating in the BHS. The HIC will largely be enrolled during the BHS at T0; however, eligible residents of the participating 20% households who are present during AHS visits (prior to the final visit) and who were not previously enrolled to the HIC (e.g. due to absence, 15 years of age, etc.) will also be asked to enroll in the HIC (i.e., the HIC is an open cohort). Therefore, the **household** inclusion criteria described above for the BHS will apply to the HIC. Eligibility criteria for **HIV Incidence Cohort members** are as follows:

Inclusion criteria:

- Documented to be HIV-uninfected according to the rapid HIV test algorithm
- Aged 16-64 years
- Permanent study community resident in any of the 30 study communities
- Household member
- Does not intend to move out of the study community in the next 12 months
- Botswana citizen or spouse of Botswana citizen
- Agrees to annual home visits, as well as other study procedures
- Provision of contact details (such as telephone numbers, physical and/or alternate addresses) that would allow the study team to trace him/her
- Able to provide informed consent if ≥18 years old, or able to provide assent to complement a guardian's consent, if a minor (aged 16 or 17)

Note: The above HIC eligibility criteria apply during the BHS (T0) and AHS visits prior to the final visit (i.e. the HIC is an open cohort).

4.4.3. Individuals Eligible for Research Blood Draw in Combination Prevention Communities

For HIV-infected residents of Combination Prevention communities who did not take part in the research blood draw through the BHS, the following eligibility criteria will apply for the blood draw for HIV-1 RNA and genotyping/incidence assay/TDR/storage:

Inclusion Criteria:

- Any individual with documented HIV infection, including newly-diagnosed individuals and HIV-infected persons already receiving care and/or antiretroviral treatment at the clinic. (note: blood draws for HIV-1 RNA, genotyping, incidence assay, TDR and storage will be obtained once from HIV-infected clinic patients throughout the study.)
- Adult aged 16-64 years
- Permanent or part-time resident of combination prevention communities
- Botswana citizen or spouse of Botswana citizen
- Able to provide informed consent for study enrollment if ≥18 years old, or able to provide assent to complement a guardian's permission, if a minor (aged 16 or 17)

4.4.4. End of Study Survey (ESS)

The ESS will be conducted at the end of the study in 3 pairs of communities (6 communities) selected out of the 30 participating communities. It will be conducted in the ~80% of the population that is not taking part in the 20% household surveys. At T2, residents of the 20% households will also contribute data toward the ESS analyses.

Inclusion criteria:

- Aged 16-64 years
- Permanent or part-time study community resident in any of the selected 3 pairs of study communities (for ~80% households to be newly enrolled at ESS), or any of the 30 communities (for ~20% BHS/HIC households having final evaluation at T2/ESS)

- Able to provide informed consent for study enrollment if ≥18 years old, or able to provide assent to complement a guardian's permission, if a minor (aged 16 or 17)

4.4.5. Economic Assessment

Participants in the 20% household surveys will be asked to answer questions to estimate healthcare costs and quality of life; these questions are included as supplemental sections in the annual resident survey that participants will complete. The eligibility criteria for this group of participants in the economic assessments are therefore the same as for the BHS.

There will be additional sampling of individuals with opportunistic illnesses or low CD4 cell count. The eligibility criteria for these assessments are as follows:

Inclusion criteria for Supplemental Economic Assessment:

- Permanent or part-time study community resident in any of the study communities
- Aged 18-64 years
- Documented to be HIV-infected

- Measured CD4<200 cells/μL or WHO stage 3 or 4 illness
- Botswana citizen or spouse of Botswana citizen
- Able to provide informed consent for study enrollment

Records for a small subset of community residents (up to 80 residents) who die during the course of the study will be abstracted to evaluate for healthcare costs at the end of life. These individuals need not necessarily be part of one of the study cohorts. This aspect of the research does not involve human subjects per the definition at 45CFR46.102(f) that a human subject is a *living* individual. The eligibility criteria for the record review is as follows:

Inclusion criteria for Record Abstraction:

- Resident in any of study communities (defined by at-home death in a study community and/or recorded as a resident in the medical record)
- Aged 18-64 years at the time of death
- Botswana citizen or spouse of Botswana citizen
- Died during the years of the course of the study
- Records available for review

4.4.6. Barriers to and Facilitators of Linkage-to-Care (LTC) and ART Initiation

A small subset of participants in the 20% household surveys will be invited to participate in a focus group discussion (FGD) or individual in-depth interview (IDI) to provide information on barriers to and facilitators of linkage-to-care (LTC) and ART initiation. Staff from the BHS (Research Assistants) and MOH (Nurse Prescribers) will be invited to participate in separate FGDs to provide insight into the dynamics surrounding linkage to care and initiation of ART.

Inclusion Criteria for BHS Participants in FGDs or IDIs

- BHS participant (enrolled in the 20% household survey prior to the FGD)
- Resident of study community in the Combination Prevention arm
- Aged 18-64 years
- Documented to be HIV-infected
- Eligible for ART initiation (to be used for purposive selection for focus group discussion and in-depth interviews)
- Able to provide informed consent for FGD or IDI participation

Inclusion Criteria for Staff Participants in FGDs

- BHS staff member or Nurse Prescriber in any of the Combination Prevention communities
- At least 18 years of age
- Able to provide informed consent for FGD participation

4.5. Rationale for Eligibility Criteria

- **Rationale Surrounding Need for Botswana Citizenship for BHS/HIC:**
  Although the Botswana Government provides free HTC services to non-citizens, free access to health services, including ART, MC and PMTCT is restricted to a) persons who can produce valid

documentation of Botswana citizenship or b) spouses of Botswana citizens. The BCPP package of intervention services will therefore not be available to non-citizens of Botswana unless married to a citizen, per regulations of the MOH (Presidential Directive Cab. 5 (b)/2004).

As the households are followed longitudinally under the auspices of the study team but non-citizens cannot access free public health services unless married to a citizen, the eligibility criterion of Botswana citizenship (or being married to a Botswana citizen) is included for BHS/HIC participants, but not for ESS participants who are not followed longitudinally.

- **Rationale for Including Non-Citizens in ESS:** The intervention package implemented in Combination Prevention communities includes the following for non-citizens: expanded HTC services, including a referral to the health clinic in their community of residence to connect them with available fee-based services including treatment; and enhanced linkage to MC services, including for men who decline HIV testing or have indeterminate results. A major goal of the ESS is to evaluate and compare coverage of the intervention package components between arms, particularly HTC coverage which cannot be evaluated in the 20% BHS cohort. Comprehensively surveying *all* potential recipients of services in the study communities is an important aspect of achieving the goal of the ESS. Other key objectives of the ESS are to improve mapping of viral genetic linkage, and to accurately estimate how viral burden and viral suppression are distributed across HIV-infected residents of the study communities. Obtaining a sample from as many HIV-infected residents of the study communities as possible at ESS is critical for these objectives. Finally, the lack of access to free ART for non-citizens residing in Botswana represents a potentially significant gap in achieving the 90-90-90 goal for viral suppression that will be important to understand and quantify if Botswana is to consider expanding its treatment program, and for potentially further reducing HIV incidence in Botswana. Non-citizens will be included in the cross-sectional survey at ESS only, and will be offered anonymous participation to protect their identity. Non-citizens living in Botswana without legal documentation and others in the same household may face the risk of penalty (see Section 11). Participation in ESS may help non-citizens by providing free HIV testing and counseling with referral to services that they would be able to access.

- **Rationale for Age Limits:**
  Persons <16 years of age will be excluded from study participation because our intervention is targeting individuals age 16–64, and we hope to measure relevant study outcomes among this population. In Botswana, all persons aged ≥16 can access medical services, including HTC, without needing guardian permission. Although individuals aged 16-17 can access medical services without guardian permission, participation in research still requires guardian permission, because the age of consent for research in Botswana is 18. Therefore, eligible 16-17 year-olds will be asked for assent to participate in research activities if/after permission is provided by the guardian.

- **Rationale for Number of Nights Spent per Month in the Community:**
  For the BHS, we propose that adults who report spending ≥3 nights each month in the community over the past year, on average, be included, because these adults will be eligible for interventions if the community is randomized to the intervention arm (please see the *Intervention Protocol* for details on rationale for use of this definition of community residence vis a vis eligibility for

interventions). We need to estimate baseline and subsequent service uptake among a similar (representative) group of adults enrolled in the BHS (and subsequent household surveys), to allow assessment of baseline use of and uptake of interventions over time, and comparison between study arms.

For the HIC, we propose that adults who report spending on average ≥14 nights each month in the community over the past 12 months, and who do not intend to move out of the community over the next 12 months be included, because we believe that these adults are more likely to:

1) have sexual relationships with individuals residing inside versus outside the study community (hopefully reducing sexual network contamination),
2) access available health services in the community versus outside the study community (hopefully reducing intervention contamination of the HIC cohorts), and,
3) be easier to retain in the study (hopefully avoiding possible selection bias introduced by excessive loss to follow-up).

For research blood draws in all HIV-infected adults in the Combination Prevention arm, investigators propose that adults who report spending on average ≥3 nights per month in the community over the past year be included in blood draws for viral phylogenetic linkage.

For the ESS, investigators propose that adults who report spending ≥3 nights per month in the community over the past year be eligible because: (1) we need to measure coverage of the CP package within this group of adults, and (2) consenting HIV-infected adults identified during the ESS will contribute blood samples to improve completeness of the HIV-1 genotype map in Enhanced Care and Combination Prevention communities (important for secondary objectives 1-3).

- **Rationale for Number of Nights Spent per Month in the Household:**
  It is important to determine that participants in household-based procedures (BHS and HIC) are indeed at least part-time residents of the approximately 20% of households that are sampled (rather than casual visitors). The definition of household membership used (spending more nights on average in the household being sampled than in any other household in the same community over the preceding 12 months) is relatively straightforward to implement, and also helps decrease the chance of a given individual being asked to take part in household procedures in more than one household per community (although this criterion will not affect the chance of a participant enrolling in more than one community).

  Note: we intend to avoid enrolling the same individual more than once in the same component of the study (the BHS or the HIC). We will try to achieve this through the following means:

  - Inclusion of the household membership eligibility criterion.
  - Avoid enrollment of residents in the study if they state that they already took part in the study, with confirmation through other means.
  - Using the unique citizen Omang number to reduce the number of instances in which a resident is enrolled twice.

5. SAMPLE SIZE AND SAMPLE SELECTION METHODS

5.1. Sample Size Calculations Related to Primary Objective #1

To estimate the required sample size to detect predicted differences in cumulative HIV incidence between combination prevention and enhanced care incidence cohorts, we: (1) modeled the potential impact of the originally proposed intervention package on adult HIV incidence, and (2) used impact estimates in standard formulae for pair-matched CRTs to estimate the number of community clusters, size of incidence cohorts, and duration of incidence cohort follow-up, required to detect the originally estimated impact with sufficient power (≥85% power).

5.1.1. Estimating Impact of Combination Prevention on Adult HIV Incidence

We used two epidemic models to estimate the potential impact on adult HIV incidence of scale-up of the CP package to high population-level coverage levels.

- **Primary model**: The primary model is an agent-based network epidemic simulation model. Using models for generating degree distribution for sexual network described by Jones and Handcock (Jones and Handcock 2003) and implemented as an R package (Handcock, Hunter et al. 2003), we constructed stochastic degree distributions based on data from Likoma Island (Helleringer and Kohler 2007). Methods in Blitzstein and Diaconis (Blitzstein and Diaconis 2006) allow for the uniform sampling of networks with the prescribed degree sequence. For the sampled networks we select only those networks that match the prescribed level of mixing between communities. Using techniques similar to Eaton et al. (Eaton, Hallett et al. 2011), the model allows investigators to take into account community characteristics, including varying coverage levels for different prevention modalities, and population sizes, as well as individual characteristics including transmission risk, disease progression, condom use, linkage to care, and circumcision status.

- **Secondary model**: We also used the published Goals model available at (http://futuresinstitute.org/Default.aspx.) which is part of the Spectrum suite of models (Stover, Bollinger et al. 2011), to estimate CP impact on population-level HIV incidence. The Goals model is widely used by national governments, including Botswana (Stover, Fidzani et al. 2008), to plan HIV prevention programs and also estimate impact on HIV incidence and other indicators (e.g. number of AIDS deaths). We used the Goals model primarily to assess whether Goals impact estimates were similar to impact estimates from the primary model. The Goals model produced comparable results when using the same set of input parameters.

Table 1A lists input parameters used in the original primary model where we assumed the expanded ART intervention would be offered to HIV-infected individuals with CD4≤350 cells/µL or viral load ≥10,000 copies/ml in the Combination Prevention arm throughout the study, and that the Botswana national guidelines for ART initiation would not change during the study period. Original estimates of model input parameters were obtained from Botswana Government data and from the population-based Mochudi Prevention Project that was conducted from May 2010 to May 2013 (see below for a detailed explanation of input parameters). Timely linkage to care and treatment was assumed to be

80% in Enhanced Care and 90% in Combination Prevention communities during the study period. We assume that sexual mixing between communities is on average 20% with a 95% confidence interval of 15% to 25%.

Table 1A: Original Model Input Parameters for the Primary Model to Estimate Impact of Combination Prevention (CP) Package Scale-Up in CP Communities Versus Enhanced Care (EC) Communities over 4 Years

	EC Arm Anticipated True Coverage of Services in the EC Community		CP Arm CP Scale-up with ART at CD4≤350 or VL≥10K in CP Arm	
Time	HTC outside of study cohort	Male Circumcision	HTC*	Male Circumcision*
Baseline Survey	37%	12.7%	37%	12.7%
End Y1	41%	31.4%	90%**	46.4%
End Y2	45%	50.0%	90%	80%
End Y3	49%	60.0%	80%	80%
End Y4	53%	70.0%	70%	80%

*Intervention Community scale-up targets
**Allowing 4 months of scale-up period

For version 5.0 of the protocol, we conducted new simulations using updated baseline parameters based upon observed data in the first several community pairs. Treatment initiation is assumed to be 80% in the Enhanced Care communities and 90% in the Combination Prevention communities during the study period. Table 1B lists input parameters used in the updated primary model. Note that the updated model was undertaken to assess power under more realistic assumptions related to baseline parameters and coverage by interventions and under different scenarios for expanded ART.

Table 1B: Model Input Parameters for the Updated Primary Model to Estimate Impact of Combination Prevention (CP) Package Scale-Up in CP Communities Versus Enhanced Care (EC) Communities over 4 Years

	EC Arm		CP Arm	
Time	**Anticipated True Coverage of Services in the EC Community**		**CP Scale-up with Universal Test and Treat in CP Arm**	
	HTC outside of study cohort	Male Circumcision	HTC	Male Circumcision
Baseline Survey		35%		35%
End Y1	27%	40%	79%	50%
End Y2	31%	45%	85%	55%
End Y3	35%	50%	80%	60%

Explanation of input parameters:

- **HTC Service Uptake**
  HTC coverage is defined as the probability of a person of unknown status being tested for HIV anytime in the previous 12 months. We assumed 79% and 85% HTC coverage for the CP arm in

the updated model, more conservative than the 90% target, in years one and two, respectively. HTC uptake will likely increase in Enhanced Care communities during the study period as well. We assumed that HTC coverage in Enhanced Care communities would increase 4% per year during the study period.

- **Male Circumcision Service Uptake**
  A 2010–2011 survey of the Mochudi district in Botswana suggests MC coverage is 12.7% (the MOH Spectrum estimate is 11%). Linkage to male circumcision services in Enhanced Care communities will be ongoing. The original model parameters assumed that MOH, and the original *Intervention Protocol* targets for MC would be met in the EC, and CP arm, respectively by the end of Year 4 post-randomization. According to BAIS reports, the prevalence of circumcision among males in Botswana aged 16–64 was approximately 13% in 2008 and 28% in 2013, an increase of only 15% in 5 years. In addition, version 4.0 of the *Intervention Protocol* includes a lower MC target of 60% in Year 3. In the updated model, we therefore set the baseline MC prevalence at 35%, revised the coverage in the EC arm to increase by 5% per year, and assumed the revised MC coverage target of 60% would be achieved by the end of Year 3 in the CP arm.

- **ART Service Uptake**
  In the primary model using either the original or updated parameters, ART coverage is an intermediate outcome of HTC coverage and linkage to care. In the updated model we assumed baseline ART coverage of 88.7%.

- **PMTCT service uptake**
  We have not listed PMTCT coverage input parameters because in both models, PMTCT coverage increases have no direct impact on adult HIV incidence. However, in real program settings, with an estimated 10% of adult women becoming pregnant each year, we expect ANC clinics to be an important avenue for adult HTC and entry into HIV care and treatment. The primary PMTCT coverage goal of the CP package is to increase the proportion of HIV-infected post-partum women who are enrolled in care clinics (linked) and are retained in care and on ART (Option B+).

- **Sexual Mixing between Study Communities**
  One of the main reasons for building the primary model was to allow for potential sexual network contamination of HIV incidence cohorts in intervention and Enhanced Care communities. Therefore, the primary model assumed that an average of 21% (SE: 2.6%) of sexual relationships occurred between persons in opposite study arms. Data from a survey of Mochudi district in Southern Botswana showed that 30% of the relationships were from outside of Mochudi, with 25% of the out-of-community relationships in Gaborone, the capital of Botswana. We decided to assume, on average, 21% sexual mixing for two primary reasons: (1) many of the study communities are more rural than Mochudi; and (2) some of the out-of-community relationships in Enhanced Care communities will not be with Combination Prevention community residents.

Table 2A below provides model estimates of differences in cumulative HIV incidence between Enhanced Care and Combination Prevention communities. Table 2A reflects modeling conducted with the original expanded ART design offered in CPCs at the time of launching the study in 2013. The input parameters are based on the Mochudi survey, Botswana government data before the study launched, and the HTC and MC coverage levels as listed in Table 1A.

Table 2A: Projected Cumulative HIV Incidence in Enhanced Care (EC) versus Combination Prevention (CP) Communities over 4 Years of Study Follow-Up Using Original Parameters and with ART for Individuals with CD4≤350 cells/μL or Viral Load ≥10,000 copies/ml in the CP Arm

Time	EC Arm	CP Arm CP Scale-up with ART at CD4≤350 or VL≥10K in CP Arm	
		Cumulative Incidence	(% Reduction vs. EC)
End of Y1	1.79%	1.40%	(22% ↓)
End of Y2	3.05%	2.01%	(34% ↓)
End of Y3	**4.05%**	**2.44%**	**(40% ↓)**

Table 2B reflects modeling conducted with change in CD4 threshold from 350 to 500 in 2015, and move to UTT in Combination Prevention communities (implemented with version 4.0 of this protocol) and Botswana's "Treat All" policy in place starting June 2016. The input parameters for the projections in Table 2B are based on observed data in the first several community pairs and HTC and MC coverage levels listed in Table 1B. In the updated models, we assumed 80% ART initiation in the EC arm and 90% in the CP arm.

The Botswana MoH adoptetd universal ART as national policy effective June 2016. Therefore, we simulated this scenario of treatment eligibility in the Enhanced Care arm compared to UTT in the Combination Prevention arm: CD4≤350 followed by universal ART in 2016. Guidelines changes are assumed to become policy in June 2016, and ramp up to full scale over the following 12 months. The CP arm was simulated as follows: launching with the original design (CD4≤350 or VL>10,000), followed by CD4≤500 or VL≥10,000 in the third quarter of 2015, followed by UTT in June 2016. These simulations were done with the updated input parameters shown in Table 1B above.

Table 2B: Projected Cumulative HIV Incidence and Study Power with Universal Test and Treat in the Combination Prevention (CP) Arm and "Treat All" in the Enhanced Care (EC) Arm

Treatment Eligibility		Length of Follow-up	Cumulative Incidence Rate (CIR) (%)		Reduction in CIR (%)	Power (%)
ECC	CPC		ECC	CPC		
CD4≤350 → universal ART in 2016	CD4≤350 or VL≥10k at study launch → CD4≤500 or VL≥10k in 2015 → UTT in 2016	2 Years	1.45	0.93	36	47
		2.5 Years	1.70	1.00	41	63
		3 Years	1.84	1.06	42	70

Since the exact timing of UTT roll-out is unknown in Enhanced Care communities under Botswana's "Treat All" program, we made a simple assumption that each Enhanced Care community will have the same (reflecting the average) amount of time of follow-up before and after UTT. This assumption was also made for the Combination Prevention communities, but these communities will receive UTT earlier. While the reality will undoubtedly depart from these assumptions, the models are useful in providing a first-order approximation of power.

5.1.2. Estimating Needed Number of Community Clusters and Size of Incidence Cohorts to Detect Estimated HIV Incidence Differences

We examined the number of communities and incidence cohort size needed to detect a 40% difference in cumulative HIV incidence (originally 4.05% in the Enhanced Care arm vs. 2.44% in the Combination Prevention arm), which we hoped would be achieved by the end of the third year of follow-up if Botswana national guidelines remain at ART initiation for individuals with CD4≤350 throughout the entire study. Required sample size was determined based on the original combination prevention package design prior to the launch of the study (see version 1.0 of the Evaluation Protocol). See Sections 5.1.1, 5.2.1, and 5.2.2 for updated study power estimates based on the current design and assumptions.

We aimed to have ≥85% power to detect the anticipated effect size with alpha set at 0.05.

We used the formula from Hayes and Moulton, appropriate for a pair-matched CRT (Hayes and Moulton 2009).

$c = 2 + \left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \frac{\pi_0(1-\pi_0)/m + \pi_1(1-\pi_1)/m + k_m^2(\pi_0^2+\pi_1^2)}{(\pi_0-\pi_1)^2}$, where,

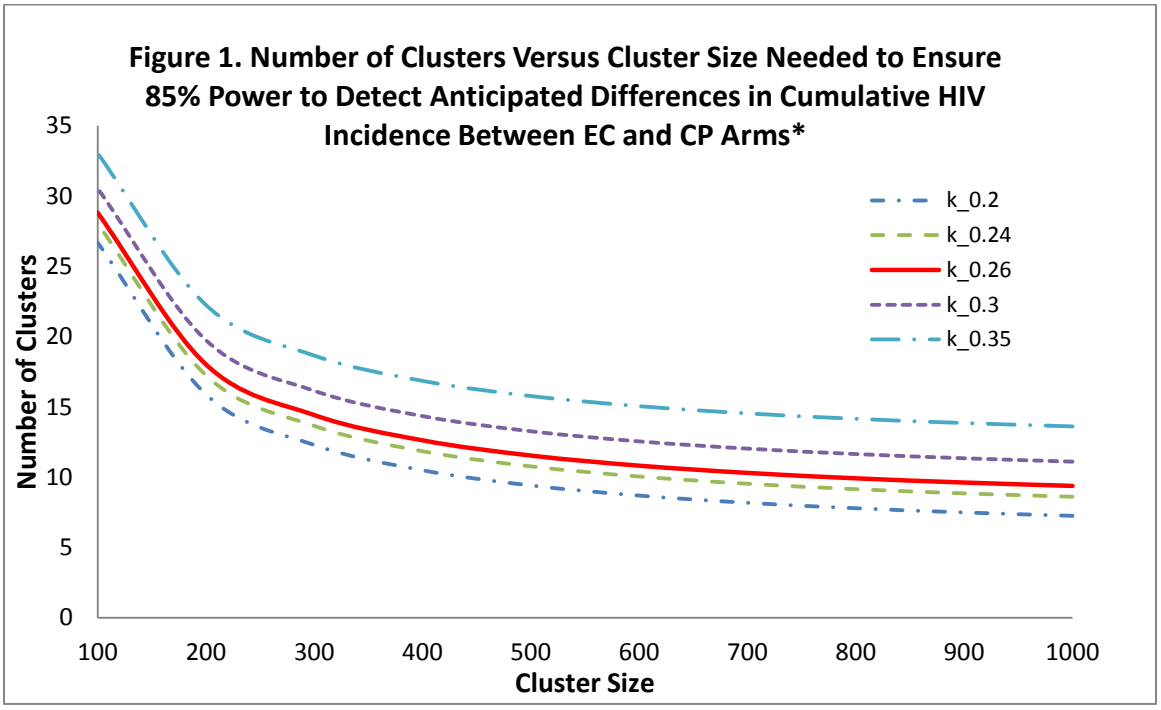
c is the number of clusters (community clusters) per arm,
$Z_{1-\alpha/2}$ is the standard normal deviate corresponding to the upper tail probability of $\alpha/2$ where α is the probability of a type I error.
$Z_{1-\beta}$ is the standard normal deviate corresponding to the upper tail probability of β where β is the probability of a type II error,
π_0 is the cumulative HIV incidence in the control arm,
π_1 is the cumulative HIV incidence in the intervention arm,
m is the cluster size in each community cluster (examined a range – see below)
k_m is the matched pair, between-cluster coefficient of variation of the true proportions in both the control and intervention arms (based on primary model simulations, the estimate of k_m is 0.24. To be conservative, we used 0.26, but we examined a range of k_m from 0.2 - 0.35).

The table and graph below illustrate the community and cluster sizes needed to ensure ≥85% power to detect the anticipated effect size by the end of the third year of follow-up.

