CURRICULUM VITAE

Kristen A. Stafford, MPH, PhD

Department of Epidemiology and Public Health

University of Maryland School of Medicine

Department of Epidemiology and Public Health
University of Maryland School of Medicine
660 West Redwood St. Howard Hall Rm. 100
Baltimore, MD 21201
Email: kristenastafford@gmail.com

Contact Information

kristenastafford@gmail.com

Education

2010 – 2015 University of Maryland School of Medicine, Baltimore
Graduate Program in Life Sciences
Department of Epidemiology and Public Health
Doctor of Philosophy

2003 – 2004 Johns Hopkins University
Bloomberg School of Public Health
Master of Public Health
Health Policy and Management

1991 – 1995 College of Notre Dame of Maryland
Bachelor of Arts, Psychology; Research Track

Employment History

University of Maryland School of Medicine; Baltimore

August 2010 to May 2015

Graduate Research Assistant – *Perform independent and mentored research while completing my Doctoral studies.*

- Dissertation Topic: The effect of age on the outcomes of combination antiretroviral treatment in resource limited settings.

Institute of Human Virology, University of Maryland; Baltimore

May 2007 to August 2010

Program Director – Outcomes & Evaluation – *Provide strategic vision and direction for data collection, analysis and use for an 8 country 400,000 patient President's Emergency Plan for AIDS Relief (PEPFAR) funded care and treatment program. Develop strategy, methodology and tools to evaluate patient and program level outcomes in over 200 treatment facilities. Direct quality improvement strategies and activities of 25 staff in 8 countries.*

- Served as the program wide Director for the Outcomes & Evaluation Program supporting 180 medical technical staff and 240 clinics in 8 resource limited countries
- Designed and implemented a comprehensive patient level outcomes project gathering health and program factors related to viral suppression from over 30,000 patients in 6 countries
- Directed analysis of patient and program factors for relationship to viral suppression and other health outcomes
- Disseminated statistical findings to both health professionals and lay persons in usable and interpretable fashion
- Designed and implemented program evaluation tools to measure the degree to which care and treatment models had been implemented in each health facility
- Designed program wide continuous quality improvement strategy and trained over 100 health professionals in Training of Trainer formats
- Managed content input and supervised 25 internationally based quality improvement staff
- Developed new partnerships and funding streams through grant and proposal writing activities
- Co-Developed and implemented the medical management and evaluation plan for sustained viral suppression including data collection, analysis and reporting
- Managed the implementation and analysis of the medical management and evaluation plan in the nine member countries

- Developed and directed continuous quality improvement programs for the use of patient health information for adaptive continuity care delivery based on real time medical outcomes
- Served as lead on the successful submission of 4 international care and treatment education grants totaling $10,000,000 for five years
- Provided direct technical assistance to Anti-Retroviral Therapy (ART) clinics in Africa and the Caribbean related to quality improvement, data use and program evaluation

May 2005 – May 2007

Technical Program Manager – PEPFAR – AIDSRelief Program. *Coordinate all administrative, policy, and logistical activities for executing international HIV/AIDS treatment training and care program for eight resource limited countries. Evaluate subcontract operations, identify and implement program improvements. Provide technical assistance including needs assessments, training, monitoring and evaluation of healthcare programs. Track agency activities, compile programmatic and financial data, prepare and submit status reports internally and to sponsoring agency. Act as a liaison between University, Division Director, subcontracted sites and sponsor. Assist in the development and implementation of training programs.*

- Serve as leadership representative to lead agency and consortium members
- Coordinate activities of the Institute with AIDSRelief consortium members
- Serve as the representative of the Institute during in country deployments
- Manage ART curriculum development and publication
- Coordinate with Senior Faculty responsible for curriculum content
- Manage curriculum work group assignments and facilitate task completion
- Provide drug forecasting for nine countries based on ARV expert and Lead Faculty drug mix for 40,000 HIV+ patients
- Monitor drug usage and provide analysis of drug mix agreement, patient scale up and supply chain needs to ensure consistent access to ART
- Edit, publish, and distribute quarterly medical technical updates to member countries
- Provide program management for training and technical assistance related to ART for the Caribbean and Africa

November 2004 – May 2005

Regional Program Manager – PEPFAR – AIDSRelief Program *Provide program management for 3 African and 2 Caribbean countries.*

- Primary liaison between in-country AIDSRelief teams and the Institute of Human Virology
- Provide technical assistance to ART clinics in Africa and the Caribbean related to adherence and program development.
- Develop scopes of work for medical and adherence technical assistance

- Provide comprehensive reporting on activities for lead agency and HRSA

July 2001 to August 2010

Independent Consultant, Baltimore, Maryland – *Provide professional consultant services on behalf of independently contracted groups related to program infrastructure, contractual requirements, service delivery assessment, quality improvement practices, program materials, and program development.*

- Performed thorough evaluation and assessment of HIV/AIDS Case Management and related services for the Chicago Eligible Metropolitan Area provided by 52 sub-contracted service providers
- Provided in-depth written analysis of the Standards of Care for Case Management Services for the Chicago Eligible Metropolitan Area including recommendations for improvement
- Performed a nationwide survey of management information systems for the tracking of client level service data provided to recipients of Federal funds
- Developed and provided recommendations regarding service area wide data tracking for the Chicago Eligible Metropolitan Area
- Developed and produced a Consumer Advisory Brochure for the state of West Virginia related to HIV+ consumer involvement in program development and analysis
- Provided consulting services to the Baltimore City Health Department

Baltimore City Health Department (BCHD), Baltimore, Maryland

May 2002 - July 2003

Quality Improvement Program Coordinator – *Responsible for the development, implementation, coordination and evaluation of the EMA wide Quality Improvement Program project for Ryan White CARE Act Title I federally funded categories.*

- Design and implement continuum wide QI program.
- Supervise and assist with the development and implementation of a Continuous Quality Improvement Plan by service category.
- Assist in the identification of indicators to be measured by category.
- Review and finalize data collection tools and methods.
- Formulate reports based on data collected.
- Establish QI project focus goals and supervise all QIP staff.
- Review data, project goals and scope.
- Document, monitor and measure solutions to issues found.
- Assess effectiveness of improvement intervention.
- Act as point of contact for both federal and local Administrative Agency.

- Provide systemic change and policy recommendations resulting from data abstraction findings.
- Coordinate Baltimore participation as one of five cities in the Federal Institute for Healthcare Improvement collaborative.
- Responsible for recruiting, educating and coordinating HIV care improvement collaborative including three Primary Medical Care providers, two Case Management providers and a policy level Response Team.
- Provide assistance and guidance to all participating agencies regarding instituting tests of change.
- Report to the HIV/AIDS Bureau of the Health Resources and Services Administration division of the Federal Government regarding all quality indicators and improvement projects.

May 2001 – May 2002

Public Health Analyst – *Responsible for 70 contracts totaling $5.2 million dollars, federal and local contract compliance, and vendor performance activities for Ryan White CARE Act Title I funded vendors in coordination with Ryan White Program Manager.*

- Review monthly and quarterly program and fiscal reports to review compliance with local and federal requirements for program operation and performance.
- Provide technical assistance to vendors to enhance quality of services, capacity and continuum of care to meet federal and local program requirements.
- Write comprehensive program reports per vendor based on findings from program report reviews, site visits and other appropriate monitoring activities.
- Inform management of program successes and deficiencies regarding vendor compliance and quality of services.
- Develop and monitor corrective action plans for noncompliant vendors as needed in collaboration with program management.
- Prepare performance summaries, spending analyzation within and across priority categories, presentations and other reports for BCHD management and oversight agencies.
- Represent BCHD at Planning Council, consortium, State AIDS Administration and other committee meetings as appropriate.
- Provided written segments and participated in the development of the Ryan White Title I grant application to HRSA for approximately $18 million dollars.

Baltimore Pediatric HIV Program, Baltimore, Maryland

March 2000 – May 2001

Site Coordinator/Family Services Worker - *Provided Case Management and service coordination to 70 families infected/affected with HIV/AIDS*

- Maintained and compiled all program data including demographics and client level service data.

- Researched and wrote reports on goal progress and achievement for all program funders, including the Baltimore City Health Department, Department of Human Resources, AIDS Administration and the Administration on Children, Youth and Families.
- Wrote three grants and obtained $45,000 in targeted funding for BPHVIP.
- Performed capacity building activities for program expansion.
- Provided supervision of program staff.
- Developed training materials for system wide presentations.
- Represented BHIVP to Ryan White Title IV and Title I Planning Council Committees.

Moveable Feast, Baltimore, Maryland

July 1999 - March 2000

Coordinator of Client Services – *Coordinated and implemented the Moveable Feast Program providing services to over 1000 people living with HIV/AIDS.*

- Provided comprehensive needs assessments at intake and quarterly thereafter for all clients.
- Coordinated applicable services based on individual client needs
- Ensured appropriate service administration based on clients specialized needs.
- Performed community outreach and education for consumers and vendors
- Monitored and reported on program compliance with all federal grant requirements and licensing administrations.
- Formulated and maintained linkage agreements with collaborative organizations.
- Provided outreach and service linkage to under-represented and difficult to access communities.
- Provided client advocacy services for all clients, and non-clients who contacted the agency for services.
- Represented MF to boards and committees throughout the state continuum of care.
- Performed grant writing for Ryan White and HOPWA funded services obtaining approximately $425,000 in program funding.
- Maintained and presented statistical information on client demographics, utilization and trends.
- Supervised Client Services staff.

Department of Public Safety and Correctional Services, Jessup, Maryland

July 1997 - July 1999

Prevention Case Manager – *Provided long term behavioral modification program focusing on risk-reduction and recidivism issues to over 500 incarcerated adults within eighteen months of their release from incarceration.*

- Facilitated and implemented AIDS Administration Prevention Case Management Program.
- Ensured facility program compliance with all grant requirements.
- Provided training for new Prevention Case Managers within the Division of Corrections.
- Provided one on one counseling and intervention services to inmates.
- Authored intervention module on feelings and emotions/degrees of response.
- Developed and implemented HIV re-entry education program for all inmates within one month of release.
- Provided resources and after-care planning to assist ex-offenders in accessing HIV/AIDS care or maintaining sero-negativity and to reduce individual recidivism.
- Provided pre and post-test counseling to inmates voluntarily tested for HIV.

ReVisions Behavioral Health System, Catonsville, Maryland

August 1996 - May 1997

Case Manager– *Provided comprehensive case management services to 50 chronically mentally ill adults including hospital release planning, resource linkage, crisis intervention, Psychosocial Assessment, development and monitoring of Individual Rehabilitation Plans and benefit application and maintenance.*

Epilepsy Association of Maryland, Towson, Maryland

July 1995 - August 1996

Resource Counselor – *Provided services related to community living, employment, housing, daily living skills for 17 individuals with epilepsy and related neurological disorders.*

Honors and Awards

2013 - Otani Award for superior academic performance and consistent service and support to the overall goals of GPILS by improving the quality of academic studies among fellow students.
University of Maryland School of Medicine

Graduate Program in Life Sciences

2013 - Trudy Bush Award for Outstanding Research in Women's Health and Epidemiology
University of Maryland School of Medicine
Department of Epidemiology and Public Health

2013 - Nomination and acceptance into the Excellence in Science Program of the American Association for the Advancement of Science (AAAS)

2010 - Outstanding Service Award
AIDSRelief Project
Catholic Relief Services

Professional Society Memberships

1995 – Present	Psi Chi; Psychology Honor Society
2004 – Present	American Public Health Association
2007 – Present	International AIDS Society
2013 – Present	The American Association for the Advancement of Science

Teaching Services

2014	Teaching Assistant for PREV 659 – Observational Epidemiology
2014	Facilitator – Medical Student Small Group Epidemiology Workshops
2014	Invited Lecturer – Towson University Physician Assistant Program; Course: *Epidemiology of HIV* Course Instructor: Theresa Neumann, PA-C
2013	Teaching Assistant for PREV 668 – Environmental and Occupational Health
2013	Teaching Assistant for PREV 659 – Observational Epidemiology
2013	Teaching Assistant for PREV 749 – Infectious Disease Epidemiology
2013	Facilitator – Medical Student Small Group Epidemiology Workshops
2013	Invited Lecturer – Towson University Physician Assistant Program; Course: *Epidemiology of HIV* Course Instructor: Theresa Neumann, PA-C
2012	Teaching Assistant for PREV 659 – Observational Epidemiology
2012	Teaching Assistant for PREV 600 – Introduction to Epidemiology
2012	Invited Lecturer - Johns Hopkins Bloomberg School of Public Health*;* Course*:Pharmaceutical Management for Under-served Populations.* Course Instructor: Dr. Maria Eng
2012	Invited Lecturer – Towson University Physician Assistant Program; Course: *Epidemiology of HIV* Course Instructor: Theresa Neumann, PA-C
2011	Invited Lecturer - Johns Hopkins Bloomberg School of Public Health*; Course:Pharmaceutical Management for Under-served Populations.* Course Instructor: Dr. Maria Eng
2011	Teaching Assistant for PREV 619 – Biostatistical Computing

2004 Teaching Assistant for Problem Solving in Public Health (JHSPH)

Grant Support

2010 – 2015 GRA support for my PhD studies provided through the Clinical Care and Research Division of the Institute of Human Virology, University of Maryland School of Medicine

Publications

(Refereed Journals)

Riedel DJ, Cox ER, **Stafford KA**, Gilliam BL. Clinical presentation and outcomes of prostate cancer in an urban cohort of predominantly african american, human immunodeficiency virus-infected patients. Urology. 2015;85(2):415-422. doi: 10.1016/j.urology.2014.09.054 [doi].

Riedel DJ, Gilliam BL, Cox ER, **Stafford KA**. Reply. Urology. 2015;85(2):422. doi: 10.1016/j.urology.2014.09.058 [doi].

Osinusi-Adekanmbi O, **Stafford K**, Ukpaka A, et al. Long-term outcome of second-line antiretroviral therapy in resource-limited settings. J Int Assoc Provid AIDS Care. 2014;13(4):366-371.

Fisher LH, **Stafford KA**, Fantry LE, Gilliam BL, Riedel DJ. Cancer knowledge and opportunities for education among HIV-infected patients in an urban academic medical center. J Cancer Educ. 2014. doi: 10.1007/s13187-014-0714-y [doi].

Stafford KA, Boutin M, Evans SR, Harris AD. Difficulties in demonstrating superiority of an antibiotic for multidrug-resistant bacteria in nonrandomized studies. Clin Infect Dis. 2014;59(8):1142-1147. doi: 10.1093/cid/ciu486 [doi].

Manheimer,E.; van der Windt,D.; Cheng,K.; **Stafford,K**.; Liu,J.; Tierney,J.; Lao,L.; Berman,B.M.; Langenberg,P.; Bouter,L.M. The effects of acupuncture on rates of clinical pregnancy among women undergoing in vitro fertilization: A systematic review and meta-analysis. Hum Reprod Update. 2013;19(6):696-713. doi: 10.1093/humupd/dmt026 [doi].

Stafford KA, Sorkin JD, Steinberger EK. Influenza vaccination among cancer survivors: Disparities in prevalence between blacks and whites. J Cancer Surviv. 2013;7(2):183-190. doi: 10.1007/s11764-012-0257-3

Anthony Amoroso, MD; Martine Etienne-Mesubi, Dr PH; Anthony Edozien, MD; Sylvia Ojoo, MBBS, MRCP, DTM&H; Robert Sheneberger, MD; Michael Obiefune, MBBS; Mian Bazle Hossain, PhD; **Kristen Stafford, MPH**; Robert R Redfield, MD. Treatment outcomes of recommended first-line antiretroviral regimens in resource-limited clinics. J Acquir Immune Defic Syndr. 2012;60(3):314-320. doi: 10.1097/QAI.0b013e31824e5256

Constance Shumba MSc, Peter Memiah DrPH, Ruth Atukunda MIPH, Richard Imakit BSc, Jairus Mugadu MBChB, **Kristen Stafford MPH**. *Continuous quality improvement in AIDSReliefsupported HIV treatment clinics in Uganda: An evaluation of "See-Try-Observe- Continue"(STOC) model in patient care*. Int. J. Med. Public Health. Vol 2 (1) 2012.

Etienne M, Hossain M, Redfield R, **Stafford K**, Amoroso A. Indicators of adherence to antiretroviral therapy treatment among HIV/AIDS patients in 5 african countries. J Int Assoc Physicians AIDS Care (Chic). 2010;9(2):98-103. doi: 10.1177/1545109710361383

(Abstracts):

Mwangi, EI., Amoroso, A., Watson, D., **Stafford, K**., Etienne-Mesubi, M., Hossain, M., Nwizu, C., Niyang, M., Enejoh, V., Olutola, A., Memiah, P., Agbor, S., Redfield, R. *Virologic outcomes of first-line antiretroviral treatment in HIV-positive children in AIDSRelief clinics in Nigeria. Abstract MOPE043.* XIX International AIDS Conference. Washington D.C., 22-27 July 2012.

Olukemi Osinusi, **Kristen Stafford**, Adiba Ukpaka, Donald Salami, Samuel Ajayi, Nicaise Ndembi, Alash'le Abimiku, Chidi Nwizu, Bruce Gilliam, Anthony Amoroso. *Long-term Durability of Second Line ART Regimens in Patients with Multiple Baseline NRTI Resistance Mutations in a Resource Limited Setting.* XIX International AIDS Conference. Abstract 1954. Washington D.C., 22-27
July 2012.

Etienne M, Amoroso A, Edozien A, Obiefune M, Sheneberger R, Bositis C, **Stafford K**, Hossain M, Redfield R. *A Global Clinical Comparison of TDF+3TC+NVP vs. TDF+3TC+EFV in Resource Constrained Populations. Paper 566.* 18th Annual Conference on Retroviruses and Opportunistic Infections. Boston, MA. 27 February - 2 March 2011.

Donahue, J., Foreit, K., **Stafford, K.,** Oluoch, M., Burrows, L., Jefferson, B. *Managing treatment and prevention programs for sustainability: the Site Capacity Assessment (SCA) Tool.* XVIII International AIDS Conference. Abstract THPE0778. Vienna, Austria. 18 – 23 July 2010.

R. Atukunda, C. Shumba, R. Imaki, P.Memiah, **K. Stafford**, A. Edozien. *Using small tests of change in continuous quality improvement: Uganda AIDSRelief experience.* XVIII International AIDS Conference. Abstract TUPE0844. Vienna, Austria. 18 – 23 July 2010.

Stafford, K., Hossain, M., Mesubi, R., Etienne, M., Oshi, R., Bositis, A., Bositis, C., Henley, Y., Zaremba, T., Seruyange, H., Amoroso, A. *Viral suppression outcomes of patients using nevirapine in combination with tenofovir and cytosine analog (3TC or FTC) as first line regimen in resource limited settings.* Abstract CDB080. 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention. 19 – 22 July 2009. Capetown, South Africa

Stafford K., Mwangi I., Medina-Moreno S., Were S., Amoroso A., *Long term durability of ART: 36 month on treatment analysis of 338 patients in Sub-Saharan Africa. :* AIDS 2008 – XVII International AIDS Conference: Abstract no. MOPE0050

A. Amoroso, I. Mwangi, S. Medina-Moreno, S. Were, **K. Stafford** *Treatment outcomes comparison of AIDSRelief supported sites initiated in the first and second years of PEPFAR. :* AIDS 2008 - XVII International AIDS Conference: Abstract no. MOPE0069

K. Stafford, I. Mwangi, M. Hossain, I. Wanyeki, N. Smith, A. Amoroso *Age at ART initiation and its impact on viral outcomes. :* AIDS 2008 - XVII International AIDS Conference: Abstract no. TUPE0097"

Stafford K, Hossain M., Amoroso, A. *Probability of patients remaining on their initial HAART regimen at one year. :* AIDS 2008 - XVII International AIDS Conference: Abstract no. TUPE0098

Mian B. Hossain, PhD, Martine Etienne, DrPH, Anthony Amoroso, MD and **Kristen A. Stafford,** MPH *Relationship between Antiretroviral Therapy Treatment (ART) knowledge and disclosure of HIV status to support groups among HIV/AIDS Patients in Uganda, Kenya and Zambia* APHA 136th Annual Meeting 2008

Martine Etienne, Mian B. Hossain, Anthony Amoroso, **Kristen Stafford**. *Relationship between Perceived Quality of Care and Adherence in Antiretroviral Therapy Treatment among HIV/AIDS Patients in Uganda, Kenya and Zambia.* Population Association of America Annual Meeting 2008.

Stafford K., Etienne M., Aina O., Lewin S., Bositis A., Bositis C., Doherty A., Sheneberger R., Amoroso A., *Time to event similarities between deceased patients and patients lost to follow up after initiating antiretroviral therapy (ART)in Zambia. :* 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention, 2007: Abstract no. CDB017

Etienne M, Aina O, Mesubi O, **Stafford K**, Kapilikisha H. Kiama B, Amoroso, A. *Provider assessment of patient adherence: a poor predictor of viral suppression in resource limited settings. Poster exhibition:* 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention, 2007: Abstract no. WEPEB100

Stafford K., Etienne M., Aina O., Lewin S., Bositis A., Bositis C., Doherty A., Sheneberger R., Amoroso A., *Immunological improvement and viral suppression after the initiation of antiretroviral therapy (ART) in Zambia. :* 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention, 2007: Abstract no. CDB292

Invited Speeches and Presentations

Stafford, K. Notre Dame of Maryland University. Psi Chi National Honor Society induction ceremony. Keynote speaker. April 2015

Stafford, K. *Developing and Implementing Quality Improvement Programs in Resource Limited Settings.* Catholic Relief Services. Annual Global Health Conference. September 21, 2011

Jefferson, B., **Stafford, K**., Burrows, L., Donahue, J., Foreit, K. *The site capacity assessment tool and other mechanisms to monitor transition status.* Eighth Annual President's Emergency Plan for AIDS Relief Track 1.0 ART Program Meeting. Maputo, Mozambique. 2010

Stafford, K. *Developing Quality Improvement Programs in Resource Limited Settings.* Walter Reed Army Medical Center – AIDS Initiative. June 2, 2010

Wanyeki, I., **Stafford, K.** *From Aggregate Indicators to Impacting Patients: Data Use to Inform Treatment and Improve Care.* Seventh Annual President's Emergency Plan for AIDS Relief Track ART Program Meeting. Dar es Salaam, Tanzania. 2009

Stafford, K. *Domestic Lessons from Ryan White: Can they be applied Internationally?* Sixth Annual President's Emergency Plan for AIDS Relief Track 1.0 ART Program Meeting. Washington, DC. 2008

Stafford, K., Bass, P. *Innovative Monitoring & Evaluation, Quality Management, and Mentoring: Monitoring & Evaluation.* Fifth Annual President's Emergency Plan for AIDS Relief Track 1.0 ART Program Meeting. Atlanta, GA. 2007.

Stafford, K. University of Delaware. Master of Business Administration Program *Public Health Programs and their Intersection with Marketing.* April 7, 2006.

Stafford, K. Gilead Sciences, Inc. *Comprehensive HIV Care in Resource Limited Settings; Progress, Challenges, and Ways Forward.* September 15, 2005.

Stafford, K. Catholic Medical Mission Board. Annual Meeting. *Providing Antiretroviral Treatment in Resource Limited Settings.* June 9, 2005

Stafford, K. Owings Mills High School. World AIDS Day Remembrance. Keynote Speaker. December 1, 2001.

Stafford, K. Maryland Congressional Black Caucus. *HIV in Maryland: Challenges faced by kinship caregivers.* May 15, 2000.

ABSTRACT

Dissertation Title: The effect of age on the outcomes of combination antiretroviral treatment in resource limited settings

Kristen A. Stafford, Doctor of Philosophy, 2015

Dissertation Directed By:

Mona Baumgarten, PhD
Professor
Department of Epidemiology and Public Health

Background: HIV is one of the most closely monitored epidemics in the world. Despite this, little attention has been placed on older adults living with HIV, especially in resource limited settings.

Objectives: The objectives of this research were to estimate 1) the association between age at combination antiretroviral therapy (cART) initiation and mean CD4 cell count over time by strata of baseline CD4 cell count as well variability of immune reconstitution, and 2) whether older age is associated with more rapid regimen change due to cART associated toxicities and side-effects.

Methods: We conducted a retrospective cohort study of adults who initiated cART between August 1, 2004 and September 1, 2012 in 157 PEPFAR funded clinics supported by AIDSRelief in four countries in sub-Saharan Africa.

Results: Of the 452,819 patients enrolled, 181,354 met the study eligibility criteria. Patients age 40 and older had significantly lower mean CD4 cell counts and less

variability as compared to patients aged 20 – 39 with each strata of baseline CD4 cell count up to five years after cART initiation. The differences in mean CD4 cell count were more pronounced in the higher strata of baseline CD4 cell count than in lower strata. Older patients progressed to regimen change due to toxicity or side-effect more rapidly than younger patients within regimens containing D4T and AZT. There was no difference in the hazard of regimen change within TDF containing regimens comparing older to younger patients.