Table 3: Cluster Size versus Cluster Number Needed to Achieve >85% Power to Detect Anticipated Effect Size by the End of the 3rd Year of Follow-up across a Range of Coefficients of Variation (k), Assuming ART is Offered to HIV-Infected Individuals with CD4≤350 or Viral

Load≥10,000 copies/ml in the Combination Prevention Arm based on the original set of parameters

Cluster size (m)	Number of clusters required, depending on the size of k				
	k=0.2	*k=0.24*	*k=0.26*	*k=0.3*	*k=0.35*
1000	7	9	9	11	14
900	7	9	10	11	14
800	8	9	10	12	14
700	8	10	10	12	15
600	9	10	11	13	15
500	9	11	12	13	16
400	10	12	13	14	17
300	12	14	14	16	19
200	16	17	18	20	22
100	27	28	29	31	33



**Assumes ART is offered to HIV-infected individuals with CD4≤350 or viral load≥10,000 copies/ml in the Combination Prevention arm based on the original set of parameters*

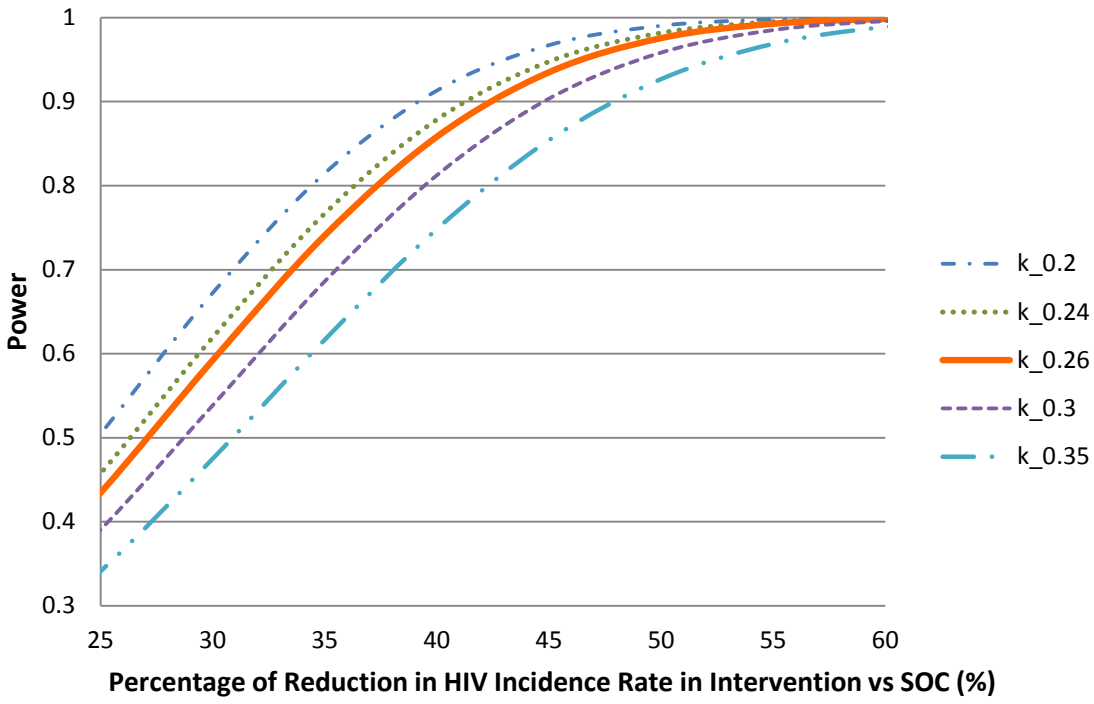
With 15 clusters per arm and on average 300 HIV-negative HIC participants per community (i.e., 4,500 HIC participants per arm or 9,000 HIC participants total), we have 86% power to detect the anticipated difference in cumulative HIV incidence between Enhanced Care and Combination Prevention communities (4.05% vs. 2.44%) by the end of the third year of follow-up, if Botswana national guidelines remain at ART initiation for individuals with CD4≤350 throughout the entire study, assuming the coefficient of variation (k) is 0.26. Under the Combination Prevention package introduced with version 4.0 of this protocol (ART for all HIV-infected individuals), the estimated

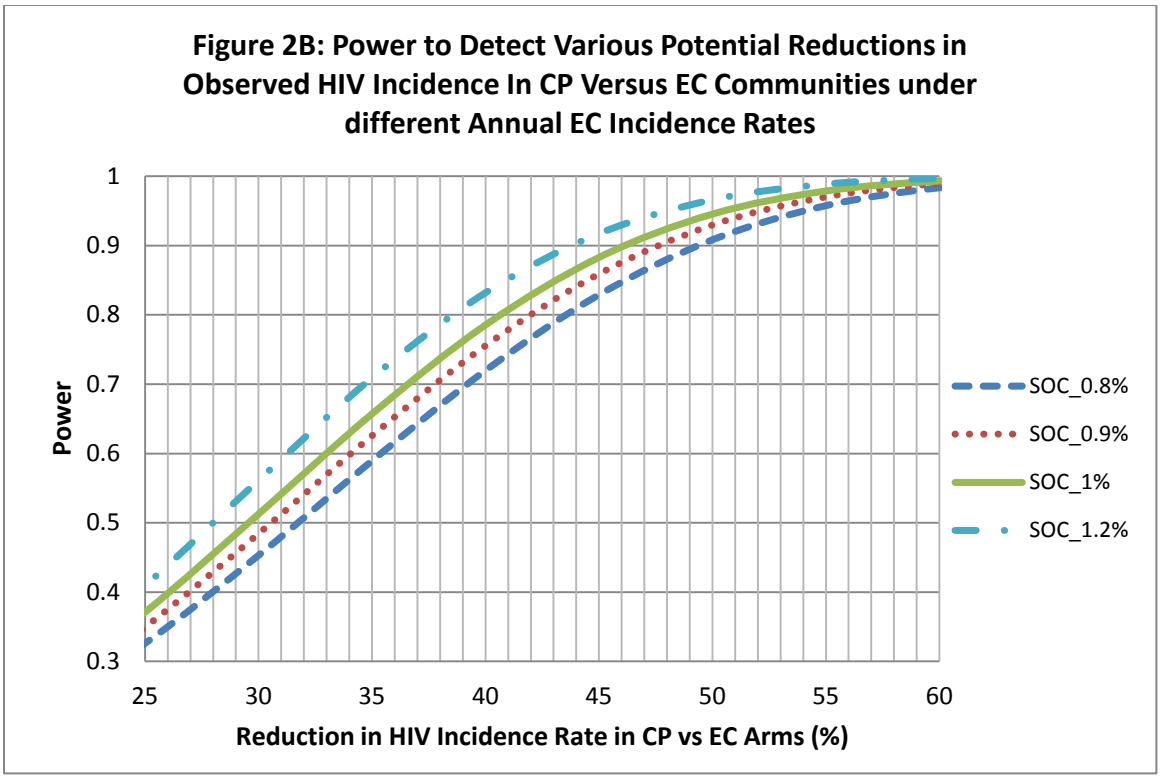
power based on more conservative assumptions (Table 1B) is >72% if national guidelines change to also offer universal ART in 2016.

Investigators note that power calculations are based on differences in proportions of the population infected over the 3-year period for each pair of communities, and do not take into account all of the variability that could arise from the interval-censoring that allows for making use of partial information for dropouts, irregular schedules of visits, and delayed entry into the cohort. However, we believe that the power calculations will be reasonable because the available information should not be sharply reduced by these factors. While some information will be lost because of dropout, the open nature of the HIC, use of the results from multiple tests throughout the study period, and the interval-censored data, which reflects the actual time of infection, would add information.

Assuming cumulative HIV incidence in the Enhanced Care arm is 4.1% at 3 years, we examined the range of effect sizes that might be detected if 30 communities are enrolled with 300 HIV-negative adults per incidence cohort (see Figures 2A and 2B below).

Figure 2A. Power to Detect Various Potential Reductions in Observed HIV Incidence In CP Communities when EC Communities have a Cumulative Incidence of 4.1%





The above power estimates suggest that, for 15 matched pairs, with average HIC size 300 per community, if cumulative incidence in the Combination Prevention arm is ≥38% lower than in the Enhanced Care arm (4.1%), we should have >80% power to detect the difference by the end of the third year of follow-up, assuming k is 0.26 (Figure 2A). If the annual incidence rate in the Enhanced Care arm turns out to be as low as 1.0%, we will still have >80% power if we can achieve a reduction of at least 41% in cumulative incidence in the Combination Prevention arm (Figure 2B).

5.1.3. Study Community Selection

Our rationale for selection of the 30 study communities is provided in Section 4.1. Here we describe in more detail how selected study communities facilitate enrollment of HIV incidence cohorts of sufficient average size (averaging 300 persons per HIC).

The total estimated population in the 30 targeted communities is 180,800, on average 6,027 individuals per community, ranging from 2,409 (Ranaka) to 12,086 (Mmadinare). The age-eligible (16-64 year olds) subset is estimated at 58% or 3,495 per community including 2,622 HIV-negative and 874 (25%) HIV-positive individuals. Assuming 20% of household members are absent, and 85% of present members consent to participate in the study, sampling 20% of households from communities of population ≤6,000 and approximately 333 households from communities of population >6,000, should facilitate identification of on average 300 HIV-negative persons per community, sufficient to meet sample size requirements for the CRT. Assuming 4.2 persons per household (Central Statistics Office 2009), 2.4 of whom are adults, and 1.8 of whom are HIV-negative adults, this would suggest we need to visit at baseline and annually on average 240 households per community.

5.1.4. Incidence Cohort Selection – the Baseline 20% Household Survey

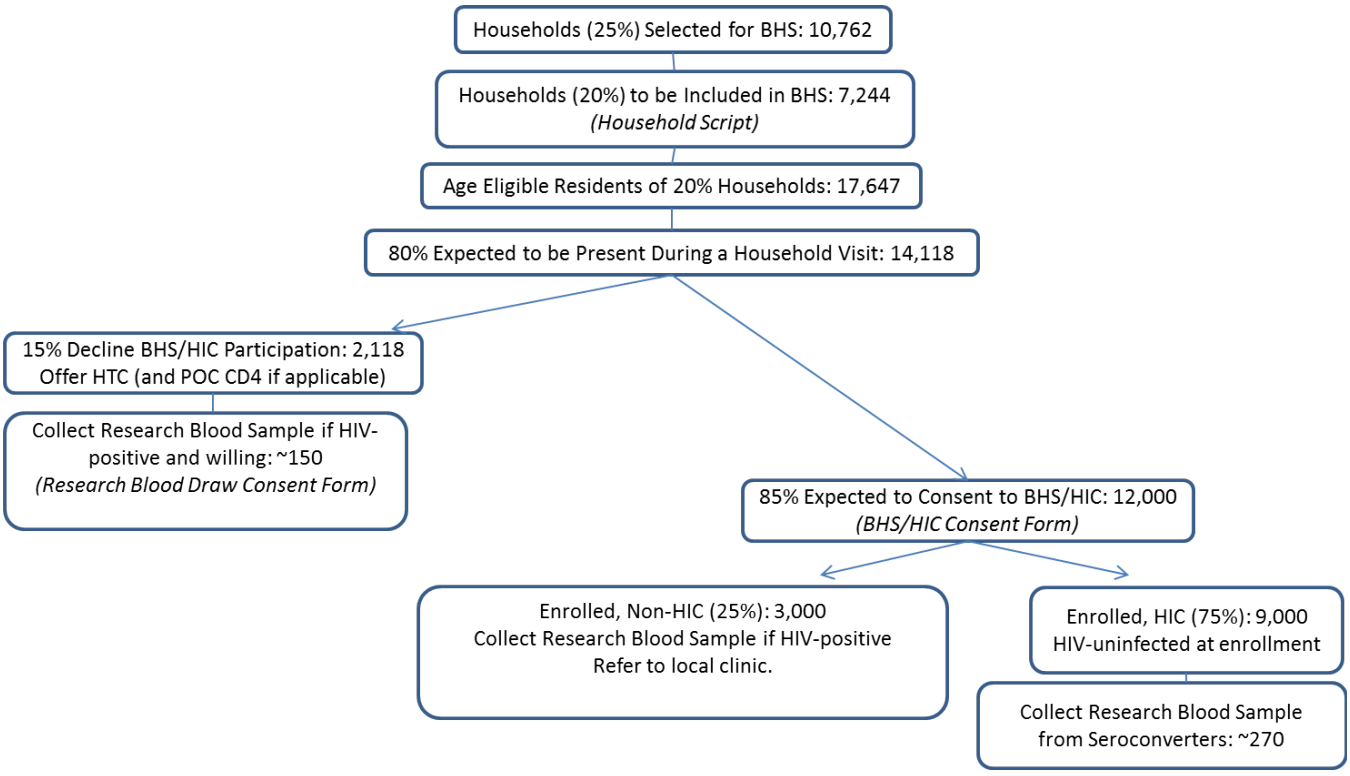
The primary purpose of the baseline 20% household survey is to identify and recruit HIV negative individuals for the HIV incidence cohort. To reach a 20% household sample size (on average 240 households per community), a 25% sample of households (possibly ranging from 115 to 576, and averaging 360 households per community) will initially be randomly selected. The initial unit of selection will be a plot using google area maps (and potentially population census and other publicly available sources); in some instances there may be more than one household on a plot and all houses on a selected plot will be asked to take part. The specifics of the household selection process are described in the BHS/HIC manual. In villages with population under 6,000, the required 20% households will be randomly selected from the 25% sample and approached first for participation in the study. In villages with population over 6,000, 333 households will be randomly selected from the 25% sample and approached first for participation in the study.

The remaining households in the 25% sample will be ordered randomly (e.g. from 1-49). If some of those households in the primary 20% sample in smaller villages (population ≤6,000) or the 333 households in larger villages (population >6,000) do not participate (e.g. refuse consent), the additional households in the full 25% sample will be approached sequentially, in the order specified by the random sampling scheme. In villages with population under 6,000, the enrollment will stop when either a 20% sample of the entire community has been achieved or the full 25% household sample list is exhausted. In villages with population over 6,000, after at least 273 households have been enrolled, the enrollment will continue until 20% of the households in the village have enrolled, or 23 working days have been reached, whichever comes first.

Based on previous survey experience in Mochudi and northern Botswana, we anticipate that we will be able to enroll 20% of households from the baseline 25% household sample. Our reason for not wishing to approach more than 25% of households is to avoid excessive intervention contamination of the Enhanced Care arm.

Figure 3 depicts the numbers and flow of participants from the 20% household sample.

Figure 3: Flow of participants from 20% Household Survey to BHS and HIC



5.2. Sample Size Calculations Related to Primary Objective #2

5.2.1. Estimating Precision Surrounding HTC, ART, VL Suppression, MC, and PMTCT Coverage Proportions

The rationale for the size of the baseline 20% household sample is provided above.

The end of study survey (ESS) will provide the primary estimates of intervention coverage. It should be noted that the model inputs related to intervention coverage used to estimate power for Primary Objective #1 (Tables 1B and 2B) differ from the intervention coverage values used to estimate power for Primary Objective #2. Modeling for Primary Objective #1 used a combination of observed coverage (from the first several communities) and targets (for MC). For Primary Objective #2, actual coverage of the intervention package will generally be assessed in distinct populations according to the service uptake indicators outlined in Section 2.2 and the targets of the Intervention Protocol (Table 4).

At the ESS we will compare coverage of HTC, ART, VL Suppression, and MC between the two study arms. We will have limited power (<60%) to detect a difference in PMTCT coverage proportions between study arms due to small sample size; the number of HIV-positive, pregnant women at ESS is estimated to be 270 per study arm. However, HIV-positive pregnant women will be included in measurement of coverage of the ART and VL suppression targets.

The ESS will be conducted in 3 pairs of communities (6 communities in total, 3 in each arm) selected out of the 30 participating communities. The ESS will attempt to survey the ~80% of the population that is not taking part in the BHS/HIC, in these 6 communities (in addition, the 20% households in these communities will participate in T2 surveys (or T3 surveys, in a small number of communities completing T3 visits prior to the roll-out of version 5.0), leading to the target of 100% community assessment in these 6 communities at the end of the study). The final surveys among residents in the 20% households in the remaining 24 communities will also provide data.

The rationale for seeking a 100% community census in three Enhanced Care and three Combination Prevention communities is as follows: (1) to measure HTC coverage in both study arms and more accurately measure study-end MC and ART coverage, (2) to improve mapping of viral genetic linkage, (3) to accurately estimate how the viral burden and viral suppression are distributed across HIV-infected people in Enhanced Care and Combination Prevention communities, (4) to compare CP package uptake within 20% households to CP package uptake in the broader communities they represent, and (5) to improve precision of cross-sectional assay-derived HIV-incidence estimates in Enhanced Care communities. The ESS was restricted to 6 (rather than 30) communities as it provides sufficient data for these analyses and requires significantly fewer resources.

The average total population size of the study communities is 6,027. Therefore, we estimate a total population in the six ESS communities of 36,162 people, with 58% (20,974) aged 16-64. Approximately 20% of these residents will be visited at T2 (or T3 in communities having this visit prior to version 5.0) as part of the BHS/HIC follow-up visits. Residents of the remaining approximately 80% will be invited to participate in the ESS as a one-time household survey. Assuming 80% of residents are present at the time of an ESS/T2 visit, and 85% consent to participate, there will be on average 2,377 participants per community, and approximately 14,262 in total, including residents of the previously enrolled 20% households and new enrollees from the remaining 80% of households.

In the table below we estimate precision of confidence intervals surrounding coverage proportions of the HIV prevention interventions to be compared (HTC, ART including VL suppression, and MC) in all study communities at baseline and, separately in Enhanced Care and Combination Prevention communities at study end.

Table 4: Estimated Precision of 95% Confidence Interval Surrounding HTC, ART (including VL suppression) and MC Coverage Proportions at Baseline and Study End

		Baseline Household Survey (BHS)	End of Study Survey (ESS)	
		All Study Communities	EC Arm	CP Arm
HTC Coverage	Denominator (n=residents aged 16–64 years)	12,000	5,931	5,931
	Numerator (n=residents aged 16–64 years who know they are HIV-positive or have tested HIV-negative within the past 12 months)	4,440	2,847	5,338
	HTC coverage proportion	37%	48%	90%
	Width of 95% CI (+-)	**±1.6%**	**±4.9%**	**±8.9%**
ART Coverage	Denominator (n=HIV-positive people aged 16–64 years)	3,000	2,971	2,971
	Numerator (n=HIV-positive people aged 16–64 years who know they are HIV-positive and are currently receiving ART)	2,190	2,405	2,763
	ART coverage proportion	73%	79.5%	93%
	Width of 95% CI (+-)	**±3.0%**	**±4.3%**	**±4.8%**
VL Suppression	Denominator (n=HIV-positive people aged 16–64 years)	3,000	2,971	2,971
	Numerator (n= HIV-positive people aged 16–64 years who know they are HIV-positive, are currently receiving ART and with HIV viral load ≤400 copies/mL)	2,100	2,264	2,822
	VL suppression coverage proportion	70%	76.2%	95%
	Width of 95% CI (+-)	**±3.0%**	**±4.1%**	**±4.9%**
MC Coverage	Denominator (n=HIV-negative men aged 16-64 years)	4,320	4,278	4,278
	Numerator (n=HIV-negative men aged 16-64 years who are circumcised)	1,512	2,139	2,567
	MC coverage proportion	35%	50%	60%
	Width of 95% CI (+-)	**±1.9%**	**±2.9%**	**±3.4%**

A detailed explanation of the contents of Table 4 is provided below. Total populations sizes may be found in Appendix C3.

- **HTC coverage numerators and denominators:**
  - The **BHS denominator** is calculated as (average number of BHS enrollees per community)*number of communities = 400*30 = **12,000**.
  - The **BHS numerator** is calculated as 12,000 (BHS enrollees)*(expected proportion of residents who either know they are HIV-positive or have tested HIV-negative within the past 12 months) = 12,000*0.37 = **4,440** (note that the definition of HTC coverage used here differs slightly from the definition used in section 5.1, due to requirements related to model structure).
  - The ESS denominator includes all residents aged 16-64 years outside of the BHS 20% sample in the 3 pairs of selected communities. The BHS 20% sample cannot be used to assess HTC coverage because all BHS participants are tested at enrollment per study design. The same proportions expected to be present and consent to BHS at T0 were assumed for the ESS at T2.

Therefore the **ESS denominator per arm** is calculated as [(average population size per community)*(proportion expected to be age-eligible)*(proportion expected to be present)*(proportion expected to consent) – (average number of BHS enrollees per community)]*(# communities per arm) = [(6,027*0.58*0.80*0.85) – (400)]*3 = (2,377–400)*3 = **5,931**.

- We assumed that HTC coverage in EC communities would increase 7% in the first year and 4% per year thereafter during the study period (note that the definition of HTC coverage used here differs slightly from the definition used in section 5.1, due to requirements related to model structure). Therefore the **ESS numerator in the EC arm** is calculated as 5,931*(expected proportion who know they are HIV-positive or have tested HIV-negative within the past 12 months at T2) = 5,931*(0.48) = **2,847**
- The **ESS numerator for the CP arm** is calculated as 5,931*(target outlined in Protocol 3) = 5,931*0.90 = **5,338**.

- **ART coverage numerators and denominators:**
  - The **BHS denominator** includes HIV-positive persons aged 16-64 years in the BHS 20% sample and is calculated as (the total number of BHS enrollees)*(proportion HIV-positive) = 12,000*0.25 = **3,000**.
  - The **BHS numerator** is calculated as 3,000*(proportion HIV-positive persons expected to know their status and currently on ART based on preliminary BHS data) = 3,000*0.73 = **2,190**.
  - The ESS denominator includes HIV-positive adults from the BHS 20% sample in the 24 communities not selected for participation in the ESS and all HIV-positive adults (100% sample) in the 6 ESS communities. Based on preliminary follow-up data at T1, we expect 90% of participants enrolled at T0 to be present at T2. In addition, we expect additional household members will be added to the cohort at T1. Based on preliminary data from T1, we assume an increase in enrollment of 10% at T1.

    The number of HIV-positive adults from the BHS 20% sample from the 24 non-ESS communities and 6 ESS communities is calculated as (average number of BHS enrollees per community)*(proportion expected to be present at T2)*(additional proportion newly enrolling at T1)*(proportion HIV-positive)*(number of communities) = 400*0.90*1.10*0.25*30 = 2,970.

    The number of HIV-positive adults in the 6 ESS communities outside the BHS 20% samples is calculated as [(average population size per community)*(proportion expected to be age-eligible)*(proportion expected to be present)*(proportion expected to consent)*(proportion HIV-positive)–(average number of HIV-positive BHS enrollees per community at T2)]*(number of ESS communities) = [(6,027*0.58*0.8*0.85*0.25) – (2,970/30)]*6 = 2,972.

    Therefore the **ESS denominator per arm** is 2,970 + 2,972 = 6,050/2 = **2,971**.
  - Based on preliminary BHS data, we found that 87% of HIV-positive participants who knew their status were on ART representing 73% of all HIV-positive persons (irrespective of prior knowledge of status). Assuming that HIV diagnosis among HIV-positives will increase by 3% over 2 years in the EC arm and that 87% of these new diagnoses will initiate ART, we expect an additional 2.6% of all HIV positive-persons will be on ART by T2. Additionally, we expect that by T2 UTT will have been scaled up and available in EC communities for approximately 6 months. Therefore, all ART-naïve individuals will be eligible for ART including those with CD4≤350 at

baseline. Based on preliminary BHS data, 7.8% of HIV-positive, ART-naïve persons have a CD4>350 cells/mm^3. Since UTT will only have been in place for approximately 6 months by T2, we assume that 50% of these ART-naïve individuals with a CD4>350 cell/mm^3 will be on ART by T2 or 7.8%*0.5 = 3.9%.

Thus, the **ESS numerator for the EC arm** is calculated as 2,971*[(proportion of HIV-positive persons expected to know their status and currently on ART at T0) + (additional proportion of HIV-positive, ART-naïve at T0 expected to be on ART by T2 due to increased diagnosis and under UTT)] = 2,971*(0.73+0.026+0.039) = 2,971*0.795 = **2,405**

- The **ESS numerator for the CP arm** is calculated as 2,971*(target outlined in Protocol 3) = 3,516*0.93 = **2,763**.

- **VL suppression numerators and denominators:**
  - The **BHS denominator** includes HIV-positive persons aged 16-64 years in the 20% BHS sample and is calculated as described above for the BHS denominator for ART coverage = **3,000**.
  - The **BHS numerator** is calculated as 3,000 * (proportion HIV-positive persons expected to know their status, be on ART and have a VL ≤400 copies/mL based on preliminary BHS data) = 3,000*0.70 = **2,100**.
  - The **ESS denominator** by arm includes all HIV-positive adults from the BHS 20% sample in the 24 communities not selected for participation in the ESS and all HIV-positive adults in the 6 ESS communities as described above for the ESS denominator for ART coverage = **2,971**.
  - Based on preliminary BHS data, we found that 96% of HIV-positive participants who knew their status and were on ART were virologically suppressed representing 70% of all HIV-positive persons (irrespective of prior knowledge of status and ART status). Therefore, the additional 3% increase in diagnosis among HIV-positive persons by T2 in the EC arm contributes to an increase in VL suppression coverage of 3%*87%*96% = 2.5%. As described above, due to UTT, there will be an increase in VL suppression coverage among previously ART-naïve individuals with a CD4>350 cells/mm^3 of 3.9%*96% = 3.7%.

    Therefore, the **ESS numerator for the EC arm** is calculated as 3,516*[(proportion of HIV-positive persons expected to know their status who are on ART and virologically suppressed) + (additional proportion on ART and virologically suppressed due to increased diagnosis and UTT)] = **2,971***(0.70+0.025+0.037) = **2,971***0.762 = **2,264**.
  - The **ESS numerator for the CP arm** is calculated as **2,971***(target outlined in Protocol 3) = **2,971***0.95 = **2,822**.

- **MC coverage numerators and denominators:**
  - The **BHS denominator** includes HIV-negative men in the BHS 20% sample and is calculated as average number of BHS enrollees per community)*(proportion who are male)*(proportion HIV-negative)*(number of communities) = 400*0.45*0.8*30 = **4,320**.
  - The **BHS numerator** is calculated as 4,320*(proportion of HIV-negative men circumcised based on preliminary BHS data) = 4,320*0.35 = **1,512**.
  - The ESS denominator includes HIV-negative men from the BHS 20% sample in the 24 communities not selected for participation in the ESS and all HIV-negative men (100% sample)

in the 6 ESS communities. The assumptions regarding additional enrollment at T1 are described in detail above for ART coverage.

The number of HIV-negative men from the BHS 20% sample in the 24 non-ESS communities and 6 ESS communities is calculated as (average number of BHS enrollees per community)*(proportion expected to be present at T2)*(additional proportion newly enrolling at T1)*(proportion male)*(proportion HIV-negative)*(number of communities) = 400*0.90*1.10*0.45*0.8*30 = 4,277.

The number of HIV-negative men in the 6 ESS communities outside the BHS 20% sample is calculated as [(average population size per community)*(proportion expected to be age-eligible)*(proportion expected to be present)*(proportion expected to consent)*(proportion male)*(proportion HIV-negative) – (average number of HIV-negative BHS male enrollees per community at T2)]*(number of ESS communities) = [(6,027*0.58*0.8*0.85*0.45*0.8) – (4,277/30)]*6 = 4,279.

Therefore the **ESS denominator per arm** is 4,277 (HIV-negative from the BHS 20% sample in 30 communities) + 4,279 (HIV-negative men from the 80% sampled in 6 ESS communities) = 8,556/2 = **4,278**.

- The **ESS numerator for the EC arm** is calculated as **4,278*** (proportion of HIV-negative men expected to be circumcised) = **4,278***0.5 = **2,139**.
- The **ESS numerator for the CP arm** is calculated as **4,278***(target outlined in Protocol 3) = **4,278***0.6 = **2,567**.

- **Widths of the 95% confidence intervals:**
  - Based on the variance formula from Hayes and Moulton (2009), the 95% CI widths are calculated as follows:

$$precision = 1.96\sqrt{\frac{p(1-p)DEFF}{n}}$$

$$DEFF = 1 + (m-1)k^2\frac{p}{(1-p)}$$

$$m = \frac{n}{c}$$

precision is the width of the 95% CI
p is the proportion for which the 95% CI is calculated
DEFF is the design effect
n is the total sample size from which *p* is calculated (i.e., denominator)
k is the between-cluster coefficient of variation of the true proportion
c is the number of clusters (i.e., number of communities)

Table 5 summarizes the inputs for calculating the widths of the 95% CIs shown in Table 4.

Table 5: Inputs for Calculating CI Widths Surrounding HTC, ART (including VL suppression), and MC Coverage Proportions at Baseline and Study End

		Baseline Household Survey (BHS)	End of Study Survey (ESS)	
	Input	All Study Communities	EC Arm	CP Arm
HTC Coverage	p	0.37	0.48	0.90
	n	12,000	5,931	5,931
	c	30	4	4
	k	**0.1**	**0.1**	**0.1**
ART Coverage	p	0.73	0.795	0.93
	n	3,000	2,971	2,971
	c	30	15	15
	k	**0.1**	**0.1**	**0.1**
VL Suppression	p	0.70	0.762	0.95
	n	3,000	2,971	2,971
	c	30	15	15
	k	**0.1**	**0.1**	**0.1**
MC Coverage	p	0.35	0.50	0.60
	n	4,320	4,278	4,278
	c	30	15	15
	k	**0.1**	**0.1**	**0.1**

5.2.2. Power to detect difference in coverages of HTC, ART, VL suppression, and MC at the end of study

The coverage of ART, VL suppression, and MC will be assessed in eligible participants in 100% of 3 pairs of randomly selected communities and 20% of the remaining 12 pairs of communities. The coverages of ART, VL suppression, and MC in the 12 pairs of communities will be adjusted based on the difference in coverage observed between the 20% cohort and the remaining 80%. HTC coverage over the past 12 months and 24 months will be assessed in eligible participants in 80% of the 3 pairs of communities, outside of the 20% cohort. This is because the 20% cohort in both arms is expected to receive 100% HTC coverage by study design.

The projected coverage of HTC, ART, VL suppression, and MC at the end of year 3 is 52%, 80%, 76% and 50%, respectively, in the Enhanced Care arm, and 90%, 93%, 95% and 60%, respectively, in the Combination Prevention arm (see Table 4 above). Assuming a coefficient of variation of 0.1 for HTC, ART, VL suppression, and MC coverage, the power in detecting the aforementioned differences in coverage in the ESS is 99%, 96%, 99% and 98% for HTC, ART, VL suppression, and MC, respectively (see Table 6).