Conclusions: While we found statistically significant differences at most time points following the initiation of cART for all strata, it was only in the highest strata of baseline CD4 cell count (> 350 cells/mm^3) that the difference between age groups was what we, *a priori*, defined as an important difference of 50 cells. Older groups may demonstrate less variability in CD4 cell reconstitution than younger groups. The faster progression to regimen change among older adults on D4T and AZT warrants a discussion on closer monitoring of older patients for toxicity and side-effects earlier after the initiation of cART.

THE EFFECT OF AGE ON THE OUTCOMES OF COMBINATION ANTIRETROVIRAL TREATMENT IN RESOURCE LIMITED SETTINGS

by
Kristen A. Stafford

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2015

DEDICATION

To my family.

ACKNOWLEDGEMENTS

First and foremost I would like to thank the patients and the staff of the AIDSRelief program for whom it was an honor to serve.

I would like to thank Dr. Mona Baumgarten for her unwavering support and guidance throughout my doctoral studies, and most especially during the entire dissertation process. I am extremely grateful for her mentorship, insight, training, compassion, countless hours of time, and commitment. Her direction made me and this study better than I could hope to express. I would additionally like to thank my committee members: Dr. Laurence Magder for all of his time and thoughtfulness in helping me craft the analysis and interpretation of this work; Dr. Laura Hungerford for her methodological expertise and helping me never lose sight of either the forest or the trees; Dr. Samer El-Kamary for his clinical insight and support of both me and this work; Dr. Jack Guralnik for his expertise in aging related research and helping me see the broader context to which we could contribute; and to Dr. Robert Redfield, my mentor for over a decade and the reason I was able to undertake this journey. His vision, commitment, passion, and emphatic belief that we can end this epidemic have inspired me throughout my career. I do not have the words to thank him adequately for his belief in me.

I would like to acknowledge and thank my friends and colleagues from the AIDSRelief consortium at Catholic Relief Services, the Catholic Medical Mission Board, the Children's AIDS Fund, International Medical Assistance World Health, and Futures Group; most especially Ms. Lanette Burrows and Mr. Bobby Jefferson. Thank you to my

friends and colleagues at the Institute of Human Virology for all of their hard work and support, especially Ms. Kathy Vardjan and Ms. Carrie Wallace. To Dr. David Riedel for his collaboration and feedback. To Dr. Anthony Amoroso for all of the hard work, sacrifice, long hours, and long flights; but most especially for his friendship.

I would like to acknowledge and thank the faculty and staff of the Department of Epidemiology and Public Health for their support, guidance, and the opportunities they offered me, particularly Dr. Anthony Harris, Dr. Kerri Thom, Dr. Sania Amr, Dr. John Sorkin, Dr. Patricia Langenberg, Dr. O. Colin Stine, Dr. Patrick McArdle, Ms. Danielle Fitzpatrick, and Ms. Jessica Kelley.

I would like to acknowledge the University of Maryland College Park for use of their GRACE server to initially clean and format my data.

Thank you to my classmates for all of their passion, hard work, dedication, and humor. Special thanks to Dr. Modupe Coker, Ms. Marie-Claude Lavoie, Mr. Max Masnick, Mr. Chris Pepin, Dr. Lindsay Croft, Ms. Danielle Abraham, Ms. Kara Moser, Ms. Shana Burrows, and Mr. Wayne Chang. This process would have been a lot harder without them.

I would like to thank all of my friends for their support throughout this journey, beginning with all the time I was overseas up through the last five years of this process. Most especially Ms. Megan Schneebaum, Mr. Jason Rosenberg, their son Felix, and their daughter Luna. Their friendship and support mean more than I can say.

And finally, I would like to acknowledge my family. To my sister, Ms. Terri Thomas, who was my first and is still my most important friend. Her unconditional support throughout my life has helped to shape who I am today and I am forever grateful. To my brother-in-law, Mr. Cole Thomas whose love, support, and humor I am lucky to have in my life. To my nephew Master Jacob Thomas whose love, kindness, and creativity inspire me every day; and for the much needed Plants vs. Zombie breaks. To my niece, Miss Anna Thomas whose love, independence, intelligence, and humor make my world a better place; and for always calling her Aunt to check on her.

To my parents, Mr. Michael Stafford and Mrs. Angela Stafford. I would like to thank them for being my first and best teachers. I thank them for supporting every crazy decision I have ever made throughout my life, including quitting a perfectly good job to go back to school. They are the two people by whom I compare the character of all others I meet. The thing I am proudest of in this world is being their daughter.

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CHAPTER I. INTRODUCTION

The objective of this research was to examine the association between age at initiation of combination antiretroviral therapy (cART) and treatment outcomes in resource limited settings. Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) is one of the most closely monitored epidemics in the world. Despite this, little attention has so far been placed on older adults living with HIV, especially in resource limited settings. Little is known about whether the response to cART in these settings varies by age, particularly within strata of baseline CD4 cell count. Similarly, whether older age is associated with more rapid development of treatment associated toxicities and side-effects is not well documented.

The President's Emergency Plan for AIDS Relief (PEPFAR) was the largest commitment by any nation in the world to combat a single disease. At the initiation of PEPFAR in 2003, there were approximately 25,000 people on cART in the 15 PEPFAR focus countries in sub-Saharan Africa, the Caribbean, South America, and Asia (OGAC, 2004). As of the end of 2010, PEPFAR had provided cART to over 3.2 million people (OGAC, 2011). This program has been an unprecedented success in increasing access to lifesaving cART and reducing mortality (Hontelez et al., 2012).

The AIDSRelief program was a Track 1.0 PEPFAR funded program. AIDSRelief was a five member consortium consisting of Catholic Relief Services, the Institute of Human Virology of the University of Maryland School of Medicine, Future Group, Catholic

Medical Mission Board, International Medical Association World Health, and the Children's AIDS Fund. AIDSRelief provided support to over 290 clinics and 700,000 individuals living with HIV in nine countries.

Despite the massive investment of resources and the global attention the HIV epidemic in sub-Saharan Africa has received, important populations have been largely neglected. Most of the HIV/AIDS indicators do not include data from adults age 50 and over. For example, the United Nations General Assembly Special Assembly on HIV/AIDS and the Joint United Nations Programme on HIV/AIDS (UNAIDS) report prevalence only for adults age 15-49 (UNAIDS, 2009). Similarly, the HIV indicators for the Millennium Development Goals only require reporting for people between the ages of 15 and 24 (UNSD, 2012). Many developing countries in sub-Saharan Africa perform demographic and health surveys. However, they often only interview women up to age 49 and men up to age 59 (MeasureDHS, 2012). While the United Nations General Assembly High Level Meeting on AIDS committed in 2011 to achieving universal cART coverage (UN, 2011), it failed to address the changing age distribution of persons affected by the HIV/AIDS epidemic worldwide (J. Negin, Mills, & Albone, 2011). This lack of focus on the older population may be due in part to how the emergency response was originally designed, with particular attention towards the most vulnerable, that is, mothers and children (Mills, Beyrer, Birungi, & Dybul, 2012). This focus was also driven by the availability of inexpensive medication which made it feasible to implement widespread mother-to-child transmission prevention programs in resource limited settings (J. Negin, Mills, Barnighausen, & Lundgren, 2012). As resources and efforts expanded worldwide, other

vulnerable populations such as children and young adults received attention. However, older adults have received little attention so far. As cART access continues to expand and the population continues to age, an understanding of HIV infection in older adults will be critical to the long-term sustainability and management of the epidemic in sub-Saharan Africa. The aims and hypotheses of this study were:

Aim 1. To determine the effect of older age at start of treatment on immunologic response to cART in HIV-infected adults in sub-Saharan Africa.

Hypothesis: Older age at the initiation of treatment (compared to younger age) will be associated with less immunologic improvement and less variability as measured by the mean and variance of CD4 cell count.

Aim 2. To determine the effect of older age at start of treatment on the time to regimen change due to antiretroviral therapy-related toxicity and side-effects, within treatment regimen, in HIV-infected adults in sub-Saharan Africa.

Hypothesis: Within each treatment regimen, older age at the initiation of treatment (compared to younger age) will be associated with a shorter time to regimen change due to the development of toxicity and side-effects.

CHAPTER II. BACKGROUND

There is little information on the prevalence of HIV infection in people age 50 and older in resource limited settings such as sub-Saharan Africa. Negin and Cummings (Joel Negin & Cumming, 2010) estimated that, at the end of 2007, there were approximately 3 million people age 50 and older living with HIV in sub-Saharan Africa, representing over 14% of all people age 15 and older living with HIV. They also estimated that the prevalence of HIV infection is 4% among the 74 million people age 50 and older in sub-Saharan Africa. Another recent study by Hontelez et al. projected that the number of HIV-positive older adults in sub-Saharan Africa will increase dramatically over the next 30 years, rising from 3.1 million in 2011 to 9.1 million by 2040 (Hontelez et al., 2012). The authors also project that this increase in the number of older adults with HIV will be accompanied by a drop in the number of HIV-infected younger adults (age 15-34) from 12.1 million to 10.8 million by 2040 (Hontelez et al., 2012). These projected changes will result in a dramatic shift in the age composition of the infected population in sub-Saharan Africa, highlighting the need for more information on HIV treatment and outcomes among older adults. It is estimated that approximately one out of every eight people living with HIV in sub-Saharan Africa is at least 50 years of age (Joel Negin & Cumming, 2010). As life expectancy continues to increase among people on combination antiretroviral therapy (cART) (Mills et al., 2011), and as older adults continue to become infected, the number of older adults living with HIV will continue to grow. This could present a significant challenge to the health care systems in these settings, as well as to the course of the epidemic in the future (Joel Negin et al., 2012).

Treatment outcomes: The provision of effective cART has been shown to increase survival among people infected with HIV in resource limited settings (Herbst et al., 2009; Mills et al., 2011; P. C. Mutevedzi, Lessells, Rodger, & Newell, 2011). Dramatic declines in HIV related mortality have been observed since access to cART has increased (Herbst et al., 2009; Mermin et al., 2008). These declines in mortality have led to increased life expectancy among people living with HIV in resource limited settings who access treatment. Prior to the expanded access to cART in resource limited settings, approximately 80% of people presenting with an AIDS defining illness died within two years (Kitayaporn et al., 1996). Since the expansion of cART access, life expectancy for HIV positive individuals on treatment has risen to approximately 80% of the life expectancy of an HIV negative individual (WHO, 2013b). In a recent study based on data from Uganda, Mills et al. estimated that people starting cART at 20 years of age are likely to live to age 47; at 35, individuals can expect to live to age 63, and at age 50, individuals can expect to live to 74 years of age (Mills et al., 2011). Because of these changes, the age distribution of those infected with HIV is beginning to shift towards older groups. This emerging age trend among people living with HIV/AIDS worldwide can be explained by two factors: the increased incidence of infection in older age groups and the increased duration of HIV infection in the presence of treatment (Hontelez et al., 2012).

Few studies to date have focused on the effect of age on treatment outcomes other than mortality, or on outcomes beyond the first year of therapy. A study from Malawi found that there was no significant difference in survival comparing patients age 50 to 59 to those age 25 to 49 (J. Negin, van Lettow, et al., 2011). Three studies from sub-Saharan Africa found that younger patients had significantly higher mean gains in CD4 cell count compared to older patients; however, these three studies only examined mean gains at six and 12 months following cART initiation (Balestre et al., 2012; Greig, Casas, O'Brien, Mills, & Ford, 2012; Maskew, Brennan, Macphail, Sanne, & Fox, 2012). The current study adds to the literature by addressing treatment outcomes beyond the first year of therapy. Additionally, this study provides a unique focus on the effect of age on treatment outcomes within specific regimen groups. This focus will assist providers in identifying the effect of age on regimen durability (i.e., how long a patient remains on their first cART regimen) and tolerability (i.e., the absence of side-effects or toxicities necessitating a regimen change) which will aid in the long term management of older HIV infected patients.

Disease progression and aging: Increasing age is well-established in the scientific literature as an independent risk factor for a number of morbidities including cardiovascular disease, cancer, bone loss, and central nervous system disorders as well as reduced kidney and liver function. The natural decline of the body's ability to produce naïve lymphocytes, as well as the loss of memory T cells that is associated with aging is

also of significant concern regarding older adults with HIV/AIDS who have compromised immune systems (Adler et al., 1997; Effros et al.).

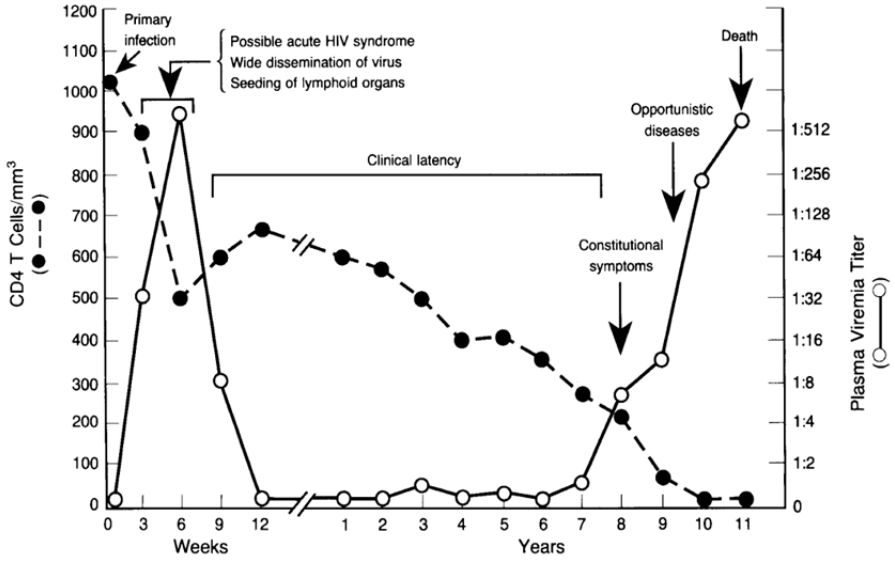
Studies have shown that chronic morbidities such as diabetes, cardiovascular disease, hypertension, kidney disease, liver disease, depression, and some cancers are common among people living with HIV, regardless of age (Christensen, Doblhammer, Rau, & Vaupel, 2009; Grabar, Weiss, & Costagliola, 2006; Mayosi et al., 2009). As people living with HIV age, and when older adults become infected with HIV, these underlying morbidities may have an important impact on the progression of HIV and on the effects of cART, potentially giving rise to higher rates of side-effects and toxicity in older than younger HIV-infected patients (Portia C. Mutevedzi & Newell, 2011).

HIV pathophysiology and immune system degradation: HIV begins its life cycle by binding to CD4 receptors and one of two co-receptors (C-C chemokine receptor 5 or C-X-C chemokine receptor 4) on the surface of a $CD4^+$ T-lymphocyte. After fusing with the cell, viral RNA is released and reverse transcription occurs, turning single stranded RNA into DNA. The DNA then moves to the nucleus of the cell and the viral enzyme integrase "hides" the virus within the cell's own DNA, thus incorporating the provirus DNA with the host cell DNA. When the host cell is activated, RNA polymerase makes copies of the genomic material and mRNA is used to make long chains of HIV proteins. Protease then cuts the chains into smaller individual proteins which join with the HIV

RNA and assemble new virus particles. The new virus particles then bud from the host cell, taking part of the CD4+ T-lymphocyte's outer envelope, and move on to infect other cells. Each host cell, once infected, can produce as many as one billion copies per day.

During the primary infection phase, there is rapid and widespread dissemination of the virus corresponding with a large acute drop in the number of CD4 cells in the blood stream. HIV elicits an immune response which works to decrease the amount of detectable circulating virus in the blood stream, which is usually followed by a long clinically latent period. Over time the CD4 T-cell count continues to decrease, often reaching a threshold below which the risk for opportunistic infections dramatically increases (Figure 1) (Pantaleo, Graziosi, & Fauci, 1993).

Figure 1. Typical Course of HIV Infection



The rate of CD4 replacement is one of the most important factors related to clinical response to cART (Grabar et al., 2000; Sabin et al., 2008). It has been shown that while patients initiating cART at lower CD4 count levels regain cells as quickly as those

initiating at higher CD4 counts, their maximum CD4 count is lower (Moore & Keruly, 2007).

It has been demonstrated that people living in sub-Saharan Africa have higher levels of immune activation as compared to people living in less resource limited environments (Dyer et al., 1998; Lawn, Butera, & Folks, 2001). This higher level of immune activation leads to a more rapid degradation of the immune system and thus a faster progression of HIV disease than is seen in the United States and Europe (Bentwich, Kalinkovich, & Weisman, 1995; Clerici et al., 2000; Dyer, Hoffman, Eron, Fiscus, & Cohen, 1999; Lawn et al., 2001; Rizzardini et al., 1998). As immune activation and HIV infection increase the propensity for apoptosis among CD4 cells (Deeks, Verdin, & McCune; Lawn et al., 2001; Molina-Pinelo et al., 2009), and the propensity for apoptosis of immune cells is also associated with aging (Sikora, 2015), it is plausible that the effects of immunosenescence may be observable at younger ages in resource limited settings as compared to richer nations.

A. The effect of age on CD4 cell count change after cART initiation

Most studies conducted on HIV infection among older adults have been based in developed countries (Althoff et al., 2010; Goetz, Boscardin, Wiley, & Alkasspooles, 2001; Greenbaum, Wilson, Keruly, Moore, & Gebo, 2008; Nogueras et al., 2006; Schmid et al.; Tumbarello et al., 2004). Several studies have reported that older patients exhibit

lower immune response after the initiation of cART compared to younger patients (Florence et al., 2003; Grabar et al., 2004; Le Moing et al., 2001; Nogueras et al., 2006; Viard et al., 2001). It has been postulated that while older adults may achieve viral suppression at the same rate as younger adults, there may be a reduced immune reconstitution in older adults (Kelly A Gebo, 2006). The effect of age identified in these studies was moderate and may be partly explained by preserved thymic function in younger patients associated with the reconstitution of naïve T cells (Cohen Stuart et al., 2002). Additionally, no clear age threshold was identified. One study found that while patients initiated on treatment at age 50 or older had robust immunologic response to therapy, they never achieved the same immunologic level as younger patients (Nogueras et al., 2006). Another study found that, among patients who had achieved viral suppression in response to treatment, a smaller proportion of patients age 55 and older achieved increases of between 50 and 150 cells in the first 18 months of therapy compared to younger patients(Goetz et al., 2001). Another study from the North American AIDS Cohort Collaboration on Research and Design investigated the hazard of gaining at least 100 cells in the first 24 months of therapy comparing patients age 18 to 30, 40 to 50, 51 to 60 and those over 60 to patients age 31 to 39 (Althoff et al., 2010). The study found a decreased hazard of gaining at least 100 cells in each of the age groups age 40 and older compared to those age 31 to 39. There was no significant interaction between age and initial treatment regimen. A study conducted in Italy found no association between age and CD4 cell count. Two smaller studies found no effect of age on immune reconstitution after the initiation of cART (Orlando et al., 2006; Tumbarello et al., 2004).

Little is known about the effect of age on immune response to cART in sub-Saharan Africa, and it is possible that the immunologic response to cART differs from what has been observed in Europe and the United States (Balestre et al., 2012). As mentioned previously, it has been shown that HIV positive patients in Africa present with higher T-cell activation compared to higher income countries (Bentwich et al., 1995; Clerici et al., 2000; Dyer et al., 1999; Lawn et al., 2001; Rizzardini et al., 1998). This higher level of activation has been linked to CD4 cell gains that are lower than observed in other settings after the initiation of cART (Hunt et al., 2003). It has therefore been postulated that there may be an association between age, decreased CD4 cell production, and increased immune activation that could result in a stronger association between age and immune response in patients treated for HIV in sub-Saharan Africa compared to the US or Europe (Balestre et al., 2012). Three studies to date have evaluated the effect of age on immunologic response in sub-Saharan Africa (Balestre et al., 2012; Maskew et al., 2012; P. C. Mutevedzi et al., 2011). First, the International epidemiological Database to Evaluate AIDS (IeDEA) in West Africa found that patients under the age of 30 gained an average of 22 cells/mm^3 more than patients at least 50 years of age (95% confidence interval (CI) 2 – 43) after 12 months on cART (Balestre et al., 2012). While this study suggests that there is a statistically significant difference in immune reconstitution after cART initiation when comparing the oldest patients to the youngest in their cohort, the study only considered the first 12 months following therapy. It is important to determine if this difference in mean CD4 cell count over time persists after the first year of therapy, or if over time the age-related difference in mean cell gain increases or diminishes.

While a difference of 22 cells is statistically significant, it may not be a clinically relevant difference among patients in the higher CD4 cell count strata who initiated cART (WHO, 2013a). Additionally, while the authors controlled for baseline CD4 cell count in the analysis, it is possible that the association between age and CD4 cell gain differs across strata of CD4 cell baseline count. These authors did not present differences in the mean and variability of CD4 cell count between older and younger patients within CD4 cell count strata as we did in our study. It has been demonstrated that individuals who start treatment at lower CD4 cell counts do not achieve the same level of immune reconstitution as individuals who begin treatment at higher CD4 cell counts (Moore & Keruly, 2007). If older patients are started on treatment with higher levels of immune suppression compared to younger patients, it would be difficult to determine if there is an association between age and immune restoration over time. By not comparing immune response between age groups among patients with the same baseline CD4 cell counts, it would be difficult to determine if differences in mean CD4 cell count observed were true age differences or if they could be attributed to differences in the degree of immune suppression at baseline. In order to determine if there is an effect of age on immune system reconstitution it is important to ensure that the comparison between older and younger patients is made among patients with the same degree of immune suppression when they start therapy.

The second study of the effect of age on immunologic response in sub-Saharan Africa examined a cohort of adult patients on cART in South Africa and found that patients 50

years of age or older were less likely to gain at least 50 cells/mm^3 compared to patients 30 years of age or younger. However, the outcome was evaluated only at six and 12 months after initiation of treatment (Maskew et al., 2012). In the third study of age and immunologic response in sub-Saharan Africa, carried out among adult patients in South Africa, a lower proportion of older patients (50 years of age or older) than younger patients (16 to 24 years of age) gained at least 50 cells/mm^3 by six months after start of therapy, and a larger proportion of older than younger patients failed to achieve a CD4 cell count of 200 or more cells/mm^3 by 12 months (P. C. Mutevedzi et al., 2011). Further investigation is needed on the difference between older and younger adults in CD4 cell reconstitution by baseline CD4 cell count, as well as beyond the first year of therapy.

Higher levels of immune activation in sub-Saharan Africa, as well as the presence of nutritional deficiencies, and widespread parasitic and bacterial infections may cause HIV disease to progress more rapidly than in settings such as the United States and Europe. Because of these factors it is plausible that differences in treatment outcomes may be observed by comparing patients age 40 and older to those under 40 as opposed to the United States and European convention of age 50 and older as the age cutoff.

B. Age and the development of cART-associated toxicity and side-effects

Overview of combination antiretroviral therapy in resource limited settings: The goals of antiretroviral therapy are multipronged as laid out by the Department of Health and Human Services Guidelines for the treatment of HIV-infected adults and in the

International Edition of the Medical Management of HIV Infection (Bartlett, Redfield, & Pham, 2013). The clinical goals of cART include prolonging life and reducing morbidity and mortality thus improving the quality of life of the patient. The virologic goal is the suppression of virus to inhibit disease progression. The immunologic goal is reconstitution of the immune system, measured both quantitatively (by CD4 cell counts reaching a normal range) and qualitatively (by pathogen specific response), so that patients' risk for infection by opportunistic pathogens is reduced. The therapeutic goal is a rational sequencing of regimens that support the previous goals while also maintaining treatment options, limiting drug toxicity and helping patients be adherent. The epidemiologic goal is to reduce HIV transmission.