Table 6. Estimated Power of Detecting Differences in Coverages in HTC, ART, VL Suppression, and MC between Enhanced Care (EC) and Combination Prevention (CP) Arms in the ESS

		HTC	ART	VL	MC
Projected Coverage at ESS	EC	48%	80%	76%	50%
	CP	90%	93%	95%	60%
Coefficient of Variation		0.1	0.1	0.1	0.1
Power		99%	96%	99%	98%

5.2.3. Sampling Strategy for End of Study Survey (ESS)

To ensure on-time completion of the ESS, which will be conducted by one team in each arm, ESS communities must be selected with consideration given to the sequence of the rollout of BHS, population size and region. To select 3 feasible pairs of ESS communities, we considered the anticipated rollout sequence and selected one pair of communities from each of the three regions represented in the study. This resulted in the inclusion of the 5th, 7th and 13th pairs as the initial list of ESS communities. No further adjustment of selection is expected to be necessary, but in the event of unanticipated changes in logistical or operational activities, the selection procedure described above may be repeated prior to any outreach for the ESS.

To sample 100% of households in 3 pairs of Combination Prevention and Enhanced Care communities at the end of the study, all households in the selected Combination Prevention and Enhanced Care communities will be approached for: (1) GPS mapping, (2) enumeration of number and demographics of persons living in households, and (3) offering of HTC services. The ESS will therefore include residents of the 80% of households not participating in the BHS/HIC in the 3 selected pairs of communities, and ESS data will be complemented by data from the residents of the 20% of households participating in the BHS/HIC in all 30 communities at T2.

Note that it is likely we will reach all 20% of households with BHS/HIC enrollees during the ESS, because HIC households will have previously agreed to study procedures. However, it is less certain what proportion of the remaining 80% of households will agree to the survey.

Possible reasons for not reaching non-HIC households in Enhanced Care and Combination Prevention communities at study end include:

- **Refusal**: We expect rates of refusal for participation in the ESS to be <15%, based on experience from northern Botswana and Mochudi district.
- **Not at home during the household visit**: This may apply particularly to men. If the survey team determines that certain household members are best reached for the survey after hours during the week or on weekends, arrangements will be made to visit the households during these convenient times.

Investigators will need to assess whether participation rates in the household survey introduces any potential selection bias.

5.3. Expected Numbers of Study Participants

Study participants will be recruited to participate in and provide data for different aspects of the study per the Table below.

Table 7. Expected Numbers of Participants in Study Components

Study component	Targeted subjects	Expected # of Participants	Data Collection
Household structure	Household representative in 20% households	7,244	• Household Composition Form (updated at each visit) • Baseline Household Survey at T0
BHS/HIC	Age-eligible members of 20% households, a subset of whom will be enrolled into the HIC (HIV-negative and meeting additional HIC eligibility critieria)	12,000 total (9,000 in HIC)	• Baseline Resident Survey at first visit • Annual Resident Survey at subsequent visits
Qualitative Research on Barriers to and facilitators of LTC and ART initiation among BHS participants	HIV-infected BHS participants BHS Research Assistants and Nurse Prescribers	60 BHS participants in FGDs 40 BHS participants in IDIs 20 Research Assistants and Nurse Prescribers inFGDs	• Focus Group Discussions • In-depth semi-structured interview guide
Blood draw for research purposes	HIV-infected individuals (or seroconverters in BHS/HIC	3,270 (part of 12,000 above)	Same as for BHS
	HIV-infected BHS residents who decline survey participation but are willing to give a blood sample	150	Research blood draw form
	All HIV-infected persons in CP community clinics	11,934	Research blood draw form
End of study survey	All eligible residents in 3 pairs of selected communities	14,262 total: ~2,400 from the 20% households at T2 and ~11,862 from the remaining 80% of households.	End of Study Resident Survey (for participants enrolling for the first time at ESS) Final Resident Survey (modified annual follow-up survey) for previous enrollees from 20% households)
Supplemental Economic Assessment	Individuals experiencing OIs seeking out- or inpatient care	Up to 150 (depending on how many respondents from 20% households meet this criteria)	Economic Assessment
	Individuals with low CD4 without OIs seeking out- or inpatient care	Up to 300 (depending on how many respondents from 20% households meet this criteria)	Economic Assessment
Point-of-care Viral Load Pilot Study	ART-naïve HIV-infected individuals in BHS	Up to 400	

5.4. Planned Interim Analysis of Data

The BCPP is divided into three interlocking protocols (two active protocols, after Protocol 2 was terminated in 2015), and the investigators expect that DSMB review will require information regarding interim study results and study conduct from all three. The combination prevention package under study includes provision of ART for all HIV-infected adults regardless of WHO Stage or CD4 count. Hence, the reports will provide information needed to evaluate the adverse and favorable effects of this intervention on individually treated patients as well as on the villages in which they reside. The study will require at least 5 reviews, DSMB 1-5, proposed to take place at baseline and at the end of years 1, 2, 3, and 4. DSMB 3 will evaluate intervention rollout and safety. To demonstrate that the study has reasonable power to answer the study questions given information up to the time of each review, investigators will provide the DSMB with assessments of the adequacy of coverage of proposed interventions; estimated conditional power given available incidence information will be provided at DSMB 4. Investigators (other than statisticians preparing DSMB reports) will remain blinded to HIV incidence data collected from the HIC, during the conduct of the study. Details of the DSMB review plan for each protocol are provided in that protocol, and the full detailed plan is provided in Appendix C4.

5.4.1. Proposed Schedule of DSMB Reviews

The incidence cohorts (HIC) will be enrolled in all 30 communities over a period of approximately two years. The enrollment of incidence cohorts for one matched pair (2 study communities) will begin simultaneously. The number of working days required to complete each community in the pair will vary depending on the sizes of the communities.The BHS/HIC households will be revisited approximately 12 months, and 30 months after they were enrolled. Figure A in Appendix C4 illustrates the planned schedule of enrollment and follow-up.

As demonstrated in Figure A in Appendix C4, there are 5 proposed DSMB reviews. The proposed components of each DSMB review are summarized in Table A in Appendix C4. Harvard investigators will provide at least 5 DSMB reports related to activities covered in this *Evaluation Protocol*: one for protocol review before enrollment, one each at DSMB 2 and 3 with reports of baseline parameters; one at DSMB 4 (after the end of calendar year 3) with assessment of conditional power based on available incidence information and relevant coverage information in intervention implementation, and one at study end at DSMB 5. At DSMB 5, we will provide reports on all data collected during the BHS, HIC follow-up, and ESS.

6. STUDY PROCEDURES

In this section we describe detailed study procedures for each of the following study segments: (1) the Baseline Household Survey (BHS), (2) the HIV Incidence Cohort (HIC), (3) Research Blood Draws, and (4) the End of Study Survey (ESS).

6.1. Baseline Household Survey (BHS)

Overview: During the BHS conducted in the 20% households, voluntary HIV testing and counseling (HTC) will be conducted, and the HIC will be identified and enrolled (note: the HIC is an open cohort, so additional eligible residents of the same 20% households may enroll in the HIC in subsequent years). HIV-positive participants will be referred to the local clinic for evaluation and if eligible, related interventions in Combination Prevention communities. All eligible consenting residents of the 20% households will be asked to take part in a survey (and, if HIV-infected, a research blood draw). All of these participating residents will be asked to respond to the same core survey (Resident Survey, Baseline).

Data collected under previous versions of the protocol with the original follow-up schedule will still be used, i.e. data and samples from participants whose communities had a T2 and/or T3 visit under the former follow-up schedule will be included in analyses. This protocol outlines the visit schedule and procedures to be implemented following the roll-out of version 5.0 but does not invalidate approved protocol procedures conducted prior to the roll-out of version 5.0.

Starting with version 5.0 of the protocol, during the subsequent visit to the household at T1, newly-identified eligible residents will be asked to enroll in the BHS/AHS, including the HIC (or, if HIV-infected, for a research blood draw); and all eligible residents will be asked to complete a survey focused on assessing uptake of key components of the combination prevention package (and changes in risk behavior), regardless of whether or not they are participating in the HIC or are referred for interventions.

6.1.1. Selection and Recruitment of Households for the BHS

- **Random Selection**: As described in Section 5, for each study community, a complete list of households will be obtained from google area maps (and potentially complemented by data from other sources). From this sample frame, 25% of households will be selected using simple random sampling. In villages with population under 6,000, the required 20% households will be randomly selected from the 25% sample and approached first for participation in the study. In villages with population over 6,000, 333 households will be randomly selected from the 25% sample and approached first for participation in the study. The remaining 5% of selected households will be considered back-up households in case some of the households in the primary 20% sample refuse to participate. Households in the back-up list will be ordered (e.g. 1-49) to indicate the order in which they should be approached to meet sample size requirements.

- **Recruitment**: Trained community liaison officers (CLOs) may do reconnaissance visits in advance of the survey team, for example, to identify households in the sample that are not actually inhabited or are not residential dwellings. These staff may also try to find out the ideal time for the survey team to visit, and the approximate size of the household so that the survey team may plan to accommodate potential participants. Research staff may perform these functions in the absence of CLOs. Each of the households in the 20% primary sample will be approached by the survey team for participation in the study. If some of these selected 20% households elect not to participate (e.g., all residents are repeatedly absent or refuse consent), the additional households in the full 25% sample will be approached, in the order specified, until either the pre-specified sample (described above) has been achieved or this 25% sample list is exhausted. All eligible residents will be asked to take part in activities related to the BHS (using the BHS/HIC Consent). Those who are confirmed HIV-uninfected and meet additional HIC eligibility criteria will be enrolled in the HIC (see Section 6.2). ART-naive HIV-infected residents will be referred to local clinics for WHO staging and in CP communities immediate ART initiation (see *Intervention Protocol).* See Figure 3.

  Training: Prior to approaching the community, survey staff will have been trained in study procedures, including the informed consent process, HTC counseling, questionnaires and sample collection/processing/transport methods. Training will occur following structured procedures, to ensure that all study staff are trained in the same procedures. Survey staff will be arranged into teams of sufficient size and number, such that the study schedule can be met.

6.1.2. Household Visit Procedures for the BHS

Interview of Household Representative: Trained study staff will approach the household representative of a selected household, which will preferably be the household head when available, but may be any other eligible household representative. Verbal consent will be requested using an IRB-approved script (Household Script). If consent is granted, general household information will be obtained from the household representative using the Household Composition Form. Information to be recorded includes:

- Household GPS
- Household address
- Household Size
- Household Composition. For each household member:
  - Age
  - First name or initials
  - Gender
  - Presence at the time of the household visit
  - Estimated number of nights spent per month outside the community over the past 12 months (which will be confirmed by individual household members)
  - Relationship to the head of the household or representative
- Common household socioeconomic indicators (Household Survey, Baseline)

Classification of Households that Cannot be Enumerated

All visited households selected for BHS need a final disposition status so that a decision can be made about whether any household is eligible for replacement. In cases where the residency status of a plot remains uncertain after a total of three visits, a research staff member will approach either a neighbor or a representative at the Village Development Committee (VDC) for assistance. This representative, who must be 18 years of age or older, will be asked whether a plot/household is regularly occupied by potentially eligible residents (Household Residency Status Assessment) to assist in making a final determination of the plots/households' residency status.

Interview with Age-Eligible Household Members (16-64):

HIV counseling and testing (also, see HIV Testing Procedures below): to avoid creating an impediment to HIV testing that would not exist for residents accessing routine health services (by requiring written informed consent for, and participation in, study procedures), HTC (and POC CD4 as needed) will be offered to household members who decline participation in the research survey. Whereas only BHS study team members in Enhanced Care communities will offer HTC testing, HTC may be offered either by the BHS study team or by the HTC campaign team in Combination Prevention communities; the BHS study team will also refer individuals who decline testing to local clinics where HIV testing is available. Note: HIV and CD4 results (and accompanying information that is routinely collected as part of public HTC programs) will be collected on the HTC Intake form (see *Intervention Protocol*) in Combination Prevention communities and made available to the linkage to care manager for monitoring and evaluation of the intervention package (and as will be done as part of the HTC campaigns in the Combination Prevention communities). The linkage to care manager and HTC campaign team will be aware of the location of households selected for BHS/HIC in Combination Prevention communities, and will be informed of numbers of those who were not reached during the household survey due to refusal or absence. Residents declining HIV testing can still take part in the BHS, if they agree.

- ***Written informed consent*** for participation in the BHS and annual follow-up visits will be obtained from each willing individual aged ≥18 who meets BHS eligibility criteria (BHS/HIC Consent). For residents aged 16 or 17 who meet BHS eligibility criteria, parental permission followed by minor assent will be obtained. Up to two revisits will be scheduled for absent individuals. The BHS consent will request permission for the study questionnaire, blood draws for research purposes for persons testing HIV-positive (including blood draw for HIV genotype, HIV incidence assay, and viral load) at the current visit or in cases of future seroconversion to HIV-positive status, optional storage of leftover blood for future testing, CD4 and viral load testing at T1 and/or T2 for HIV-positive individuals who are ART-naïve at enrollment or who seroconvert and are ART-naive after enrolling, and annual household questionnaire completion (at T1, T2--and T3 [for any communities completing T3 prior to the roll-out of version 5.0]) by all participants. With the elimination of the T3 visit in version 5.0 of this protocol, a Dear Participant letter will be used to inform enrolled participants that T2 will be their final visit. HIV-infected participants who decline participation in the BHS survey can still provide informed consent to donate blood for HIV VL and genotyping/incidence assay, using a separate consent form (Research Blood Draw Consent).

- ***Questionnaire:***
  Each consenting eligible household member will be interviewed/complete a questionnaire in a private environment within the household or at an agreed upon space. A questionnaire will be completed at the BHS enrollment visit (Resident Survey, Baseline); in addition, a shorter follow-up questionnaire (Resident Survey, Annual) will be administered to BHS participants, including HIC members, at subsequent visits. During the final T2 visit, a Final Resident Survey will be administered to BHS/HIC participants. The first questionnaire (Resident Survey, Baseline) will include questions on basic demographic variables, current health status, medical history, prior coverage of HTC, current coverage of MC, prior or current pregnancies, sexual risk behavior, basic screening for active tuberculosis by symptoms-questions, and (among HIV-infected persons), prior/current use of antiretrovirals for treatment or PMTCT. All residents will complete a core questionnaire. When possible, residents may be randomly assigned to complete different short supplemental questionnaires in order to keep the duration of the interview as short as possible while still collecting key variables. Skip patterns will ensure that appropriate questions are asked depending on the self-reported HIV status, gender, and prior participation of the consenting adult. Subsequent questionnaires will include variables related to CP package intervention uptake, general health (e.g. history of serious illnesses, and in a sample of willing respondents, blood pressure and hip/waist circumference), and sexual risk behavior. Data will be collected on a mobile electronic device (e.g. notebook); these devices will use authentication/password protection and encryption to render information electronically unusable, unreadable, or indecipherable to unauthorized individuals. Mobile devices and the staff operating them will not be equipped with the encryption keys to decrypt select sensitive data fields. Secure and lockable rooms will be used to store mobile devices when not in use.

- **HIV testing procedures, BHS:**
  HIV testing will be offered to all adults who do not have documentation of HIV-positive status. Documentation of HIV-positive status is participant self-report of positive HIV status, in combination with: (1) an official MOH HIV test result card indicating HIV-positive status, (2) documentation of prior ART or PMTCT ARV prescription, or (3) documentation of pre-ART care including either a CD4 count, a WHO Stage, or some specific mention of medical care for the HIV-infected patient by a clinical care provider. When in doubt, HIV status will be considered "undocumented".

  a) **Adults with no Documentation of HIV-Positive Status, BHS:**
     These individuals include those reporting being HIV-negative at their last test, individuals reporting never having HIV tested, or individuals reporting being HIV-infected but not having documentation of HIV-positive status as described above.
     - Pre- and post-test counseling will be provided by trained staff, as per Botswana Government guidelines and standards.
     - Capillary blood samples will be collected by fingerprick using a sterile disposable lancet. Blood will be aliquoted into a microtube. This blood will be used for both the rapid HIV test and subsequently (among HIV-positive individuals), for point-of-care CD4 cell count (POC CD4). If a capillary blood sample cannot be obtained a venous blood sample (4-5 ml) may be drawn instead for the rapid HIV test and subsequently (among those testing HIV-positive), used for POC CD4.

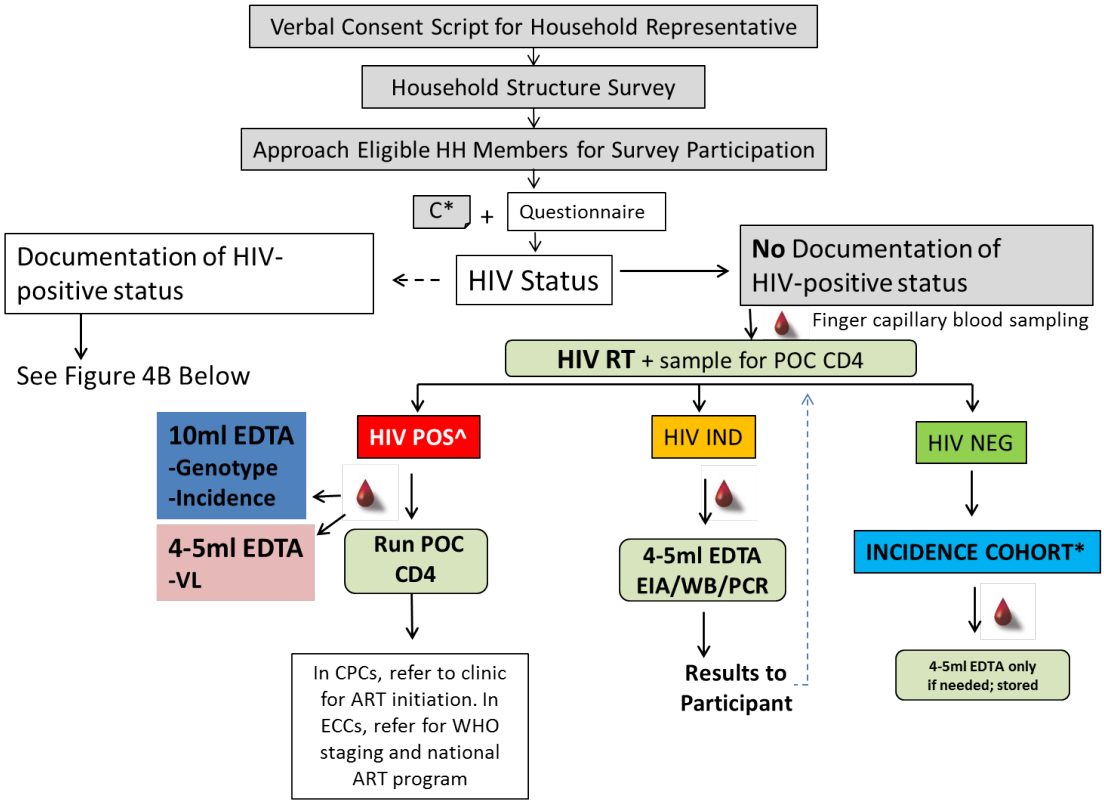
- The capillary (or venous) blood sample will first be tested using parallel HIV rapid testing algorithms (see Section 8.4). HIV rapid test results will be released to the participant at the time of the visit.
- Discordant HIV test results will be resolved by collection of a venous blood sample (~ 4-5 ml) that will be sent to the laboratory for HIV enzyme immune-assay (EIA) testing as per the National Algorithm. Contact details of individuals with sero-discordant results will be obtained and when HIV EIA results are available, the study personnel make arrangements provide the results (either in the person's home or in the clinic, depending on the participant's preference) and perform the appropriate post-test counseling and remaining study procedures.
- Subsequent management of the individual will depend on the HIV test result:

  - ***HIV Negative Participants****:* HIV negative household members meeting eligibility criteria for the **HIV Incidence Cohort (HIC)** will be enrolled in the HIC (see Sections 4.4 and 6.2)**.** HIV-negative participants meeting criteria for the BHS but not the HIC will participate only in the BHS and subsequent surveys.

  - ***HIV-Positive Participants:*** Residual blood remaining after HIV testing will be used to perform a POC CD4 test using the PIMA CD4 machine at the household. If insufficient blood remains from the sample taken for the HIV rapid test, venous blood should be drawn (4-5 ml) along with the tubes for VL and genotyping/incidence assay (see below) to avoid a separate prick. POC CD4 results will be provided to the participant immediately following testing. Participants will have venous blood drawn for viral load/HIV incidence assay/genotyping/storage. The blood for viral load, HIV genotyping, and HIV incidence assay testing will be collected in 2 tubes: 4-5 ml for viral load and up to 10 ml for HIV genotype/incidence assay/storage. The viral load results will be returned to HIV care clinics. The pre-ART viral load is not standard of care and is not required to determine ART eligibility in Botswana. Therefore, while it will be shared with clinics, it will be of limited clinical use. The incidence assay and viral phylogenetic tests are being performed primarily for research purposes, and will not be run in real time; therefore results will not be returned to the patient. Efforts will be made to avail results indicating transmitted drug resistance to participants. However, this testing will be done retrospectively and therefore may be of limited clinical utility.

    All HIV-infected persons who are not yet in HIV care and/or on ART will be referred for immediate HIV care and treatment at the study community clinic according to national guidelines in all Enhanced Care and Combination Prevention communities. If the patient is treatment-naive, the patient will also be referred to the study community clinic in Combination Prevention communities for immediate ART initiation. Access to universal ART is described in the separate *Intervention Protocol* that is part of the BCPP.

Figure 4A: BHS Procedures for Individuals with no Documentation of HIV-Positive Status



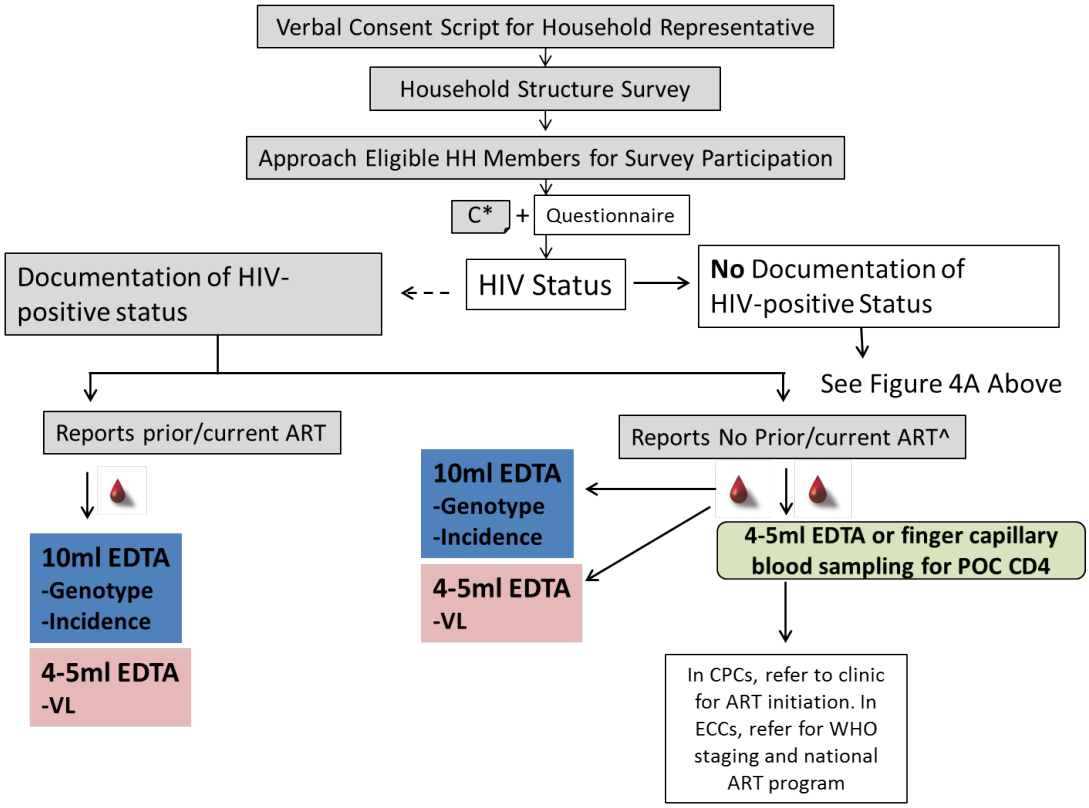
Abbreviations: CPC, Combination Prevention Community; ECC, Enhanced Care Community; HH, Household; HTC, HIV Testing and Counseling; RT, Rapid Test; POC, Point-of-Care; C, consent procedures; IND, indeterminate; VL, viral load; EIA, enzyme immuno-assay; WB, Western Blot.
*Eligible persons only (See section 4.4).
^Leftover sample from either the fingerprick or 4-5mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).

b) **Adults who Have Documentation of HIV-Positive Status, BHS:**
These individuals include residents who report being HIV-positive and have documentation supporting this (MOH HIV test card, documentation of prior ART, CD4, WHO staging, or specific mention of HIV-related clinical care). Subsequent management of the participant will depend on whether the participant reports taking ART:

- **All BHS participants documented to be HIV-infected (regardless of ART status)**: A venous blood draw for a 4-5ml EDTA tube for HIV viral load, and a 10ml EDTA tube for genotyping/HIV incidence assay will be requested.
- **Residents documented to be HIV-infected but ART-naive:** these residents will have a POC CD4 test run (and will be provided with the result). Generally, the sample for this POC CD4 in ART-naive residents documented to be HIV-infected will be drawn along with the venous blood for VL and genotyping/incidence assay, to avoid a separate prick. For residents declining the research blood draw, sample for POC CD4 can be obtained by finger stick for capillary blood. All HIV-infected persons who are not yet in HIV care and/or on ART will be referred for immediate HIV care and treatment at the study community clinic according to national guidelines in all Enhanced Care and Combination Prevention communities. Patients will also be

referred to the study community clinic in Combination Prevention communities, for universal ART, which is described in the separate *Intervention Protocol* that is part of the BCPP.

Figure 4B: BHS Study Procedures for Individuals with Documentation of HIV-Positive Status



Abbreviations: CPC, Combination Prevention Community; ECC, Enhanced Care Community; HH, Household; HTC, HIV Testing and Counseling; POC CD4, Point-of-Care CD4; C, consent procedures; VL, viral load.
*Consent administered to eligible household members only (see section 4.4).
^Leftover sample from either the fingerprick or 4-5mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).

Note about Test Results During BHS: *Individuals participating in HTC will be offered the result of their rapid HIV test with post-test counseling. During the BHS, POC CD4 results will be given to the participants in all communities. The viral load results will be returned to HIV care clinics. The pre-ART viral load is not standard of care and is not required to determine ART eligibility in Botswana. Therefore, while it will be shared with clinics, it will be of limited clinical use. The incidence assay and viral phylogenetic tests are being performed primarily for research purposes; therefore results will not be returned to the patient. Genotyping will not be run in real time and therefore will be of limited utility for patient care. However, if results indicate transmitted drug resistance, efforts will be made to avail this information to the participant upon completion of testing.*

- **Compensation:**
  BHS participants will receive 20 pula in cell phone airtime as compensation for their time to participate in the survey.

6.1.3. Household Visit Procedures for Non-HIC Members at T1:

These procedures pertain to consenting residents (both HIV-uninfected and HIV-infected) in 20% households who are not participating in the HIC. This group will include residents who took part in the original BHS, as well as residents newly-identified at T1 who are not enrolled in the HIC.

For residents who already consented to and took part in the BHS (and are not in the HIC), a short survey will be administered at T1, primarily to assess combination prevention intervention uptake (and to assess risk behavior/risk compensation and general health). HTC will be offered to those without documentation of HIV-positive status. Point-of-care CD4 and viral load testing will be offered at T1 to HIV-positive individuals who were ART-naïve upon enrollment into BHS, or who seroconvert and are ART-naive after enrolling in BHS. POC CD4 results from T1 will be shared with participants in both arms, and viral load results will be shared with clinics (or with patients, if a validated point-of-care viral load test is introduced). A research blood draw (for HIV genotype/incidence assay testing/optional storage) will be obtained from anyone newly identified with HIV infection. Procedures for the final visit to BHS/HIC households at T2 are described with those for the end of study survey (ESS). See Figure 5B.