The regimens used in the settings we investigated are primarily comprised of three drugs. The most common regimens contain a backbone of two drugs from the nucleoside reverse transcriptase inhibitor (NRTI) class and one drug from the non-nucleoside reverse transcriptase inhibitor (NNRTI) class. Alternately, a drug from the protease inhibitor (PI) class could be used as the third drug in the regimen. Lamivudine (3TC) or Emtricitabine (FTC), which are NRTIs, are present in all of the drug regimens used. Both drugs are generally well tolerated and are associated with minimal toxicity and low side-effect proportions (Bartlett et al., 2013). There are three drugs that are commonly used in the context of our study as the other NRTI in the backbone of the regimen. Stavudine (D4T) was recommended as the first-line drug throughout most of the resource limited world until 2010 (WHO, 2006). However, D4T has been shown to be an important cause of

mitochondrial toxicity including peripheral neuropathy, lipoatrophy, lactic acidosis, and hepatic steatosis (Cote et al., 2002). Clinical side-effects of D4T, such as nausea or diarrhea, are generally infrequent. Zidovudine (AZT) is known to cause severe anemia in some patients (Richman et al., 1987). AZT is also associated with gastrointestinal (GI) intolerance, myalgias, malaise and headaches (Tokars et al., 1993). Tenofovir disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor (NtRTI) often grouped with the NRTI class of drugs. Medications like TDF may cause injury to the renal system and nephrotoxicity is of greatest concern with regimens containing TDF (Lai et al., 2014; Peyriere et al., 2004). While GI symptoms have also been reported with this drug, they appear to be infrequent. Nevirapine (NVP) is an NNRTI commonly used in these settings. A risk for early hepatotoxicity, generally within the first few months of treatment, has been demonstrated with NVP (Haas et al., 2006). Severe rash requiring discontinuation of NVP has also been reported (van Leth et al., 2005). Efavirenz (EFV) is the other NNRTI commonly used in these settings. EFV has been associated with central nervous system side-effects such as confusion, abnormal dreams, and dizziness, and the side-effects may be dose related (van Luin et al., 2009). Lopinavir boosted with ritonavir (LOP) is a coformulated PI. The most common side-effect is diarrhea which can be of moderate severity. LOP has also been associated with fat accumulation (Bartlett et al., 2013).

Age and cART associated toxicity and side-effects: Through the natural process of aging, both liver and kidney function decline resulting in a reduction in the metabolism and

excretion of toxic substances, such as antiretroviral medications (Portia C. Mutevedzi & Newell, 2011). Due to this reduced functioning, older adults may develop toxicity and side-effects from cART at an increased rate compared to younger adults on the same regimens. We are unaware of any studies that focus specifically on the effect of age on the development of toxicity and side-effects after the initiation of cART, particularly in resource limited settings such as sub-Saharan Africa. Understanding the durability of cART is important, particularly in resource limited settings where treatment options are limited. Determining whether there are age-associated differences in the rates of toxicity and side-effect development by regimen, which lead to regimen changes, could affect how providers in resource limited settings monitor patients after the initiation of cART and thus improve the management of patients with HIV.

CHAPTER III. STUDY DESIGN AND METHODS

A. The AIDSRelief program

The President's Emergency Plan for AIDS Relief (PEPFAR) is the largest commitment by any nation in the world to combat a single disease (OGAC, 2004). At the initiation of PEPFAR in 2003 there were approximately 25,000 people on combination antiretroviral therapy (cART) in the 15 PEPFAR focus countries (OGAC, 2004). As of the end of 2010 PEPFAR had provided cART to over 3.2 million people (OGAC, 2011). AIDSRelief, a PEPFAR Track 1.0 partner, supported rapid expansion of HIV care and treatment services for poor and underserved people in 10 countries (Ethiopia, Guyana, Haiti, Kenya, Nigeria, Rwanda, South Africa, Tanzania, Uganda and Zambia). Valued at approximately $740 million over nine years, the program served more than 713,000 people, including more than 395,000 who received antiretroviral therapy through 276 treatment centers.

AIDSRelief worked largely through rural facilities and established basic packages of care and treatment that exceeded what many thought possible in a resource-constrained environment. Instead of merely offering HIV tests and dispensing medicine, AIDSRelief helped broad cadres of health workers to identify and manage treatment failure or other adverse drug events; to diagnose, treat, and prevent opportunistic infections such as tuberculosis or pneumonia; and to provide patients with adherence counseling and support, empowering them to effectively manage their own treatment.

AIDSRelief consortium partners included Catholic Relief Services as prime grantee; the University of Maryland School of Medicine Institute of Human Virology as technical lead for clinical care and treatment; Futures Group as lead agency for strategic information; IMA World Health and Catholic Medical Mission Board as implementing partners; and Children's AIDS Fund as a key sub-grantee supporting sites in three countries. By building clinical capacity and regularly monitoring patient outcomes, AIDSRelief endeavored to support its partners in delivering high-quality, sustainable HIV care.

AIDSRelief's medical model was rooted in the approach that care delivery of HIV treatment must be, first and foremost, medically driven. AIDSRelief's medical model incorporated the following components:

- Advocating for the most tolerable, durable, and efficacious therapeutic drug regimens available
- Adherence as a vital therapeutic intervention
- Disclosure
- Defined catchment area
- Longitudinal medical records
- On-site laboratory capacity
- Continuity of care with service extension from the clinic to the community
- Continuous quality improvement

B. Study design

This study was a retrospective cohort study of adults who initiated cART between August 1, 2004 and September 1, 2012 in 157 PEPFAR funded program facilities supported by the Institute of Human Virology's International HIV treatment program (AIDSRelief) in four countries in sub-Saharan Africa.

C. Data source

Electronic databases for AIDSRelief-supported facilities have been maintained since the inception of AIDSRelief. These databases contain all clinical, demographic, and programmatic information collected in the medical records. Patients were generally seen monthly, and the information from their monthly visits was recorded by clinicians on standardized patient management forms and then entered in the electronic databases at each facility by trained data entry staff. Data quality was maintained through monthly report generation, which allowed information from the electronic database at each facility to be compared to a random sample of the patient management forms. This helped to ensure the data entry was accurate and complete. Additionally, to aid in follow up of patients, a missed medication refill report was generated at each facility if a patient was seven or more days late for his or her refill. This report was then provided to community based adherence staff to ascertain patient status and promote medication continuity. Among patients who were traced by community based adherence staff, the primary reasons for having been originally classified as lost to follow-up were mortality and migration to another area of the country.

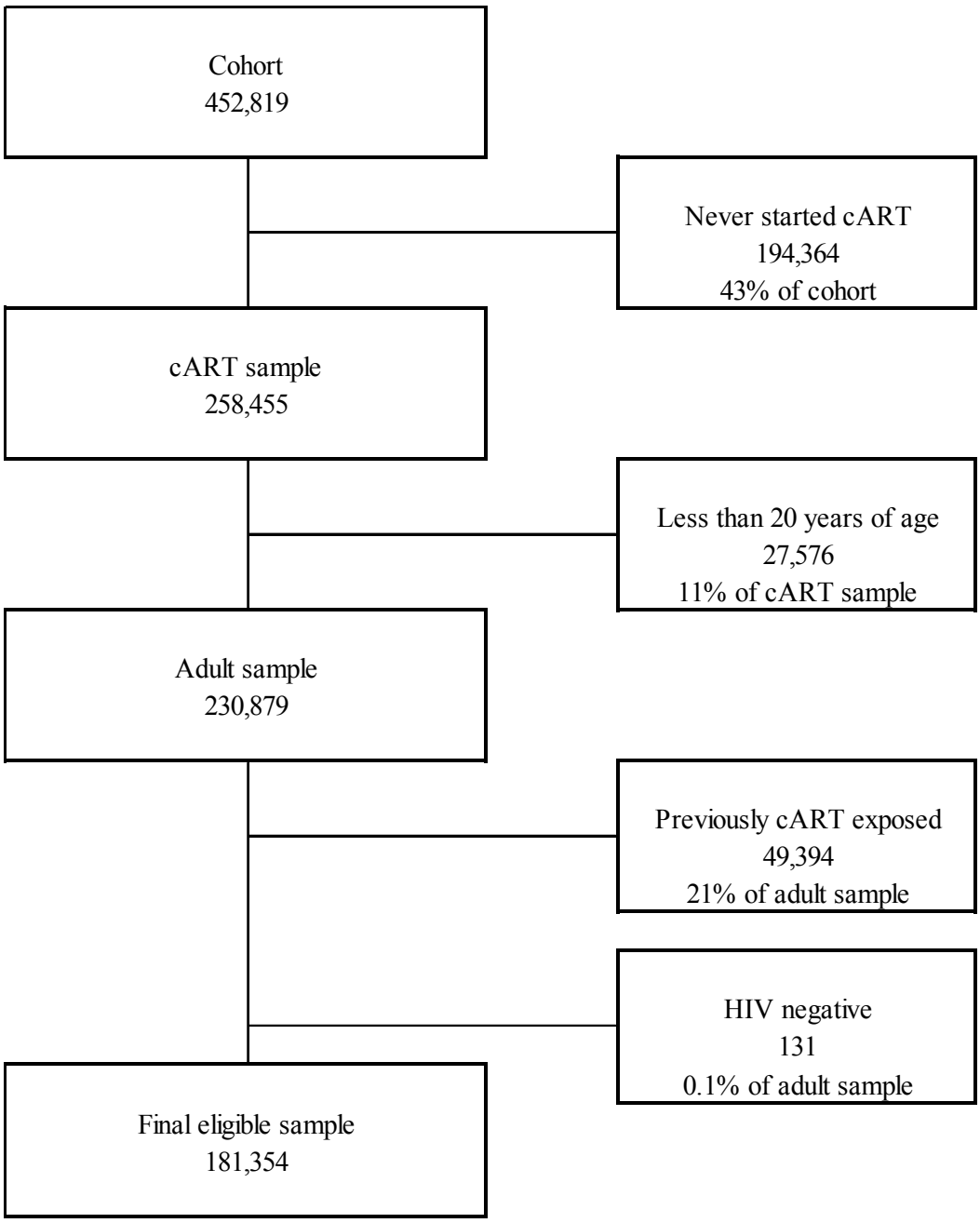
To ensure protection of patients' confidentiality while allowing for secondary analysis, a set of de-identified and anonymized databases of patient medical records from each AIDSRelief sites was created. These de-identified databases used standard definitions and met requirements specified in the Code of Federal Regulations for Human Subjects Research (45 CFR 46), revised January 15, 2009. All patients registered in the cART program at each facility were included, regardless of their status. All identifying patient information was stripped from the investigational databases and patients were assigned randomly-generated identification numbers and the key was not maintained. Clinic names were replaced with a randomly-assigned clinic identification number and a limited number of key variables were retained, including type of facility, type of location, province and district, and year activated. Patient level data were extracted from each facility database and combined into a country specific database. These country specific databases were then combined into one dataset for this study. Use of the de-identified data for research purposes was approved by the University of Maryland School of Medicine Institutional Review Board and by the ethics committees in each of the four countries where patients were enrolled.

Study patients were selected from the aforementioned de-identified study dataset. To be eligible for inclusion in the study sample, patients must have been HIV-positive, have a non-missing date of birth, have been at least 20 years of age at cART initiation, have had no previous exposure to antiretroviral therapy including single-dose nevirapine, and have had started on cART on or after August 1, 2004.

D. Study sample

The de-identified dataset contained 452,819 individuals enrolled in the program across all facilities in the four countries (see Figure 2). Of these, 181,354 (40%) were at least 20 years of age, were initiated on cART, and were treatment naïve at enrollment.

Figure 2. Study sample flow chart



E. Outcome variables

Aim 1: The outcome variable for Aim 1 was the number of CD4 cells/mm^3 at six month intervals following the initiation of cART.

CD4 cell count observations were obtained from the patient medical records. CD4 cell counts after baseline observation were binned by six month intervals based on the date of the initiation of cART. CD4 cell counts that were documented no earlier than 60 days before each six month time point or no later than 60 days after each six month time point were classified as having occurred at each six monthly time point. In the event that more than one CD4 cell count was recorded within the six monthly bin time window, the value recorded closest to the six month time point was used.

Aim 2: The outcome variable for Aim 2 was time to the occurrence of cART associated toxicities or cART-associated side-effects necessitating a regimen change after the cART initiation date.

Toxicity and side-effects necessitating a regimen change were documented in the patient medical records and were based on either laboratory values or clinician determination. cART toxicities included abnormal hemoglobin, abnormal liver functioning, abnormal renal functioning, central nervous system adverse events, hepatitis, jaundice, pancreatitis, skin rash, and clinically assessed peripheral neuropathy. Variables in the data set will be described below. Accuracy of information about regimen changes due to toxicity was evaluated by randomly selecting 50 patients who had toxicity documented as the reason

for regimen change and determining the associated laboratory values in the database. Significant side-effects were defined as nausea, vomiting, diarrhea, dizziness, suicidal ideation, or fat loss in the arms, legs, or face that resulted in a cART regimen change.

F. Predictor variable

The predictor variable for this study was the age group of study patients at the initiation of cART (40 years of age or older versus 20-39). Age was calculated as the cART initiation date minus the date of birth.

G. Covariates

Variables chosen for inclusion in the multivariable models were selected based on their effect on the primary measure of association in stratified analysis as well as the extent to which they improved model fit. Variables that were investigated as potential confounders in this study, included sex, CD4 count at cART initiation, baseline World Health Organization (WHO) disease stage (Appendix 1), baseline functional status (categorized as Working Ambulatory Bedridden) Stage, as assessed by the clinician (Appendix 2), first treatment regimen, baseline presence of active opportunistic infection (OI), baseline body mass index (BMI), and year of cART initiation (Amoroso et al., 2012; M. W. Brinkhof et al., 2008; Chi et al., 2010; Egger et al., 2002; Etard et al., 2006; Etienne, Hossain, Redfield, Stafford, & Amoroso, 2010; Greig et al., 2012). For our study, the choice of variables treated as confounders depended primarily on the results of the exploratory analyses and differed by study aim. Conceptual models for each study aim

based on findings from the literature and clinical knowledge are presented below (Figure 3 and 3b). The lines indicate the variables hypothesized to influence the age at which an individual started cART as well as the relationship between covariates. Appendix 3 includes the proposed minimally sufficient adjustment sets based on each conceptual model for each study aim, as well as the testable implications for conditional independence of variables.

Figure 3. Conceptual model of the association between age at cART initiation and mean CD4 count (Textor, Hardt, & Knuppel, 2011)

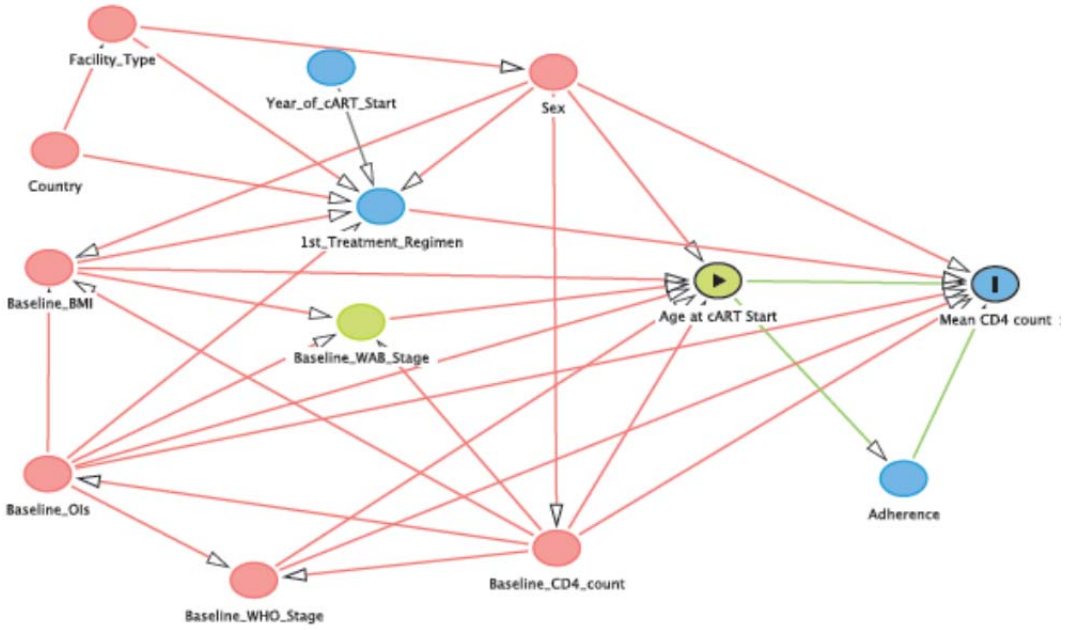
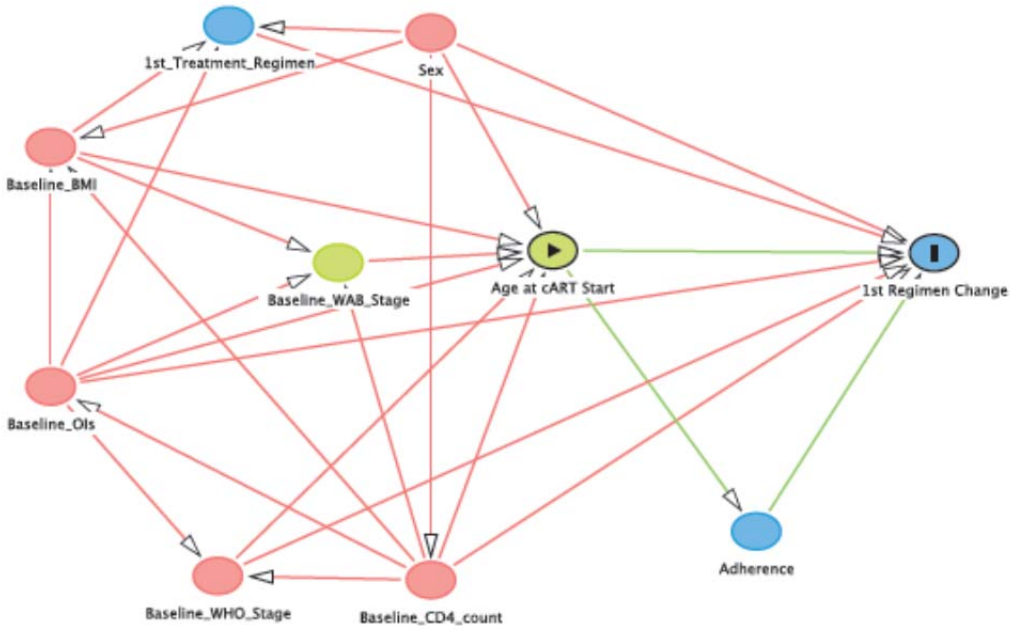


Figure 4. Conceptual model for the association between age at initiation of cART and time to first regimen change due to toxicity or significant side-effect



Baseline variables were included if they were obtained no more than three months prior to and no more than two weeks after cART initiation. Where more than one measurement was available, the measurement closest to the start of cART was used. BMI was analyzed in four categories: underweight (BMI less than 18.5 kg/m^2); normal (BMI 18.5-24.9); overweight (BMI 25.0-29.9); and obese (BMI 30.0 or greater) (CDC, 2011). Patients whose BMI was less than 10.0 or 50.0 or greater were treated as having missing baseline BMI.

Baseline CD4 count was classified into the five standard clinically relevant categories: 0-50, 51-100, 101-200, 201-350, and > 350 $cells/mm^3$ (DHHS, 2010).

Sex was classified as a binary variable (male or female).

Hepatotoxicity was categorized into six levels based on the grading of hepatotoxicity by the AIDS Clinical Trials Group (ACTG)(Bartlett et al., 2013). Laboratory values of alanine transaminase (ALT) between 10 and 40 international units per liter (IU/L) or aspartate aminotransferase (AST) between 10 and 34 IU/L were categorized as normal. AST or ALT levels one to two and a half times the upper limit of normal (ULN) were categorized as grade one, two and a half to five times the ULN were categorized as grade two, five to 10 times ULN were categorized as grade three, and values greater than 10 times ULN were categorized as grade four. Patients without a documented AST or ALT value were categorized as missing. Creatinine clearance was calculated using the Cockcroft-Gault equation and adjusted for female sex (Bartlett et al., 2013). Creatinine clearance was then categorized into seven levels with sex and age appropriate normal levels. For males, a creatinine clearance between 77-160 milliliters per minute (mL/min)

was categorized as normal. For females normal creatinine clearance is age specific. Normal values for females by age were 78-161 mL/min for 20 – 29 years of age, 72-154 mL/min for 30 – 39 years of age, 67-146 mL/min for 40 – 49 years of age, 62-139 mL/min 50 – 59 years of age, 56-131 mL/min for 60 – 90 years of age. Patients with creatinine clearance rates above the normal values were categorized as high. Other non-normal rates were categorized based on levels associated with progressing kidney disease that warrant ART dose adjustment. Patients without a documented serum creatinine value were categorized as missing. Hemoglobin was categorized into six levels. Hemoglobin values greater than 10 grams per deciliter (g/dL) were considered normal. Values between 8.5 and 10 g/dL were categorized as grade one, 7.5 to less than 8.5 g/dL were categorized as grade two, 6.5 to less than 7.5 g/dL were categorized as grade three, and less than 6.5 g/dL were categorized as grade four. Patients without a documented hemoglobin value were categorized as missing.

Because laboratory tests outside of CD4 cell count are clinically rather than protocol driven in these settings, we anticipated a large amount of missing data for AST, ALT, creatinine, and hemoglobin. However, it is important to note that missing values for these laboratory tests most likely indicate that performing the test was not clinically warranted and patients with missing values could be considered to have normal renal and hepatic functioning and no clinical symptoms of anemia. We ran the inferential models with patients with missing values categorized as missing as well as with patients with missing values categorized as normal and all abnormal values categorized as non-normal for each of these and found no difference in the primary estimate of effect.

Tuberculosis (National Center for HIV/AIDS), cryptococcal disease, and pneumocystis *jiroveci* pneumonia (PCP) were each categorized as binary (yes/no) variables. Patients without documentation of the presence of TB, cryptococcal disease, and PCP were categorized as not having active disease. Other OIs were grouped and categorized as a binary yes/no variable. Patients without documentation of the presence of an OI were categorized as not having an active OI.

H. Adherence

As adherence is thought to be differentially associated with age, is subsequent to initiation of cART, and is associated with the outcomes of interest, it is a possible mediator of the associations of interest. To determine whether adherence mediated the relationship between age and the outcomes of interest we ran each model with and without a variable for adherence and compared the estimates of effect for the association of age with the outcomes. If the estimate of effect was importantly different it would indicate the effect of age is mediated by adherence. Adherence was quantified by calculating the proportion of medication refills that were filled on time in the first year after the initiation of cART with a potential range of 0% to 100%. "On time" was defined as a positive difference of no more than seven between the number of pills dispensed and the number of days between medication refills.

I. Treatment regimens

The association between age and side-effects and toxicities was investigated within regimens because most side-effects and toxicities are regimen specific. For our study, a cART regimen for a treatment naïve adult was defined as a three drug combination with two drugs from the NRTI class and one drug from the NNRTI or PI class based on guidelines used in each country at the time that the data for this study were collected. Each of the regimens contains 3TC as one of the NRTIs. Patients receiving FTC were considered to have received 3TC because these two drugs are largely interchangeable. Patients on a first regimen who did not meet the above definition were excluded from analysis. Nine drug regimens were eligible for inclusion in our study. These combinations arose from a combination of 3TC and one other NRTI paired either with a NNRTI or a PI (Table 1). Regimens that did not meet our definition (e.g., only one or two drugs documented) were classified as other and were not analyzed.

Table 1. List of cART regimens to be included in this study

Regimen Number	First Drug	Second Drug	Third Drug
1	Lamivudine	Stavudine	Nevirapine
2	Lamivudine	Stavudine	Efavirenz
3	Lamivudine	Stavudine	Lopinavir/Ritonavir
4	Lamivudine	Zidovudine	Nevirapine
5	Lamivudine	Zidovudine	Efavirenz
6	Lamivudine	Zidovudine	Lopinavir/Ritonavir
7	Lamivudine or emtricitabine	Tenofovir	Nevirapine
8	Lamivudine or emtricitabine	Tenofovir	Efavirenz
9	Lamivudine or emtricitabine	Tenofovir	Lopinavir/Ritonavir

J. Sample size and power

The calculation for Aim 1 was performed using the formula for clustered data. Due to the very large study sample available to address our aims, we chose to explore how differences in our postulated within-cluster correlation and the average number of CD4 cell measurements expected for each subject could impact the power available to detect the minimum clinically relevant difference we have specified (Table 2).

Aim 1: We aimed to detect a clinically informative difference of 50 cells/mm^3 comparing older to younger patients. For this calculation we assumed a dichotomous exposure of less than 40 years of age versus 40 years of age or older. For younger patients, we first assumed a within-subject standard deviation (SD) of 25 cells/mm^3, a between-subject SD of 50 cells/mm^3, and a maximum sample size of 85,770 patients. For older patients, we postulated a within-subject SD of 35 cells/mm^3, a between-subject SD of 60 cells/mm^3, and a maximum sample size of 46,970 patients. These assumptions were made based on a review of the current literature and consultation with clinical experts. The SDs were used to calculate the within- and between-cluster correlation (ρ) for each age group. The initial calculation assumed an average of seven CD4 cell measurements per subject and a difference of 50 cells/mm^3. We then postulated a range of alternate within- and between-cluster SDs for 1,000 patients per group with fewer CD4 cell measurements per patient to assess how power to detect a minimum difference of 50 cells/mm^3 would be affected.

Table 2. Power to detect a minimum mean difference of 50 cells/mm^3 in mean CD4 cell count comparing older to younger patients based on varying calculations of rho (ρ) and the average number of repeated measurements per subject (n_i).