For residents who did not take part in the BHS at T0 (and are not in the HIC), the same procedures as outlined for the BHS will be offered at T1.

6.1.4. Data Collection and Handling:

- **Questionnaires**: For consenting BHS participants, all questionnaire data, HIV test results, POC CD4 test results, and information regarding collection of sample for genotyping/incidence assay/storage will be collected electronically using notebook computers. These devices will use authentication/password protection and encryption to render information electronically unusable, unreadable, or indecipherable to unauthorized individuals. Mobile devices and the staff operating them will not be equipped with the encryption keys to decrypt select sensitive data fields. Secure and lockable rooms will be used to store mobile devices when not in use.

- **Consent Forms:** Investigators will document consent procedures on the notebook application. One copy of the signed consent form will be kept by investigators while a second copy will be kept by the participant.

- **Blood samples** will be labeled with barcoded stickers and transported to the study clinic or lab, as appropriate.

6.1.5. Criteria for Study Discontinuation

Participants will be discontinued from longitudinal follow-up in the BHS (or HIC) if the participant (or guardian if < age 18) requests to withdraw.

6.1.6. Data Collection on Cause-of-Death for Deceased Study Participants

If study staff learn that an enrolled study participant has died during the course of the study, basic information related to the cause of death will be recorded. A deceased study participant is no longer a human subject per the definition at 45CFR46.102(f); a human subject is a *living* individual. Data for the Cause-of-Death Form may be collected from official sources, such as medical records, a death certificate, or from health care providers.

6.2. HIV Incidence Cohort (HIC)

Methods for household selection and identification of eligible persons for HIC enrollment are described above (Section 6.1).

6.2.1. Enrollment Procedures, HIC

- **Consent**: As described in Figure 4A above, residents of the 20% households will be asked for consent for survey participation (BHS/HIC Consent). This consent will request permission for the baseline study questionnaire, subsequent HIV testing and questionnaire at follow-up visits, future blood draw for research purposes if seroconversion to HIV-positive status occurs (blood for HIV viral load/genotype/incidence assay/optional storage), POC CD4 and viral load at T1 and/or T2 (among a subset, as described above) if seroconversion occurs, and storage of blood remaining from the HIV rapid test microtube finger prick draw. The latter will be used for confirmation of negative HIV status at enrollment, if future HIV seroconversion occurs. Consenting residents who are determined to meet HIC eligibility criteria will be enrolled in the HIC.
- **Venous blood draw**: In the event that insufficient blood remains from the sample drawn for the HIV rapid test, HIC enrollees will have a venous blood sample drawn for storage at the research lab. If the participant sero-converts during HIC follow-up the baseline sample will be available to confirm that the participant was truly HIV-negative at enrollment. The storage of this baseline blood sample is indicated for this protocol and covered in the main body of the BHS/HIC consent form. This blood draw will also mitigate the potential for inadvertent HIV status disclosure that could occur if venous blood draws only pertain to HIV-infected individuals.
- **Questionnaire and Forms**: All consenting residents will be administered an enrollment questionnaire (Resident Survey, Baseline), and contact information (mobile phone numbers, alternate addresses, and addresses of a friend/family member) will be obtained to facilitate tracing (Locator Form). At T1, a shorter questionnaire (Resident Survey, Annual) including questions related to CP package intervention uptake, general health, and sexual risk behavior will be administered. Those who decline enrollment in the HIC will be asked to take part in the BHS only. Residents declining both the HIC and BHS will be thanked for their time and asked, via an IRB-approved verbal consent script (Refusal Script) to provide basic information about their reason for declining via the Refusal Survey Form. This will assist in characterizing the impact of refusal on study outcomes.

6.2.2. HIC Follow-up

- **HIC Follow-up Schedule**: Subsequent household surveys of the HIC will be conducted at approximately 12 and 30 months after enrollment (T1 and T2, respectively). Procedures for the final visit at T2 visit are described with those for the end of study survey (ESS). Therefore, in this section, we restrict description of procedures to those that apply to T1. HIC activities conducted during the T1 follow-up household visits include: (1) updating the household structure to assess who might be newly eligible for BHS/HIC enrollment, (2) repeat HIV testing and questionnaire administration to HIC enrollees, and (3) screening non-HIC enrollees for HIC eligibility. These three activities are described in detail below. Wherever possible, via telephone or other communication channels, study teams will arrange a suitable time and venue to meet, and perform study procedures, for HIC household members. When necessary, study interviews may be conducted by phone.

- **Activity #1, HIC Follow-up: Update Household Structure**: Study staff will approach the household head or representative of the selected household. A previously enrolled household member can serve as the household representative, where appropriate. Updated household information will be obtained. Information to be recorded includes:
  - Household GPS (confirmed – not re-measured)
  - Household address updated if there have been changes
  - Household Composition (updated)

- **Activity #2, HIC Follow-up: Questionnaire, Repeat HIV Testing, and Other Lab Draws in Existing HIC Enrollees (Figure 4C):**
  HIC participants will have previously consented to the questionnaires and blood draws described here (as part of the BHS/HIC consent process). Previous enrollees of the HIC will be engaged in a follow-up questionnaire (Resident Survey, Annual), administered using devices such as notebook computers; these devices will use authentication/password protection and encryption. In addition, HIC enrollees will be re-tested for HIV using point of care rapid tests (two different rapid tests in parallel) performed on blood collected from a fingerstick in a microtube. Even if the HIC enrollee indicates that he/she has tested HIV-positive and enrolled in care since the earlier negative HIV test, repeat HTC will be offered to existing HIC enrollees to confirm HIV positive status one time.

  If the existing HIC enrollee tests HIV-negative, the enrollee will be offered routine post-test counseling, and, in Combination Prevention communities only, those services for HIV-negative adults that constitute part of the CP package (TB screening and referral of uncircumcised HIV-negative males for MC).

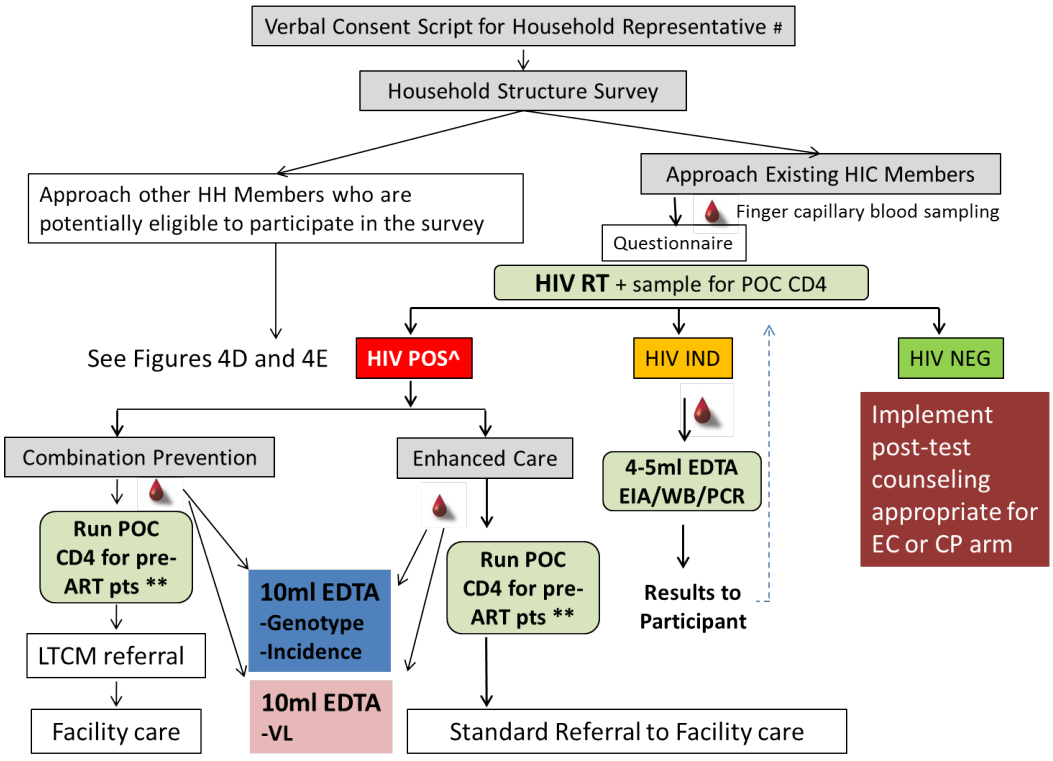
  If the participant tests HIV-positive, (or if the participant reports knowing HIV-positive status and refuses repeat HTC), a venous blood draw will be performed for HIV viral load (10mL EDTA tube) and for genotyping/incidence assay/optional storage (10mL EDTA tube) among HIC participants in both the Enhanced Care and Combination Prevention communities.

  Procedures will differ slightly for HIC members in the Enhanced Care vs. Combination Prevention communities with regard to linkage-to-care services:

- **<u>Procedures For HIC Members Testing Positive for HIV During Follow-up</u>:**
  - **Participants Reporting no Previous ART**: a POC CD4 test will be run on residual sample from the HIV test, and the CD4 result shared with the participant. If the residual sample is insufficient a venous blood draw (4-5 ml) may be performed. Linkage-to-care services will be provided in Combination Prevention Communities. Participants in Enhanced Care communities will receive the Enhanced Care referral to care and treatment services described in the BCPP *Intervention Protocol*.
  - **Participants Reporting Prior ART Initiation:** Management will be the same as for pre-ART participants, except no POC CD4 will be run.
  - **HIC Members who test positive for HIV at T1:** These participants will still have a follow-up visit at T2. Participants who seroconvert between T0 and T1, and report no previous ART at T1 will have point-of-care CD4 and viral load testing again at T2. See Figure 5B.

***Note about Test Results During Follow-up:** Individuals participating in HTC during T1 and T2 will be offered the result of their rapid HIV test with post-test counseling. During follow-up, POC CD4 results will be given to the participant during the study visit. Viral load results will be returned to HIV care clinics. The pre-ART viral load is not standard of care and is not required to determine ART eligibility in Botswana. The incidence assay and viral phylogenetic tests are being performed primarily for research purposes; therefore results will not be returned to the patient. Genotyping will not be run in real time and therefore will be of limited utility for patient care. However, if results indicate transmitted drug resistance, efforts will be made to avail this information to the participant upon completion of testing.*

Figure 4C: Follow-up Procedures for HIC Enrollees During T1

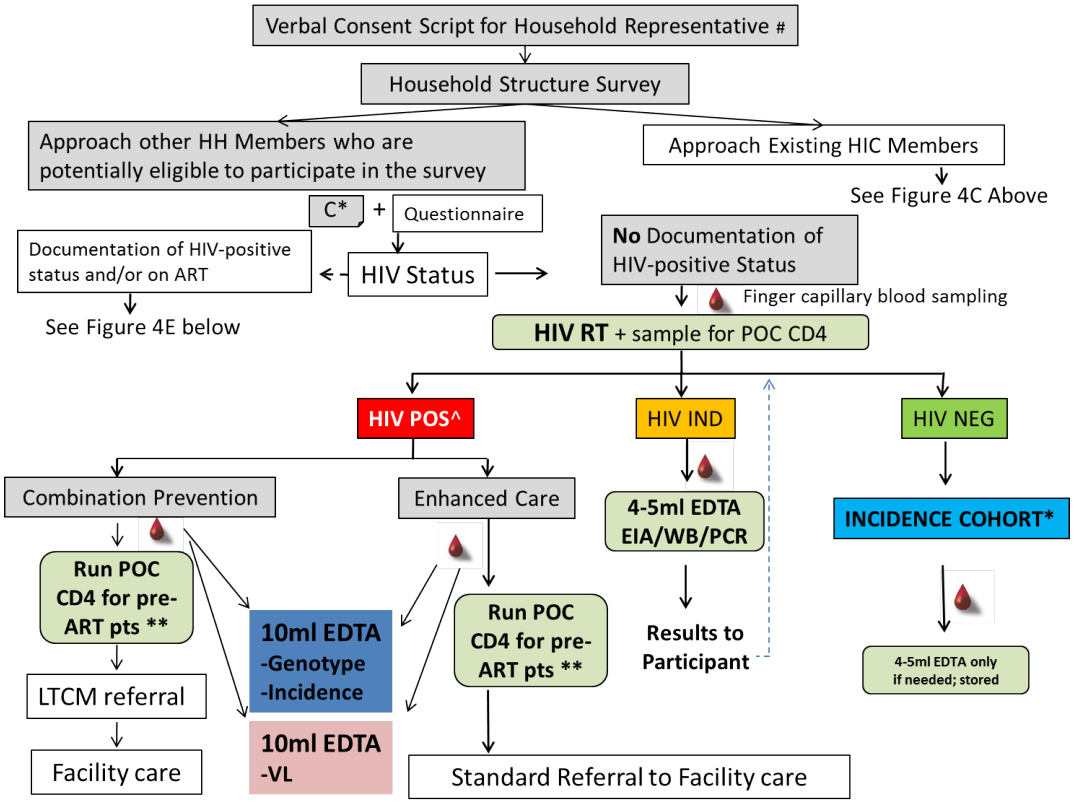


Abbreviations: CP, Combination Prevention; EC, Enhanced Care; HH, Household; RT, Rapid Test; POC CD4, Point-of-Care CD4; IND, indeterminate; VL, viral load; EIA, enzyme immuno-assay; WB, Western Blot.
#Verbal consent script need not be re-administered if the respondent is the same person at follow-up visits, or an enrolled survey participant.
^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).
**At T1, previous HIC enrollees who have seroconverted since T0 will have POC CD4 run if they are ART-naïve. At T2, these HIC enrollees who had POC CD4 at T1, i.e. those who were ART-naïve at T1, will have another POC CD4 at T2, even if they have started ART between the T1 and T2 visits (see Figure 5B).

- **<u>Activity #3, HIC Follow-up</u>: Screening of non-HIC Members for HIC Eligibility:** Adults identified as possibly being newly eligible for the HIC, based on the interview with the household head/representative, will be approached at T1. For example, residents in the 20% of households originally selected who were too young to enroll in the HIC during the BHS may turn 16 years of age during follow-up, and now be eligible for the HIC, or a new resident may be present. These adults will be invited to participate in the research, asked to sign the BHS/HIC Consent, respond to the questionnaire, and then be offered HTC services, similar to procedures conducted during the BHS. Similar to the BHS, adults in the HIC household will either have documentation of HIV status, or not. Subsequent management depends on whether the adult already has documentation of HIV-positive status or not:

    - **No documentation of HIV-positive Status:** These procedures are summarized in Figure 4D below. Potential new participants will be asked consent to join the research study. Eligible HIV-negative adults will be offered the opportunity to enroll in the HIC (refusers and those who are not eligible for the HIC will be asked to participate in the same procedures summarized in the BHS (see Section 6.1). If consent is granted, the same questionnaire administered during the BHS (Resident Survey, Baseline) will be administered to these newly identified individuals. HTC will be offered. HIV-infected adults will be asked for research blood draws, and further managed according to whether the patient is in a Combination Prevention or Enhanced Care community (Figure 4D).

Figure 4D: Procedures for Individuals in 20% Households who are Newly Identified During T1 and who have No Documentation of HIV-Positive Status



Abbreviations: HH, Household; HTC, HIV Testing and Counseling; RT, Rapid Test; POC CD4, Point-of-Care CD4; C, consent procedures; IND, indeterminate; VL, viral load; EIA, enzyme immuno-assay; WB, Western Blot.

#Verbal consent script need not be re-administered if the respondent is the same person at follow-up visits, or an enrolled survey participant.

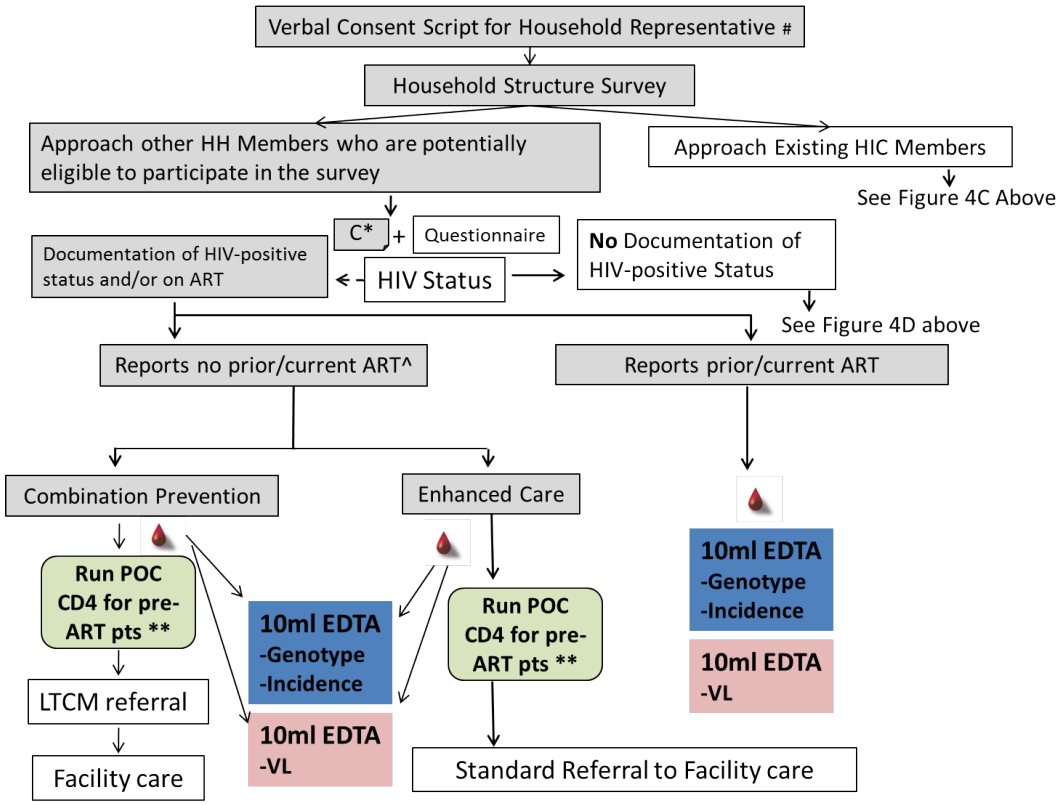
*Eligible persons only (See section 4.4).

^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).

**HIV-positive pre-ART participants enrolled at T1 will have POC CD4 and viral load tests at T1 (as shown above) and again at T2 (see section 6.1.3 for follow-up procedures of non-HIC enrollees).

Documented HIV-Positive Status: Newly identified household members with a documented HIV-positive status will be interviewed and asked for research blood draws and further managed according to whether the individual is in an Combination Prevention or Enhanced Care community (Figure 4E). These participants will still have a follow-up visit at T2. HIV-positive participants who enroll at T1, and report no previous ART will have point-of-care CD4 and viral load testing again at T2 (see section 6.1.3 and Figure 5B).

Figure 4E: Procedures for Individuals in 20% Households who are Newly Identified During T1 and who HAVE Documentation of HIV-Positive Status



Abbreviations: HH, Household; HTC, HIV Testing and Counseling; POC CD4, Point-of-Care CD4; C, consent procedures; VL, viral load.
#Verbal consent script need not be re-administered if the respondent is the same person at follow-up visits, or an enrolled survey participant.
*Consent administered to eligible household members only (see section 4.4).
^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).
**HIV-positive pre-ART participants enrolled at T1 will have POC CD4 and viral load tests at T1 (as shown above) and again at T2 (see section 6.1.3 for follow-up procedures of non-HIC enrollees and Figure 5B).

- HIV Incidence Cohort Retention Procedures: Three attempts to make appointments for follow-up visits are made by phone for each study participant before the survey team returns to enrolled households. Once the survey resumes in the community, three attempts, either household visits or calls, will be performed in an effort to reach each enrolled member and complete follow-up study procedures.

- Data Collection: Questionnaires to be administed to HIC enrollees will be approved by IRBs prior to use in the field. All data will be collected electronically on devices such as notebook computers that are encrypted and password protected. Investigators will store signed paper consent forms and will also indicate electronically whether participant consent has been obtained. Paper copies of informed consent forms will be provided to the HIC enrollees. Blood samples may have bar codes linked to study IDs that are applied at the point of sample collection.

- Compensation: Each HIC participant will receive 20 Pula (about $3) worth of air time as compensation for time dedicated to the study at each scheduled follow-up study home visit.

6.3. Focus Group Discussions and Individual In-Depth Interviews related to barriers to and facilitators of linkage to care and ART initiation among a subset of BHS participants

In order to understand locally-relevant barriers to and facilitators of linkage to care and ART initiation, including initiation of "expanded" ART outside of local guidelines, we will conduct focus group discussions (FGDs) and individual in-depth interviews (IDIs) with a subset of HIV-infected BHS participants. The goal is to improve/optimize approaches to linkage to care and ART initiation in the context of the overall trial and ultimately in Botswana.

1) Recruitment

Recruitment will occur at or after T1, because at that time we will be assessing whether HIV-infected persons referred for care and ART at T0 actually linked/started ART.

We will invite HIV-infected BHS participants 18 years of age or older who, within their own community: 1) have not linked to care since initiation of the BHS, 2) have linked to care and were found to be eligible for treatment but did not start ART, or 3) linked to care, started and continued ART. We will convene up to two focus groups for each one of the three categories to learn about barriers to linkage and ART initiation as well as attitudes and approaches that can facilitate linkage to care and ART adherence. We aim to include in the FGDs a subset of individuals who were eligible to start ART by current local guidelines and a subset who were eligible by expanded ART guidelines. These FGDs will be semi-structured and done using an FGD guide. We will also conduct semi-structured IDIs focusing on the three groups described above.

We will convene up to 6 FGDs among BHS participants from CPCs that will each include between 8 and 10 participants. Participants will be purposefully selected to participate in one of the three categories of focus groups based on demographic and laboratory values (data collected in BHS). The BHS/HIC consent form states that participants may be invited to participate in additional aspects of the research. Using the BHS database and the eligibility criteria (see section 4.4.6) a Research Assistant will contact potential participants and ask to schedule a dedicated home visit to discuss participation in the FGD or IDI. Potential participants will be given a consent form and, if selected for a FGD, a confidentiality agreement to review. If a participant agrees to participate in a FGD, he/she will be informed of the date and time of the scheduled event. IDIs will be scheduled with participants based on their and staff member availability. Written informed consent will be obtained at the time the participant arrives for the scheduled FGD or IDI, and before any study procedures take place.

The BHS field team and the MOH nurse prescribers may have vital insight into the dynamics surrounding linkage to care and initiation of ART. Therefore, we will invite up to 20 members of these two groups to participate in separate FGDs. These FGDs will be semi-structured and done using an FGD guide. Findings from these FGDs will be used to finalize the FGD guides for HIV-infected BHS participants.

In general, different individuals will participate in FGDs and IDIs. We will continue to invite participants for IDIs until thematic saturation is achieved. We plan to conduct up to 40 IDIs. As

above, we will aim to include participants who met the eligibility criteria for expanded treatment (to investigate their understanding of expanded treatment, and motivators/barriers to starting ART) as well as those who were eligible by current local guidelines. Findings from the FGDs will be used to finalize the IDI guides.

2) Consent
All individuals taking part in the qualitative research will sign a separate written consent form (ART Start Focus Group Consent for BHS, ART Start Focus Group Consent for Staff, or ART Start Interview Consent). Those participating in FGDs will also sign a confidentiality agreement.

3) Data Collection and Handling
FGDs and IDIs will be conducted in a private setting by a trained research assistant in the local language (usually Setswana). Focus group discussions and interviews will be audio recorded, transcribed and translated; all study records will be collected using study identification numbers (without personal identifiers), and will be kept locked and accessible only to relevant study staff. Overall results (without identifiers) will be shared with the study team and implementing partners, in an effort to enhance linkage to care and ART initiation.

4) Compensation
Participants in FGDs or IDIs will receive 30 Pula to compensate them for their time and up to 30 pula in transport reimbursement to attend the FGD or IDI.

6.4. Research Blood Draw Procedures in Combination Prevention Communities, Outside of the BHS

Procedures for the HTC campaigns are detailed in the *Intervention Protocol* and described in brief below. Under that protocol, community residents identified as HIV-infected in Combination Prevention communities will be referred by counselors and linked by Linkage-to-Care Managers (LTCMs) to HIV care and treatment clinics. These patients will be asked to consent to research participation including a blood draw for viral load/HIV genotype/incidence assay testing/optional storage. In addition, all other HIV-infected residents of Combination Prevention communities (including those already in HIV care; those eligible for ART by Government guidelines; and those already on ART) will be invited at the clinic to participate in the research blood draw.

1) Recruitment

- **Research blood draw eligibility:** All HIV-infected adult residents of Combination Prevention communities are eligible for the research blood draw. All eligible adults presenting to the HIV care and treatment clinics will be offered the opportunity to participate in the research, including those already in care (even those on ART). During the study (prior to the ESS), HIV-infected Combination Prevention community residents will be asked/consented to give a sample for genotyping/phylogenetics/transmitted drug resistance. Each consenting participant will be asked a few basic questions (e.g. age, gender, ART status) that will permit interpretation of results even if the participant did not take part in other surveys/questionnaires.

- **Sample size:** Based on the average population size of the study communities and 25% HIV prevalence, 11,608 HIV-infected individuals are estimated to reside in the 15 Combination

Prevention communities at the time the study starts. These individuals will be invited at local clinics to participate in the research blood draw. The expected number of additional participants in the clinic-based research blood draws following the first year is based on incident cases and therefore expected to be much lower (approximately 326).

- **Consent:** Eligible research participants will be consented for a venous blood draw at the time of their visit to the clinic that will include a 10mL tube for HIV viral load and a 10ml EDTA tube for HIV genotyping/incidence assay/storage (Research Blood Draw Consent). The participant may elect to receive the program blood draws (for viral loads only) without the research blood draw (blood draw for HIV genotyping/incidence assay/storage), if he/she chooses.

  Blood draw: Trained staff will draw the relevant blood samples from consenting patients. Patients will receive their viral load result. Results of phylogenetic analysis and other research assays will not be returned to participants or their providers. Genotyping will not be run in real time and therefore will be of limited utility for patient care. However, if results indicate transmitted drug resistance, efforts will be made to avail this information to the participant upon completion of testing.

2) Follow-up and Retention: Program follow-up and retention of HIV-infected patients is described in the *Intervention Protocol*.

3) Data Collection and Handling
   All research data will be collected electronically on devices such as notebook computers that are encrypted and password protected. Investigators will: (1) securely store original written signed consent pages, (2) record participant granting of consent electronically, and (3) ensure paper copies of informed consent forms are provided to the research participants. Blood samples may have barcodes linked to study IDs applied at the point of sample collection.

4) Compensation
   There will be no compensation for participating in the HTC campaign nor for participating in blood/data collection for this research component of the study.

6.5. The End of Study Survey (ESS) and Final T2 Study Visit for Previously Enrolled 20% Households

1) ESS Recruitment

- **ESS Community Selection:** 3 pairs of communities (6 communities in total) will be selected to take part in the ESS. Section 5.2.3 details the process for selecting the ESS communities. As noted previously, residents of the 20% households in all 30 communities will undergo final follow-up evaluations at this T2 time as well.

- **ESS Household Selection**: 100% of households in the 3 pairs of Enhanced Care and Combination Prevention communities (6 total communities) will be targeted for the ESS--the 20% households already participating in the repeat surveys (T2 survey), and the 80% of households not yet approached for participation in the evaluation research. Google maps (and other publicly available sources) will be used to identify eligible households.