ρ Older	ρ Younger	n_{Older}	$n_{Younger}$	n_i	Mean Difference	Power
0.75	0.8	46,970	85,770	7	50	1
0.75	0.8	1,000	1,000	7	50	0.87
0.75	0.8	1,000	1,000	5	50	0.87
0.75	0.8	1,000	1,000	3	50	0.84
0.65	0.7	1,000	1,000	7	50	0.94
0.65	0.7	1,000	1,000	5	50	0.93
0.65	0.7	1,000	1,000	3	50	0.90
0.55	0.6	1,000	1,000	7	50	0.98
0.55	0.6	1,000	1,000	5	50	0.97
0.55	0.6	1,000	1,000	3	50	0.94

Aim 2: If the rate of cART associated toxicity or significant side-effect necessitating regimen change among younger patients was 30% per year, to detect a 20% increased rate with 90% power and a two sided alpha level of 0.01, we would need a total of 4,580 patients in each of the 9 regimen categories, accounting for a maximum of 25% loss to follow-up.

The size of the study sample available to address each of the aims far exceeds the number of patients needed and may result in very small differences being considered statistically significant. While an alpha level of 0.01 to assess the strength of the evidence against the null hypothesis was used for the calculations, p-values may not be the most appropriate way to determine the significance of differences detected in this study. Because of the large sample size, an observed difference that provides very strong evidence against the null hypothesis might be much smaller than would be clinically important. On the other hand, the large sample size will provide very precise estimates of effect. In the

interpretation of results, we placed more emphasis on confidence intervals and the standard errors of the estimates than on significance testing.

K. Statistical analysis

Aim 1: Age group and CD4 cell count

Exploratory Analysis: To determine the effect of age on immunologic response, the initial exploratory analyses focused on the mean and the variance of CD4 counts over time, stratified by baseline CD4 group. We sought to go beyond the population means to address quantiles and variance, and therefore used quantile regression in the exploratory phase of our analyses. We modified the least squares criterion to estimate the 25th, 50th, and 75th percentiles of CD4 cell count. We used quantile regression to determine population average slopes to inform how we structured the parametric models. The linearity of the relationship of age with the outcome was assessed by 1) using scatter plot estimates with locally weighted scatter plot smoothing and categorization of age and 2) plotting the crude beta estimates from mixed model linear regression. Given that mean CD4 counts varied greatly by baseline CD4 level, these analyses were stratified by baseline CD4 group. Patients without a CD4 laboratory value at baseline were coded as missing and analyzed separately.

Assessing Interaction: Two covariates were selected *a priori* to be investigated as modifiers of the effect of age on CD4 count: sex and baseline presence of active TB at the initiation of cART, which may inhibit immune reconstitution. Interactions were

assessed by creating a term that was the product of the primary exposure variable and the pre-specified variables. While the p-value of the interaction term was used to determine if the interaction was significant, we also considered the strength of the association based on the beta estimate for the interaction term in combination with the change in the estimate of the main effect with the addition of the interaction term.

Assessing Confounding: Stratified analyses were utilized to determine whether sex, CD4 cell count at cART initiation, WHO disease stage, functional status, treatment regimen, TB, cryptococcal disease, pneumocystis *jiroveci* pneumonia, presence of other active OI, BMI, and year of cART initiation were potential confounders in this data set. Covariates that changed the crude estimate of the association between age and mean CD4 cell count by more than 25% were investigated further in multivariable models. We also included covariates that did not change the crude estimate of association by more than 25% but that did significantly improve model fit as assessed by subtracting the -2 log likelihood score of the nested model from that of the full model and comparing the difference to a chi-square distribution with degrees of freedom based on the difference between the models in the number of covariates. While the effect of cART on CD4 cell count over time is not expected to be different by regimen, we additionally ran adjusted models both with and without regimen and compared the effect of age on CD4 cell count estimates between the models.

Analyses for Statistical Inference: As the outcome variable was a repeated measure, we accounted for within-subject correlation using random effects linear mixed models. The

correlation matrices from preliminary unstructured models were assessed to determine the appropriate correlation structure to reduce the number of parameters to be estimated in the final model.

Using linear mixed models allowed us to model the mean response for the population as a combination of characteristics shared by all of the patients in the study as well as subject-specific effects unique to each individual (Fitzmaurice, Laird, & Ware, 2011). We utilized no-intercept models with an unstructured covariance for all crude associations. We determined that the correlation between observations decreased over time. We were not willing to assume a strictly exponential decrease in correlation for observations that were farther apart in time, nor were we willing to assume a homogeneous variance between groups. We therefore used a heterogeneous Toeplitz covariance structure which allowed for the correlation to decrease over time without assuming exponential decline and which also allowed for the variance to be heterogeneous over time.

Models were created by adding one variable, or category of variables, at a time and assessing 1) changes in beta estimates 2) -2 log likelihood and 3) Akaike's information criterion. We then fit the adjusted model and summarized the results in terms of the variance of the data and mean over time.

Aim 2: Age and regimen change due to toxicity or significant side-effects

Analyses of the effect of age on the incidence of cART-related significant side-effects and toxicity were conducted within each regimen group because the types and timing of toxicity and side-effect were expected to vary between regimen. Investigating the effect of age within regimen group helped to control for confounding by indication that may have been associated with treatment selection. Six of the nine standard regimens previously defined were analyzed. Regimens with a PI as the third drug did not have a sufficient number of patients in each age group to make reliable inferences. A small proportion of patients were started on regimens that did not meet our regimen definition (e.g. documentation of only two drugs) and were not analyzed.

The primary endpoint for this analysis was the first regimen change of cART due to toxicity or significant side-effect.

Exploratory Analysis: Kaplan-Meier methods were used to explore differences in time to first regimen change due to toxicity or side-effect comparing older to younger patients. We stratified by the covariates of interest to identify variables to be included in the inferential analyses. Patients who died or were lost to follow-up were censored at the date of their last visit. Patients without an event were censored at the end of study period.

Time zero for all patients was the date of cART initiation. We investigated regimen change in the first five years after cART initiation. Time to event was compared between older and younger patients using the Wilcoxon test.

Assessing Interaction: Sex was selected *a priori* to be investigated as a modifier of the effect of age on the hazard of regimen change due to toxicity or side-effect. Interaction was assessed by creating a term that was the product of the age group and sex. While the p-value of the interaction term was used to determine if the interaction was significant, we also considered the strength of the association based on the beta estimate for the interaction term in combination with the change in the estimate of the main effect with the addition of the interaction term.

Assessing Confounding: Stratified analyses were utilized to determine whether sex, CD4 cell count at cART initiation, baseline WHO disease stage, baseline functional status, baseline TB, baseline cryptococcal disease, baseline pneumocystis *jiroveci* pneumonia, presence of other active OIs at baseline, baseline BMI, baseline AST and ALT, baseline creatinine clearance, baseline hemoglobin level, and year of cART initiation were potential confounders in this data set. Covariates that changed the crude estimate of association between age and time to regimen change by more than 25% were investigated further in multivariable mixed models. Covariates that did not change the crude estimate

of association by more than 25% but that did significantly improve model fit were also included.

Analysis for Statistical Inference: To estimate the rate of the regimen change by age, multivariable Cox models were used. The previously identified covariates were included in the multivariable models and the assumption of proportionality was investigated by plotting Shoenfeld residuals to assess whether the intercept and slope of the residuals is approximately zero, and through the introduction of a term representing the interaction of age and time. Confounders were assessed by determining the difference in the -2 log likelihood between nested models and changes to the magnitude of the crude estimate of effect.

Adherence: As adherence is thought to be differentially associated with age, is subsequent to initiation of cART, and is associated with the outcomes of interest, it is a possible mediator of the associations of interest. To determine whether adherence mediated the relationship between age and the hazard of regimen change we ran each model with and without a variable for adherence and compared the estimates of effect for the association of age with the outcome. If the estimate of effect was importantly different it would indicate the effect of age is mediated by adherence. Adherence was quantified by calculating the proportion of medication refills that were filled on time in the first year after the initiation of cART with a potential range of 0% to 100%. "On time" was defined as a positive difference of no more than seven between the number of pills dispensed and the number of days between medication refills.

L. Missing outcome data

Patients who die or are lost to follow-up are likely to have outcome values that are not missing at random. Failure to account for this nonignorable missingness can lead to bias (Little & Rubin, 2002; Magder, 2003; Rubin, 1976; Shardell, Scharfstein, Vlahov, & Galai, 2008). To address this challenge we performed a sensitivity analysis to determine how robust our findings were to deviations from the missing at random assumption.

Aim 1: We performed a closed cohort analysis within each stratum of baseline CD4 among all patients who had both a baseline and a 48 month CD4 observation. The closed cohort analysis was used to determine if the association between age group and CD4 mean and variance over time that we observed in the open cohort persisted when only patients present at the final time point were considered in the analysis.

Aim 2: For time to first regimen change due to toxicity or significant side-effect, we originally proposed to perform a sensitivity analysis whereby we would assign the 25^{th} percentile time to regimen change from the Kaplan-Meier curves to patients in the younger age group who died or were lost to follow-up before experiencing a regimen change and the 75^{th} percentile to patients in the older group whom had died or were lost to follow-up before experiencing a regimen change and then the reverse as well. However, the Kaplan-Meier method was unable to estimate the 25^{th} and 75^{th} percentiles of time to change due to low number of regimen changes in most of the regimens. We

therefore performed a sensitivity analysis within each regimen stratum in which we imputed an event for all patients who had died or were lost to follow up before experiencing a regimen change at the time of their last visit.

CHAPTER IV RESULTS

A total of 181,354 adults between the ages of 20 and 90 initiated cART between August 1, 2004 and September 30, 2012. The baseline characteristics of the entire cohort by age group are presented below in Table 3. A summary table of the difference in mean CD4 cell count over all strata can be found in Appendix 4. In our study sample, 25% were age 20 to 29, 40% were age 30 to 39, 23% were age 40 to 49, 9% were age 50 to 59 and 3% were age 60 and older.

Table 3. Baseline characteristics of patients initiated on cART by age group (n=181,354)

Characteristic	**Older n (%) n=64,431**	**Younger n (%) n=116,923**	**p-value***
Median age (years) (IQR)**	47 (43 – 52)	32 (28 – 36)	
Median CD4 cell count (IQR)	173 (87 – 270)	182 (87 – 283)	<0.0001***
Sex			<0.0001
Female	36,061 (56)	85,862 (73)	
Male	28,369 (44)	31,061 (27)	
WHO stage			<0.0001
I	18,334 (28)	41,555 (36)	
II	16,055 (25)	28,281 (24)	
III	19,010 (30)	29,146 (25)	
IV	6,517 (10)	7,983 (7)	
Missing	4,515 (7)	7,983 (7)	
Functional status			<0.0001
Working	54,801 (85)	100,674 (86)	
Ambulatory	5,321 (8)	8,542 (7)	
Bedridden	572 (1)	898 (1)	
Missing	3,737 (6)	6,809 (6)	
BMI			<0.0001
Normal	16,082 (25)	30,230 (26)	
Underweight	8,008 (12)	13,952 (12)	
Overweight	2,814 (4)	4,741 (4)	
Obese	1,005 (2)	1,570 (1)	
Missing	36,522 (57)	66,430 (57)	
Tuberculosis			0.04
No	60,171 (93)	109,481 (94)	
Yes	4,260 (7)	7,442 (6)	
Cryptococcal disease			0.61
No	64,235 (99)	116,583 (99)	
Yes	196 (1)	340 (1)	
Pneumocystis *jiroveci pneumonia*			0.35
No	63,387 (98)	115,095 (98)	
Yes	1,044 (2)	1,828 (2)	

Characteristic	Older n (%) n=64,431	Younger n (%) n=116,923	p-value*
Table 3 continued			
Other opportunistic infection			0.87
No	55,997 (87)	101,586 (87)	
Yes	8,434 (13)	15,337 (13)	
First cART regimen			<0.0001
D4T/3TC/NVP	16,760 (26)	27,007 (23)	
D4T/3TC/EFV	14,997 (23)	22,410 (19)	
D4T/3TC/LOP	17 (<1)	142 (<1)	
AZT/3TC/NVP	9,825 (15)	24,021 (21)	
AZT/3TC/EFV	13,314 (21)	17,441 (15)	
AZT/3TC/LOP	132 (<1)	1,081 (1)	
TDF/3TC/NVP	5,333 (8)	17,185 (15)	
TDF/3TC/EFV	14,997 (23)	22,410 (19)	
TDF/3TC/LOP	335 (1)	843 (1)	
Other	1,352 (2)	3,155 (3)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

A. Baseline CD4 cell count between 0 and 50 cells/mm^3

Of the study patients initiating cART during the study period 20,167 had a documented baseline CD4 cell count between 0 and 50 cells/mm^3 (Table 4). In the older group, 19% had died and 9% had been lost to follow-up by the end of the study period, leaving approximately 72% of older patients still active at the original or another cART facility. In the younger group, 17% had died and 11% had been lost to follow-up by the end of the study period, leaving approximately 73% still active at the original or another cART facility. The proportion of females was significantly smaller in the older age group than the younger age group while the distribution of WHO stage, WAB stage, BMI, presence of OIs, active TB, active cryptococcal disease, and active pneumocystis *jiroveci* pneumonia were similar between the two groups. Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the

multivariable models. The median CD4 cell count at cART initiation was 25 cells/mm^3 in the older age group and 24 cells/mm^3 in the younger age group. The median time of follow-up among older patients was 12.6 months (IQR 1.9 – 32.7) and 12.7 months (IQR 2.3 – 31.5) among younger patients (p=0.79).

Table 4. Baseline characteristics of patients initiated on cART with a CD4 count of 0 to 50 cells/mm^3 (n=20,167)

Characteristic	Older n (%) n=6,898	Younger n (%) n=13,269	p-value*
Median age (IQR)**	46 (43 – 51)	32 (28 – 36)	
Median CD4 (IQR)	25 (12 – 37)	24 (11 – 36)	<0.0001***
Sex			<0.0001
Female	3,224 (47)	8,800 (66)	
Male	3,674 (53)	4,469 (34)	
WHO stage			0.001
I	1,526 (22)	3,257 (25)	
II	1,495 (22)	2,887 (22)	
III	2,434 (35)	4,487 (34)	
IV	1,084 (16)	1,919 (15)	
Missing	359 (5)	719 (5)	
Functional Status			0.34
Working	5,553 (81)	10,729 (81)	
Ambulatory	976 (14)	1,831 (14)	
Bedridden	128 (2)	210 (2)	
Missing	241 (4)	499 (4)	
BMI			0.003
Normal	1,360 (20)	2,649 (20)	
Underweight	1,115 (16)	2,286 (17)	
Overweight	194 (3)	271 (2)	
Obese	61 (1)	101 (1)	
Missing	4,168 (60)	7,962 (60)	
Tuberculosis			0.29
No	6,214 (90)	11,890 (90)	
Yes	684 (10)	1,379 (10)	
Cryptococcal disease			0.74
No	6,835 (99)	13,154 (99)	
Yes	63 (1)	115 (1)	
Pneumocystis *jiroveci pneumonia*			0.87
No	6,751 (98)	12,991 (98)	
Yes	147 (2)	278 (2)	
Other opportunistic infection			0.02
No	5,760 (84)	10,904 (82)	
Yes	1,138 (16)	2,365 (18)	
First cART regimen			<0.0001
D4T/3TC/NVP	2,174 (32)	4,037 (30)	
D4T/3TC/EFV	1,301 (19)	2,422 (18)	
D4T/3TC/LOP	4 (<1)	8 (<1)	
AZT/3TC/NVP	846 (12)	1,959 (15)	
AZT/3TC/EFV	1,653 (24)	2,374 (18)	
AZT/3TC/LOP	14 (<1)	27 (<1)	
TDF/3TC/NVP	401 (6)	1,491 (11)	
TDF/3TC/EFV	1,301 (19)	2,422 (18)	
TDF/3TC/LOP	57 (1)	87 (1)	
Other	132 (2)	278 (2)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

There was strong evidence, in both the crude analysis and the adjusted analysis, that older patients had consistently lower mean CD4 cell counts over time following the initiation of cART. In the adjusted analysis, the period over which the mean CD4 cell counts were significantly lower in older compared to younger patients was from six to 48 months (Table 5) (Figure 5). While immune system reconstitution was more variable in younger patients than in older patients after the first six months of treatment ($p<0.00001$) (Figure 6), the standard deviation was large in both groups. By 12 months, approximately 75% of older patients had failed to achieve a CD4 cell count of 200 cells/mm^3 or greater compared to 50% of younger patients. By 36 months only 15% of older patients had a CD4 cell count of 450 cells/mm^3 or higher compared to 25% of younger patients.

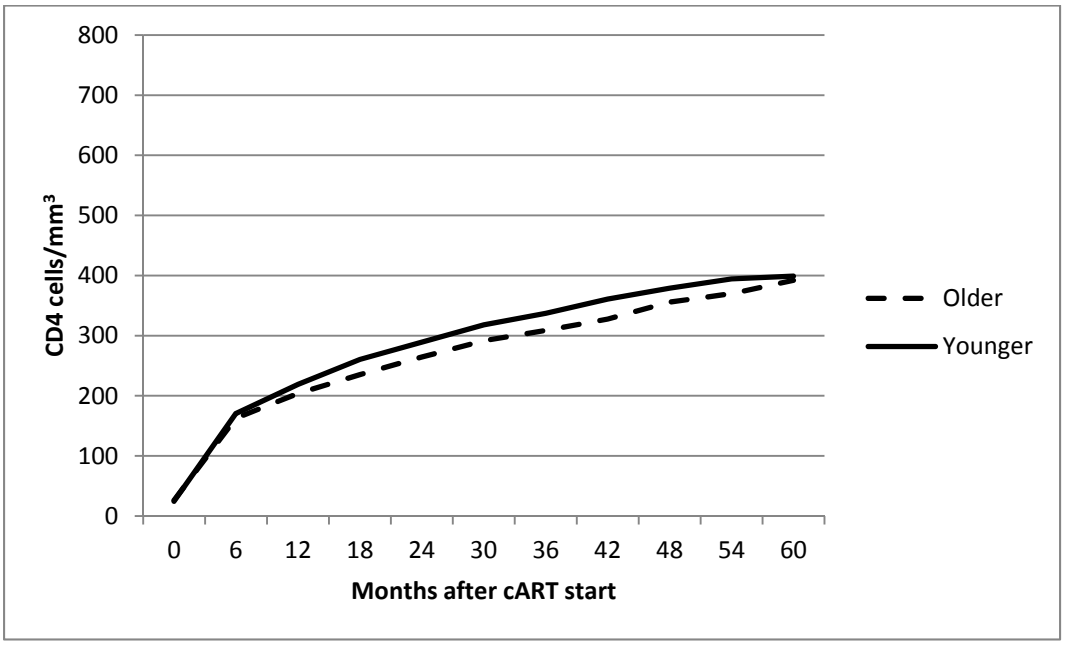
Table 5. Model based estimates of mean CD4 cell counts[*] and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 0 and 50 cells/mm^3

Months since initiation of cART	Older age			Younger age			p-value[**]
	n	Mean	SD	n	Mean	SD	
0	6,699	26	15	12,879	24	15	<.0001
6	2,747	163	142	5,326	170	148	0.02
12	2,174	204	142	4,179	219	151	<.0001
18	1,791	235	140	3,364	260	156	<.0001
24	1,487	265	141	2,611	289	158	<.0001
30	1,165	292	149	2,087	318	162	<.0001
36	895	309	152	1,493	337	167	<.0001
42	629	328	156	1,091	361	176	<.0001
48	396	356	166	687	379	174	0.02
54	238	370	180	387	394	200	0.08
60	152	392	186	273	399	193	0.70

[*] Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

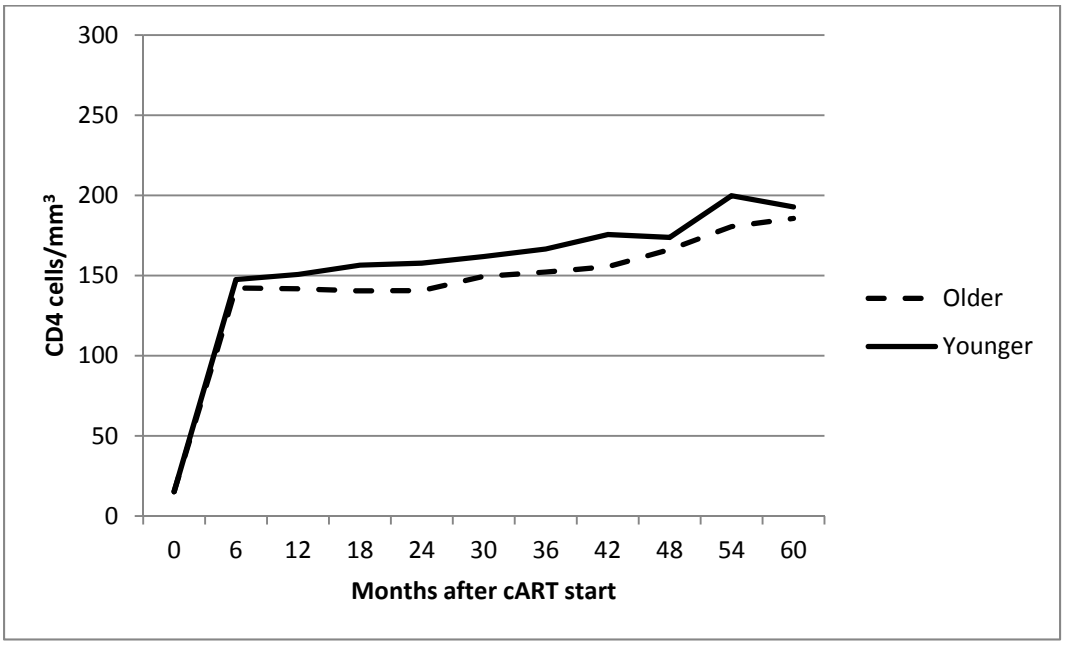
[**] p-value for the difference in mean CD4 cell count between older and younger groups

Figure 5. Model based mean CD4 cell count*, by age group and months since cART initiation, among patients initiated on cART with a baseline CD4 cell count between 0 and 50 cells/mm^3



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 6. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 0 and 50 cells/mm^3



Sensitivity analysis: Of the patients with a baseline CD4 cell count between 0 and 50 cells/mm^3, 1,081 patients had both a baseline and 48 month CD4 cell count (Table 6) (Figure 7). The differences in mean CD4 cell count between older and younger patients were similar over time to the differences in the open cohort analysis. CD4 cell count variability was also similar across all time periods to the open cohort analysis for both older and younger patients. There was consistently less variability of CD4 cell count at each time point after six months following cART initiation among older patients compared to younger patients.

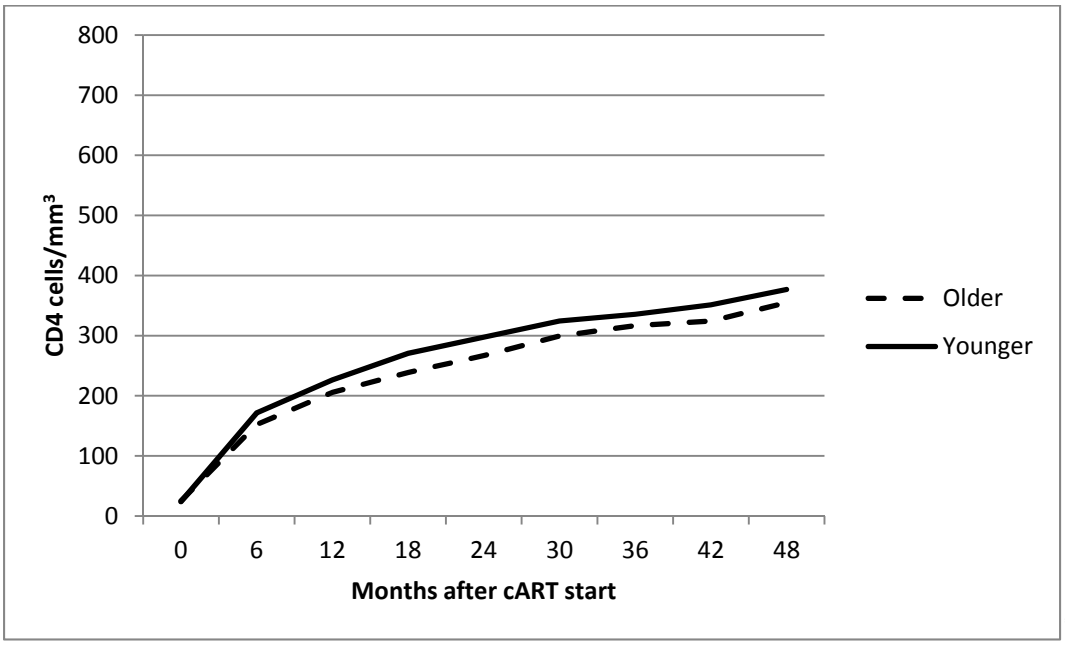
Table 6. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 0 and 50 cells/mm^3 (closed cohort)

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	396	25	17	685	24	17	0.35
6	264	152	140	478	171	145	0.05
12	270	206	133	458	227	140	0.03
18	263	239	128	463	271	156	0.001
24	279	267	132	441	297	141	0.002
30	278	299	147	453	324	160	0.02
36	262	317	155	441	336	164	0.10
42	247	324	156	432	351	165	0.02
48	396	355	171	685	377	177	0.04

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

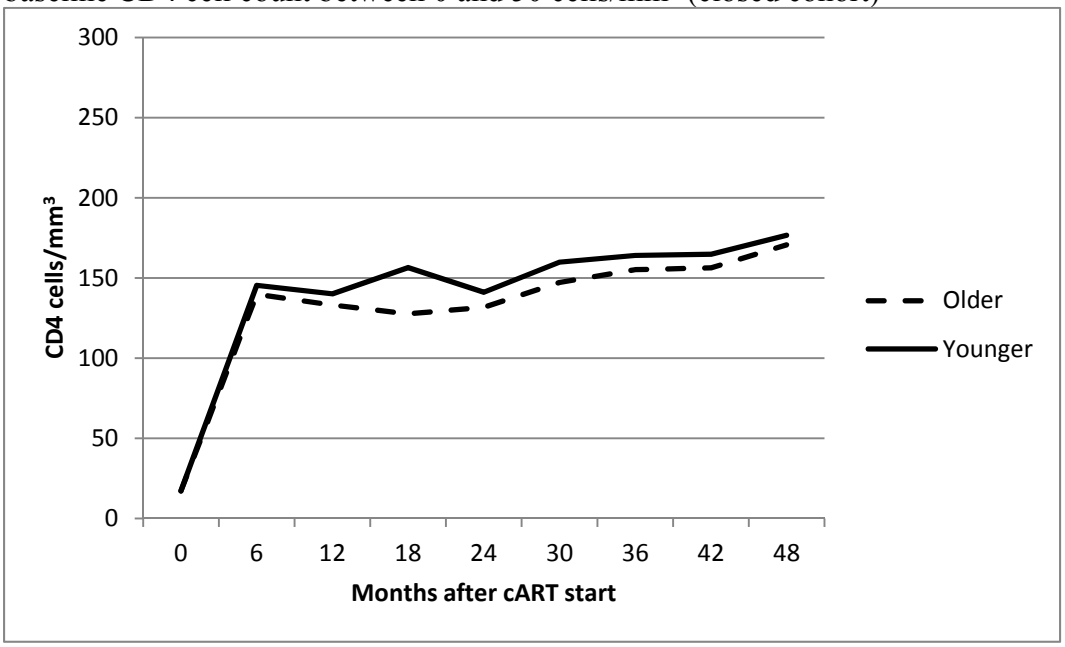
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 7. Model based mean CD4 cell count*, by age group and months since cART initiation, among patients initiated on cART with a baseline CD4 cell count between 0 and 50 cells/mm^3 (closed cohort)



*Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 8. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 0 and 50 cells/mm^3 (closed cohort)



B. Baseline CD4 cell count between 51 and 100 cells/mm^3

Of the study patients initiating cART during the study period, 18,050 had a documented baseline CD4 cell count between 51 and 100 cells/mm^3 (Table 7). In the older group, 13% had died and 8% had been lost to follow-up by the end of the study period, leaving 79% of older patients were still active at the original or another ART facility. In the younger group, 11% had died and 10% had been lost to follow-up by the end of the study period, leaving 79% still active at the original or another cART facility. The median time on cART among older patients was 17.1 months (IQR 3.9 – 35) and 17 months (IQR 4 – 34) among younger patients (p=0.89).

Table 7. Baseline characteristics of patients initiated on cART with a CD4 count of 51 to 100 cells/mm^3 (n=18,050)

Characteristic	Older n (%) n=6,725	Younger n (%) n=11,325	p-value*
Median age (IQR)**	46 (43 – 52)	32 (28 – 36)	
Median CD4 (IQR)	75 (63 – 88)	75 (63 – 88)	0.02***
Sex			<0.0001
Female	3,471 (52)	7,849 (69)	
Male	3,252 (48)	3,476 (31)	
WHO stage			<0.0001
I	1,859 (28)	3,575 (32)	
II	1,582 (24)	2,773 (25)	
III	2,195 (33)	3,378 (30)	
IV	753 (11)	1,080 (10)	
Missing	336 (5)	519 (5)	
WAB stage			0.18
Working	5,680 (84)	9,659 (85)	
Ambulatory	729 (11)	1,184 (11)	
Bedridden	80 (1)	101 (1)	
Missing	236 (4)	381 (3)	
BMI			0.25
Normal	1,534 (23)	2,598 (23)	
Underweight	1,001 (15)	1,727 (15)	
Overweight	219 (3)	303 (3)	
Obese	60 (1)	104 (1)	
Missing	3,911 (58)	6,593 (58)	
Tuberculosis			0.27
No	6,127 (91)	10,262 (91)	
Yes	598 (9)	1,063 (9)	
Cryptococcal disease			0.48
No	6,691 (99)	11,276 (99)	
Yes	34 (1)	49 (1)	
Pneumocystis *jiroveci pneumonia*			0.40
No	6,579 (98)	11,100 (98)	
Yes	146 (2)	225 (2)	
Other opportunistic infection			0.29
No	5,636 (84)	9,422 (83)	
Yes	1,089 (16)	1,903 (17)	
First cART regimen			<0.0001
D4T/3TC/NVP	2,053 (31)	3,119 (28)	
D4T/3TC/EFV	283 (4)	438 (4)	
D4T/3TC/LOP	0 (0)	2 (<1)	
AZT/3TC/NVP	884 (13)	2,034 (18)	
AZT/3TC/EFV	1,401 (21)	1,736 (15)	
AZT/3TC/LOP	11 (<1)	23 (<1)	
TDF/3TC/NVP	459 (7)	1,601 (14)	
TDF/3TC/EFV	1,475 (22)	2,107 (19)	
TDF/3TC/LOP	23 (<1)	49 (<1)	
Other	136 (2)	216 (2)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

There was strong evidence in both the crude analysis and the adjusted analysis (Table 8) that older patients had consistently lower mean CD4 cell counts over time following the initiation of cART compared to younger patients. Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the multivariable models. In the adjusted analysis, the period over which the difference in mean CD4 cell counts were significantly lower in older compared to younger patients was from 12 to 42 months (Figure 9). While immune system reconstitution was more variable in younger patients than in older patients after the first six months of treatment ($p<0.00001$) (Figure 10), the standard deviation was large in both groups. By 12 months, approximately 50% of both older and younger patients had failed to achieve a CD4 cell count of 200 cells/mm^3 or greater. By 36 months only 15% of older patients had a CD4 cell count of 450 cells/mm^3 or higher compared to 35% of younger patients.

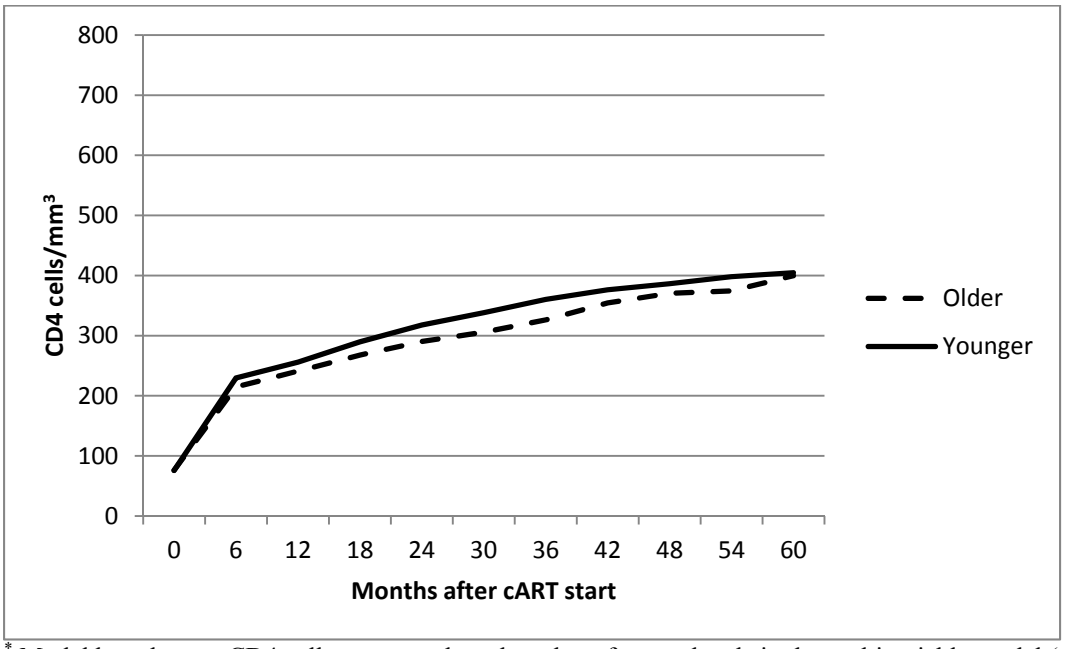
Table 8. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 51 and 100 cells/mm^3

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	6,527	76	15	11,064	76	15	0.10
6	3,065	215	144	5,212	230	153	<.0001
12	2,476	241	135	4,142	256	150	<.0001
18	2,075	268	144	3,373	290	158	<.0001
24	1,613	290	147	2,683	318	159	<.0001
30	1,329	306	149	2,030	338	160	<.0001
36	985	326	152	1,511	360	162	<.0001
42	677	354	162	1,064	376	171	0.003
48	428	370	167	691	386	173	0.09
54	235	374	171	337	398	179	0.08
60	142	400	183	213	405	190	0.79

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

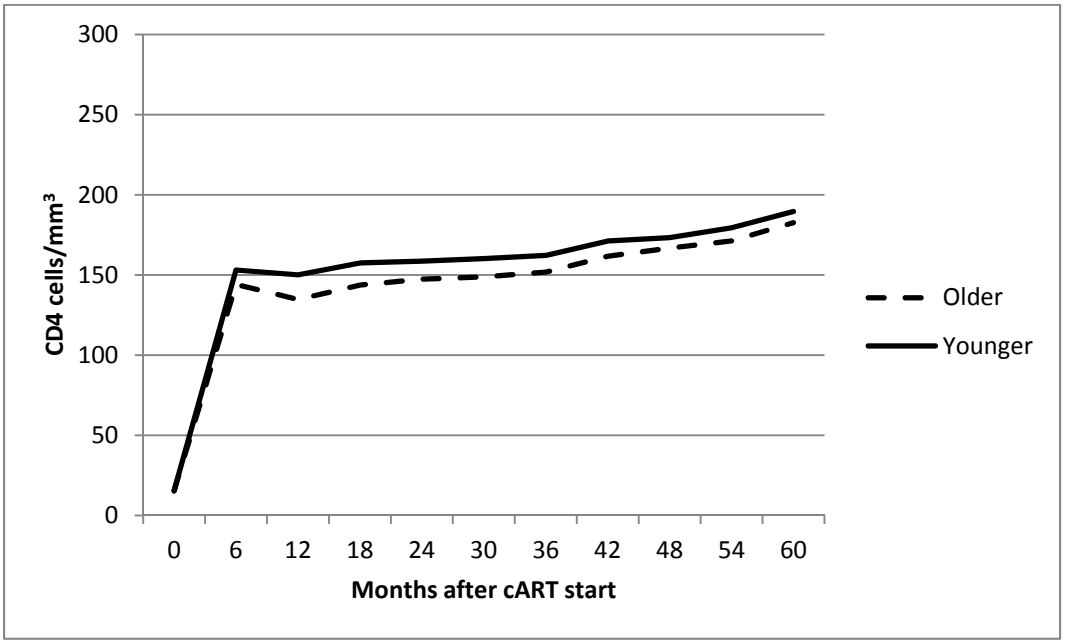
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 9. Model based mean CD4 cell count*, by age group and months since cART initiation, among patients initiated on cART with a baseline CD4 cell count between 51 and 100 cells/mm^3



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 10. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 51 and 100 cells/mm^3



Sensitivity analysis: Of the patients with a baseline CD4 cell count between 51 and 100 cells/mm^3, 1,116 patients had both a baseline and 48 month CD4 cell count (Table 9) (Figure 11). The differences in mean CD4 cell count between older and younger patients were similar over time to the results of the open cohort analysis. CD4 cell reconstitution followed a similar pattern as in the open cohort analysis for both older and younger patients. CD4 cell reconstitution was less variable among older patients after cART initiation from 12 months on compared to younger patients.

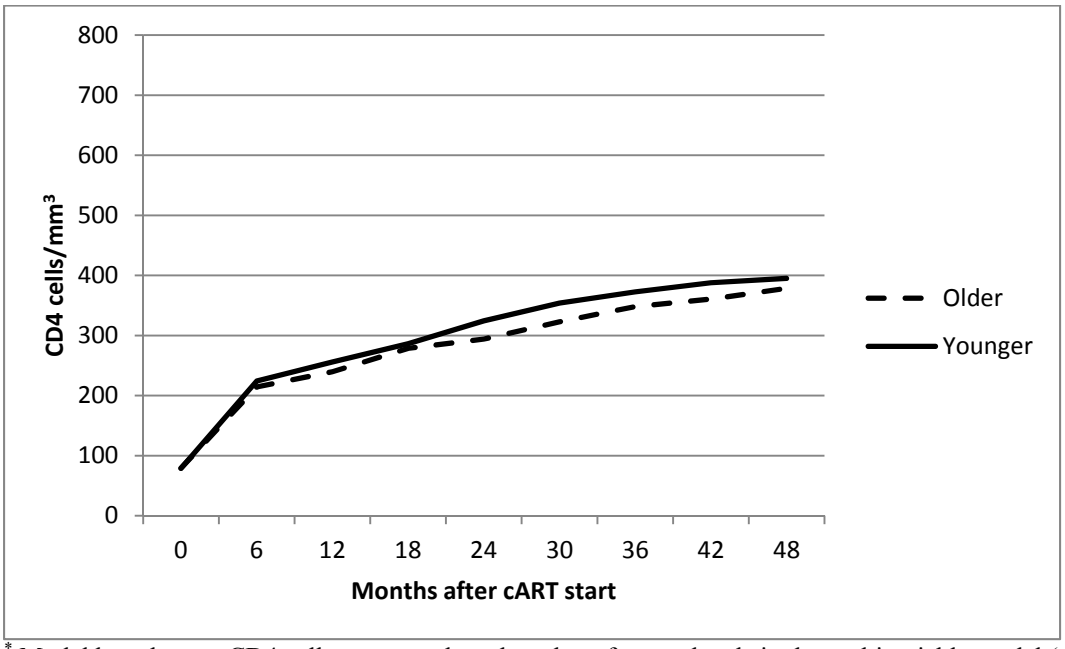
Table 9. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 51 and 100 cells/mm^3 (closed cohort)

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	427	79	17	689	79	17	0.66
6	305	214	154	478	224	142	0.34
12	300	240	134	479	256	139	0.08
18	300	279	140	457	286	145	0.43
24	270	294	142	454	324	145	0.003
30	291	323	152	462	354	165	0.005
36	293	348	151	439	373	165	0.03
42	257	361	152	423	388	179	0.02
48	427	379	167	689	395	178	0.12

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

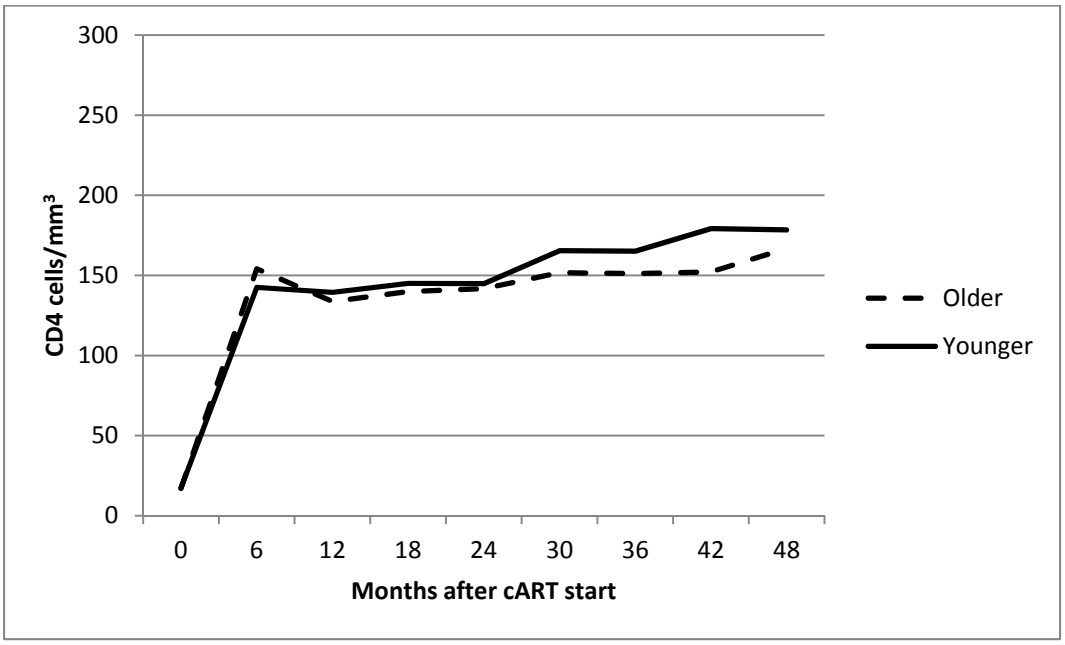
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 11. Model based mean CD4 cell count*, by age group and months since cART initiation, among patients initiated on cART with a baseline CD4 cell count between 51 and 100 cells/mm^3 (closed cohort)



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 12. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 51 and 100 cells/mm^3 (closed cohort)



C. Baseline CD4 cell count between 101 and 200 cells/mm^3

Of the study patients initiating cART during the study period, 36,399 had a documented baseline CD4 cell count between 101 and 200 cells/mm^3 (Table 10). The median time on cART among older patients was 20.6 months (IQR 6.5 – 37.2) and 20 months (IQR 6.3 – 36.4) among younger patients (p=0.07). By the end of the study period, nine % of older patients and seven % of younger patients had died, and seven % of older patients and 10% of younger patients had been lost to follow-up. Eighty-five % of older patients and 83% of younger patients were still active or had transferred to another ART facility by the end of the study period.

Table 10. Baseline characteristics of patients initiated on cART with a CD4 count of 101 to 200 cells/mm^3 (n=36,399)

Characteristic	Older n (%) n=13,561	Younger n (%) n=22,838	p-value*
Median age (IQR)**	47 (43 – 52)	32 (28 – 36)	
Median CD4 (IQR)	151 (125 – 175)	151 (126 – 176)	0.24***
Sex			<0.0001
Female	7,491 (55)	16,608 (73)	
Male	6,070 (45)	6,230 (27)	
WHO stage			<0.0001
I	4,090 (30)	8,502 (37)	
II	3,745 (28)	6,159 (27)	
III	3,981 (29)	5,645 (25)	
IV	1,053 (8)	1,440 (6)	
Missing	692 (5)	1,092 (5)	
WAB stage			0.001
Working	11,814 (87)	20,168 (88)	
Ambulatory	1,123 (8)	1,667 (7)	
Bedridden	104 (1)	137 (1)	
Missing	520 (4)	866 (4)	
BMI			<0.0001
Normal	3,313 (24)	5,895 (26)	
Underweight	1,679 (12)	2,798 (12)	
Overweight	560 (4)	773 (3)	
Obese	202 (2)	259 (1)	
Missing	7,807 (58)	13,113 (57)	
Tuberculosis			0.13
No	12,600 (93)	21,313 (93)	
Yes	961 (7)	1,525 (7)	
Cryptococcal disease			0.75
No	13,538 (99)	22,796 (99)	
Yes	23 (1)	42 (1)	
Pneumocystis *jiroveci Pneumonia*			0.48
No	13,311 (98)	22,440 (98)	
Yes	250 (2)	398 (2)	
Other opportunistic infection			0.31
No	11,665 (86)	19,558 (86)	
Yes	1,896 (14)	3,280 (14)	
First cART regimen			<0.0001
3TC/D4T/NVP	3,965 (29)	6,127 (27)	
3TC/D4T/EFV	463 (3)	643 (3)	
3TC/D4T/LOP	1 (<1)	5 (<1)	
3TC/AZT/NVP	2,093 (15)	4,963 (22)	
3TC/AZT/EFV	2,701 (20)	3,099 (14)	
3TC/AZT/LOP	19 (<1)	54 (<1)	
3TC/TDF/NVP	1,107 (8)	3,742 (16)	
3TC/TDF/EFV	2,934 (22)	3,714 (16)	
3TC/TDF/LOP	31 (<1)	96 (<1)	
Other	247 (2)	395 (2)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

In the crude analysis there was strong evidence that older patients had lower mean CD4 cell counts following the initiation of cART from six months to 60 months. Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the multivariable models.

After adjustment for covariates, the relationship between older age group at the initiation of cART and CD4 cell count over time persisted for months six through 60 and demonstrated very strong evidence against the null hypothesis of no difference in mean CD4 cell count comparing older to younger patients (Table 11) (Figure 13).

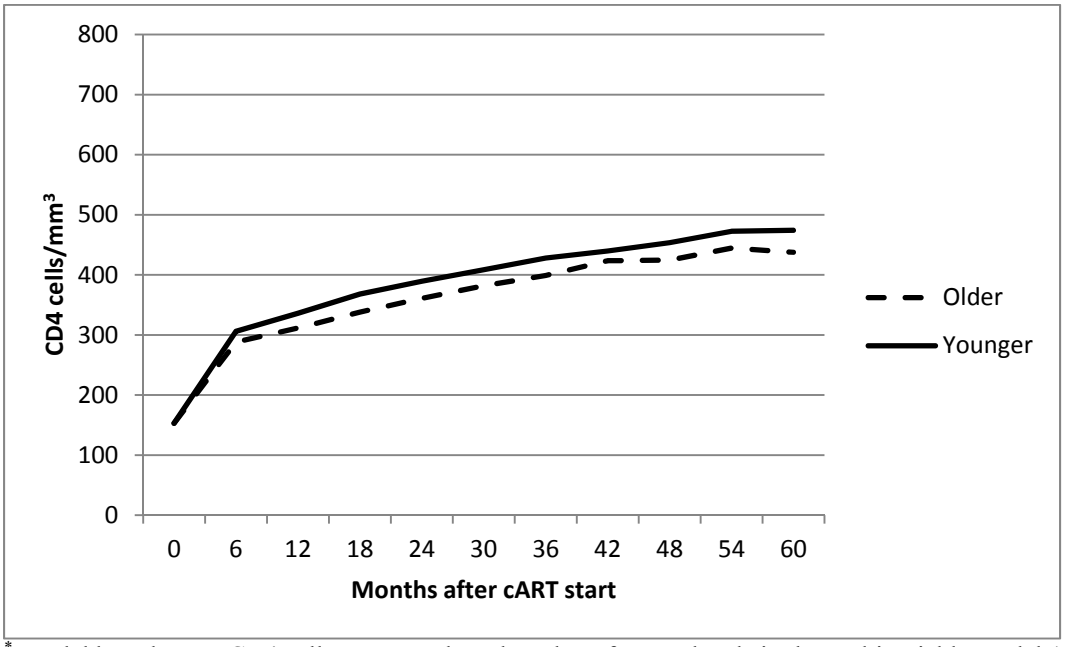
Table 11. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 101 and 200 cells/mm^3

Months since cART initiation	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	13,182	153	31	22,316	153	31	0.40
6	6,688	288	155	11,321	306	163	<.0001
12	5,534	312	153	9,195	336	166	<.0001
18	4,635	338	156	7,637	368	167	<.0001
24	3,715	361	157	6,120	389	170	<.0001
30	2,824	382	160	4,807	409	172	<.0001
36	2,218	399	162	3,587	428	170	<.0001
42	1,544	424	168	2,533	440	179	0.001
48	996	424	174	1,524	454	181	<.0001
54	493	444	185	802	473	193	0.004
60	285	437	188	425	474	195	0.005

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

** p-value for the difference in mean CD4 cell count between older and younger groups

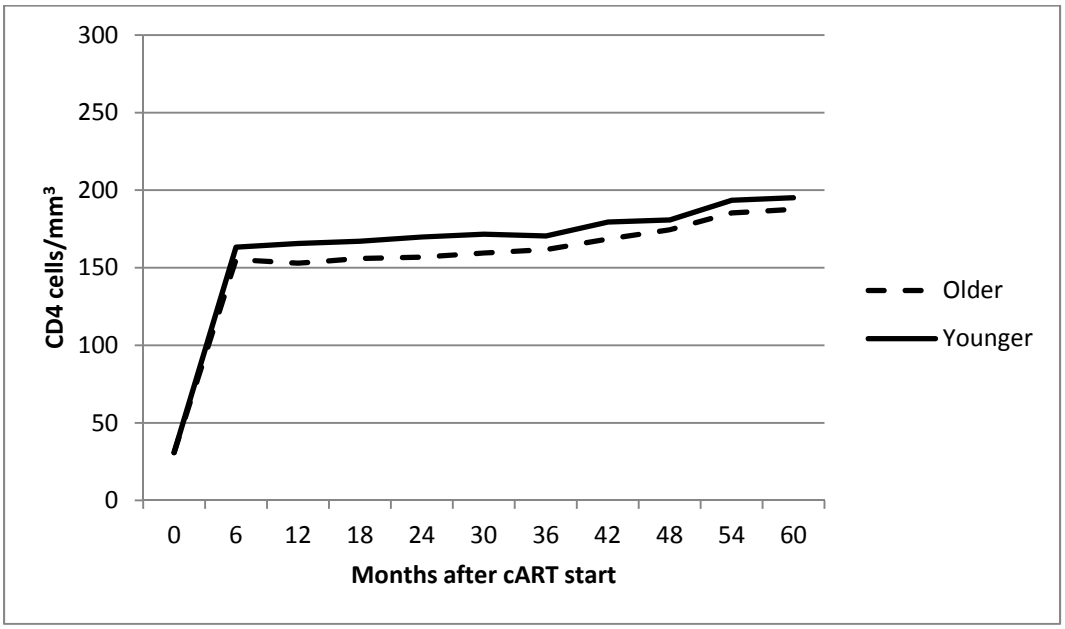
Figure 13. Model based mean CD4 cell count*, by age group and months since cART initiation, among patients initiated on cART with a baseline CD4 cell count between 101 and 200 cells/mm^3



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

While immune system reconstitution was more variable in younger patients than in older patients after the first six months of treatment ($p<0.00001$) (Figure 14), the standard deviation was large in both groups. By 12 months, approximately 25% of older patients had failed to achieve a CD4 cell count of 200 cells/mm^3 or greater compared to 20% of younger patients. By 36 months only 30% of older patients had a CD4 cell count of 450 cells/mm^3 or higher compared to 40% of younger patients.