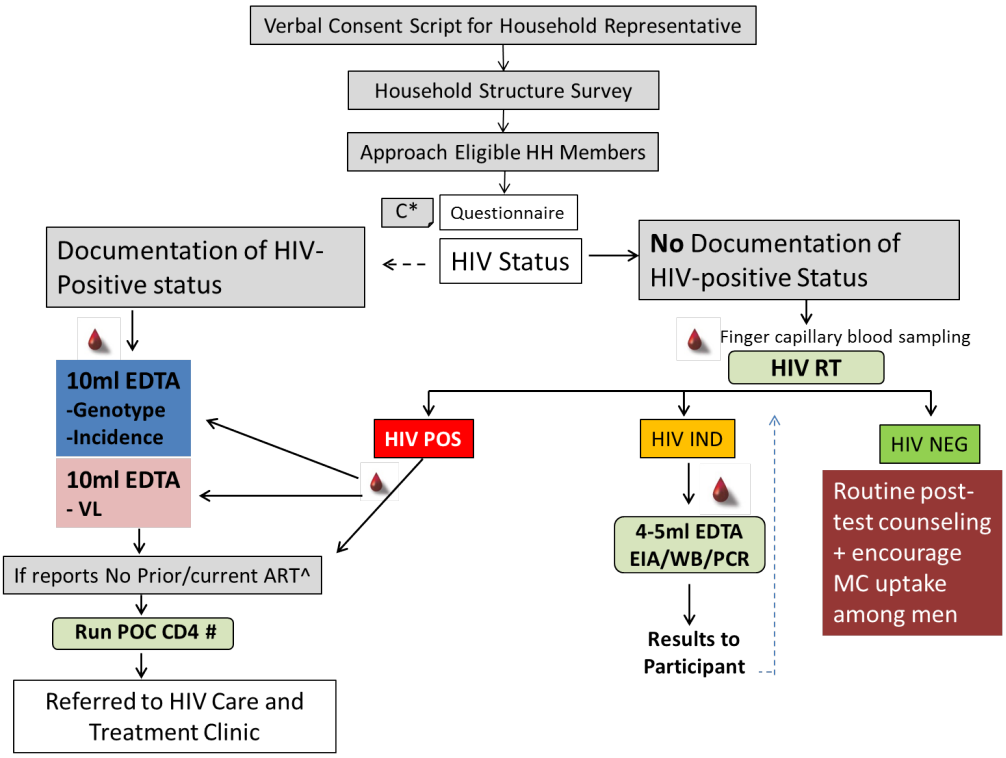
- **Recruitment**: Each of the study community households will be approached for participation in the study. If the heads of eligible households, or a suitable household representative, are not available at the time of the first home visit, up to two re-visits to the household will be scheduled.

Schedule: The ESS will be scheduled to coincide with the T2 visit to 20% households.

2) The remaining ESS study activities (training; household visit procedures; data collection and handling) will be very similar to those in the BHS, with a few exceptions:
    - Participants will be consenting to a one-time questionnaire and blood draw, and not longitudinal follow-up in any cohort.
    - Participants will be referred to local treatment facilities for HIV care and treatment (if HIV-infected) and male circumcision (if HIV-uninfected), and no linkage to care interventions will be implemented.
    - Non-citizens will be eligible to participate anonymously in this one-time survey.

    In sum, the ESS will consist of HTC (if not documented to be HIV-infected); informed consent of eligible residents of the 80% households in the participating 6 communities (or newly-eligible residents of the 20% households in the participating 6 communities), followed by completion of a questionnaire that focuses on combination prevention intervention uptake; and (among consenting HIV-infected residents) collection of a sample for HIV viral load/genotyping/incidence assay/optional storage (see Figure 5A).

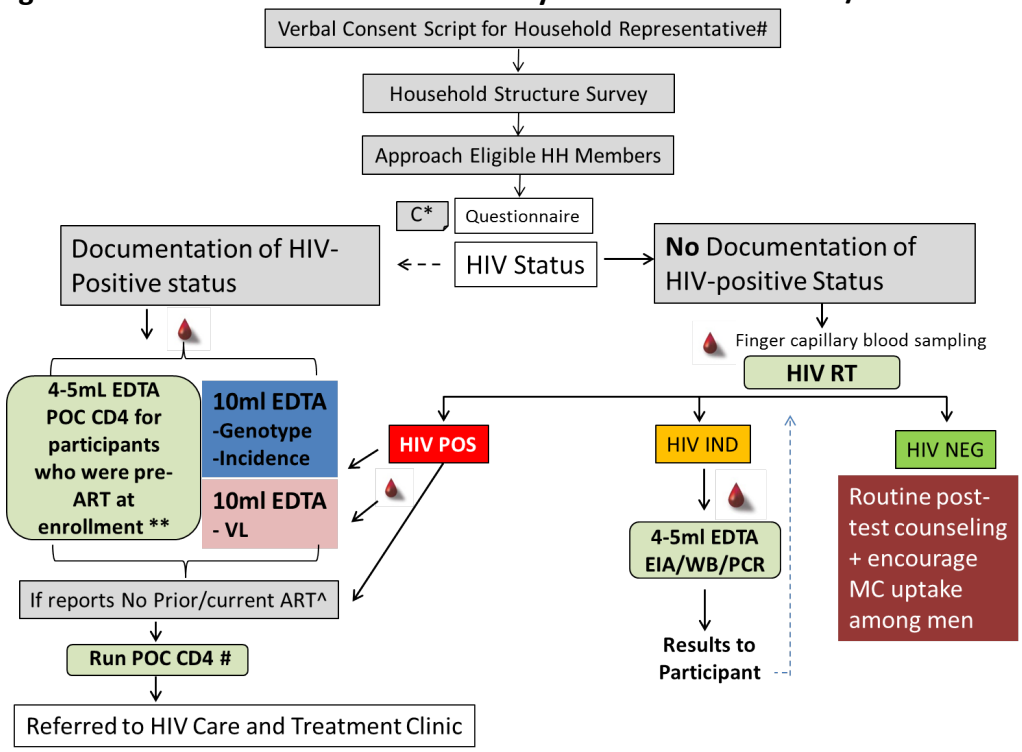
Figure 5A. Procedures for the End of Study Survey in 80% Households in 6 Communities



Abbreviations: HH, Household; C, consent procedures; RT, rapid test; VL, viral load; MC male circumcision; IND, indeterminate; EIA, enzyme immuno-assay; WB, Western Blot.
*Consent administered to eligible household members only (see section 4.4).
^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).
Sample for the POC CD4 in previously documented HIV-positive participants may be drawn along with the venous blood for VL, to avoid a separate finger prick. Otherwise, capillary blood sample leftover from rapid HIV test may be used.

3) T2 visit: procedures during the T2 visit among 20% household residents will be very similar to those in the ESS participants (with the exception that participants who previously consented to taking part in the BHS or HIC will not need to provide additional consent for study procedures). Note that eligible consenting residents of 20% households in the 30 communities who did not previously participate in the BHS/AHS will complete the ESS consent and survey/procedures.

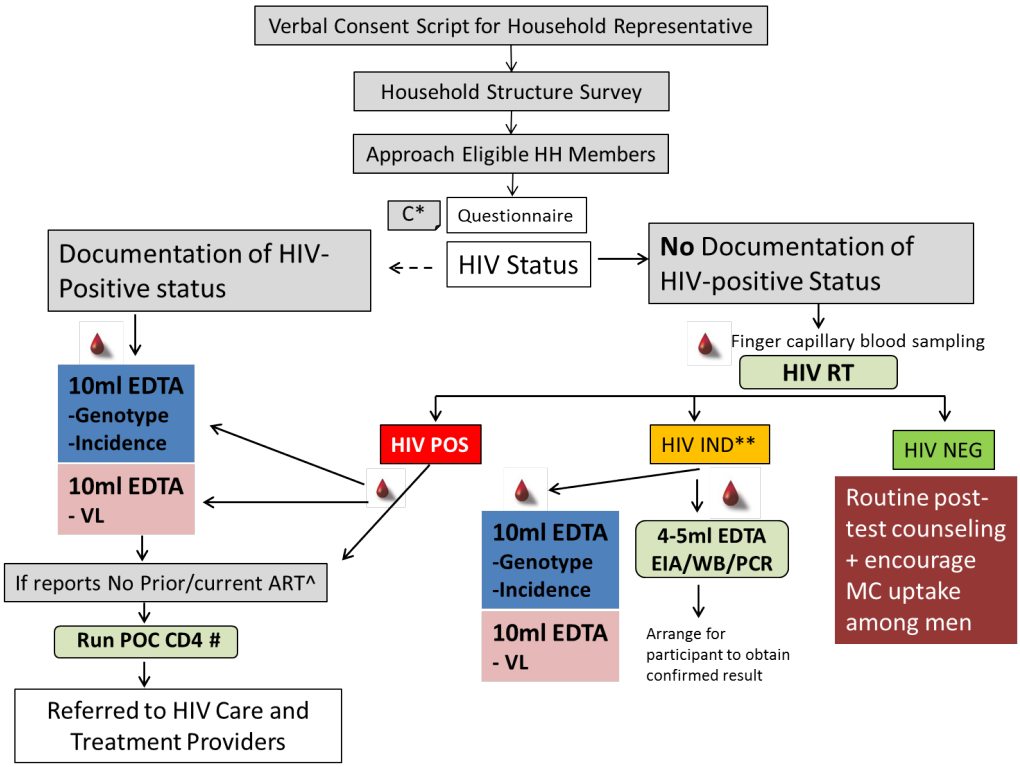
Figure 5B. Procedures for the Final Survey Visit at T2 in 20% BHS/HIC Households



Abbreviations: HH, Household; C, consent procedures; RT, rapid test; VL, viral load; MC male circumcision; IND, indeterminate; EIA, enzyme immuno-assay; WB, Western Blot.
#Verbal consent script need not be re-administered if the respondent is the same person at follow-up visits, or an enrolled survey participant.
*Consent required from newly identified residents who did not enroll and consent previously at BHS or T1. Consent administered to eligible household members only (see section 4.4.4).
^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).
**HIV-positive participants who were ART-naïve upon enrollment at a prior visit will have POC CD4 repeated at T2, even if they have started ART since enrollment. Generally, the sample for the POC CD4 at T2 in previously documented HIV-positive participants will be drawn along with the venous blood for VL, to avoid a separate finger prick.

Newly identified HIV-positive participants (i.e. no prior study documentation of HIV-positive status) who are ART-naïve will have POC CD4 run on leftover capillary blood. If a capillary blood sample cannot be obtained, a venous blood sample (4-5 ml) may be drawn instead.

Figure 5C. Procedures for the End of Study Survey for Non-Citizen/Spouse of Citizen Respondents



Abbreviations: HH, Household; C, consent procedures; RT, rapid test; VL, viral load; MC male circumcision; IND, indeterminate; EIA, enzyme immuno-assay; WB, Western Blot.
*Waiver of requirement for written documentation of informed consent. Administer Consent Script/Written Statement (see section 11.2).
^Leftover sample from either the fingerprick or 10mL sample will be run in a small subset of HIV+ participants not currently on ART to validate POC VL (run in parallel with standard VL) (see Section 8.5).
Sample for the POC CD4 in previously documented HIV-positive participants may be drawn along with the venous blood for VL, to avoid a separate finger prick. Otherwise, capillary blood sample leftover from rapid HIV test may be used.
**Sample will be collected for confirmatory testing and study staff will arrange for participant to obtain confirmed result. Real-time result will be made available to participant at mobile lab/clinic. Research Blood Draw and Viral Load will be collected simultaneously in the event the confirmed result is positive.Similar to other study participants, anonymous non-citizen participants may obtain their viral load results. Non-citizen participants will be provided with a receipt matching their coded specimen. They may present the receipt to the mobile lab/clinic to obtain their result.

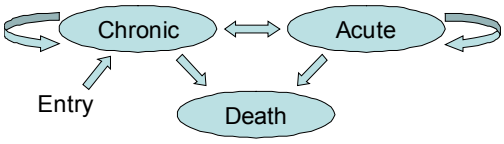
7. COST EFFECTIVENESS ANALYSIS

In this section we describe the Cost-Effectiveness of Preventing AIDS Complications International (CEPAC-I) model, the novel data collection, and the analysis plan to determine the cost per HIV infection averted under the intervention and related secondary objectives of estimating cost per quality adjusted life year (QALY) saved under the observed and ideal conditions.

7.1 CEPAC Model Structure

The CEPAC model is comprised of a CEPAC-I state-transition model of HIV disease progression linked to a dynamic CEPAC model of HIV transmissions.

1) CEPAC-I state transition model
   CEPAC-I, as a *state-transition* model characterizes disease progression as a sequence of monthly transitions from one "health state" to another as depicted in the following diagram: chronic, acute, and death (Kreke, Schaefer et al. 2004; Walensky, Wood et al. 2008).



   Health states are defined by current and setpoint HIV RNA, current and nadir CD4 count, and history of opportunistic infections (OIs). OIs are divided into groups based on country-specific data and are classified as severe or mild. From each health state in each month, individuals face competing mortality risks from non-HIV causes. The efficacy of sequential ART regimens is characterized by their impact on HIV RNA and CD4 count (Cole, Hernan et al. 2003; Losina, Yazdanpanah et al. 2007). A random number generator and set of estimated probabilities determine the clinical pathway that a patient follows until death. The entry characteristics of simulated patients (age, sex, CD4 count, HIV RNA) are randomly drawn from a distribution based on data from the setting of interest. A tally of clinical events, time spent in each health state, and cost associated with each health state is maintained. Upon the patient's death, summary statistics for that person are recorded. Results from large numbers of individual simulations are averaged to develop stable population estimates. Simulation sizes of 1 million patients are typically sufficient for treatment strategies.

2) CEPAC HIV dynamic model
   We have completed a pilot transmission model incorporated into CEPAC-I (Walensky, Ross et al. 2012) and are developing a full agent-based, dynamic transmission model of the HIV epidemic linked to the CEPAC-I Model. This transmission model will be incorporated into this analysis to estimate the additional potential population-wide impact of HIV treatment interventions on incidence. While several dynamic transmission models have been published (Alam, Meyer et al. 2009; Leclerc, Matthews et al. 2009; Bendavid, Brandeau et al. 2010; Enns and Brandeau 2011; Gray, Ghaus et al. 2011; Hallett, Baeten et al. 2011; Phillips, Pillay et al. 2011; Prabhu, Farnham et al. 2011; Schneider, Puthanakit et al. 2011; Bershteyn, Klein et al. 2012; Ventelou, Arrighi et al. 2012), the CEPAC Dynamic Model combines behavioral, demographic, and biological parameters to project HIV transmission from and to individuals on a population level and links to the clinical detail of CEPAC-I (natural history, treatment outcomes, and costs of care). Outcomes include incidence and prevalence over user-defined time periods. Using a fitting procedure based on Bayesian melding methods (Poole and Raftery 2000; Alkema, Raftery et al. 2008; Johnson, Alkema et al. 2010), we have fit the model to HIV prevalence in South Africa

from 1990-2002 prior to the introduction of ART, and are currently fitting it to 2003-2012 in the presence of ART (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2010).

7.2 Data Inputs

1) Natural History

We have derived CD4-stratified model input parameters from Côte d'Ivoire and South Africa to describe the natural history of HIV in sub-Saharan Africa (Holmes, Wood et al. 2006; Losina, Anglaret et al. 2006; Wang, Kumarasamy et al. 2006). Natural history data from South Africa are primarily from the Cape Town AIDS Cohort (CTAC), a cohort of 2,088 patients followed by Wood et al. (Holmes, Wood et al. 2006). These model parameters will be compared with observed CD4 trajectories and clinical outcomes in the Baseline Household Survey to confirm that the model accurately describes the experience of the Botswana population.

2) ART Efficacy

Efficacy data for 1st-line NNRTI- or PI-based regimens (66-88% viral suppression at 48 weeks) are from both clinical trials and observational cohorts in Africa with similar efficacy for 2nd-line regimens (Coetzee, Hildebrand et al. 2004; Nachega, Stein et al. 2004; DART Virology Group and Trial Team 2006; Charalambous, Innes et al. 2007; Barth, van der Meer et al. 2008; Boulle, Van Cutsem et al. 2008; Delfraissy, Flandre et al. 2008; Hammond and Harry 2008; Losina, Chang et al. 2008; Wouters, Van Damme et al. 2008; Bierman, van Agtmael et al. 2009; Gandhi, Moll et al. 2009; Fox, Ive et al. 2010). CD4 increase on 1st-line ART averages 113-236 cells/µl at 48 weeks, with the sharpest increase occuring in the first two months, followed by a more gradual increase (Charalambous, Innes et al. 2007; Seyler, Adje-Toure et al. 2007; Barth, van der Meer et al. 2008; Hammond and Harry 2008; Pujades-Rodriguez, O'Brien et al. 2008; Wouters, Van Damme et al. 2008; Gandhi, Moll et al. 2009; Fox, Ive et al. 2010). While data from clinical trials are more complete in terms of virologic and CD4 response, as well as loss to follow up (LTFU) and toxicity, they generally provide higher estimates of suppression than data from large community cohorts (DART Virology Group and Trial Team 2006; Delfraissy, Flandre et al. 2008; Hammond and Harry 2008). We will therefore use data from trials to represent primarily best-case scenarios in terms of efficacy and LTFU (Cohen, Chen et al. 2011; Anglaret and Eholié 2012; Grinsztejn and Mugyenyi 2012) and cohorts (Cornell, Schomaker et al. 2012; Wandeler, Keiser et al. 2012) for more likely generalizable programmatic outcomes. Efficacy and effectiveness data will be updated as studies emerge with data from newer regimens (1st-, 2nd-, and 3rd- line) as treatment provision, regimens, and experience matures (Pujades-Rodriguez, O'Brien et al. 2008; Fox, Ive et al. 2010; Saravanan, Madhavan et al. 2012). We will assess both trial- and cohort-reported efficacies to assess how differences may change policy conclusions.

3) Costs

We will fully update the cost parameters for direct medical care in the CEPAC-I model. We will carry out a facility-based costing study in Botswana using an 'ingredients-based' methodology (UNAIDS 2011) that leverages longitudinal utilization records over a retrospective two-year period in several of the community clinics. This will allow us to quantify utilization of services for routine care, OI treatment, treatment of HIV-related co-morbidities, and end-of-life care (see Table 8).

We will use facility-based staffing records, inventories, procurement and accounting records, and other site-specific data to generate a unit cost per outpatient visit, per inpatient day, per procedure, per laboratory test, and per monthly medication cost. To determine the total unit cost for each service we will define each facility's service centers and measure their output, for example an inpatient day. We will then define and measure the input required by the service center in terms of capital resources (buildings, equipment and other one-off costs such as consultant fees) and recurrent resources (staff salaries, consumables and monthly costs such as electricity bills). Once we have determined the total input we will calculate the monetary value of each item to establish the current financial price and to calculate the unit cost of each service center output. Through this UNAIDS-recommended (UNAIDS 2011) costing methodology we will reflect the most current utilization patterns and unit costs, and will tailor HIV care recommendations to Botswana cost structures. All costs will be updated to constant year dollars and discounted at 3%/year (Gold, Siegel et al. 1996).

Table 8. Resource Utilization and Cost Data Needs

Parameter	Stratified by
Start-up costs	Intervention, Site, Monthly for first 3 months
Scale-up costs	Incremental cost of increasing coverage levels by 10% increments; Quarterly
Personnel costs	FTEs for all hired staff per implemented intervention* Salaries for each staff member
Medications	1st and 2nd line ART Cotrimoxazole OI treatment
Patient visits to healthcare setting	Average frequency on ART Average frequency by CD4 stratum (off ART) Urgent care visits (by ART status) Hospitalizations and length of stay (by ART status and with diagnosis) Death costs Costs by visit type †
Laboratory‡	HIV test, Confirmatory HIV test, CD4 monitoring, HIV-1 RNA, Screening chemistries, Screening CBCs

* Including but not limited to: home visits, nurses, care and treatment, doctors for ART, surgical sMC
† Urgent care, per diem hospital stay, routine visit
‡ Includes anticipated frequencies and associated costs

The measurements of healthcare utilization and personal health care costs are included in the questionnaire to be used at T1 (Resident Survey, Annual) and during the ESS (Resident Survey, ESS). These supplementary items will be requested from at least 1,500 participants drawn from both Enhanced Care and Combination Prevention communities at T1 and T2.

In order to more accurately estimate changes in health resource utilization during periods of illness and during periods with low CD4 cell count (CD4200 cells/µL), supplementary sampling of individuals in clinics and hospitals serving study communities will be conducted. Up to 150

consenting participants experiencing opportunistic illnesses and 300 without opportunistic illness but with low CD4 counts will be recruited from outpatient and inpatient settings.

Possible participants will be approached in three settings. First, individuals participating in the 20% household surveys with low CD4 count or current opportunistic illness will be identified and selected for completion of these supplemental items. Second, qualifying individuals receiving care in the Combination Prevention community clinics will be identified and referred for possible participation in these questionnaires. Finally, if these settings are inadequate to achieve the target sample size, other patients seeking care in clinics or inpatient facilities serving study communities will be identified through review of care logs and conversations with clinical staff. Participants recruited from the clinics, and those not previously part of a study cohort, will be required to provide written informed consent prior to completion of these questionnaires (Economic Consent). Consenting participants will be interviewed for health resource utilization and quality of life (Economic Assessment).

Health care costs frequently are concentrated at the end of life and these costs may be missed during community surveys and during a focused clinical survey described above. Consequently, we will abstract health resource utilization during the 30 days before death from existing medical records in up to 80 residents of study communities (40 HIV-infected, 40 HIV-uninfected) who die during the course of the study (Death Economic Abstraction). Of these, 20 non-accidental deaths that occur outside of the hospital will be targeted as costs associated with these deaths are anticipated to be different. Study teams will learn of deaths of community residents from the community clinic staff, and from review of inpatient care logs for facilities providing care for study communities. Family members or contacts of the decedent will not be interviewed. No identifiable information will be recorded during record abstraction. This aspect of the research does not involve human subjects per the definition at 45CFR46.102(f) that a human subject is a *living* individual.

Table 9. Individual Healthcare Utilization, Cost, and Quality of Life Assessments

Instruments	Sample Size	Timing	Recruitment
Annual Resident Survey and End of Study Survey. These instruments include EQ5D5L, resource utilization (including HIV-related costs for HIV-infected), lost economic activity	At least 3,000 (at least 1,500 at each time point)	T1 and T2	Participants in BHS/AHS
Economic Assessment: EQ5D5L, resource utilization (including HIV-related costs for HIV-infected), lost economic activity CEA Enrollment checklist for participants outside BHS/ESS	150 with opportunistic illness 300 with low CD4 ≤200 cells/μL without opportunistic illnesses	Throughout study	1) Participants in BHS/ESS 2) Participants in IDCC 3) Other patients treated in facilities caring for residents of study communities
Death Economic Abstraction: Resource utilization	80	Throughout study	Clinics and hospitals caring for residents of study communities

4) Quality of Life

Quality of life implications of HIV treatment in resource-limited settings are becoming more important in the evaluation of innovations that incrementally improve clinical management of HIV than a decade ago, when survival gains were appropriately the overriding concern. To enable the CEPAC-I model to estimate QALYs as an outcome, we will synthesize existing evidence on preference-based measures of QOL (EuroQol Group 1990; World Health Organization Division of Mental Health and Prevention of Substance Abuse 1997; Brazier, Usherwood et al. 1998; Brazier, Roberts et al. 2002; Sullivan, Lawrence et al. 2005) for HIV health states from the literature (Jelsma, Maclean et al. 2005; Louwagie, Bachmann et al. 2007; Zimpel and Fleck 2007; Lara, Wakholi et al. 2008; Robberstad and Olsen 2010; Rajeev, Yuvaraj et al. 2012; Tran, Ohinmaa et al. 2012). The largest study to date of QALY-weights in HIV used the EQ5D instrument in a trial involving 21,000 US patients and has been used in model-based analyses (Simpson, Luo et al. 2004; Pitter, Kahn et al. 2007; Meyer-Rath and Over 2012). To produce estimates that can be adapted to the CEPAC-I model structure, we will adjust the QALY-weights as needed by collecting EQ5D data at T1 and T2 and use new methods for synthesizing evidence on QALYs (Tengs and Lin 2002; Isogai, Rueda et al. 2012) and predicting health-related QOL among HIV-infected persons (Isogai, Rueda et al. 2012).

The EQ5D is included in the questionnaire instrument used at T1 (Resident Survey, Annual) and at T2 to estimate QOL in the study communities. Supplementary sampling of individuals with opportunistic infections and low CD4 will be conducted. The EQ5D will be administered to up to 150 consenting individuals with opportunistic illnesses and 300 individuals with CD4<200 cells/μL receiving care in inpatient and outpatient facilities serving study communities (Economic Assessment), as described above for medical cost estimation.

7.3 Model validation and approach to uncertainty

Medical decisions and guidelines for care are nearly always formulated without perfect information. This is particularly true with rapid advances in knowledge and, thus, justifies both the use of model-based approaches and an inclusive attitude toward the data used in modeling studies. Because hypotheses cannot be tested using traditional measures of statistical significance in microsimulation modeling, we have a particular responsibility to both validate models and to investigate the impact of uncertainty with care and transparency. Here we describe approaches to model validation and sensitivity analysis.

1) Validation

We ascertain internal consistency in the model in several steps: First, we examine the face validity of randomly selected, individual patient "traces" (Figure 5). These detailed views of a patient's month-to-month experience offer a check on the reasonableness of the output and a mechanism for debugging. Second, we check "internal validity," verifying that model output accurately approximates the data used to derive input parameters. This is an area of increasing importance in disease policy modeling. Figure 6 illustrates the validation of model runs using HPTN 052 trial data inputs with trial outcomes (Losina, Yazdanpanah et al. 2007; Walensky, Ross et al. 2012).

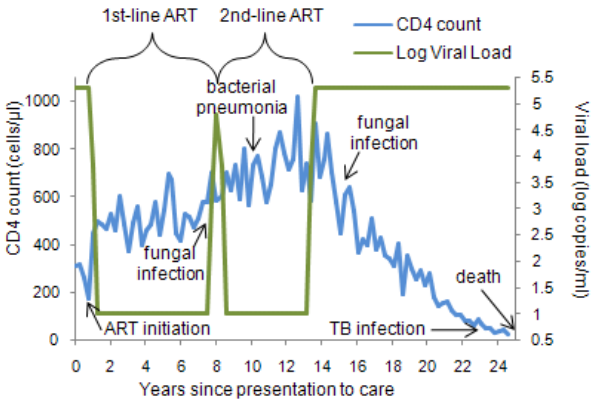


Figure 5: A simulated patient trace from South Africa

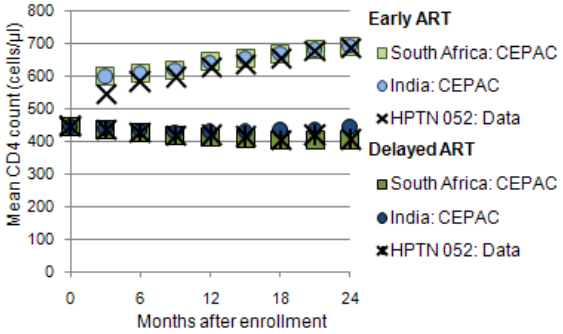


Figure 6: CD4 Trajectory in the CEPAC-I model as compared to the HPTN-052 trial

2) Deterministic sensitivity analysis

The US Panel on Cost-Effectiveness in Health and Medicine and the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) Task Force on Good Modeling Practice both recommend deterministic sensitivity analysis to provide insight about uncertainty where small changes in model input data produce large changes in model results (Weinstein, Siegel et al. 1996; Paltiel 2001; Drummond, Brown et al. 2003; Weinstein, O'Brien et al. 2003; Ades, Claxton et al. 2006; Briggs, Weinstein et al. 2012). We will use deterministic methods (one-way, multi-way, best-case, worst-case, and threshold approaches) to assess how uncertainty in current data or lack of data about treatments still in development may affect policy conclusions, or when application of data from alternative regions or countries yields different results (Gold, Siegel et al. 1996; Paltiel 2001; Uhler, Kumarasamy et al. 2010).