Figure 14. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 101 and 200 cells/mm^3



Sensitivity analysis: Of the patients with a baseline CD4 cell count between 101 and 200 cells/mm^3, 2,512 patients had both a baseline and 48 month CD4 cell count. Older patients had consistently lower mean CD4 cells counts at each time point after the initiation of cART compared to younger patients in the sensitivity analysis. The variability of immune system reconstitution was similar across all time periods to the open cohort analysis with both older and younger patients displaying a similar trend. CD4 cell count reconstitution was less variable among older patients at each time point after cART initiation from six months to 60 months compared to younger patients. The differences in absolute CD4 cell count comparing older to younger patients was similar to what was observed in the open cohort analysis.

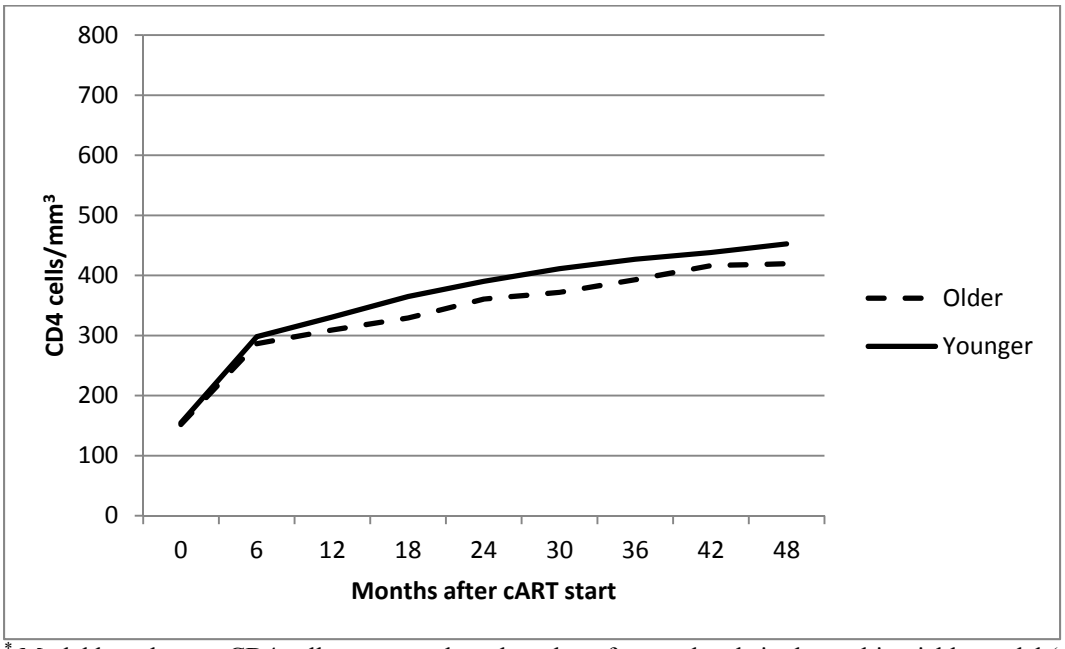
Table 12. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 101 and 200 cells/mm^3 (closed cohort)

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	993	152	32	1,519	155	32	0.02
6	709	286	150	994	298	158	0.10
12	642	309	150	994	331	164	0.003
18	686	329	158	998	365	167	<.0001
24	653	361	153	1,008	390	171	<.0001
30	616	372	160	1,022	411	175	<.0001
36	689	393	167	1,052	427	171	<.0001
42	625	416	173	956	438	183	0.009
48	993	419	178	1,519	452	184	<.0001

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

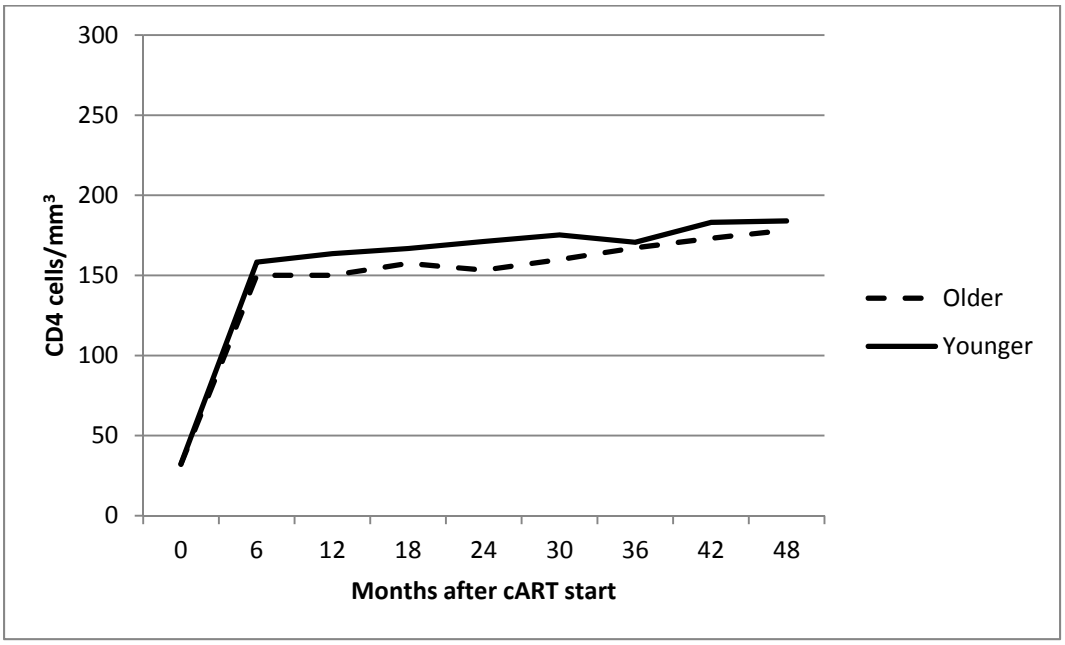
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 15. Model based estimates of mean CD4 cell counts*, by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 101 and 200 cells/mm^3 (closed cohort)



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 16. Variability of model based mean CD4 cell count presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 101 and 200 cells/mm^3 (closed cohort)



D. Baseline CD4 cell count between 201 and 350 cells/mm^3

Of the study patients initiating cART during the study period, 44,152 had a documented baseline CD4 cell count between 201 and 350 cells/mm^3 (Table 13.). The median time on cART among older patients was 19.4 months (IQR 7.2 – 34.2) and 18 months (IQR 6.4 – 33.3) among younger patients ($p<0.0001$). By the end of the study period, six % of older patients and four % of younger patients had died, and six % of older patients and eight % of younger patients had been lost to follow-up. 88% of older patients and 87% of younger patients were still active or had transferred to another ART facility by the end of the study period.

Table 13. Baseline characteristics of patients initiated on cART with a CD4 count of 201 to 350 cells/mm^3 (n=44,152)

Characteristic	Older n (%) n=15,552	Younger n (%) n=28,600	p-value*
Median age (IQR)**	47 (43 – 52)	31 (27 – 35)	
Median CD4 (IQR)	268 (233 – 307)	270 (234 – 308)	0.05***
Sex			<0.0001
Female	9,343 (60)	21,623 (76)	
Male	6,208 (40)	6,977 (24)	
WHO stage			<0.0001
I	5,400 (35)	12,457 (44)	
II	4,388 (28)	7,542 (26)	
III	4,130 (27)	6,026 (21)	
IV	784 (5)	1,156 (4)	
Missing	850 (5)	1,419 (5)	
WAB stage			<0.0001
Working	13,955 (90)	26,006 (91)	
Ambulatory	858 (6)	1,305 (5)	
Bedridden	47 (<1)	85 (<1)	
Missing	692 (4)	1,204 (4)	
BMI			<0.0001
Normal	4,625 (30)	8,794 (31)	
Underweight	1,790 (12)	2,895 (10)	
Overweight	857 (6)	1,492 (5)	
Obese	313 (2)	441 (2)	
Missing	7,967 (51)	14,978 (52)	
Tuberculosis			0.04
No	14,706 (95)	27,171 (95)	
Yes	846 (5)	1,429 (5)	
Cryptococcal disease			0.14
No	15,527 (99)	28,569 (99)	
Yes	25 (1)	31 (1)	
Pneumocystis *jiroveci pneumonia*			0.57
No	15,334 (99)	28,180 (99)	
Yes	218 (1)	420 (1)	
Other opportunistic infection			0.23
No	13,542 (87)	25,020 (87)	
Yes	2,010 (13)	3,580 (13)	
First cART regimen			<0.0001
3TC/D4T/NVP	2,882 (19)	4,500 (16)	
3TC/D4T/EFV	556 (4)	785 (3)	
3TC/D4T/LOP	6 (<1)	31 (<1)	
3TC/AZT/NVP	2,565 (16)	6,658 (23)	
3TC/AZT/EFV	3,188 (21)	4,039 (14)	
3TC/AZT/LOP	24 (<1)	227 (1)	
3TC/TDF/NVP	1,628 (10)	5,094 (18)	
3TC/TDF/EFV	4,349 (28)	6,513 (23)	
3TC/TDF/LOP	39 (<1)	138 (<1)	
Non-standard	315 (2)	615 (2)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

In the crude analysis there was strong evidence that older patients had lower mean CD4 cell counts following the initiation of cART from six months to 36 months and from 48 to 54 months. Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the multivariable models.

After adjustment for covariates the relationship between older age group at the initiation of cART and CD4 cell count over time persisted for months six through 54, but did not demonstrate evidence against the null hypothesis of no difference in mean CD4 cell count comparing older to younger patients at 60 months (Table 14) (Figure 17).

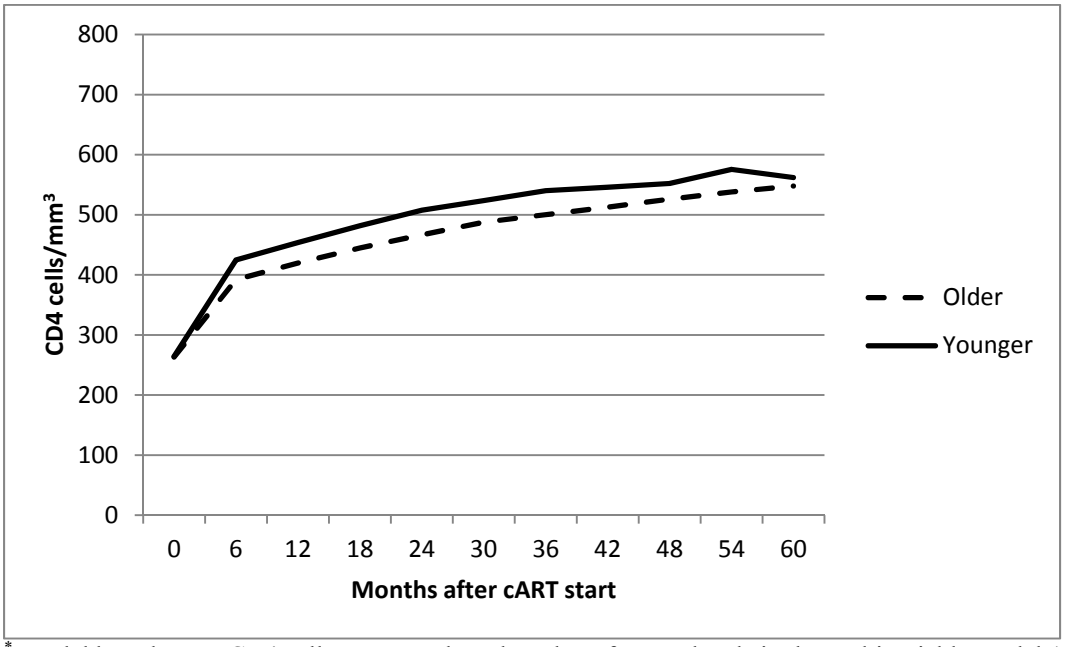
Table 14. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 201 and 350 cells/mm^3

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	15,148	263	44	27,977	264	45	0.01
6	8,526	392	176	15,360	425	198	<.0001
12	6,821	420	185	12,101	454	205	<.0001
18	5,638	445	190	9,708	482	208	<.0001
24	4,309	467	190	7,574	508	214	<.0001
30	3,387	488	197	5,630	524	217	<.0001
36	2,346	500	195	4,106	540	219	<.0001
42	1,604	513	202	2,754	546	219	<.0001
48	820	526	204	1,558	552	224	0.001
54	335	538	218	608	576	226	0.005
60	165	548	210	256	562	235	0.47

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

** p-value for the difference in mean CD4 cell count between older and younger groups

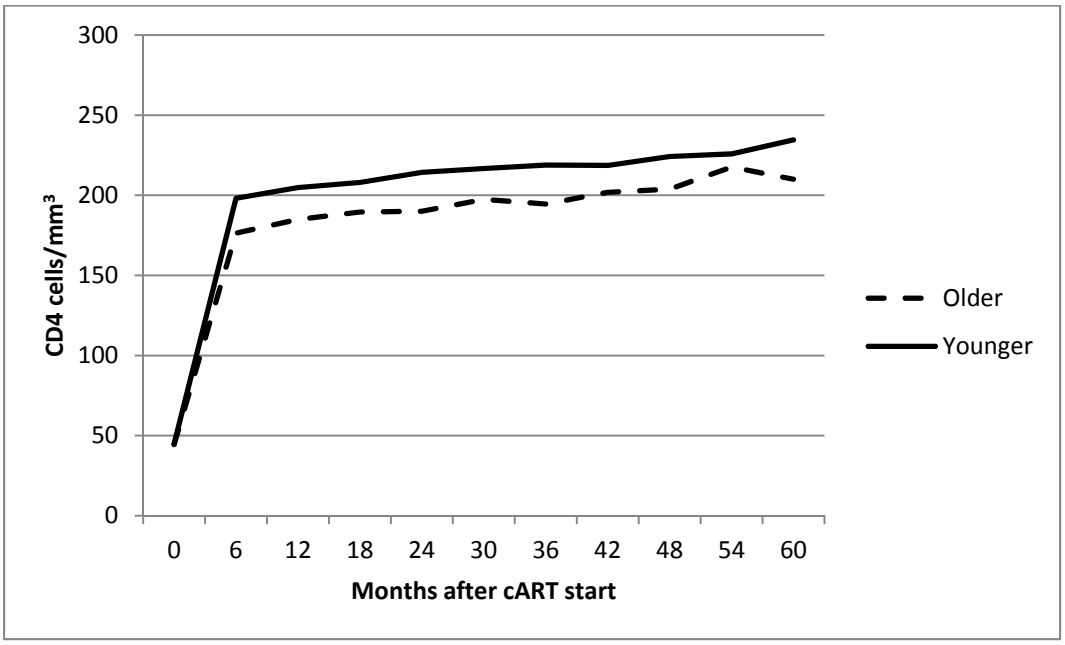
Figure 17. Model based estimates of mean CD4 cell counts*, by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 201 and 350 cells/mm^3



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

While immune system reconstitution was less variable over time among older patients compared to younger patients (Figure 18), the standard deviations in both groups were large. By 12 months after cART initiation only 40% of older patients had to achieved a CD4 cell count of 450 cells/mm^3 or higher compared to 50% of younger patients. By 36 months 45% of older patients had still not reached a CD4 cell count of 450 cells compared to approximately 30% of younger patients.

Figure 18. Variability of CD4 cell reconstitution presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 201 and 350 cells/mm^3



Sensitivity analysis: Of the patients with a baseline CD4 cell count between 201 and 350 cells/mm^3, 2,360 patients had both a baseline and 48 month CD4 cell count. Older patients had consistently lower mean CD4 cell counts at each time point after the initiation of cART compared to younger patients in the sensitivity analysis. Variability of immune system reconstitution was consistent across all time periods with the open cohort analysis with both older and younger patients displaying a similar trend. Immune system reconstitution was consistently less variable at each time point after cART initiation compared to younger patients. The differences in absolute CD4 cell count comparing older to younger patients was similar to what was observed in the open cohort analysis.

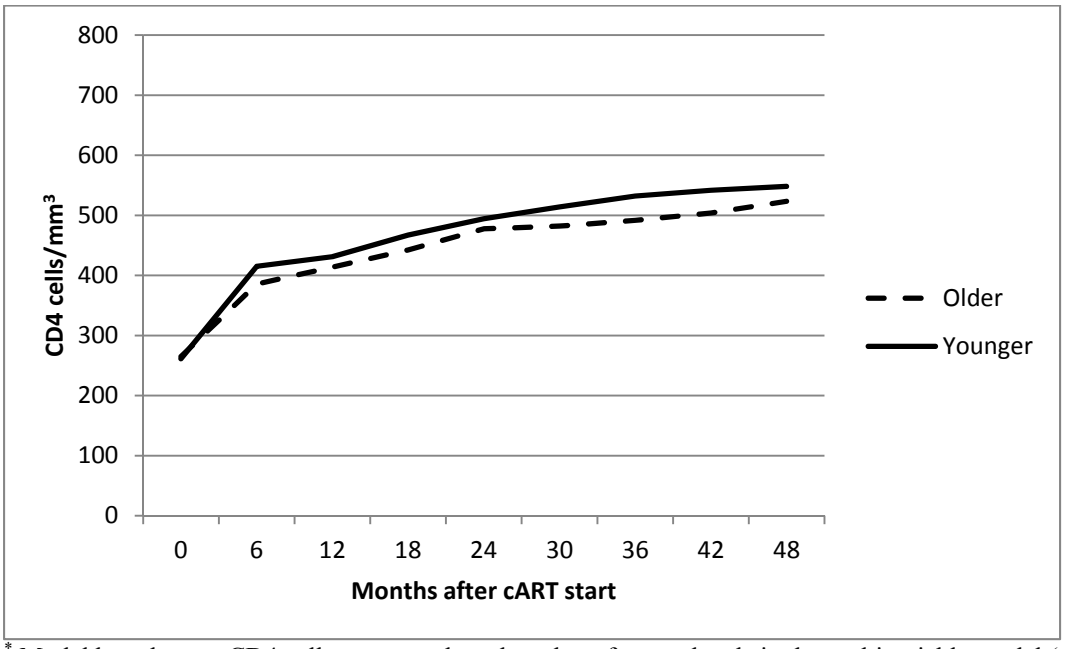
Table 15. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 201 and 350 cells/mm^3 (closed cohort)

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	813	265	46	1,547	261	48	0.08
6	564	386	170	1,016	415	211	0.001
12	564	414	207	1,036	431	220	0.10
18	580	442	206	1,068	467	223	0.02
24	576	478	214	1,118	494	220	0.11
30	607	482	212	1,094	514	221	0.002
36	570	492	189	1,110	532	219	<.0001
42	556	504	203	1,084	542	222	0.0002
48	813	523	209	1,547	548	229	0.009

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

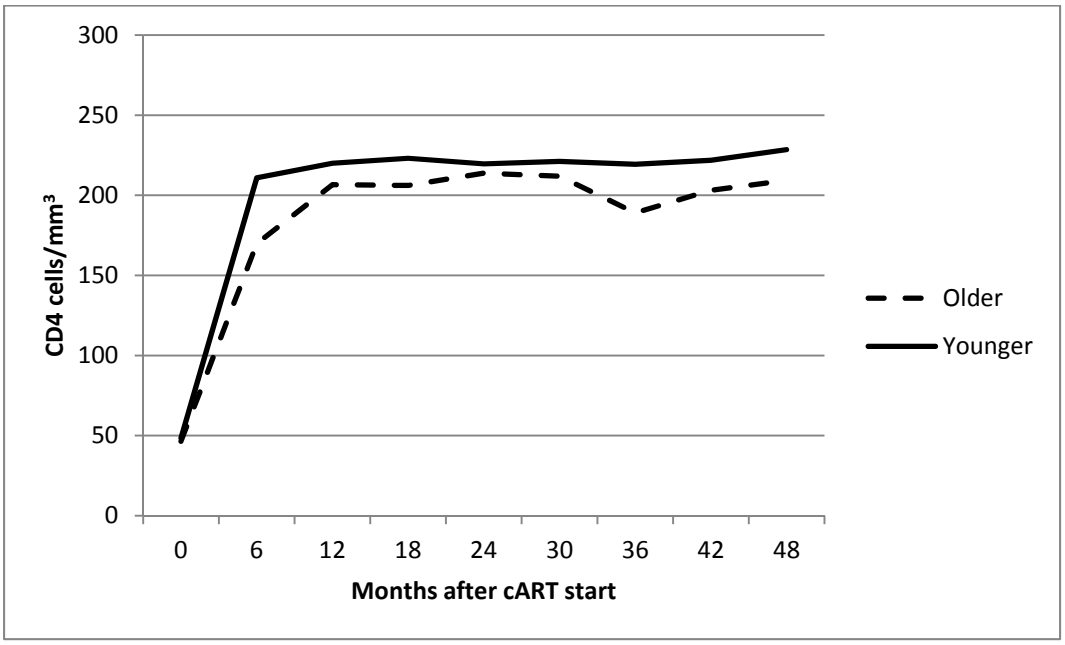
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 19. Model based estimates of mean CD4 cell counts*, by age group and months since initiation of cART, among patients with a baseline CD4 cell count between 201 and 350 cells/mm^3 (closed cohort)



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 20. Variability of CD4 cell reconstitution presented as standard deviation over time comparing older to younger patients who initiated cART with a baseline CD4 cell count between 201 and 350 cells/mm^3 (closed cohort)



E. Baseline CD4 cell count > 350 cells/mm^3

Of the study patients initiating cART during the study period, 13,906 had a documented baseline CD4 cell count > 350 cells/mm^3 (Table 16). The median time on cART among older patients was 16.3 months (IQR 5.6 – 31.3) and 13.2 months (IQR 3.8 – 27.3) among younger patients ($p<0.0001$). By the end of the study period, six % of older patients and four % of younger patients had died, and seven % of older patients and nine % of younger patients had been lost to follow-up. Approximately 90% of older patients and 87% of younger patients were still active or had transferred to another ART facility by the end of the study period.

Table 16. Baseline characteristics of patients initiated on cART with a CD4 count of greater than 350 cells/mm^3 (n=13,600)

Characteristic	Older n (%) n=4,217	Younger n (%) n=9,689	p-value*
Median age (IQR)**	47 (43 – 52)	30 (26 – 35)	
Median CD4 (IQR)	438 (380 – 558)	459 (385 – 603)	<0.0001***
Sex			<0.0001
Female	2,603 (62)	8,004 (83)	
Male	1,614 (38)	1,685 (17)	
WHO stage			<0.0001
I	1,293 (31)	4,553 (47)	
II	997 (24)	2,005 (21)	
III	1,180 (28)	1,843 (19)	
IV	440 (10)	614 (6)	
Missing	307 (7)	674 (7)	
WAB stage			0.0001
Working	3,698 (88)	8,701 (90)	
Ambulatory	244 (6)	397 (4)	
Bedridden	23 (1)	54 (1)	
Missing	252 (6)	537 (6)	
BMI			0.0005
Normal	1,204 (29)	2,767 (29)	
Underweight	478 (11)	874 (9)	
Overweight	275 (7)	637 (7)	
Obese	91 (2)	248 (3)	
Missing	2,169 (51)	5,163 (53)	
Tuberculosis			0.001
No	3,936 (93)	9,179 (95)	
Yes	281 (7)	510 (5)	
Cryptococcal disease			0.62
No	4,208 (99)	9,664 (99)	
Yes	9 (<1)	25 (<1)	
Pneumocystis *jiroveci Pneumonia*			0.03
No	4,151 (98)	9,581 (99)	
Yes	66 (2)	108 (1)	
Other opportunistic infection			0.05
No	3,661 (87)	8,528 (88)	
Yes	556 (13)	1,161 (12)	
First cART regimen			<0.0001
3TC/D4T/NVP	845 (20)	1,256 (13)	
3TC/D4T/EFV	151 (4)	260 (3)	
3TC/D4T/LOP	4 (<1)	48 (1)	
3TC/AZT/NVP	670 (16)	2,085 (22)	
3TC/AZT/EFV	852 (20)	1,510 (16)	
3TC/AZT/LOP	22 (1)	441 (5)	
3TC/TDF/NVP	312 (7)	1,102 (11)	
3TC/TDF/EFV	1,212 (29)	2,187 (23)	
3TC/TDF/LOP	39 (1)	169 (2)	
Other	39 (1)	169 (2)	

*p-value from chi-square test of proportions
**Interquartile range
***p-value from nonparametric two-sample test of medians

In the crude analysis there was strong evidence that older patients had lower mean CD4 cell counts following the initiation of cART from baseline to 42 months and at 54 months. Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the multivariable models.

After adjustment for covariates there was strong evidence against the null hypothesis of no difference in mean CD4 cell count from six to 36 months and 54 months and some evidence at 54 and 60 months. These data did not demonstrate evidence against the null hypothesis of no difference in mean CD4 cell count comparing older to younger patients at 42 and 48 months (Table 17) (Figure 21).

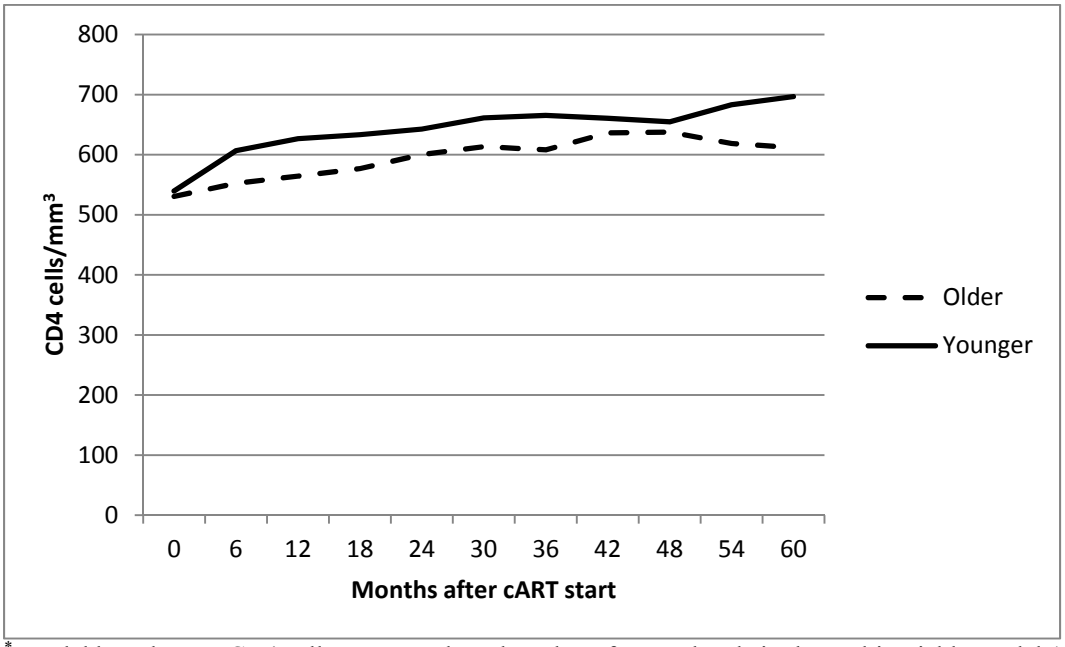
Table 17. Model based estimates of mean CD4 cell counts[*] and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count between CD4 cell count greater than 350 cells/mm^3

Months since initiation of cART	Older age			Younger age			p-value[**]
	n	Mean	SD	n	Mean	SD	
0	4,078	531	201	9,448	540	205	0.02
6	2,198	552	239	4,994	607	274	<.0001
12	1,677	564	233	3,740	627	271	<.0001
18	1,350	577	232	2,893	633	257	<.0001
24	1,019	600	255	2,143	643	257	<.0001
30	762	613	236	1,530	661	267	<.0001
36	517	608	238	998	665	263	<.0001
42	329	636	259	601	660	254	0.1
48	164	638	272	347	655	273	0.5
54	65	619	253	134	683	274	0.06
60	38	612	248	62	697	279	0.07

[*] Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

[**] p-value for the difference in mean CD4 cell count between older and younger groups

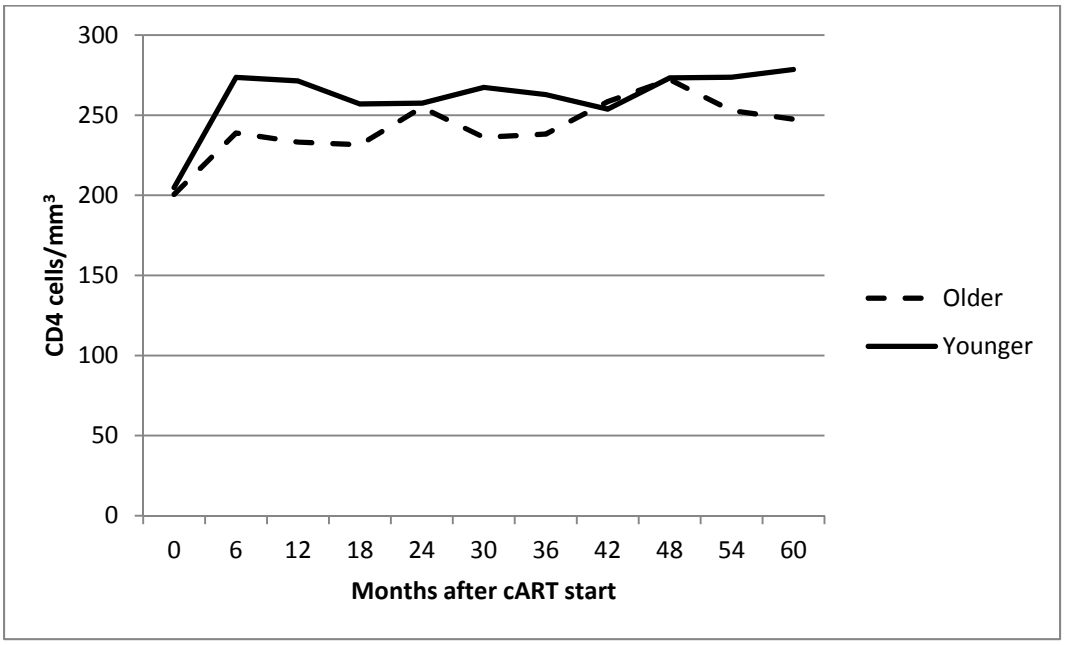
Figure 21. Model based estimates of mean CD4 cell counts*, by age group and months since initiation of cART, among patients with a baseline CD4 cell count greater than 350 cells/mm^3



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

While immune system reconstitution was more variable in younger patients than in older patients from baseline to 36 months and again at 54 and 60 months after treatment ($p<0.00001$) (Figure 22), the standard deviation was large in both groups. By 12 months, approximately 30% of older patients had failed to achieve a CD4 cell count of 450 cells/mm^3 or higher compared to 25% of younger patients. By 36 months 35% of older patients had failed to achieve a CD4 cell count of at least 450 cells/mm^3 compared to 25% of younger patients.

Figure 22. Variability of CD4 cell reconstitution presented as standard deviation of the mean CD4 cell count over time comparing older to younger patients who initiated cART with a baseline CD4 cell count greater than 350 cells/mm^3



Sensitivity analysis: There were insufficient patients with observations beyond 36 months. We therefore conducted the sensitivity analysis for this stratum for patients with a baseline and 36 month CD4 cell count. Of the patients with a baseline CD4 cell count > 350 cells/mm^3, 1,500 patients had both a baseline and 36 month CD4 cell count. Older patients had consistently lower mean CD4 cell counts at each time point after the initiation of cART compared to younger patients in the sensitivity analysis. The variability of immune system reconstitution displayed a similar trend as in the open cohort. There was less variability of CD4 cell reconstitution among older patients at each six and 12 months after cART initiation and from 24 to 36 months compared to younger patients. The differences in mean CD4 cell count comparing older to younger patients was similar to what was observed in the open cohort analysis.

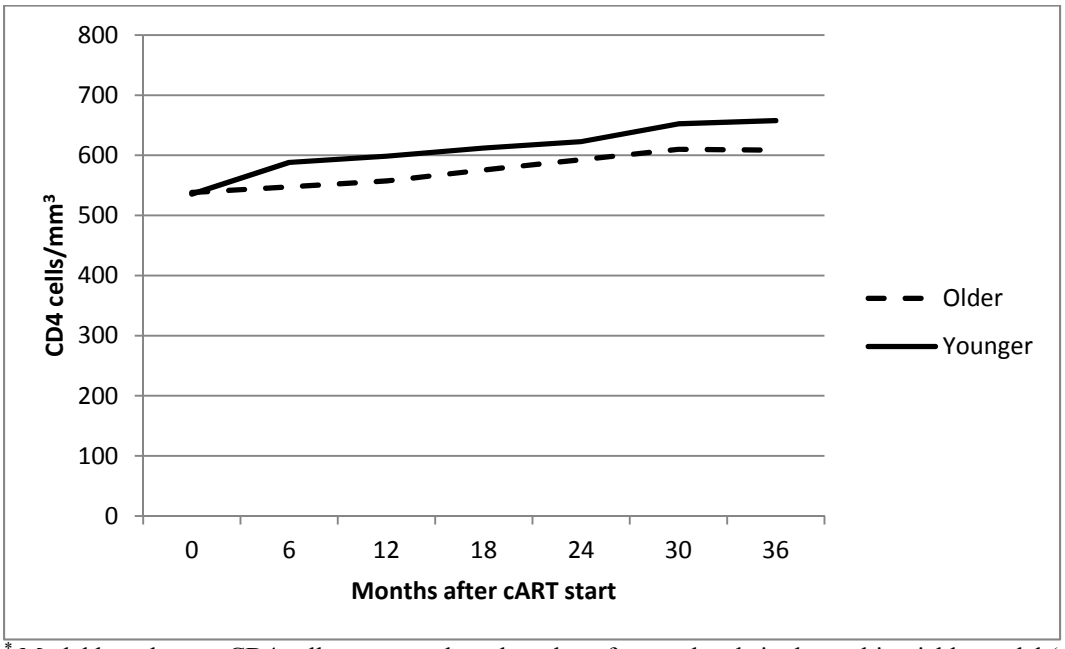
Table 18. Model based estimates of mean CD4 cell counts* and standard deviations (SDs), by age group and months since initiation of cART, among patients with a baseline CD4 cell count greater than 350 cells/mm^3 (closed cohort)

Months since initiation of cART	Older age			Younger age			p-value**
	n	Mean	SD	n	Mean	SD	
0	509	538	222	991	535	205	0.82
6	384	547	262	773	588	279	0.01
12	373	557	235	728	599	250	0.005
18	388	576	253	746	612	242	0.02
24	370	593	240	716	623	255	0.05
30	362	610	252	725	652	269	0.008
36	509	609	242	991	658	265	0.0005

* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

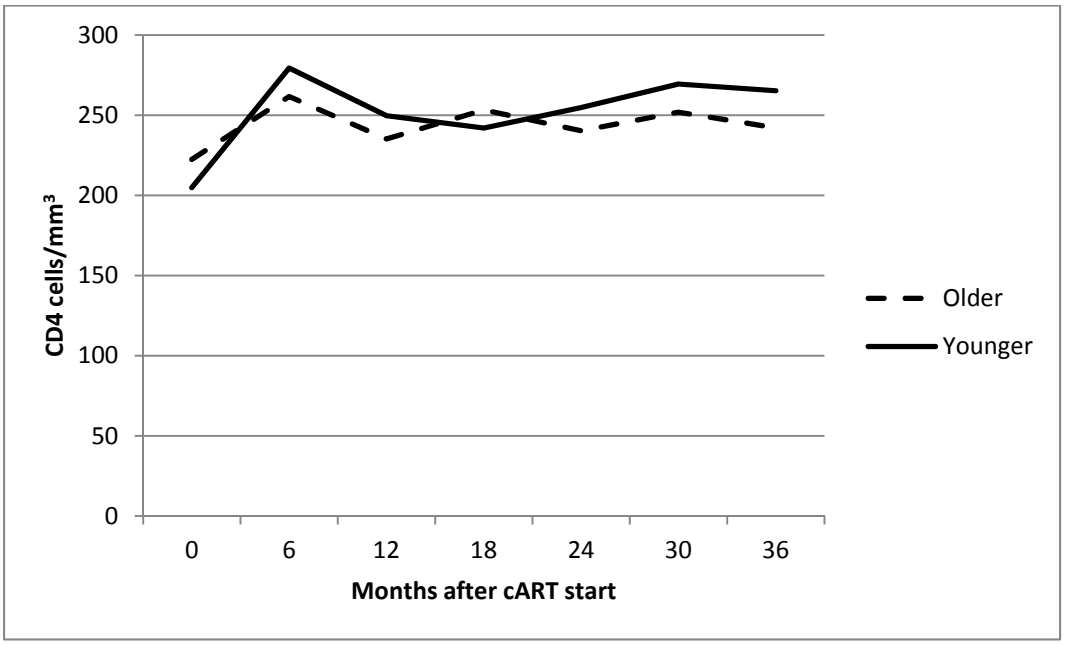
** p-value for the difference in mean CD4 cell count between older and younger groups

Figure 23. Model based estimates of mean CD4 cell counts*, by age group and months since initiation of cART, among patients with a baseline CD4 cell count greater than 350 cells/mm^3 (closed cohort)



* Model based mean CD4 cell counts are based on the reference levels in the multivariable model (women, WHO stage I, working functional status, no active TB, no active cryptococcal disease, no active pneumocystis *jiroveci pneumonia*, no other active OIs, and initiated on D4T/3TC/NVP). Model based mean CD4 cell counts for other groups (e.g., males with TB) may be somewhat different from the means shown but the mean difference between older and younger will be the same as for the reference group shown here.

Figure 24. Variability of CD4 cell reconstitution presented as standard deviation of the mean CD4 cell count over time comparing older to younger patients who initiated cART with a baseline CD4 cell count greater than 350 cells/mm^3 (closed cohort)



F. Baseline CD4 cell count missing

Of the study patients initiating cART during the study period, 48,680 did not have a documented baseline CD4 cell count (Table 19). The median time on cART among older patients was 30 months (IQR 18 – 43) and 29 months (IQR 17 – 43) among younger patients ($p<0.0001$). By the end of the study period, four % of older patients and three % of younger patients had died, and four % of older patients and five % of younger patients had been lost to follow-up. Approximately 92 % of older patients and 92 % of younger patients were still active or had transferred to another ART facility by the end of the study period. The proportion of females was significantly smaller in the older age group as compared to the younger age group (58% vs. 75% respectively). The distribution of WHO stage, WAB stage, BMI, presence of OIs, active TB, active cryptococcal disease,

active pneumocystis *jiroveci* pneumonia, and first treatment regimens were similar between the two groups.

Table 19. Baseline characteristics of patients initiated on cART with a missing CD4 count (n=48,680)

Characteristic	Older n (%) n=17,478	Younger n (%) n=31,202	p-value*
Median age (IQR)**	47 (43 – 52)	32 (28 – 36)	
Median CD4 (IQR)	-	-	
Sex			<0.0001
Female	9,929 (57)	22,978 (74)	
Male	7,549 (43)	8,224 (26)	
WHO stage			<0.0001
I	4,166 (24)	9,211 (30)	
II	3,848 (22)	6,915 (22)	
III	5,090 (29)	7,767 (25)	
IV	2,403 (14)	3,749 (12)	
Missing	1,971 (11)	3,560 (11)	
WAB stage			0.0002
Working	14,101 (81)	25,411 (81)	
Ambulatory	1,391 (8)	2,158 (7)	
Bedridden	190 (1)	311 (1)	
Missing	1,796 (10)	3,322 (11)	
BMI			0.03
Normal	4,046 (23)	7,527 (24)	
Underweight	1,945 (11)	3,372 (11)	
Overweight	709 (4)	1,265 (4)	
Obese	278 (2)	417 (1)	
Missing	10,500 (60)	18,621 (60)	
Tuberculosis			0.41
No	16,588 (95)	29,666 (95)	
Yes	890 (5)	1,536 (5)	
Cryptococcal disease			0.84
No	17,436 (99)	31,124 (99)	
Yes	42 (<1)	78 (<1)	
Pneumocystis *jiroveci pneumonia*			0.72
No	17,261 (99)	30,803 (99)	
Yes	217 (1)	399 (1)	
Other opportunistic infection			0.44
No	15,733 (90)	28,154 (90)	
Yes	1,745 (10)	3,048 (10)	
First cART regimen			<0.0001
3TC/D4T/NVP	4,841 (28)	7,968 (26)	
3TC/D4T/EFV	597 (3)	926 (3)	
3TC/D4T/LOP	2 (<1)	48 (<1)	
3TC/AZT/NVP	2,767 (16)	6,322 (20)	
3TC/AZT/EFV	3,519 (20)	4,683 (15)	
3TC/AZT/LOP	42 (<1)	309 (1)	
3TC/TDF/NVP	1,426 (8)	4,155 (13)	
3TC/TDF/EFV	3,726 (21)	5,467 (18)	
3TC/TDF/LOP	146 (1)	304 (1)	
Other	412 (2)	1,020 (3)	

*p-value from chi-square test of proportions
**Interquartile range

The baseline characteristics of these patients without a baseline CD4 was similar to the distribution of baseline characteristics among patients with a documented baseline CD4. The pattern of mean CD4 cell count over time comparing older to younger patients was also similar to what was observed in the other strata (data not shown).

G. Time to first regimen change among patients initiated on D4T/3TC/NVP

Of the study patients initiating cART during the study period, 41,048 had a first regimen of D4T/3TC/NVP (Table 20). There was a substantially lower proportion of female patients among the older age group compared to the younger age group. Older and younger patients had similar distributions of baseline covariates including CD4 cell count, liver functioning, creatinine clearance, hemoglobin, BMI, WHO stage, functional status, active TB, active cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections.

Table 20. Baseline characteristics by age group among patients initiated on D4T/3TC/NVP (n=41,048)

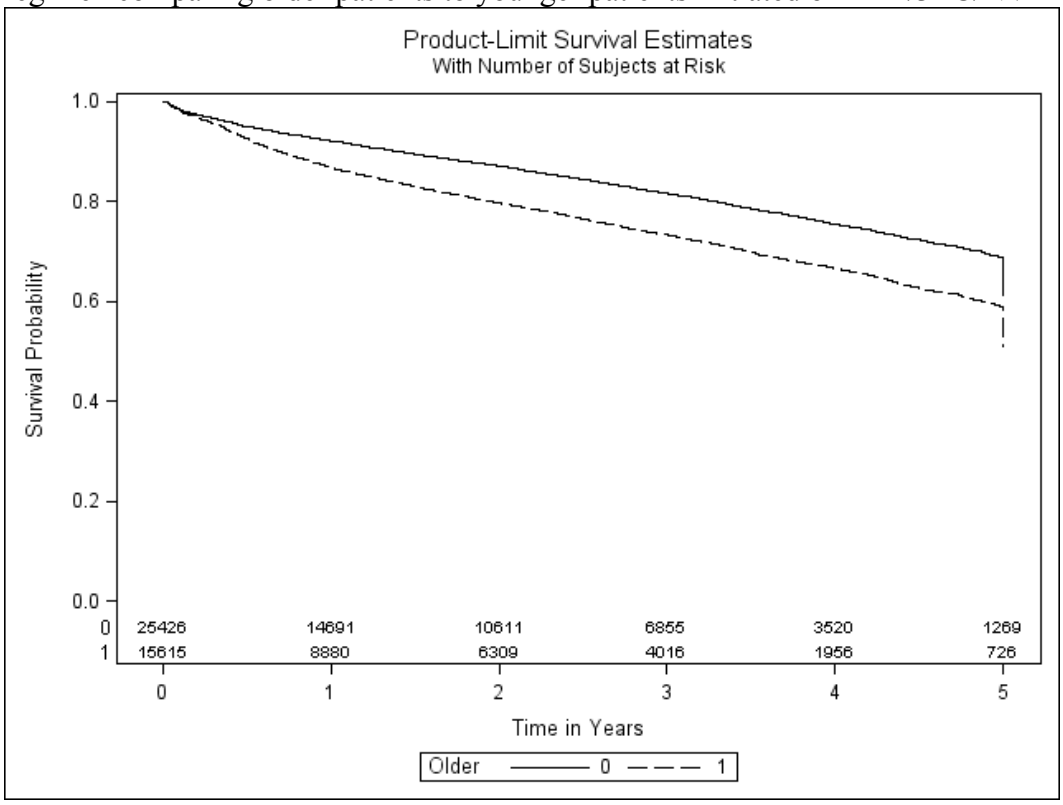
Characteristic	Older n (%) n=15,616	Younger n (%) n=25,432	p-value*
Sex			<0.0001
Female	9,064 (58)	18,991 (75)	
Male	6,552 (42)	6,441 (25)	
CD4 cells/mm^3			<0.0001
0 - 50	2,065 (13)	3,827 (15)	
51 – 100	1,940 (12)	2,989 (12)	
101 – 200	3,767 (24)	5,880 (23)	
201 – 350	2,689 (17)	4,236 (17)	
>350	788 (5)	1,179 (5)	
Missing	4,367 (28)	7,321 (29)	
Hepatotoxicity			0.12
None	2,393 (15)	4,140 (16)	
Grade 1	186 (1)	331 (1)	
Grade 2	10 (<1)	22 (<1)	
Grade 3	7 (<1)	11 (<1)	
Grade 4	2 (<1)	2 (<1)	
Missing	13,018 (83)	20,926 (82)	
Creatinine clearance			<0.0001
Normal	633 (4)	1,217 (5)	
30 – 49 mL/min	343 (2)	574 (2)	
15 – 29 mL/min	89 (<1)	97 (<1)	
5 – 14 mL/min	16 (<1)	22 (<1)	
<5 mL/min	5 (<1)	4 (<1)	
High	142 (1)	327 (1)	
Missing	14,388 (92)	23,191 (91)	
Hemoglobin			<0.0001
Normal	3,308 (21)	5,025 (20)	
Grade 1	913 (6)	1,585 (6)	
Grade 2	323 (2)	705 (3)	
Grade 3	217 (1)	479 (2)	
Grade 4	150 (1)	354 (1)	
Missing	10,705 (69)	17,284 (68)	
BMI			<0.0001
Normal	2,814 (18)	5,152 (20)	
Underweight	1,190 (8)	2,073 (8)	
Overweight	494 (3)	790 (3)	
Obese	140 (1)	219 (1)	
Missing	10,978 (70)	17,198 (68)	
WHO stage			<0.0001
I	2,493 (16)	4,877 (19)	
II	4,026 (26)	6,786 (27)	
III	6,083 (39)	9,044 (36)	
IV	2,420 (15)	3,668 (14)	
Missing	594 (4)	1,057 (4)	
WAB stage			0.0007
Working	13,021 (83)	21,205 (83)	
Ambulatory	1,973 (13)	3,104 (12)	
Bedridden	262 (2)	381 (2)	
Missing	360 (2)	742 (3)	

Characteristic	Older n (%) n=15,616	Younger n (%) n=25,432	p-value*
Table 20 continued			
Tuberculosis			0.46
No	15,181 (97)	24,692 (97)	
Yes	435 (3)	740 (3)	
Cryptococcal disease			0.94
No	15,578 (99)	25,371 (99))	
Yes	38 (<1)	61 (<1)	
Pneumocystis *jiroveci* pneumonia			0.44
No	15,515 (99)	25,283 (99)	
Yes	101 (1)	149 (1)	
Other opportunistic infection			0.63
No	12,841 (82)	20,865 (82)	
Yes	2,775 (18)	4,567 (18)	

*p-value from chi-square test of proportions

There were a total of 3,180 (20%) regimen changes due to toxicity or side-effect among the patients in the older group and 3,542 (14%) in the younger group (Figure 25).