Probabilistic approaches. In contrast to deterministic sensitivity analyses, probabilistic sensitivity analysis (PSA) generates values for multiple inputs simultaneously based on predefined probability distributions for each input. PSA is recommended under guidelines elaborated by the ISPOR-Medical Decision Making Task Force (Society for Medical Decision Making and International Society for Pharmacoeconomics and Outcomes Research ; Briggs, Weinstein et al. 2012). We have developed the capacity to conduct PSA on output from the CEPAC-I model. Parameters of interest will be chosen based on importance to the results (as identified by deterministic sensitivity analyses described above) and also on whether data are available to specify distributions (Ades, Claxton et al. 2006). We will use these analyses to calculate the expected cost-effectiveness of each prevention strategy, the uncertainty surrounding that result, and the number of times each strategy is optimal.

7.4 Cost-Effectiveness Analysis Plan

The CEPAC International model will be used to assess expected outcomes from the proposed combination prevention programs. Table 10 shows the prevention strategies and outcomes of interest that will be examined; the main outcome of interest will be the comparison of the three strategies to each other. An additional “no regular screen, no ART” strategy will be modeled for historical comparison only. In addition to life expectancy, cost, and cost-effectiveness of alternative HIV combination prevention strategies, the impact of these strategies on secondary transmission and HIV incidence will also be examined by estimating the number of secondary cases per infected individual in

each combination prevention scenario. Results will be stratified showing life expectancy for the entire population, life expectancy for only the HIV-infected residents, and the number of new HIV infections averted. Table 8 shows resource utilization and cost data needs.

Table 10. Prevention Strategies and Outcomes of Interest

Prevention strategy	Outcomes of interest
Enhanced Care communities (Current Standard of Care)	Total population QALYs Mortality at 2 years Number of new HIV infections at 2 years Reduction in HIV incidence Per person total direct costs Cost-effectiveness ($/HIV infection averted) Cost-effectiveness ($/YLS*, $/QALY)
Combination Prevention communities (expanded HTC, MC, PMTCT, and universal testing and treatment)	
"Optimized Package" (model-based scenario based on the Combination Prevention arm, but will assess the case where linkage to routine HIV care is ideal, ART is optimized, HIV screening is more frequent, and pre-exposure prophylaxis is administered)	

*year of life saved: YLS

Incremental cost-effectiveness ratios – both dollars per year of life saved ($/YLS or $/QALY), and dollars per HIV infection averted ($/infection averted) – will be used to assess the comparative value of these alternative combination HIV prevention strategies. The incremental cost-effectiveness ratios will be determined by ranking strategies in order of increasing cost. Incremental cost-effectiveness is defined as ΔC/ΔE, where ΔC is the difference in costs between two rank-ordered strategies, and ΔE is the difference in effectiveness, as defined either by life expectancy (when calculating $/YLS or $/QALY) or number of new HIV infections (when calculating $/infection averted). Before assessing the incremental costs and benefits of increasingly expensive strategies, we will eliminate strategies that are less effective and more costly. Analyses will be conducted from the modified societal perspective (not including time and productivity costs), and discounted at 3% per annum. Additional analyses will be done to include time and productivity costs, with data derived from external sources and consistent with other combination prevention trials.

8. LABORATORY METHODS

Detailed laboratory procedures, including procedural guides during actual specimen and data collection and study-specific SOPs are described in the BCPP Manual and outlined in brief below.

8.1. Overview of Laboratory Methods

An overview of the types of laboratory tests, sample types, and the location of the testing is provided below. The study will use the following laboratories and locations:

1. BHHRL: Botswana Harvard HIV Reference Laboratory (Gaborone)
2. NHHRL: MOH regional HIV Reference Lab (Francistown)
3. MOH Local Program Labs (Botswana, multi-sites)

4. Field sites: Households and community venues conducting surveys and HTC campaigns
5. Harvard Chan: Harvard T.H. Chan School of Public Health (Boston)
6. CDC: International Laboratory Branch at Centers for Disease Control and Prevention (Atlanta)

Table 11: BCPP Evaluation Protocol Laboratory Testing Responsibilities:

Test	Location of Testing*
HIV Rapid Tests	Field Sites
CD4	MOH Local Program Lab and Field Sites (POC)
HIV-1 RNA Viral Load	BHHRL/NHHRL
Laboratory-based incidence assays	BHHRL
Viral linkage	Harvard Chan/BHHRL or collaborators
Minor drug-resistant variant genotyping	Harvard Chan/BHHRL or collaborators
QA/QC Procedures	BHHRL and NHHRL
Tandem mass spectrometry/HPLC	Harvard Chan/BHHRL or collaborators
Point of care viral load	Field sites

*****Collaborating institutions may participate in testing activities if requested by the responsible institution listed above.

8.2. Training of Laboratory Personnel

All laboratory personnel will participate in study-specific training prior to study initiation. Training will include an overview of the BCPP study design and study-specific laboratory methodologies in addition to Research Ethics, GCLP (Good Clinical and Laboratory Practice), QA/QC (quality assurance/quality control), and laboratory record keeping procedures. Study staff responsible for providing HIV testing and counseling will complete the required training curriculum recognized by the MOH. Staff will also be trained on the use and completion of all study forms related to these services. Study staff members who will collect blood samples or conduct laboratory analyses will receive training in universal precautions, sample collection, and testing of study samples. Additional refresher trainings will be scheduled during the study to ensure consistency and address training needs as they arise.

8.3. Specimen Collection, Transport, and Processing

Specimens will be collected from participants at study field sites and samples transported to designated laboratories for processing, testing and storage. Testing may also occur at study field sites.

Sample types:

a) *Capillary blood samples:* Finger prick samples will be collected in a microtube using a sterile disposable lancet, by trained staff at study field sites according to the appropriate blood collection SOP and used for the HIV rapid test, POC CD4 test, and (in a subset), POC viral load test.

b) *Venous blood samples:* Samples will be collected by trained phlebotomists according to policies and procedures described in a SOP for blood collection. The number and types of samples will vary by the study group as described in Section 6. Tube sizes have been standardized to streamline collection of specimen in the field and integration into existing laboratory testing sites. Samples will be labeled with participant ID/barcodes and transported using coolers or portable refrigerators to the designated research laboratory, processed within 24 hours, and aliquots at -70^{o}C. In brief, the following specimen sizes will be used:

- 4-5 ml EDTA tube or 10ml EDTA tube: Viral Load
- 10 ml EDTA tube: Genotyping, TDR, incidence assays

In cases in which sample for POC CD4 is obtained from venous blood (rather than lancet fingerstick)--e.g. among ART-naive patients with documented HIV infection in whom a rapid test is not being performed—an additional 4-5 ml EDTA tube will be collected.

Note: consenting pre-ART participants may also have blood drawn for baseline safety labs as part of the "research blood draw" in Combination Prevention communities, to avoid multiple sticks and to help expedite time to treatment initiation. More detailed procedures related to this phlebotomy are described in the *Intervention Protocol*.

c) *Plasma and buffy coats:* Plasma and buffy coats will be collected from whole blood specimens (EDTA tube) by centrifugation (1,500 x g or ~3,000 rpm for 10 min), then aspirate plasma into 1 ml aliquots, followed by aspirate of buffy coat into 1 ml aliquots. Both plasma and buffy coats will be stored frozen (-70°C).

8.4. Laboratory testing for BHS, HIC, and ESS (returned to participant and/or participant file)

a) *HIV Diagnostic Testing:* HIV diagnostic testing will be conducted by the study sites as per National HIV Testing guidelines using Botswana-government–approved parallel rapid tests. The current algorithm includes KHB and Unigold™ HIV rapid test in parallel. If results are discordant, a 4-5 ml blood specimen will be taken at the visit and sent to a reference laboratory for further testing using ELISA or Nucleic Acid testing per BHHRL HIV testing algorithm (e.g. Murex HIV 1.2.0 test, Genetic Systems Biorad HIV-1/2) and/or Western blot and/or DNA/RNA PCR. The results of EIA and/or Western Blot and/or DNA/RNA PCR at the BHHRL lab will supersede the discordant and/or indeterminate results obtained in the field. All HIV tests will be conducted according to the manufacturer's guidelines.

If results of anonymous HIV diagnostic testing in the household are discordant, the confirmatory HIV testing will be performed at the mobile lab in real-time. The Cepheid Xpert POC HIV-1 Qual will be used according to the manufacturer's instruction. Xpert HIV-1 Qual provides a total nucleic acid based test for viral RNA and proviral DNA in one integrated cartridge by using 100 ul of whole blood. If the result of Xpert HIV-1 Qual test is negative, the person is considered to be HIV-negative. If result of Xpert HIV-1 Qual test is positive, the person is considered to be HIV-positive.

b) *POC CD4*: Capillary blood samples (obtained from the lancet fingerprick blood draw also used for the HIV rapid test) will be tested using the Pima™ CD4 with the Pima analyzer (Alere, Inc.) described previously (Mtapuri-Zinyowera, Chideme et al. 2010; Jani, Sitoe et al. 2011) to obtain an absolute count of CD4 cells.

c) *HIV-1 Viral Load:* Testing will be conducted by NHHRL at Francistown and BHHRL at Gaborone using licensed assays. HIV-1 RNA VL in plasma will be performed by Roche TaqMan Assay using the Roche COBAS AmpliPrep/COBAS TaqMan version 2 HIV-1 Test or Abbott m2000sp/m2000rt. The BioMeriuex platforms are also available in the reference or national laboratories and may be used for viral load testing. All results will be made available to be keyed or electronically transfered to the protocol database after appropriate user validation.

Several POC VL devices are currently undergoing field performance characteristic evaluations [ALERE HIV NAT POC system (Alere, Inc.), Cepheid Xpert POC VL]. The potential future use of the POC VL tests through an amendment to the BCPP *Evaluation Protocol* may be considered depending on successful evaluation of the POC VL device. POC VL using best available devices will be conducted on a subset of treatment-naïve individuals and may also be conducted on individuals receiving ART.

d) *Transmitted Drug Resistance:* Efforts will be made to return results indicating transmitted drug resistance to participants upon completion of testing. Note that these results will only be available retrospectively (e.g. about 1 year or later after sampling), and may therefore be of limited clinical utility.

8.5. Research laboratory testing (results not returned to participant)

a) *Viral Genotyping*

Plasma and buffy coats will be the primary sample source for viral genotyping. The algorithm of viral genotyping based on the V1C5 region of HIV-1 *env* gp120 and HIV-1 *gag* are in place in the Harvard Chan lab (Novitsky, Lagakos et al. 2009; Novitsky, Wang et al. 2009; Novitsky, Wang et al. 2010; Novitsky, Wang et al. 2011). A newly developed technique of long-range HIV genotyping (Novitsky, Zahralban-Steele et al. 2015) will be also applied, as it provides better resolution of HIV transmission clusters (Novitsky, Moyo et al. 2015).

- Details of sample processing, amplification, sequencing and troubleshooting are summarized in (Novitsky, Zahralban-Steele et al. 2015).
- The “gray zone” samples will be resolved by applying more sensitive methods such as Single Genome Amplification and Sequencing and 454-based next generation “deep sequencing.”

The viral linkage analysis includes all generated viral sequences. QA/QC procedures will be applied routinely.

Cluster analysis using generated viral sequences from each participating community will be performed as described recently (Novitsky, Bussmann et al. 2013; Novitsky, Moyo et al. 2014; Novitsky, Kuhnert et al. 2015). Potential links and clustering will be analyzed for all available

prevalent and incident HIV infections. The optimal evolutionary model will be identified for the aligned set of query sequences using jModelTest (Posada 2008).

HIV clusters will be identified by a combination of bootstrapped ML (Felsenstein 1985; Nei and Kumar 2000; Felsenstein 2004) and internode certainty (Salichos and Rokas 2013; Salichos, Stamatakis et al. 2014). The ML phylogeny will be inferred in RAxML (Stamatakis 2006; Stamatakis 2014) under the GAMMA model of rate heterogeneity. To account for phylogenetic uncertainty, from 100 to 1,000 bootstrap trees will be inferred using the rapid bootstrap in RAxML (Stamatakis 2006). The bootstrap threshold will be set to 0.80, and we will perform the sensitivity analysis at 0.70 and 0.90. We will use 0.70 as the internode certainty threshold (Salichos and Rokas 2013; Salichos, Stamatakis et al. 2014).

To estimate effective reproductive number R, HIV transmission rate λ, and the rate of becoming non-infectious δ for the identified HIV lineages, we will use the BDSKY model in BEAST2 (Bouckaert, Heled et al. 2014; Novitsky, Kuhnert et al. 2015). The chain length of Markov Chain Monte Carlo runs will be set to $2x10^8$–$3x10^8$ with the first 25% of states of each run burned-in. The generated trees will be sampled to estimate effective reproductive number, R, HIV transmission rate, λ, rate of becoming non-infectious, δ, effective population size, N_e, and the tMRCA. Convergence will be evaluated by Tracer v1.5 (http://beast.bio.ed.ac.uk/Tracer) and effective sample sizes of >200.

Transmitted drug resistance will be analyzed using samples from new incident cases and samples identified as recent HIV infections using laboratory-based incidence assays (see below). Up to 200 samples will be analyzed annually. Identified incident cases will be prioritized for inclusion into TDR analysis.

Viral mutations associated with HIV drug-resistance will be analyzed in bulk using plasma and buffy coat specimens (Novitsky, Zahralban-Steele et al. 2015). Analysis of viral mutations associated with HIV drug-resistance will be performed at the BHHRL lab using the IAS recommendations for ART-experienced patients (Johnson, Calvez et al. 2011) and WHO-recommendations (Bennett, Camacho et al. 2009). Drug resistance testing at BHHRL will use the validated resistance testing assay developed at International Laboratory Branch, CDC, USA (Zhou, Wagar et al. 2011), or an alternative assay (Wallis, Papathanasopoulos et al. 2010). In addition, more sensitive methods such as next-generation sequencing and allele-specific PCR will be used for analysis of HIV-1 drug-resistant mutations at low frequency in a subset of samples at investigators discretion and this testing will be conducted at a Harvard Chan School lab.

b) Laboratory-based tests for HIV Incidence

Results of cross-sectional HIV incidence testing obtained at the BHHRL will be compared with the longitudinal HIV incidence data. The study may also compare HIV incidence estimates derived from multi-assay algorithms including available laboratory and clinical data. HIV incidence tests and algorithms will be validated for accuracy and scale-up.

HIV seropositive specimens will be tested using the LAg-Avidity assay in combination with additional information on antiretroviral treatment exposure (ART status) and HIV-1 RNA viral load for incidence estimate as described below. Contemporary methods for estimating incidence may be used as well, or instead, as these methods evolve.

- *LAg-Avidity EIA:* The LAg-Avidity EIA is a CDC-developed new incidence assay (Duong, Qiu et al. 2012; Duong, Kassanjee et al. 2015) now commercially available from Sedia BioSciences (Portland, OR). The LAg-Avidity assay uses a multi-subtype recombinant protein that covers the immunodominant region (IDR) of gp41 of HIV-1, group M, rIDR-M. Briefly, microwell plates are coated with limiting concentration of rIDR-M antigen. Limiting the antigen allows binding of only high-avidity antibodies which are further dissociated by a pH 3.0 buffer step. ODn values are calculated for each specimen using a pre-defined calibrator specimen (CAL) tested on the same plate as follows: ODn = specimen OD/Median CAL OD. Specimens with an ODn value less than or equal to 2.0 during initial testing are confirmed by further testing of the sample in triplicate, where the median value of the three results is considered the final ODn for the specimen. All specimens with ODn values greater than 1.5 are classified as long-term infections. The HIV-1 sero-positive status of specimens with ODn values of <0.4 is confirmed with EIA, as any HIV-negative samples would incorrectly score as recent in the assay. Any HIV seronegative specimens identified at this step are excluded from the total HIV positives and incorporated into the total number of negative specimens (N) for the incidence estimation. Recent (ODn<1.5) infections are likely to have seroconverted within a mean duration of recency of 130 days (Rehle, Johnson et al. 2015).

  ART status is collected routinely during the household surveys. Individuals who can provide evidence of being on ART during the household survey are considered long-term, not recent, cases of HIV infection for the purposes of cross-sectional estimates of HIV incidence. To rule out false recent infections, specimens with ODn in LAg-Avidity assay below 1.5 may be tested for the presence of ARV drugs, including but not limited to Tenofovir, Emtricitabine, Zidovudine, Nevirapine, Efavirenz, Lopinavir, Atazanavir and Darunavir by High Performance Liquid Chromatography (HPLC) coupled to Tandem Mass Spectrometry (Rehle, Johnson et al. 2015). Specimens with detectable traces of ARV drugs are considered long-term cases. Testing for the presence of ARVs is an evolving field and other methods will be considered and possibly used as they become available locally and regionally.

  HIV-1-positive specimens from ART-naïve individuals with an ODn value less than or equal to 1.5 will be subjected to HIV-1 viral load (VL) analysis as an indirect indicator of ART usage or a natural ability to control HIV infection (elite controllers). Specimens with a LAg ODn ≤1.5 from ART-naïve individuals and a VL ≥400 copies/mL will be classified as recent infections. Results from the LAg-Avidity testing, including ART status and VL testing, will be maintained in a computer-based data management file. Therefore, a separate false recent rate (FRR) correction is not relevant or necessary, and will be set to zero (Rehle, Johnson et al. 2015). The use of multi-assay algorithms (see below) continues to provide evidence that use of additional data or assays improves accuracy of HIV recency testing.

- *Multi-assay algorithms*:
  Other algorithms for cross-sectional estimation of HIV incidence may include a combination of BED-capture EIA, Avidity assay (AxSYM or BioRad),(Braunstein, Nash et al. 2011; Laeyendecker, Brookmeyer et al. 2012) and/or multi-assay algorithms that include a combination of BED and Avidity assays and accompanied by CD4 and VL data. These algorithms will be used in a subset of samples for validation and comparison with the data generated previously in the country and/or with other studies.

Incidence calculations are assay- and algorithm-specific. Methods for calculating estimated incidence are described in section 10.

c) POC viral load assay

A pilot study of POC viral load testing will be performed to permit assessment of accuracy of POC viral load vs. standard viral load assays as well as assessment of operating characteristics of POC viral load assay(s). The first component will include POC viral load testing in the mobile lab settings by XPERT HIV-1 Viral Load from Cepheid. The second component will include POC viral load testing in households. Both components of POC viral load testing will be accompanied by HIV-1 RNA testing in the Botswana-Harvard HIV Reference Lab (BHHRL) using standard protocol. Currently, we are awaiting a viable potential candidate to be used in the pilot study in the second component. Each component of POC viral load testing will use only one POC viral load platform (and it is possible that both components will use the same POC viral load platform).

Mobile lab POC viral load testing procedures: The same blood sample that is already drawn for standard viral load testing by phlebotomy in households (and processed in the mobile lab placed in each participating community) will be used for POC viral load testing. Approximately 200 POC viral load tests will be performed in mobile labs in real-time (the same day) using whole blood and plasma from this blood draw. Results of POC viral load testing from this pilot study will not be returned to participants, as the platform will not yet have been validated; results of the standard viral load assay will be returned to the local clinic. The same plasma sample tested in the mobile lab will also be tested at BHHRL in Gaborone using standard procedures. The targeted population for this portion of the pilot will be approximately 200 (but up to 400) ART-naïve HIV-infected individuals. The range in sample size is necessary because investigators do not know the precise viral load distribution of participants and we need to ensure that 1/3 of the participants have a viral load below the threshold of 10,000 copies/ml to allow for assessment of the performance of POC viral load tests at lower viral loads.

POC viral load testing in households procedures: Blood for POC viral load testing will be collected according to the manufacturer's instructions in households. Approximately 200 POC viral load tests will be performed in households in real-time using these blood samples. Results of POC viral load testing from this pilot study will not be returned to participants, as the platform will not yet have been validated; results of the standard viral load assay will be returned to the local clinic. A parallel sample drawn in the household will be tested in BHHRL in Gaborone using standard procedures. The targeted population for this portion of the pilot will be approximately 200 (but up to 400) ART-naïve HIV-infected individuals. The range in sample size is necessary because investigators do not know the precise viral load distribution of participants and we need to ensure that 1/3 of the participants have a viral load below the threshold of 10,000 copies/ml to allow for assessment of the performance of POC viral load tests at lower viral loads.

If high concordance is observed between POC viral load assays and standard viral load assays, POC viral load may be used in lieu of standard viral load assays, among pre-ART BHS participants, as noted previously in Section 8.4c). If this is done, then viral load results will be shared with clinics.

Operational characteristics will be evaluated in both components. We will record the time it takes to obtain viral load result in each test, reported ease of use by field operators, and stability under different conditions.

8.6. Quality Assurance

A quality management plan with monitoring and evaluation of key indicators will be implemented at the BHHRL and NHHRL. Standard operating procedures (SOPs) will be used for all tests as well as QA/QC procedures. Laboratory staff conducting testing will participate in proficiency testing and 5% of all tests will be subjected to internal QC processes. The QA/QC plan will include periodic monthly reviews of EQA performance and corrective actions implemented. Procedures will be in place for performing and documenting the quality of a specimen, including storage and transport conditions, monitoring of equipment and temperatures, and function indicators. The QA/QC plan will include routine collaboration with the CDC laboratories, enrollment in appropriate EQA schemes, and inter-laboratory comparisons. Field sites will participate in EQA and proficiency testing for all POC assays used.

Certification status for laboratory tests will be maintained including: HIV ELISA, POC HIV Rapid EIA, POC CD4 count, and HIV-1 RNA load. Laboratories will be monitored throughout the study by the study team and external monitors as requested by the study sponsor.

8.7. Biosafety and Waste Management

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC. All infectious specimens will be transported in accordance with the IATA (International Air Transport Association) guidelines using designated courier services.

8.8. Specimen Storage and Shipment

Specimens will be stored until all protocol-related and quality assurance testing have been completed. In addition, study participants will be asked to provide written informed consent for long term storage and potential future testing of their specimens. Specimens without consent for long-term storage and additional testing will be discarded at the end of the study and destruction documented in the study files. Future human subjects research on stored specimens will require submission and approval of a separate study protocol to all engaged IRBs and appropriate study team committee.

The study site will coordinate shipment of specimens as required. Samples (buffy coats, plasma, and/or isolated nucleic acid) for research components of viral genotyping will be shipped to a Harvard Chan School lab in Boston, MA. Selected samples may be transferred to the CDC Laboratories and HPTN Central Laboratory for cross-studies analyses. Other specimens will be stored at BHHRL.

9. DATA COLLECTION AND MANAGEMENT

9.1. Data Management team

A project data management team, chaired by representatives from MOH, BHP, Harvard Chan School, and CDC, will be formed to build the research data collection systems. The data management team will:
(1) Design the data collection and management systems required for BCPP;
(2) Coordinate data usage for project progress reports to funders;
(3) Coordinate data usage by the DSMB (as described in Section5.4), and,
(4) Coordinate data usage for analysis of primary and secondary study objectives.

This section outlines data collection and management guidelines; detailed methodologies for data collection and management will be developed and placed into SOPs or described in the manual.

9.2. Data Flow

Research data will be collected on mobile electronic devices during household surveys. Research data that are not collected during household surveys include routine clinical monitoring data collected in the electronic medical record system (PIMS2 or IPMS) for Combination Prevention community clinic patients (described in the *Intervention Protocol*); data from participants who consent to research-related blood draws (see section 6.3); and data on health resource utilization and quality of life from a subset of participants experiencing opportunistic illnesses (see Section 7).

9.3. Data Flow From BHS, HIC, ESS, and Research Blood Draws

Data entry: Field teams will be trained in the use of appropriate mobile devices (e.g. notebooks) for data entry during the BHS, HIC, ESS, and research blood draws. Skip patterns, legal ranges, and consistency checks will help to ensure high quality data are collected.

From the point of entry, BHS study data will be synchronized from the field devices into the community server at the end of each shift. Data from the community server will be synchronized over encrypted virtual private network (VPN) channels to the central server at the BHP Data Management Center (DMC) in Gaborone nightly. Backups of the community server are transferred to the BHP DMC nightly and then included in the DMC's general backup routine. Database backups will be encrypted and stored on encrypted drives before transfer to removable media. Secure and lockable rooms will be used to house all workstations and servers and to store mobile devices when not in use.

All information will be collected and transmitted electronically except consent forms and laboratory labels. A paper copy of the informed consent/assent forms will be offered to the participants. The consent forms with participants' signatures and Omang numbers will be collected and, at the end of the day, secured in locked file cabinets at the base sites. Laboratory labels with bar code will be used for sample tracking. The sample labels will contain a computer-generated Subject Identifier, sample date, date of birth, and coded HIV status, and the type of sample. No personal identifying information can be derived from the labels.

Management of Personally Identifying Information (PII): The national identity card, Omang, required by the MoH for access to health care services including ART, is also needed to: (1) help identify study participants during research follow-up, and (2) link study data to HIV care and treatment data collected in routine HIV care and treatment systems.

However, once entered into the data system, observations with unique Omang numbers will be allocated, and referred to thereafter, by a unique computer-generated Subject Identifier. PII (such as first and last name, and omang number) may be viewed in the context of the BHP Electronic Data Capture (EDC) system by authorized EDC users. PII will only be used outside of the EDC in sending results to healthcare staff providing care who are required to reliably identify a patient. When EDC data are transferred to partners for operational purposes, the data will be transmitted through an encrypted VPN network between servers deployed with full disk encryption (FDE). When EDC data are transferred to partners for analytical purposes, PII values are not decrypted, if included, and the data will be transmitted through an encrypted VPN between servers deployed with FDE.

Protection of Electronically Stored Subject Data: Security of electronic records and data is a significant concern. All components of the distributed data systems will use authentication and encryption to render electronically stored subject identity and personal health information unusable, unreadable, or indecipherable to unauthorized individuals for "data in use" (e.g. data being analyzed by BCPP investigators), "data in motion" [e.g. data being transferred between data entry points and the Data Management Center (DMC) in Gaborone] and "data at rest" (e.g. data in storage at DMC in Gaborone).

Full Drive Encryption will be implemented at the hardware layer of all devices storing protected health information. A three-factor scheme will be used to authenticate users through the hardware layer to the application layer where personal health information is available. The applications will have user profiles to control access to certain data and reports. The application and database layers will use a combination of hashing and encryption for sensitive and personal data. Mobile devices and the staff operating them will not be equipped with the encryption keys to decrypt select sensitive data fields.

Special EDC/Data Considerations for Anonymous Participation of Non-citizens

In the ESS, enumerated subjects who are non-citizens not married to a citizen of Botswana are eligible to participate anonymously. The EDC will be used to collect survey data for these subjects without collecting any PII should they choose to participate. Following enumeration, each subject will be asked about their citizenship status prior to completing the Eligibility Checklist. If the subject is willing to participate anonymously given their non-citizen status, the EDC will redirect the RA to the Anonymous Eligibility Checklist. If the non-citizen subject is eligible for the survey and accepting of verbal consent, the EDC will do the following to protect the anonymity of the participant and maintain data integrity and completeness across anonymous and identified participants: 1) de-link the participant's enumeration record from the household; 2) generate an electronic consent record filling all PII variables with computer-generated ones; 3) allocate a Subject Identifier; 4) redirect the RA to the survey tool for completion with the participant. In this way there is no requirement for the RA to have proof of identification in order to proceed with data collection, electronically captured data will not be associated with a location in the community and PII will not be collected into the EDC nor in any manual manner.

9.4. The BCPP Research Data Management Center (DMC)

All research data collected from the BHS, AHS, HIC, and ESS will be housed centrally at the BCPP Research Data Management Centre (DMC), a secure facility within the Botswana Harvard HIV Reference Laboratory (BHHRL) that is co-owned by the MOH and BHP. Designated representatives from Harvard, MOH, and CDC will manage these data, and keep record of data management progress and access.

Sharing of program clinical data, that are stored in the MOH M&E unit, and which reflect routine care of consenting research participants with BCPP investigators, will be described in a data sharing agreement.

9.5. Reporting Study Progress

On a monthly basis, a data manager at the Harvard Chan School will be responsible for providing indicators of project progress to project investigators and directors.

10. ANALYSIS PLANS

10.1. Analysis Plan to Meet Primary Objectives

- **1st Primary Objective**
  To estimate the impact of the CP package on cumulative HIV incidence, we will perform pairwise comparisons within matched pairs of communities using a method that estimates the incidence ratio between the two communities and allows for interval censored time to event data (Finkelstein 1986). Interval censored time to HIV infection will arise from the fact that only the time interval in which subjects have become infected will be known and that some subjects will enter the incidence cohort after the start of the study, miss a follow-up survey, or drop-out. A permutation test will be used to test the null hypothesis of no intervention effect on incidence ratio. The test statistic will be an inverse-variance weighted average of the incidence ratio of every pair. The null distribution of the test statistic will be obtained by calculating the test statistic with the treatment assignment permuted within each pair (resulting in a sign change of the log of the incidence ratio estimate for each pair). The permutation test is valid even if the variances for the incidence ratio estimates are not accurately estimated; however, efficiency increases as the variance estimates get closer to the truth. Assuming independence of the incidence ratio among different pairs, the variance of the overall estimate of the incidence ratio can be estimated as a weighted sum of the variance of incidence ratio for each pair, where the weights are the inverse of the variance squared.

  Secondary analyses will be performed to investigate the potential impact of informative censoring, which would arise if the probability of being tested was associated with risk of infection. Doubly robust methods based on weighting by the inverse of the probability of a subject receiving a test will be used for each pairwise comparison, and the method above will be employed for inference. To compare our results with those from other approaches, a secondary analysis will be performed

based on the method of Zhang and Sun (Zhang, Sun et al. 2007) for interval-censored community-level data. Missing data will be handled using approaches described in Tsiatis (Tsiatis 2006) and Little and Rubin (Little and Rubin 2002). Sensitivity analysis will be performed to assess the robustness of inference to various assumptions.

Covariate-Adjusted Analyses: Pair-matching in this CRT controls for certain factors associated with the outcome of interest, at the design stage. Nonetheless, there could be imbalance between arms for a number of other baseline community covariates (e.g., despite best attempts at matching there may be baseline imbalances in HIV prevalence between arms). By adjusting for these covariates, precision and power of the trial can be improved.

Covariate adjustment through a two-stage procedure has been proposed by Gail et al. (Gail, Byar et al. 1992). First, a proportional hazard model for interval-censored data will be fit, including the covariates of interest, as well as terms for the matched pairs, but excluding the intervention effect. The expected incidence rate of each community will be estimated based on the fitted model. We will then perform a permutation test on the difference between the expected and observed incidence rate to test the null hypothesis of no intervention effect while adjusting for imbalances in these covariates.

- **2nd Primary Objective**
  To estimate coverage of HTC, ART (including viral suppression), and MC at baseline and study end in Enhanced Care and Combination Prevention communities, the following proportions will be estimated with 95% confidence intervals:
  - The proportion of 16-64 year-old community residents who report knowing that they are HIV-infected, or report testing HIV-negative in the preceding 12 months.
  - The proportion of 16-49 year-old HIV-negative men resident in the study communities who are circumcised.
  - The proportion of HIV-infected 16-64 year-old community residents who know they are HIV-positive and are receiving ART.
  - The proportion of HIV-infected 16-64 year-old community residents who know they are HIV-positive, are receiving ART, and have HIV-1 RNA ≤400 copies/mL.

  To compare proportions between arms at the end of study, we will use cluster-level analysis. The main outcome measure will be the proportion of eligible persons successfully covered by the intervention. Cluster-specific proportions will first be calculated for each cluster within a pair. Then within-pair coverage rate ratios (r_{1j}/r_{0j}), coverage rate differences $(r_{1j} - r_{0j})$, and their

  standard errors will be estimated (where "r_1" and "r_0" are the coverage proportions for Combination Prevention and Enhanced Care clusters respectively, and "j" represents the jth cluster pair). The change in coverage between ESS and baseline will also be compared.

  To estimate the overall coverage proportion, we calculate coverage proportion among all eligible survey participants within each arm at baseline and ESS. The ratio and difference of coverage proportions between Enhanced Care and Combination Prevention communities at baseline and ESS are then calculated. We will additionally consider inclusion of data collected from non-citizens in

analyses of the second primary objective. To this end, we will conduct both stratified and pooled analyses.

Baseline characteristics of the two treatment arms will be examined to characterize the population and to identify any imbalance between the arms. Where a large difference in baseline characteristics is observed between treatment groups the relevant covariate may be included in an adjusted analysis.

- **3rd Primary Objective**
  The estimated cost per additional HIV infection averted will be estimated using the CEPAC-I model.

10.2. Analysis Plan to Meet Key Secondary Objectives

- **1st Secondary Objective: To estimate the extent to which incident HIV-infections in Combination Prevention and in Enhanced Care communities arise from HIV strains circulating within communities randomized to the same study arm as the incident case or from strains circulating outside those communities (sexual network mixing).**

  Statistical concepts applicable to assessing genetic linkage of incident infections within the HIC are described in section 8. Using these analysis techniques, incident infections observed in the HIC will be classified as either phylogenetically linked to HIV-1 variants in the same study arm, or unlinked. The proportion of incident infections that are unlinked will be provided with 95% confidence intervals.

- **2nd Secondary Objective: To estimate the efficacy of the Combination Prevention package on reducing the rate of new infections with HIV strains circulating within communities in the same study arm.**

  Maps of circulating HIV-1 genotypes will be generated in Enhanced Care and Combination Prevention communities through sample collection during the BHS, HIC and ESS. In addition, HIV genotyping will be done through blood sample collection among all HIV-infected persons attending clinics in Combination Prevention communities. However, to avoid biased comparisons between Combination Prevention and Enhanced Care arms, only HIV-1 genotype maps generated from the BHS, HIC, and ESS in both Combination Prevention and Enhanced Care arms will be used to categorize incident infections as either linked or unlinked for analysis of this secondary objective. To meet this secondary objective, pairwise comparison of HIV incidence between Enhanced Care and Combination Prevention arms (as described in section 10.1 above), limited only to linked infections in each arm, will be performed.

- **3rd Secondary Objective: In the Combination Prevention arm only, to estimate over time, the proportion of recent HIV-1-infections that can be linked to HIV-infected adults of the same study arm.**

  In Combination Prevention communities, we will carry out phylogenetic clustering using data collected at baseline and all subsequent timepoints. By comparing all newly diagnosed cases each

year and at ESS against clusters established at baseline, we will be able to assess the trend in newly diagnosed cases linking to existing cases or with each other.

- **4th Secondary Objective: During the ESS, to compare Combination Prevention (CP) package uptake within 20% HIC households to CP package uptake in the broader communities they represent.**

  Coverage of ART, MC, and PMTCT at the ESS will be estimated separately for participants within the 20% households in all 30 communities, and in the remaining 80% of households in each of the 6 communities selected for the ESS. The proportions for the coverage are as defined in 2nd primary objective. We will do pair-wise comparisons of the proportions between the study cohort and those in the same community outside of the cohort using cluster-level analysis stratified by treatment status. The within-community coverage ratio and difference and their standard errors will be calculated for ART, MC, and PMTCT, in Enhanced Care and Combination Prevention arms separately.

- **5th Secondary Objective: To obtain secondary cross-sectional estimates of community HIV incidence at baseline and study end in Enhanced Care and Combination Prevention communities through use of HIV-incidence assays.**

  Assay-based HIV incidence is calculated as the number of recent infections divided by the population at risk (those testing HIV-negative plus those recently seroconverting), then annualized by multiplying by 365 divided by the accepted mean seroconversion duration for the assay.
  To calculate the incidence as an annual instantaneous rate (I_r) the following formula, or a contemporary equivalent, may be used:

$$I_r = \frac{R - \varepsilon P}{(1-\varepsilon)\omega N}$$

  Where the survey counts (N, P, R) are as follows:

  N = number of HIV negative individuals in the survey
  P = number of HIV positive individuals in the survey
  R = number of individuals classified incidence assay positive

  The calibration parameters are as follows:

  ω = mean incidence assay duration specified in units of years
  ε = false recent rate (FRR) of the incidence assay. In the presence of more data such as viral load, documented ART status and documented testing history, FRR adjustments may not be neccessary as per the WHO guideline on recent infection testing algorithm (World Health Organization 2011; UNAIDS 2013; Rehle, Johnson et al. 2015).

Confidence intervals are computed using a delta method approximation which may include the error, assumed to be normally distributed, associated with calibration parameters. The coefficient of variation (C_v C_v) is computed as follows:

$$C_v = \sqrt{\frac{1}{P}\left(\frac{N+P}{N} + \frac{(P-R)R[1+\varepsilon/(1-\varepsilon)]^2}{[R-\varepsilon/(1-\varepsilon)(P-R)]^2}\right) + \frac{\sigma_\omega^2}{\omega^2} + \frac{\sigma_\varepsilon^2(P-R)^2}{(1-\varepsilon)^4[R-\varepsilon/(1-\varepsilon)(P-R)]^2}},$$

where

σ_ω is the standard deviation of the mean RITA duration (assumed normally distributed)

σ_ε is the standard deviation of the FRR (assumed normally distributed).

The 95% confidence interval (CI) for I_r is then computed as:

$$I_r \pm 1.96 \times I_r C_v$$

Sampling weights will be applied accordingly in the national adult incidence estimate calculation (15-59 years and 15-49 years), and survey design effects will be computed to estimate the variance for calculating the 95% CI around the estimates. Overall incidence estimates and sub-group incidence estimates (15-59 years and 15-49 years), where possible, will be calculated to highlight peak incidences that can be targeted by HIV prevention programs. Bi- and multivariate analysis using STATA and or SAS software will examine associations between demographic, behavioral or other characteristics and HIV recency status.

- **6th Secondary Objective: At baseline, during study conduct, and at study end, to estimate the proportion of recent HIV infections in combination prevention and enhanced care communities with evidence of transmitted HIV drug resistance.**

  The proportion of patients with evidence of transmitted HIV drug resistance will be calculated as the proportion of patients identified with drug resistance through drug resistance assays among patients with new or recent HIV infections identified through assay-based testing. Testing for TDR will be performed on samples from HIV-infected persons residing in the 20% BHS/HIC households and from ESS participants in both arms; and from consenting HIV-infected persons identified through the HTC campaigns in Combination Prevention communities. The proportion and its standard error will be calculated for each arm at baseline, annually, and at study end.

- **7th Secondary Objective: To estimate the proportion of HIV-infected adults with undetectable viral load (VL<400 copies/ml) at baseline, during study conduct, and study end in Combination Prevention and Enhanced Care communities.**

  This analysis will be performed using measurements of HIV-1 RNA throughout the study. The threshold of 400 copies/ml will be used for enumeration of HIV-infected individuals either above or below the threshold. Testing for HIV-1 RNA will be performed on samples from HIV-infected persons residing in the 20% BHS/HIC households and from ESS participants in both arms; and from consenting HIV-infected persons identified throughout the study in Combination Prevention

communities. The proportion and the 95% confidence intervals with transmitted drug resistance will be estimated and compared between Combination Prevention and Enhanced Care communities at baseline, annually, at the study end.

- **8th Secondary Objective: To estimate the association between HIV-1 viral load and HIV transmissions using viral linkage data.**

  The hypothesis to be tested is that individuals with high HIV-1 RNA load are responsible for the majority of new HIV transmissions. To estimate HIV-1 RNA load within clusters we will use two sets of data, results of phylogenetic clustering and measurements of HIV-1 RNA load in HIV-positive individuals participating in the study. Specifically, HIV-infected individuals will be grouped based on clustering patterns of their viral sequences, and cluster-specific (within each cluster) HIV-1 RNA load will be estimated. We will compare HIV-1 RNA load between two groups of clusters, clusters *with* and *without* incident cases identified over the study. The assumption is that HIV-1 RNA is higher within clusters with incident cases.

  Analysis plans for **Secondary Objectives 9-11** are covered in Section 7 (concerning cost-effectiveness).

- **12th Secondary objective: To compare CD4 and viral load trajectories over time, among HIV-infected ART-naive persons who reside in Combination Prevention vs. Enhanced Care communities.**

  We will compare CD4 count and viral load over time between randomized arms, among HIV-infected persons who enroll into BHS with initial CD4>350 and while still ART-naive. The underlying rationale for this comparison is the assessment of the immunologic and virologic implications of expanded/early ART. The primary endpoints for this analysis will be the change in CD4 count over time, and the proportion of participants with HIV-1 RNA < 400 copies/mL by the final visit, by randomized arm.

- **13th Secondary objective: To assess the accuracy and feasibility of point-of-care viral load testing in comparison to standard viral load platforms.**

  Sensitivity and specificity of the POC viral load testing against BHHRL standard procedure using the threshold of viral load at 10,000 copies/ml will be evaluated.

  The POC and BHHRL measurements will be compared using Bablok and Passing regression analysis (Bablok and Passing 1985), which includes evaluation of the systematic and proportional errors, as well as correlation. For each phase, the difference of the POC value and the BHHRL standard procedure on the same sample will be plotted against the mean of the two measurements (Bland-Altman plot) to assess the comparability of the POC method with the BHHRL standard procedure, and estimate the mean bias (with 95% confidence interval) (Bland and Altman 1986).

  Statistics and qualitative reports on operational characteristics of the POC procedures will be reported.

- **14th Secondary objective: To understand (through qualitative interviews) barriers to and facilitators of linkage to care and ART initiation among BHS participants.**

  Qualitative data related to linkage to care and initiation of ART will be gathered through focus group discussions (FGDs) and in-depth interviews (IDIs). Data will be collected in the CPCs from members of the BHS field team, the MOH nurse prescribers as well as from a subset of HIV-infected BHS participants who are eligible for ART. FGDs and IDIs will be semi-structured and based on a standardized guide. All FGDs and IDIs will be audio-recorded, transcribed and translated (when not in English). Investigators will read the transcripts and develop and agree on a codebook. An iterative process will be used to read transcripts, apply codes, compare and revise codes until consensus is reached about salient motivators and barriers for linking to care and initiating ART.

- **15th Secondary objective: To use BCPP data in analyses of the rates of and risk factors for major AIDS and non-AIDS illnesses/injuries/mortality among persons living with and without HIV in Botswana.**

  Measurements and survey responses from participants in the BHS, AHS, and ESS will be utilized to estimate the distribution of risk factors and frequency of health events in Botswana. The combined cohort (BHS, AHS, ESS) will also serve as a source for controls in case-control analyses and an estimate of population exposures in standardized incidence/morbidity/mortality analyses. Cases will be obtained from other sources, such as national registries, publicly available data, or other studies, with separate IRB approval where needed. Case-control and standardized incidence/mortality/morbidity analyses do not require release or sharing identifiable information outside of BCPP.

11. ETHICAL CONSIDERATIONS

11.1. Ethical Review

The protocol, informed consent/assent forms, research participant education and recruitment materials, and other requested documents — and any subsequent modifications — will be reviewed and approved by the ethical review bodies responsible for oversight of research conducted at the study sites. Subsequent to initial review and approval, the responsible Institutional Review Boards/Ethical Committees (IRBs/ECs) will review the protocol at least annually.

11.2. Informed Consent

Please see Table 12 below for a summary of the various approaches to informed consent for this protocol.

Potential participants will be given the opportunity to ask questions about the study. It will be emphasized that participation in any component of the research is completely voluntary. The informed consent process will occur in the language most comfortable for participants (usually in

Setswana). Consent materials will be translated into the local language and back-translated to ensure accuracy. Staff will obtain informed consent who have been designated this responsibility in study staff signature logs. Usually staff obtaining informed consent during household surveys and research blood draws will be healthcare auxiliaries and research assistants.

- ***BHS/AHS, HIC and ESS Household Representatives - Verbal Consent for Household Representative*:** Consent will be obtained from the household representative to obtain the household structure. Informed consent for this aspect of study participation will be obtained from research volunteers aged 18 or older, without documenting the process in writing. A waiver of documentation of informed consent will be requested from IRBs and the informed consent script approved prior to use. The consent script (Household Script) will describe the purpose of the research activity, procedures to be followed, and the risks and benefits of participation. Only participants who verbally agree to participate will be asked to provide the requested data for this part of the study. A copy of the informed consent script will be offered to the participant. The reading level of the script has been estimated using Flesch-Kincaid and is below 6.0.

- ***BHS/AHS, HIC, ESS – Citizen/Spouse of Citizen Participants in Household Surveys and Cohort, and individuals consenting to research blood draws or qualitative interviews***: Written informed consent will be obtained from each eligible individual before completion of individual questionnaires and/or blood draws for study purposes. Residents aged 16-17 years old, if eligible, will be asked to provide written assent with a guardian's written permission. The informed consent/assent form will be given to the participant to read (for literate participants), or read by a trained study staff member (for illiterate participants). Required steps in the process will be driven electronically in household visits to ensure quality, consistency and completeness. Consent/assent will be documented by having the participant sign the informed consent/assent form or make a mark if the participant is illiterate; the latter will be witnessed by a third party. A copy of the informed consent/assent form will be offered to the participant, and if applicable, parent or legal guardian giving permission for a minor to participate. Staff will ensure that the participant takes a copy of a study contact card that contains study information and telephone numbers if the participant does not wish to take a copy of the consent. The reading levels of each consent/assent form have been estimated using Flesch-Kincaid and are below 6.0.

- ***ESS – Non-Citizen Adult Participants in ESS:*** A waiver of the requirement to obtain a signed consent/assent form is requested from IRBs for non-citizen participants in the ESS. The request for this waiver is based on the U.S. Code of Federal Regulations Title 45 Part 46.117(c)(1). All data and specimens will be collected anonymously from these participants. Therefore, the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. A written statement regarding the ESS will be IRB-approved and provided to non-citizen participants prior to conducting survey procedures, but it will not bear the participant's name or signatures. The written statement will be given to the participant to read (for literate participants), or read by a trained study staff member (for illiterate participants in the presence of a witness). Parental permission will be sought with assent from any non-citizen participants 16-17 years old, but signatures will not be obtained per the waiver. Required steps in the process will be recorded with a checklist.

- **Special Populations**

  Minors: Minors (participants ages 16-17) will only be enrolled in research activities if they can provide informed assent and with the written permission of a parent/legal guardian.

  - ✓ Under circumstances where study staff suspect, or there is a reported incident of sexual abuse of a person under age 18, the study staff member must inform the relevant authorities; this is stated in the informed consent/assent forms.
  - ✓ Referrals will be made to youth services if needed.

  Institutionalized populations: Persons residing in institutions such as prisons will not be included in the study as they will not be available for household and community surveys. An enrolled participant who becomes imprisoned or involuntarily confined in a medical facility (e.g. for psychiatric illness) during the study will not undergo any study procedures during confinement. Visits missed due to absence will be recorded. Study procedures would only resume if the participant is released from confinement during the study period and is available and willing to complete any remaining study visits.

  Non-citizens: Non-citizens who are not married to Botswana citizens are eligible to participate in the ESS, a one-time household survey at the end of the study. Increased likelihood of experiencing problems with securing housing, travel, exploitation in employment, and access to healthcare make non-citizens a potentially vulnerable population. Anonymous participation in the ESS will be offered to ensure protection for non-citizen participants. In particular, the sociodemographic information collected from non-citizens will be limited, including only age (year of birth, but not date of birth), gender, immigration status, place of primary residence (at the community level, not a physical address), for example. No names or personal identifiers will be collected from participants who are non-citizens. Referrals will be made to services that are available to non-citizens.

Table 12: Summary of Approach to Consent, Various Components of the Study

Project component	Targeted subjects	Activity	Consent Type/Waiver Requested	Consent Material to be Used	Provision for Parental Permission with Assent?	Timing of Obtaining Consent
Household structure	Head of household/representative in 20% households	Questionnaire regarding household structure and socioeconomic indicators	Verbal/Waiver requested for documentation of consent	Household Script	No	BHS T0
BHS	Age-eligible members of 20% households, including HIV-infected persons and HIV-uninfected persons	Baseline and follow-up questionnaires Repeat HTC if not documented HIV-positive Research blood draws, if applicable Repeat CD4/VL, if applicable POC VL, if applicable	Written	BHS/HIC Consent	Yes	BHS T0 New participants may consent at T1 New participants at T2 will sign ESS consent
HIC	Age-eligible HIV-uninfected members of 20% households who meet HIC eligibility criteria (a subset of BHS participants)	Baseline and follow-up questionnaires Repeat HTC Research blood draws, CD4/VL/POC VL if applicable (i.e. for seroconverters)	Written	BHS/HIC Consent	Yes	BHS T0, New participants may consent at T1
Blood draw for research purposes	• All HIV-infected persons in Combination Prevention communities • BHS residents who decline survey participation but are willing to give a blood sample	Blood for HIV-1 RNA, stored sample for HIV genotyping/resistance testing and incidence assay	Written	Research Blood Draw Consent	Yes	Upon referral to or recruitment by research staff
End of study survey	All eligible residents (new enrollees from 20% BHS/HIC householdsand ~80% of remaining community members will be newly enrolled to ESS in 3 pairs of selected communities)	ESS questionnaire Research blood draw, if applicable CD4/VL/POC VL if applicable	Written for Citizens/Spouses of Citizens; Waiver requested for non-citizens	ESS Consent	Yes for Citizens/Spouses of Citizens No for Non-Citizens	T2/ESS
Supplemental Economic Assessment for Low CD4/OI	Residents with low CD4 or OIs seeking care (out- or inpatient)	Questionnaire regarding economic activity and quality of life	Written	Economics Consent	No	Upon referral to research staff

Barriers to and facilitators of LTC and ART initiation	HIV-infected BHS participants BHS Research Assistants and Nurse Prescribers	Focus group discussions Individual in-depth interviews	Written	ART Start Interview Consent; ART Start Focus Group Consent for BHS; ART Start Focus Group Consent for Staff	No	At T1 or later

11.3. Risks

The risks, discomforts, and inconveniences that could be posed to subjects participating in this study include the following:

- **Survey Participants in the BHS, HIC, research blood draw after HTC, and ESS**:
  - Blood drawing may cause pain and bruising, and rarely, infection at the site where blood is drawn. Study participants may feel lightheaded or faint as a result.
  - Repeated HIV testing and counseling in the HIC participants may cause stress, anxiety, or embarrassment. Participants are likely to become distressed if they learn they are HIV positive.
  - Participants may become embarrassed, worried, or anxious when discussing their sexual practices, HIV risk factors, ways to protect their partner(s) against HIV, and/or ways to prevent acquiring HIV infection.
  - Participants may experience discrimination as a result of being perceived by others to be HIV-infected or at high risk for HIV infection.
  - Participants may have difficulty or conflict in their relationships if they choose to participate couples HIV testing, particularly if this results in perceived or disclosed infidelity.

11.4. Benefits of Research Participation

Survey Participants in the BHS, HIC, research blood draw after HTC, and ESS:
Potential health benefits for 20% household residents include: earlier HTC delivered to their place of residence, earlier diagnosis of HIV-infection (and access to point-of-care CD4 result if ART-naive) with subsequent earlier enrollment in care and treatment or PMTCT, and earlier awareness of the importance of annual HTC and risk reduction practices for adults who test HIV-negative. HIV-infected individuals who consent to participate in the household surveys will also have access to a pre-ART viral load result at BHS enrollment and then repeat CD4 and viral load if ART-naïve at enrollment or at the study visit when HIV-positive status is recorded.

Those who participate in research blood draws independently of the household surveys may also benefit from a pre-ART viral load result; however, the test may not be run in real-time.

11.5. Confidentiality

No identifiable study data will be released to any unauthorized third party, without prior written approval of the participant, except as necessary for monitoring by the IRBs, the study sponsor, external monitors, OHRP, or the Botswana Ministry of Health.

During BHS, HIC, research blood draw after HTC, and ESS study procedures:
Project staff visiting households for study visits will identify an appropriately private space to counsel participants, perform HIV tests, and collect individual data. If private space is not available, or the participant does not wish to carry out study activities in the home, an appointment will be made to visit one of the study clinics instead. All study documents will be stored in a secure location and electronic data will be password protected. Full Drive Encryption will be implemented at the hardware layer of all devices storing protected health information. Only designated study staff will have access to study records.

De-identified data:
When de-identified datasets are created the following variables will be excluded: name, national identity number, phone numbers, address (other than community of residence), GPS coordinates, and all elements of dates (except year) for events related to the individual (birth, death, hospitalizations, clinic visits, etc.). BCPP does not collect data on persons >89 years, nor any biometric data or other identifiers.

11.6. Compensation and Incentives

- Baseline Household Survey (BHS) and HIV Incidence Cohort (HIC): Each participating resident will receive 20 Pula (about $3) worth of air time as compensation for time dedicated to the study at each attended scheduled study visit.
- End of Study Survey (ESS): Each participating resident will receive 20 Pula (about $3) worth of air time as compensation for time dedicated to the study at the End of Study Survey.

11.7. Protocol Compliance and Compliance with UN Convention on the Rights of the Child

Study staff will adhere to the ethical principles that have their origin in the Declaration of Helsinki (e.g., ICH E6, 45 CFR 46). The study will be conducted in accordance with the regulations of Botswana and the sponsor.

The US government is a signatory on the UN Convention on the Rights of the Child (defined as anyone under the age of 18) which requires that any research study or program activity which includes children that are identified as engaged in sex work, trafficked or victims of violence make special provisions for referral of these individuals to services. BCPP will report suspected cases to service providers (local police or social workers) as outlined in the Botswana National Guidelines for reporting child abuse or neglect.Contact information for the local police and social workers, and procedures for reporting suspected cases of child abuse or neglect are described in the study-specific SOP in the BCPP Study Manual. Local police and social workers will be contacted prior to the initiation of study activities in the community to ensure they are aware of the study activities in the community and the porcedures that will be followed should BCPP staff encounter suspected cases of abuse or neglect. As per national guidelines, reports of suspected abuse or neglect will be made to local authorities within 24 hours. Study personnel who may come in contact with these study participants will receive training on the appropriate procedures for handling these cases if they arise during the course of the study and training on the SOP documented in the regulatory files. The study will retain documentation of all reports that includes date of report, age of participant, and the name and type of service provider. No identified information will be retained in the documentation; documentation will be retained by the study for 2 years after study close-out. The informed consents for the study contain language that makes clear to the participant what is required to be reported to authorities and the impact on confidentiality.

11.8. Future Use of Stored Samples

Study participants will be asked to provide written informed consent for their specimens to be stored after the end of the study for possible future research testing. Any residual specimens of participants

who do not consent to long-term storage and additional testing will be destroyed at the end of the study after all protocol-required and quality assurance testing has been completed.

12. STUDY SAFETY AND MONITORING PLAN

12.1. Reporting Adverse Events

The *Evaluation Protocol* does not involve the administration of any study products.

The study team will report in real-time, to IRB's and to sponsors, any serious adverse events (SAEs) experienced by a participant or other individual, that in the opinion of the investigator are **BOTH unexpected and at least possibly related** to the research procedures. An SAE is defined as any untoward occurrence that:

- Results in death
- Is life-threatening (grade 4)
- Requires hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect, or
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

This level of reporting is chosen for this observational aspect of the BCPP to avoid real-time reporting of all events (e.g. deaths, hospitalizations etc.) occurring in study communities that are definitely not related to study procedures (but to remain compliant with reportable new information generally required by IRBs).

Timing of reporting:
Reportable events will be reported in accordance with institutional, sponsor, and study specific guidelines delineated in standard operating procedures for the study.

AE reporting period:
The AE reporting period for this study is from the time of enrollment to the end of study follow-up for a participant.

AE reporting process
Reportable events will be documented by the in-country study team who will then notify the IRBs, MoH, CDC-Atlanta, and Harvard teams. The incident will be discussed and a written action plan will be devised and implemented within 1 week of the initial report, if needed. Written documentation of all reportable events and event resolution will be retained in the study file. Reporting of events to IRBs and the study sponsor will follow institutional and IRB policies. If necessary, a formal report will be sent to the appropriate IRBs using the CDC's Incident Report Form 1254.

12.2. DSMB

DSMB review will take place prior to the start of the study and annually thereafter. Details of recommended interim analyses by the DSMB are provided in Section 5.4.

12.3. Protocol Violations

The study will be conducted in full compliance with the protocol. The research team will ensure that volunteers are informed of and understand the risks of their participation in research. Protocol violations and cases of social harm will be documented using the appropriate study forms and reported to IRBs and sponsors in accordance with the institutional policies. Minor protocol violations that do not pose a threat to the health of participants will be compiled and reported to the IRBs at the time of annual IRB review. Incident reporting is the responsibility of the Principal Investigators of this study. Emergency departures from protocol that eliminate an apparent immediate hazard to a participant and are deemed crucial for the safety and well-being of that participant may be instituted for that participant only. In that case the Investigator will contact the IRBs and sponsor as soon as possible to document the reasons for violation and ensuing events.

12.4. Study Monitoring Plan

A complete monitoring plan will be developed and the site will complete a site activation checklist prior to study initiation. Internal (sponsor) and external monitors will travel to the study site according to a pre-determined monitoring schedule specified in the monitoring plan to conduct general monitoring. The study site will maintain a monitoring log to document all monitoring visits. Investigators and staff will grant access to the monitors to allow inspection and review study documents (e.g., consent forms, data collection forms, other source documents) and pertinent clinic records for confirmation of the study data. Study monitors will also inspect study facilities and documentation and may observe the performance of study procedures.

13. ADMINISTRATIVE PROCEDURES AND INFORMATION DISSEMINATION

13.1. Study Records and Document Retention

Complete, accurate, and current study records will be maintained and stored in a secure manner throughout the duration of the study and additionally for five years after study close-out. Study records include administrative documentation— including all reports and correspondence relating to the study — as well as documentation related to each participant screened and/or enrolled in the study, including informed consent forms, locator forms, case report forms, notations of all contacts with the participant, and all other source documents. Necessary documents to be maintained by the site before, during and after the study will be in accordance with GCP regulations and guidance. A complete list of needed documentation will be provided prior to initiation of the study and will appear in the study manual.

13.2. Information Dissemination and Publication Plan

Study findings will be disseminated through presentations, reports and publication in peer-reviewed journals and other publications. In-country data and country specific information will be made available to national policy-makers, organizational, and implementing partners. A detailed publication and communication plan will be developed that describes the number and types of reports and manuscripts planned for the study and criteria for authorship. Formal presentations at conferences and scientific publications will follow CDC, Harvard, and MOH guidelines. A data sharing plan will also be developed that details the requirements and process for requesting access to stored data and samples for sub-studies and future analysis.

14. REFERENCES

Ades, A. E., K. Claxton, et al. (2006). "Evidence synthesis, parameter correlation and probabilistic sensitivity analysis." Health Econ 15(4): 373-381.

Alam, S., R. Meyer, et al. (2009). A model for HIV spread in a South African village, Lecture Notes in Computer Science. 5269: 33-45.

Alkema, L., A. E. Raftery, et al. (2008). "Bayesian melding for estimating uncertainty in national HIV prevalence estimates." Sex Transm Infect 84 Suppl 1: i11-i16.

Anglaret, X. and S. Eholié. (2012). "Early antiretroviral treatment and/or early isoniazid prophylaxis against tuberculosis in HIV-infected adults (ANRS 12136 TEMPRANO)." from http://clinicaltrials.gov/ct2/show/NCT00495651.

Attia, S., M. Egger, et al. (2009). "Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis." AIDS 23(11): 1397-1404.

Auvert, B., D. Taljaard, et al. (2005). "Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial." PLoS Med 2(11): e298.

Bablok, W. and H. Passing (1985). "Application of statistical procedures in analytical instrument testing." J Automat Chem 7(2): 74-79.

Bailey, R. C., S. Moses, et al. (2007). "Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial." Lancet 369(9562): 643-656.

Barth, R. E., J. T. van der Meer, et al. (2008). "Effectiveness of highly active antiretroviral therapy administered by general practitioners in rural South Africa." Eur J Clin Microbiol Infect Dis 27(10): 977-984.

Bendavid, E., M. L. Brandeau, et al. (2010). "Comparative effectiveness of HIV testing and treatment in highly endemic regions." Arch Intern Med 170(15): 1347-1354.

Bennett, D. E., R. J. Camacho, et al. (2009). "Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update." PLoS One 4(3): e4724.

Bershteyn, A., D. J. Klein, et al. (2012). "Description of the EMOD-HIV Model v0. 7." arXiv preprint arXiv:1206.3720.

Bierman, W. F., M. A. van Agtmael, et al. (2009). "HIV monotherapy with ritonavir-boosted protease inhibitors: a systematic review." AIDS 23(3): 279-291.

Bland, J. M. and D. G. Altman (1986). "Statistical methods for assessing agreement between two methods of clinical measurement." Lancet 1(8476): 307-310.

Blitzstein, J. and P. Diaconis (2006). "A sequential importance sampling algorithm for generating random graphs with prescribed degrees. Available at: http://www-stat.stanford.edu/~cgates/PERSI/papers/GraphAlgorithm.pdf."

Bouckaert, R., J. Heled, et al. (2014). "BEAST 2: a software platform for Bayesian evolutionary analysis." PLoS Comput Biol 10(4): e1003537.

Boulle, A., G. Van Cutsem, et al. (2008). "Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy." JAMA 300(5): 530-539.

Braunstein, S. L., D. Nash, et al. (2011). "Dual testing algorithm of BED-CEIA and AxSYM Avidity Index assays performs best in identifying recent HIV infection in a sample of Rwandan sex workers." PLoS One 6(4): e18402.

Brazier, J., J. Roberts, et al. (2002). "The estimation of a preference-based measure of health from the SF-36." J Health Econ 21(2): 271-292.

Brazier, J., T. Usherwood, et al. (1998). "Deriving a preference-based single index from the UK SF-36 Health Survey." J Clin Epidemiol 51(11): 1115-1128.

Briggs, A. H., M. C. Weinstein, et al. (2012). "Model parameter estimation and uncertainty analysis: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force Working Group-6." Med Decis Making 32(5): 722-732.

Central Statistics Office (2009). Botswana AIDS Impact Survey III (BAIS III), 2008. C. Kebonyethebe Johane. Gaborone, Botswana.

Central Statistics Office (2009). Botswana Demographic Survey 2006. Gaborone, Central Statistics Office.

Central Statistics Office (2009). Project Document: 2011 Population and Housing Census. Available at: http://unstats.un.org/unsd/censuskb20/Attachment453.aspx. Gaborone, Central Statistics Office.

Charalambous, S., C. Innes, et al. (2007). "Evaluation of a workplace HIV treatment programme in South Africa." AIDS 21 Suppl 3: S73-78.

Coetzee, D., K. Hildebrand, et al. (2004). "Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa." AIDS 18(6): 887-895.

Cohen, M. S., Y. Q. Chen, et al. (2011). "Prevention of HIV-1 infection with early antiretroviral therapy." N Engl J Med 365(6): 493-505.

Cole, S. R., M. A. Hernan, et al. (2003). "Effect of highly active antiretroviral therapy on time to acquired immunodeficiency syndrome or death using marginal structural models." Am J Epidemiol 158(7): 687-694.

Cornell, M., M. Schomaker, et al. (2012). "Gender differences in survival among adult patients starting antiretroviral therapy in South Africa: a multicentre cohort study." PLoS Med 9(9): e1001304.

Curran, K., E. Njeuhmeli, et al. (2011). "Voluntary medical male circumcision: strategies for meeting the human resource needs of scale-up in southern and eastern Africa." PLoS Med 8(11): e1001129.

Danel, C., R. Moh, et al. (2015). "A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa." N Engl J Med 373(9): 808-822.

DART Virology Group and Trial Team (2006). "Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa." AIDS 20(10): 1391-1399.

Delfraissy, J. F., P. Flandre, et al. (2008). "Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naive HIV-infected patients." AIDS 22(3): 385-393.

Drummond, M., R. Brown, et al. (2003). "Use of pharmacoeconomics information--report of the ISPOR Task Force on use of pharmacoeconomic/health economic information in health-care decision making." Value Health 6(4): 407-416.

Duong, Y. T., R. Kassanjee, et al. (2015). "Recalibration of the limiting antigen avidity EIA to determine mean duration of recent infection in divergent HIV-1 subtypes." PLoS One 10(2): e0114947.

Duong, Y. T., M. Qiu, et al. (2012). "Detection of Recent HIV-1 Infection Using a New Limiting-Antigen Avidity Assay: Potential for HIV-1 Incidence Estimates and Avidity Maturation Studies." PLoS One 7(3): e33328.

Eaton, J. W., T. B. Hallett, et al. (2011). "Concurrent sexual partnerships and primary HIV infection: a critical interaction." AIDS Behav 15(4): 687-692.

Enns, E. A. and M. L. Brandeau (2011). "Inferring model parameters in network-based disease simulation." Health Care Manag Sci 14(2): 174-188.

EuroQol Group (1990). "EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group." Health Policy 16(3): 199-208.

Fauci, A. S. and G. K. Folkers (2012). "Toward an AIDS-free generation." JAMA 308(4): 343-344.

Felsenstein, J. (1985). "Confidence Limits on Phylogenies: An Approach Using the Bootstrap." Evolution 39(4): 783-791.

Felsenstein, J. (2004). Inferring Phylogenies. Sunderland, MA, Sinauer Associates.

Finkelstein, D. M. (1986). "A proportional hazards model for interval-censored failure time data." Biometrics 42(4): 845-854.

Fox, M. P., P. Ive, et al. (2010). "High rates of survival, immune reconstitution, and virologic suppression on second-line antiretroviral therapy in South Africa." J Acquir Immune Defic Syndr 53(4): 500-506.

Fox, M. P. and S. Rosen (2010). "Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review." Trop Med Int Health 15 Suppl 1: 1-15.

Gail, M. H., D. P. Byar, et al. (1992). "Aspects of statistical design for the Community Intervention Trial for Smoking Cessation (COMMIT)." Control Clin Trials 13(1): 6-21.

Gandhi, N. R., A. P. Moll, et al. (2009). "Successful integration of tuberculosis and HIV treatment in rural South Africa: the Sizonq'oba study." J Acquir Immune Defic Syndr 50(1): 37-43.

Gardner, E. M., M. P. McLees, et al. (2011). "The spectrum of engagement in HIV care and its relevance to test-and-treat strategies for prevention of HIV infection." Clin Infect Dis 52(6): 793-800.

Gold, M. R., J. E. Siegel, et al., Eds. (1996). Cost effectiveness in health and medicine. New York, Oxford University Press.

Gray, R. H., G. Kigozi, et al. (2007). "Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial." Lancet 369(9562): 657-666.

Gray, R. T., M. H. Ghaus, et al. (2011). "Expected epidemiological impact of the introduction of a partially effective HIV vaccine among men who have sex with men in Australia." Vaccine 29(36): 6125-6129.

Grinsztejn, B. and P. Mugyenyi. (2012). "Management using the latest technologies in resource-limited settings to optimize combination therapy after viral failure (MULTI-OCTAVE)." from http://clinicaltrials.gov/ct2/show/record/NCT01641367.

Habicht, J. P., C. G. Victora, et al. (1999). "Evaluation designs for adequacy, plausibility and probability of public health programme performance and impact." Int J Epidemiol 28(1): 10-18.

Hallett, T. B., J. M. Baeten, et al. (2011). "Optimal uses of antiretrovirals for prevention in HIV-1 serodiscordant heterosexual couples in South Africa: a modelling study." PLoS Med 8(11): e1001123.

Halperin, D. T. (2009). "Combination HIV prevention must be based on evidence." Lancet 373(9663): 544-545.

Hammond, R. and T. C. Harry (2008). "Efficacy of antiretroviral therapy in Africa: effect on immunological and virological outcome measures -- a meta-analysis." Int J STD AIDS 19(5): 291-296.

Handcock, M. S., D. R. Hunter, et al. (2003). "Software tools for the Statistical Modeling of Network Data. available at: http://statnet.org."

Hayes, R., F. Mosha, et al. (1995). "A community trial of the impact of improved sexually transmitted disease treatment on the HIV epidemic in rural Tanzania: 1. Design." AIDS 9(8): 919-926.

Hayes, R. and L. H. Moulton (2009). Cluster Randomized Trials. Boca Raton, CRC Press.

Helleringer, S. and H. P. Kohler (2007). "Sexual network structure and the spread of HIV in Africa: evidence from Likoma Island, Malawi." AIDS 21(17): 2323-2332.

Holmes, C. B., R. Wood, et al. (2006). "CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: implications for prophylaxis and treatment." J Acquir Immune Defic Syndr 42(4): 464-469.

Hussey, M. A. and J. P. Hughes (2007). "Design and analysis of stepped wedge cluster randomized trials." Contemp Clin Trials 28(2): 182-191.

Isogai, P. K., S. Rueda, et al. (2012). "Prediction of health preference values from CD4 counts in individuals with HIV." Med Decis Making: Epub 8 Aug 2012.

Jani, I. V., N. E. Sitoe, et al. (2011). "Accurate CD4 T-cell enumeration and antiretroviral drug toxicity monitoring in primary healthcare clinics using point-of-care testing." AIDS 25(6): 807-812.

Jelsma, J., E. Maclean, et al. (2005). "An investigation into the health-related quality of life of individuals living with HIV who are receiving HAART." AIDS Care 17(5): 579-588.

Johnson, L. F., L. Alkema, et al. (2010). "A Bayesian approach to uncertainty analysis of sexually transmitted infection models." Sex Transm Infect 86(3): 169-174.

Johnson, V. A., V. Calvez, et al. (2011). "2011 update of the drug resistance mutations in HIV-1." Top Antivir Med 19(4): 156-164.

Joint United Nations Programme on HIV/AIDS (UNAIDS) (2010). Report on the Global AIDS Epidemic: HIV Estimates with Uncertainty Bounds. Geneva, UNAIDS.

Jones, J. H. and M. S. Handcock (2003). "An assessment of preferential attachment as a mechanism for human sexual network formation." Proc Biol Sci 270(1520): 1123-1128.

Kim, A. A., T. Hallett, et al. (2011). "Estimating HIV Incidence among Adults in Kenya and Uganda: A Systematic Comparison of Multiple Methods." PLoS One 6(3): e17535.

Kreke, J. E., A. J. Schaefer, et al. (2004). "Simulation and critical care modeling." Curr Opin Crit Care 10(5): 395-398.

Laeyendecker, O., R. Brookmeyer, et al. (2012). "Specificity of four laboratory approaches for cross-sectional HIV incidence determination: Analysis of samples from adults with known non-recent HIV infection from five African countries." AIDS Res Hum Retroviruses.

Lancet (2010). "Evaluation: the top priority for global health." Lancet 375(9714): 526.

Lara, A. M., B. N. Wakholi, et al. (2008). "Utility assessment of HIV/AIDS-related health states in HIV-infected Ugandans." AIDS 22 Suppl 1: S123-130.

Leclerc, P. M., A. P. Matthews, et al. (2009). "Fitting the HIV epidemic in Zambia: a two-sex micro-simulation model." PLoS One 4(5): e5439.

Little, R. J. and D. B. Rubin (2002). Statistical Analysis with Missing Data. New York, John Wiley and Sons.

Losina, E., X. Anglaret, et al. (2006). "Impact of opportunistic diseases on chronic mortality in HIV-infected adults in Côte d'Ivoire." S Afr Med J 96(6): 526-529.

Losina, E., Y. Chang, et al. (2008). Immunologic benefits of complete vs partial virologic suppression in patients initiating ART in Gugulethu, South Africa. Abstract 823. 15th Conference on Retroviruses and Opportunistic Infections. Boston, MA.

Losina, E., Y. Yazdanpanah, et al. (2007). "The independent effect of highly active antiretroviral therapy on severe opportunistic disease incidence and mortality in HIV-infected adults in Cote d'Ivoire." Antivir Ther 12(4): 543-551.

Louwagie, G. M., M. O. Bachmann, et al. (2007). "Highly active antiretroviral treatment and health related quality of life in South African adults with human immunodeficiency virus infection: A cross-sectional analytical study." BMC Public Health 7: 244.

Lundgren, J. D., A. G. Babiker, et al. (2015). "Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection." N Engl J Med 373(9): 795-807.

Merson, M., N. Padian, et al. (2008). "Combination HIV prevention." Lancet 372(9652): 1805-1806.

Meyer-Rath, G. and M. Over (2012). "HIV treatment as prevention: modelling the cost of antiretroviral treatment--state of the art and future directions." PLoS Med 9(7): e1001247.

Modjarrad, K., E. Chamot, et al. (2008). "Impact of small reductions in plasma HIV RNA levels on the risk of heterosexual transmission and disease progression." AIDS 22(16): 2179-2185.

Montaner, J. S., V. D. Lima, et al. (2010). "Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study." Lancet 376(9740): 532-539.

Mtapuri-Zinyowera, S., M. Chideme, et al. (2010). "Evaluation of the PIMA point-of-care CD4 analyzer in VCT clinics in Zimbabwe." J Acquir Immune Defic Syndr 55(1): 1-7.

Nachega, J. B., D. M. Stein, et al. (2004). "Adherence to antiretroviral therapy in HIV-infected adults in Soweto, South Africa." AIDS Res Hum Retroviruses 20(10): 1053-1056.

Nei, M. and S. Kumar (2000). Molecular Evolution and Phylogenetics. New York, NY, Oxford University Press.

Novitsky, V., H. Bussmann, et al. (2013). "Phylogenetic relatedness of circulating HIV-1C variants in Mochudi, Botswana." PLoS One 8(12): e80589.

Novitsky, V., D. Kuhnert, et al. (2015). "Phylodynamic analysis of HIV sub-epidemics in Mochudi, Botswana." Epidemics 13: 44-55.

Novitsky, V., S. Lagakos, et al. (2009). "Evolution of proviral gp120 over the first year of HIV-1 subtype C infection. NIHMSID # 79286." Virology 383(1): 47-59.

Novitsky, V., S. Moyo, et al. (2014). "Impact of sampling density on the extent of HIV clustering." AIDS Res Hum Retroviruses 30(12): 1226-1235.

Novitsky, V., S. Moyo, et al. (2015). "Importance of Viral Sequence Length and Number of Variable and Informative Sites in Analysis of HIV Clustering." AIDS Res Hum Retroviruses 31(5): 531-542.

Novitsky, V., T. Ndung'u, et al. (2011). "Extended high viremics: a substantial fraction of individuals maintain high plasma viral RNA levels after acute HIV-1 subtype C infection." AIDS 25(12): 1515-1522.

Novitsky, V., R. Wang, et al. (2011). "Evolutionary gamut of in vivo Gag substitutions during early HIV-1 subtype C infection." Virology 421(2): 119-128.

Novitsky, V., R. Wang, et al. (2010). "HIV-1 subtype C-infected individuals maintaining high viral load as potential targets for the "test-and-treat" approach to reduce HIV transmission." PLoS One 5(4): e10148.

Novitsky, V., R. Wang, et al. (2009). "Timing constraints of in vivo gag mutations during primary HIV-1 subtype C infection." PLoS One 4(11): e7727.

Novitsky, V., R. Wang, et al. (2010). "Dynamics and timing of in vivo mutations at Gag residue 242 during primary HIV-1 subtype C infection." Virology 403(1): 37-46.

Novitsky, V., M. Zahralban-Steele, et al. (2015). "Long-Range HIV Genotyping Using Viral RNA and Proviral DNA for Analysis of HIV Drug Resistance and HIV Clustering." J Clin Microbiol 53(8): 2581-2592.

Padian, N. S., S. I. McCoy, et al. (2011). "Evaluation of large-scale combination HIV prevention programs: essential issues." J Acquir Immune Defic Syndr 58(2): e23-28.

Paltiel, A. (2001). "Model-based drug evaluation in chronic disease: promise, pitfalls, and positioning." Drug Information Journal 35: 131-139.

Phillips, A. N., D. Pillay, et al. (2011). "Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings." AIDS 25(6): 843-850.

Pitter, C., J. G. Kahn, et al. (2007). "Cost-effectiveness of cotrimoxazole prophylaxis among persons with HIV in Uganda." J Acquir Immune Defic Syndr 44(3): 336-343.

Poole, D. and A. E. Raftery (2000). "Inference for deterministic simulation models: The Bayesian melding approach." J Am Stat Assoc 95(452): 1244-1255.

Posada, D. (2008). "jModelTest: phylogenetic model averaging." Mol Biol Evol 25(7): 1253-1256.

Prabhu, V. S., P. G. Farnham, et al. (2011). "Cost-effectiveness of HIV screening in STD clinics, emergency departments, and inpatient units: a model-based analysis." PLoS One 6(5): e19936.

Pujades-Rodriguez, M., D. O'Brien, et al. (2008). "Second-line antiretroviral therapy in resource-limited settings: the experience of Medecins Sans Frontieres." AIDS 22(11): 1305-1312.

Rajeev, K. H., B. Y. Yuvaraj, et al. (2012). "Impact of HIV/AIDS on quality of life of people living with HIV/AIDS in Chitradurga district, Karnataka." Indian J Public Health 56(2): 116-121.

Rehle, T., L. Johnson, et al. (2015). "A Comparison of South African National HIV Incidence Estimates: A Critical Appraisal of Different Methods." PLoS One 10(7): e0133255.

Robberstad, B. and J. A. Olsen (2010). "The health related quality of life of people living with HIV/AIDS in sub-Saharan Africa - a literature review and focus group study." Cost Eff Resour Alloc 8: 5.

Rosen, S. and M. P. Fox (2011). "Retention in HIV Care between Testing and Treatment in Sub-Saharan Africa: A Systematic Review." PLoS Med 8(7): e1001056.

Salichos, L. and A. Rokas (2013). "Inferring ancient divergences requires genes with strong phylogenetic signals." Nature 497(7449): 327-331.

Salichos, L., A. Stamatakis, et al. (2014). "Novel information theory-based measures for quantifying incongruence among phylogenetic trees." Mol Biol Evol 31(5): 1261-1271.

Saravanan, S., V. Madhavan, et al. (2012). "Darunavir Is a Good Third-Line Antiretroviral Agent for HIV Type 1-Infected Patients Failing Second-Line Protease Inhibitor-Based Regimens in South India." AIDS Res Hum Retroviruses.

Schneider, K., T. Puthanakit, et al. (2011). "Economic evaluation of monitoring virologic responses to antiretroviral therapy in HIV-infected children in resource-limited settings." AIDS 25(9): 1143-1151.

Seyler, C., C. Adje-Toure, et al. (2007). "Impact of genotypic drug resistance mutations on clinical and immunological outcomes in HIV-infected adults on HAART in West Africa." AIDS 21(9): 1157-1164.

Shapiro, R. L., M. D. Hughes, et al. (2010). "Antiretroviral regimens in pregnancy and breast-feeding in Botswana." N Engl J Med 362(24): 2282-2294.

Simpson, K. N., M. P. Luo, et al. (2004). "Cost-effectiveness of lopinavir/ritonavir versus nelfinavir as the first-line highly active antiretroviral therapy regimen for HIV infection." HIV Clin Trials 5(5): 294-304.

Society for Medical Decision Making and International Society for Pharmacoeconomics and Outcomes Research. "Joint modeling good research practices task force." Retrieved December 30, 2012, from http://www.ispor.org/taskforces/GRPModelingTf.asp.

Stamatakis, A. (2006). "RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models." Bioinformatics 22(21): 2688-2690.

Stamatakis, A. (2014). "RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies." Bioinformatics 30(9): 1312-1313.

Stover, J., L. Bollinger, et al. (2011). "Estimating the Impact and Cost of the WHO 2010 Recommendations for Antiretroviral Therapy." AIDS Res Treat 2011: 738271.

Stover, J., B. Fidzani, et al. (2008). "Estimated HIV trends and program effects in Botswana." PLoS One 3(11): e3729.

Stover, J., E. L. Korenromp, et al. (2011). "Long-term costs and health impact of continued global fund support for antiretroviral therapy." PLoS One 6(6): e21048.

Sullivan, P. W., W. F. Lawrence, et al. (2005). "A national catalog of preference-based scores for chronic conditions in the United States." Med Care 43(7): 736-749.

Tanser, F., T. Bärnighausen, et al. (2013). "High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa." Science 339(6122): 966-971.

Tengs, T. O. and T. H. Lin (2002). "A meta-analysis of utility estimates for HIV/AIDS." Med Decis Making 22(6): 475-481.

Tran, B. X., A. Ohinmaa, et al. (2012). "Quality of life profile and psychometric properties of the EQ-5D-5L in HIV/AIDS patients." Health Qual Life Outcomes 10(1): 132.

Tsiatis, A. A. (2006). Semiparametric Theory and Missing Data. New York, Springer.

Uhler, L. M., N. Kumarasamy, et al. (2010). "Cost-effectiveness of HIV testing referral strategies among tuberculosis patients in India." PLoS One 5(9).

UNAIDS (2010). "Global Report: UNAIDS Report on the Global AIDS Epidemic. Available at: http://www.unaids.org/globalreport/global_report.htm. Accessed February 2012.".

UNAIDS. (2011). "Manual for costing HIV facilities and services." Retrieved December 14, 2012, from http://www.unaids.org/en/media/unaids/contentassets/documents/document/2011/20110523_manual_costing_HIV_facilities_en.pdf.

UNAIDS (2013). WHO/UNAIDS Technical Update on HIV Incidence Assays for Surveillance and Epidemic Monitoring. Geneva, Joint United Nations Programme on HIV/AIDS: 6 pages.

UNAIDS (2014). 90-90-90: An ambitious treatment target to help end the AIDS epidemic. Geneva.

Ventelou, B., Y. Arrighi, et al. (2012). "The macroeconomic consequences of renouncing to universal access to antiretroviral treatment for HIV in Africa: a micro-simulation model." PLoS One 7(4): e34101.

Walensky, R. P., E. L. Ross, et al. (2012). The cost-effectiveness of treatment as prevention: analysis of the HPTN 052 trial. Abstract FRLBC01. XIX International AIDS Conference. Washington DC, USA.

Walensky, R. P., R. Wood, et al. (2008). "Scaling up antiretroviral therapy in South Africa: the impact of speed on survival." J Infect Dis 197(9): 1324-1332.

Wallis, C. L., M. A. Papathanasopoulos, et al. (2010). "Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1." J Virol Methods 163(2): 505-508.

Wandeler, G., O. Keiser, et al. (2012). "Tenofovir in second-line ART in Zambia and South Africa: collaborative analysis of cohort studies." J Acquir Immune Defic Syndr 61(1): 41-48.

Wang, B., N. Kumarasamy, et al. (2006). Incidence of opportunistic infections (OIs) within specific CD4 strata in HIV-infected patients in Southern India. Abstract WEPE0279. XVI International AIDS Conference, Toronto, Canada.

Weinstein, M. C., B. O'Brien, et al. (2003). "Principles of good practice for decision analytic modeling in health-care evaluation: report of the ISPOR Task Force on Good Research Practices--Modeling Studies." Value Health 6(1): 9-17.

Weinstein, M. C., J. E. Siegel, et al. (1996). "Recommendations of the Panel on Cost-effectiveness in Health and Medicine." JAMA 276(15): 1253-1258.

World Health Organization (2011). When and how to use assays for recent infection to estimate HIV incidence at a population level. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. Geneva, World Health Organization: 48 pages.

World Health Organization (2015). Guideline on When to Start Antiretroviral Therapy and on Pre-exposure Prophylaxis for HIV. Geneva, Switzerland.

World Health Organization Division of Mental Health and Prevention of Substance Abuse (1997). WHOQOL: Measuring Quality of Life. Geneva, Switzerland.

Wouters, E., W. Van Damme, et al. (2008). "Impact of baseline health and community support on antiretroviral treatment outcomes in HIV patients in South Africa." AIDS 22(18): 2545-2548.

Zhang, Z., L. Sun, et al. (2007). "Regression analysis of failure time data with informative interval censoring." Stat Med 26(12): 2533-2546.

Zhou, Z., N. Wagar, et al. (2011). "Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings." PLoS One 6(11): e28184.

Zimpel, R. R. and M. P. Fleck (2007). "Quality of life in HIV-positive Brazilians: application and validation of the WHOQOL-HIV, Brazilian version." AIDS Care 19(7): 923-930.