Figure 25. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on D4T/3TC/NVP



Older patients changed away from the D4T/3TC/NVP regimen due to toxicity or side-effects at approximately 1.5 times the rate of younger patients, in both the crude and adjusted hazard analysis (Table 21). Year of cART initiation did not satisfy our criteria for inclusion as a confounder and was therefore not included in the multivariable models. Covariates that did not improve model fit or change the estimate of the association of age and rate of regimen change were not included in the multivariable model.

Table 21. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing older to younger patients initiated on D4T/3TC/NVP (n=41,048)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age group (years)			
20 – 39	1	-	-
≥ 40	1.56	1.48 – 1.64	<0.0001
Sex			
Female	1	-	-
Male	0.80	0.75 – 0.84	<0.0001
CD4 cells/mm^3			
0 – 50	1	-	-
51 – 100	0.93	0.86 – 1.01	0.06
101 – 200	0.93	0.85 – 1.00	0.05
201 – 350	0.95	0.87 – 1.03	0.22
>350	0.98	0.87 – 1.12	0.79
Missing	0.81	0.75 – 0.88	<0.0001
Hepatotoxicity			
None	1	-	-
Grade 1	0.76	0.60 – 0.96	0.02
Grade 2	0.98	0.44 – 2.20	0.97
Grade 3	2.35	1.12 – 4.94	0.02
Grade 4	5.11	1.28 – 20.35	0.02
Missing	0.94	0.87 – 1.02	0.12
Creatinine clearance			
Normal	1	-	-
30 – 49 mL/min	1.10	0.91 – 1.34	0.33
15 – 29 mL/min	0.87	0.58 – 1.31	0.50
5 – 14 mL/min	1.40	0.69 – 2.82	0.35
<5 mL/min	1.19	0.30 – 4.80	0.81
High	1.68	1.35 – 2.08	<0.0001
Missing	1.24	1.09 – 1.42	0.001
Hemoglobin			
Normal	1	-	-
Grade 1	0.95	0.86 – 1.06	0.33
Grade 2	0.92	0.78 – 1.08	0.31
Grade 3	0.96	0.79 – 1.16	0.64
Grade 4	0.79	0.61 – 1.01	0.06
Missing	0.82	0.77 – 0.87	<0.0001
WHO stage			
I	1	-	-
II	1.04	0.97 – 1.13	0.21
III	1.14	1.06 – 1.22	0.001
IV	1.02	0.93 – 1.12	0.74
Missing	1.12	0.93 – 1.35	0.24
Functional status			
Working	1	-	-
Ambulatory	1.10	1.02 – 1.19	0.02
Bedridden	0.84	0.64 – 1.09	0.19
Missing	0.61	0.48 – 0.78	<0.0001

The hazard ratio for time to regimen change comparing older to younger patients was not appreciably different when comparing the adjusted model including the covariate for adherence in the first year to the model without the covariate for adherence (data not shown), indicating that the effect of age is not mediated by adherence. We additionally assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found that sex was not an effect measure modifier (data not shown). We therefore controlled for sex in the adjusted multivariable model.

Sensitivity analysis

Of patients initiated on D4T/3TC/NVP, 1,970 (13%) older patients and 2,6,79 (11%) younger patients died without changing regimen. Additionally 1,318 older patients (8%) and 2,804 (11%) younger patients were lost to follow up before a regimen change occurred. After imputing a change in regimen at the last visit for each of the patients previously censored due to death or being lost to follow up, older patients progressed to regimen change at 1.17 times the rate of younger patients.

H. Time to first regimen change among patients initiated on D4T/3TC/EFV

Of the study patients initiating cART during the study period, 5,954 had a first regimen of D4T/3TC/EFV (Table 22). Of these 2,343 (39%) were age 40 or older and 3,611 (61%) were age 20 to 39. The median age among patients in the older group was 46.3 (IQR 42.6 – 51.7) and 32.4 (IQR 28.3 – 35.8) among patients in the younger group. There was a substantially lower proportion of female patients among the older age group

compared to the younger age group (53% vs. 66% respectively). Older and younger patients had similar distributions of baseline covariates including CD4 cell count strata, liver functioning, creatinine clearance, hemoglobin, BMI, WHO stage, WAB stage, active TB, active cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections.

Table 22. Baseline characteristics by age group among patients initiated on D4T/3TC/EFV (n=5,954)

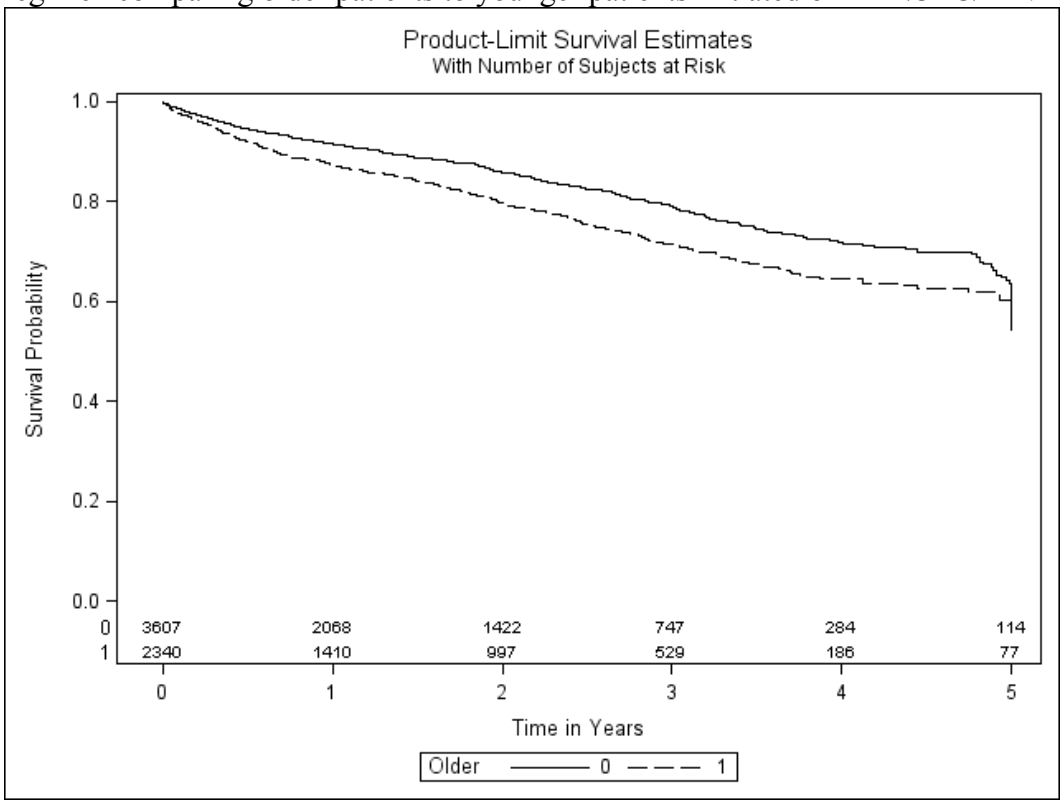
Characteristic	Older n (%) n=2,343	Younger n (%) n=3,611	p-value*
Sex			<0.0001
Female	1,238 (53)	2,399 (66)	
Male	1,105 (47)	1,212 (44)	
CD4 cells/mm^3			0.02
0 - 50	314 (13)	584 (16)	
51 – 100	281 (12)	434 (12)	
101 – 200	459 (20)	641 (18)	
201 – 350	551 (24)	780 (22)	
>350	151 (6)	259 (7)	
Missing	587 (25)	913 (25)	
Hepatotoxicity			0.13
None	857 (37)	1,269 (35)	
Grade 1	81 (3)	174 (5)	
Grade 2	12 (1)	18 (1)	
Grade 3	5 (<1)	6 (<1)	
Grade 4	0 (0)	0 (0)	
Missing	1,388 (60)	2,142 (59)	
Creatinine clearance			.01
Normal	349 (15)	451 (12)	
30 – 49 mL/min	171 (7)	216 (6)	
15 – 29 mL/min	57 (2)	57 (2)	
5 – 14 mL/min	5 (<1)	7 (<1)	
<5 mL/min	0 (0)	2 (<1)	
High	83 (4)	157 (4)	
Missing	1,678 (72)	2,721 (75)	
Hemoglobin			<0.0001
Normal	574 (25)	747 (21)	
Grade 1	346 (15)	583 (16)	
Grade 2	137 (6)	264 (7)	
Grade 3	74 (3)	152 (4)	
Grade 4	33 (1)	113 (3)	
Missing	1,179 (50)	1,752 (49)	
BMI			0.18
Normal	844 (36)	1,303 (36)	
Underweight	470 (20)	781 (22)	
Overweight	126 (5)	158 (4)	
Obese	28 (1)	31 (1)	
Missing	875 (37)	1,338 (37)	
WHO stage			0.14
I	415 (18)	680 (19)	
II	441 (19)	601 (17)	
III	912 (39)	1,386 (38)	
IV	359 (15)	607 (17)	
Missing	216 (9)	337 (9)	
Functional status			0.25
Working	1,961 (84)	2,964 (82)	
Ambulatory	268 (11)	467 (13)	
Bedridden	34 (1)	44 (1)	
Missing	80 (3)	136 (4)	

Characteristic	Older n (%)	Younger n (%)	p-value
Table 22 continued			
Tuberculosis			0.01
No	1,776 (76)	2,627 (73)	
Yes	567 (24)	984 (27)	
Cryptococcal disease			0.15
No	2,316 (99)	3,553 (98)	
Yes	27 (1)	58 (2)	
Pneumocystis *jiroveci* pneumonia			0.73
No	2,326 (99)	3,582 (99)	
Yes	17 (1)	29 (1)	
Other opportunistic infection			<0.0001
No	1,830 (78)	2,643 (73)	
Yes	513 (22)	968 (27)	

*p-value from chi-square test of proportions

At four years after initiation of cART, older patients had approximately a 36 % probability of switching from D4T/3TC/EFV due to toxicity or a significant side-effect compared to a 28 % probability for younger patients ($p<0.0001$) (Figure 26).

Figure 26. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on D4T/3TC/EFV



The crude association between age group and the hazard of regimen change due to toxicity or side-effect was 1.36 (95% CI 1.20 – 1.54). There were a total of 486 (21%) regimen changes due to toxicity or side-effect among the older group and 524 (15%) among the younger group. After controlling for covariates either known to be associated with D4T or EFV associated toxicity and side-effects, or that improved model fit, older patients progressed to regimen change for toxicity or side-effect 1.39 (95% CI 1.22 – 1.57) times as fast as younger patients (Table 23). Covariates that did not improve model fit or change the estimate of the association of age and rate of regimen change, such as year of cART initiation, were not included in the multivariable model.

Table 23. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing older to younger initiated on D4T/3TC/EFV (n=5,954)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age Group			
20 – 39	1	-	-
≥ 40	1.39	1.22 – 1.57	<0.0001
Sex			
Female	1	-	-
Male	0.85	0.74 – 0.97	0.01
CD4 cells/mm^3			
0 - 50	1	-	-
51 – 100	0.95	0.75 – 1.20	0.67
101 – 200	0.78	0.63 – 0.97	0.02
201 – 350	0.92	0.75 – 1.13	0.41
>350	0.88	0.65 – 1.17	0.38
Missing	0.96	0.78 – 1.18	0.72
Creatinine clearance			
Normal	1	-	-
30 – 49 mL/min	1.09	0.83 – 1.42	0.53
15 – 29 mL/min	1.06	0.68 – 1.65	0.81
5 – 14 mL/min	0.35	0.05 – 2.48	0.29
<5 mL/min	-	-	-
High	0.96	0.70 – 1.33	0.82
Missing	0.80	0.67 – 0.95	0.01
WHO stage			
I	1	-	-
II	1.06	0.86 – 1.31	0.56
III	1.06	0.88 – 1.27	0.56
IV	1.35	1.09 – 1.67	0.01
Missing	0.57	0.43 – 0.74	<0.0001
Tuberculosis			
No	1	-	-
Yes	1.13	0.97 – 1.33	0.11
Cryptococcal disease			
No	1	-	-
Yes	1.58	1.01 – 2.47	0.05
Other opportunistic infection			
No	1	-	-
Yes	1.23	1.07 – 1.41	0.005

In order to assess the impact of adherence on the association between age group and time to regimen change we ran the adjusted model twice, once including adherence as a covariate and once without adherence and compared the estimates of effect of age on the hazard of regimen change. The hazard ratios for time to regimen change comparing older to younger patients were the same in both models (data not shown). We additionally

assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found sex to not be an effect measure modifier (data not shown). We therefore controlled for it as a binary variable in the adjusted multivariable model.

Sensitivity analysis

Of the patients initiated on D4T/3TC/EFV, 275 (12%) older patients and 420 (12%) younger patients died before having a regimen change. Additionally 185 older patients (8%) and 449 (12%) younger patients were lost to follow up while still on their initial regimen. After imputing a regimen change at the last visit for each of the patients previously censored due to death or loss to follow up, older patients progressed to regimen change 1.01 times as fast as younger patients.

I. Time to first regimen change among patients initiated on AZT/3TC/NVP

Of the study patients initiating cART during the study period, 33,113 had a first regimen of AZT/3TC/NVP (Table 24). Of these 9,567 (29%) were age 40 or older and 23,546 (71%) were age 20 to 39. The median age among patients in the older group was 46.5 (IQR 43 – 52) and 30.8 (IQR 24 – 31) among patients in the younger group. There was a significantly lower proportion of female patients among the older age group compared to the younger age group (62% vs. 84% respectively). Older and younger patients had similar distributions of baseline covariates including CD4 cell count strata, liver functioning, creatinine clearance, hemoglobin, BMI, WAB stage, active TB, active

cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections. There were differences in the distribution of WHO stage between older and younger patients with 31 % of older patients at stage I compared to 42 % of younger patients and 27% of older patients at stage III compared to younger patients.

Table 24. Baseline characteristics by age group among patients initiated on AZT/3TC/NVP (n=33,113)

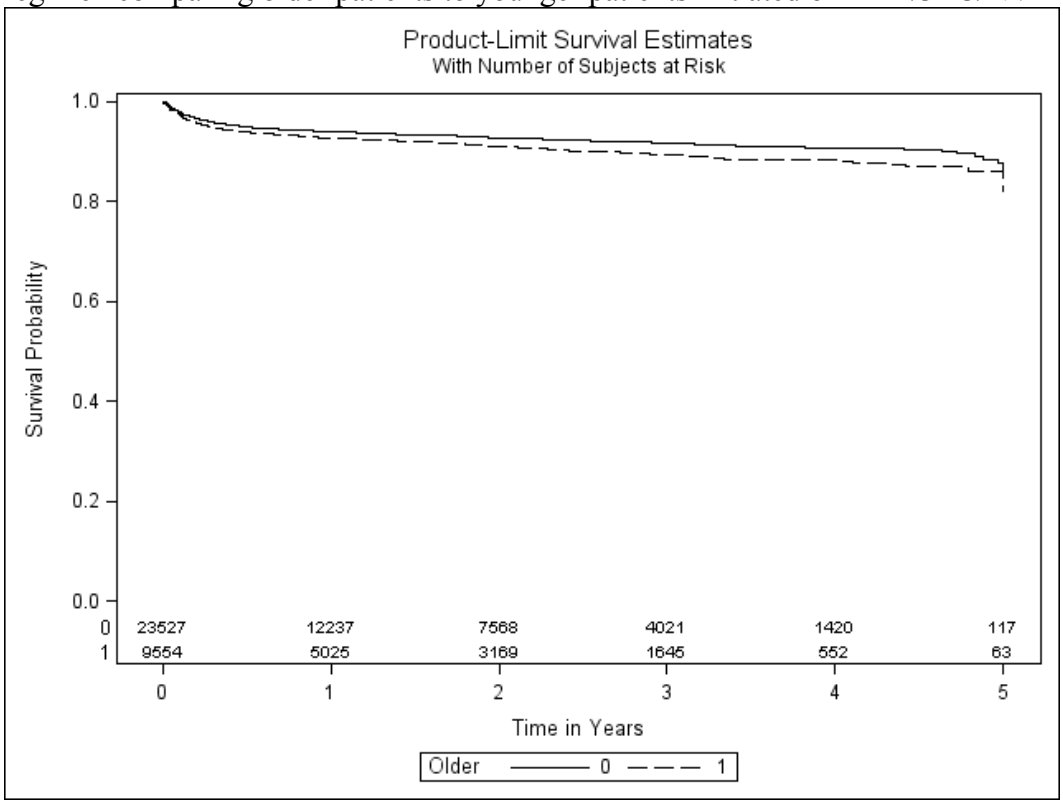
Characteristic	Older n (%) n=9,567	Younger n (%) n=23,546	p-value*
Sex			<0.0001
Female	5,969 (62)	19,791 (84)	
Male	3,598 (38)	3,755 (16)	
CD4 cells/mm^3			<0.0001
0 - 50	832 (9)	1,924 (8)	
51 – 100	865 (9)	2,010 (9)	
101 – 200	2,054 (21)	4,914 (21)	
201 – 350	2,529 (26)	6,587 (28)	
>350	658 (7)	2,054 (9)	
Missing	2,629 (27)	6,057 (26)	
Hepatotoxicity			<0.0001
None	2,761 (29)	8,106 (34)	
Grade 1	176 (2)	430 (2)	
Grade 2	6 (<1)	23 (<1)	
Grade 3	9 (<1)	21 (<1)	
Grade 4	6 (<1)	18 (<1)	
Missing	6,609 (69)	14,948 (63)	
Creatinine clearance			0.0008
Normal	961 (10)	2,410 (10)	
30 – 49 mL/min	460 (5)	1,228 (5)	
15 – 29 mL/min	95 (1)	138 (1)	
5 – 14 mL/min	9 (<1)	18 (<1)	
<5 mL/min	3 (<1)	4 (<1)	
High	251 (3)	707 (3)	
Missing	7,788 (81)	19,041 (81)	
Hemoglobin			<0.0001
Normal	2,336 (24)	5,292 (23)	
Grade 1	497 (5)	1,434 (6)	
Grade 2	129 (1)	386 (2)	
Grade 3	45 (<1)	160 (1)	
Grade 4	59 (1)	217 (1)	
Missing	6,501 (68)	16,057 (68)	
BMI			<0.0001
Normal	2,683 (28)	6,473 (28)	
Underweight	996 (10)	1,987 (8)	
Overweight	537 (6)	1,349 (6)	
Obese	224 (2)	497 (2)	
Missing	5,127 (54)	13,240 (56)	
WHO stage			<0.0001
I	3,003 (31)	9,782 (42)	
II	2,902 (30)	6,424 (27)	
III	2,563 (27)	4,890 (21)	
IV	711 (7)	1,241 (5)	
Missing	388 (4)	1,209 (5)	
WAB stage			<0.0001
Working	8,487 (89)	1,774 (89)	
Ambulatory	698 (7)	1,374 (6)	
Bedridden	51 (<1)	104 (<1)	
Missing	331 (3)	1,069 (5)	

Characteristic	Older n (%) n=9,567	Younger n (%) n=23,546	p-value*
Table 24 continued			
Tuberculosis			0.08
No	9,344 (98)	23,069 (98)	
Yes	223 (2)	477 (2)	
Cryptococcal disease			0.02
No	9,539 (99)	23,508 (99)	
Yes	28 (<1)	38 (<1)	
Pneumocystis *jiroveci* pneumonia			0.89
No	9,555 (99)	23,515 (99)	
Yes	12 (<1)	31 (<1)	
Other opportunistic infection			0.002
No	8,621 (90)	21,472 (91)	
Yes	946 (10)	2,074 (9)	

*p-value from chi-square test of proportions

At four years after initiation of cART, older patients had approximately a 12 % probability of switching from AZT/3TC/NVP due to toxicity or a significant side-effect compared to a little under a 10 % probability for younger patients ($p<0.0001$) (Figure 27).

Figure 27. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on AZT/3TC/NVP



The crude association between age group and the hazard of regimen change due to toxicity or side-effect was 1.27 (95% CI 1.16 – 1.39). There were a total of 716 (7%) regimen changes due to toxicity or side-effect among the older group and 1,382 (6%) among the younger group. After controlling for covariates either known to be associated with AZT or NVP associated toxicity and side-effects, or that improved model fit, older patients progressed to regimen change for toxicity or side-effect at 1.20 (95% CI 1.09 – 1.32) times the rate of younger patients (Table 25). Covariates that did not improve model fit or change the estimate of the association of age and rate of regimen change, such as year of cART initiation, were not included in the multivariable model.

Table 25. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing older to younger patients initiated on AZT/3TC/NVP (n=33,113)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age			
20 – 39	1	-	-
≥ 40	1.23	1.12 – 1.35	<0.0001
Sex			
Female	1	-	-
Male	0.81	0.72 – 0.90	0.0001
CD4 cells/mm^3			
0 - 50	1	-	-
51 – 100	0.68	0.57 – 0.82	<0.0001
101 – 200	0.62	0.37 – 0.72	<0.0001
201 – 350	0.58	0.50 – 0.67	<0.0001
>350	0.55	0.44 – 0.77	<0.0001
Missing	0.49	0.42 – 0.57	<0.0001
Hepatotoxicity			
None	1	-	-
Grade 1	1.04	0.75 – 1.43	0.81
Grade 2	2.00	0.75 – 5.40	0.17
Grade 3	1.47	0.47 – 4.60	0.50
Grade 4	0.92	0.13 – 6.57	0.94
Missing	1.70	1.53 – 1.89	<0.0001
Hemoglobin			
Normal	1	-	-
Grade 1	1.21	1.02 – 1.43	0.03
Grade 2	1.85	1.45 – 2.40	<0.0001
Grade 3	1.52	1.00 – 2.31	0.05
Grade 4	1.07	0.68 – 1.67	0.77
Missing	1.04	0.75 – 1.43	0.86
WHO stage			
I	1	-	-
II	1.46	1.30 – 1.64	<0.0001
III	1.81	1.61 – 2.03	<0.0001
IV	1.77	1.47 – 2.14	<0.0001
Missing	0.87	0.56 – 1.36	0.43
Functional status			
Working	1	-	-
Ambulatory	1.12	0.96 – 1.31	0.17
Bedridden	1.11	0.60 – 2.08	0.74
Missing	1.09	0.69 – 1.73	0.70
Other opportunistic infection			
No	1	-	-
Yes	1.08	0.94 – 1.23	0.29

In order to assess the impact of adherence on the association between age group and time to regimen change we ran two models, one including a variable for adherence in the first year and one without. We compared the estimate of effect for age and time to regimen

change and found them to be the same in both models (data not shown) indicating the effect of age was not mediated by adherence. We additionally assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found sex did not modify the association between age and regimen change on the hazard ratio scale. We therefore controlled for sex in the model.

Sensitivity analysis

Patients who died or were lost to follow up before experiencing a switch event are likely to represent informative censoring. That is the reason for their having missing outcome data is likely related informatively to both the exposure of interest (age) and the outcome (regimen change due to toxicity or side-effect). Of the patients initiated on AZT/3TC/NVP, 601 (6%) older patients and 1,090 (5%) younger patients died before experiencing a regimen change. Additionally 602 older patients (6%) and 2,137 (9%) younger patients were lost to follow up while still on their initial regimen. After imputing a regimen change at the last visit for each of the patients previously censored due to death or loss to follow up before a regimen change, older patients progressed to regimen change at 0.96 (95% CI 0.90 – 1.00) times the rate of younger patients.

J. Time to first regimen change among patients initiated on AZT/3TC/EFV

Of the study patients initiating cART during the study period, 29,591 had a first regimen of AZT/3TC/EFV (Table 26). Of these 12,803 (43%) were age 40 or older and 16,788 (57%) were age 20 to 39. The median age among patients in the older group was 47.3 (IQR 43 – 53) and 32.6 (IQR 29 – 36) among patients in the younger group. There was a significantly lower proportion of female patients among the older age group compared to the younger age group (52% vs. 64% respectively). Older and younger patients had similar distributions of baseline covariates including CD4 cell count strata, liver functioning, creatinine clearance, hemoglobin, BMI, WHO stage, WAB stage, active TB, active cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections (Table 24).

Table 26. Baseline characteristics by age group among patients initiated on AZT/3TC/EFV (n=29,591)

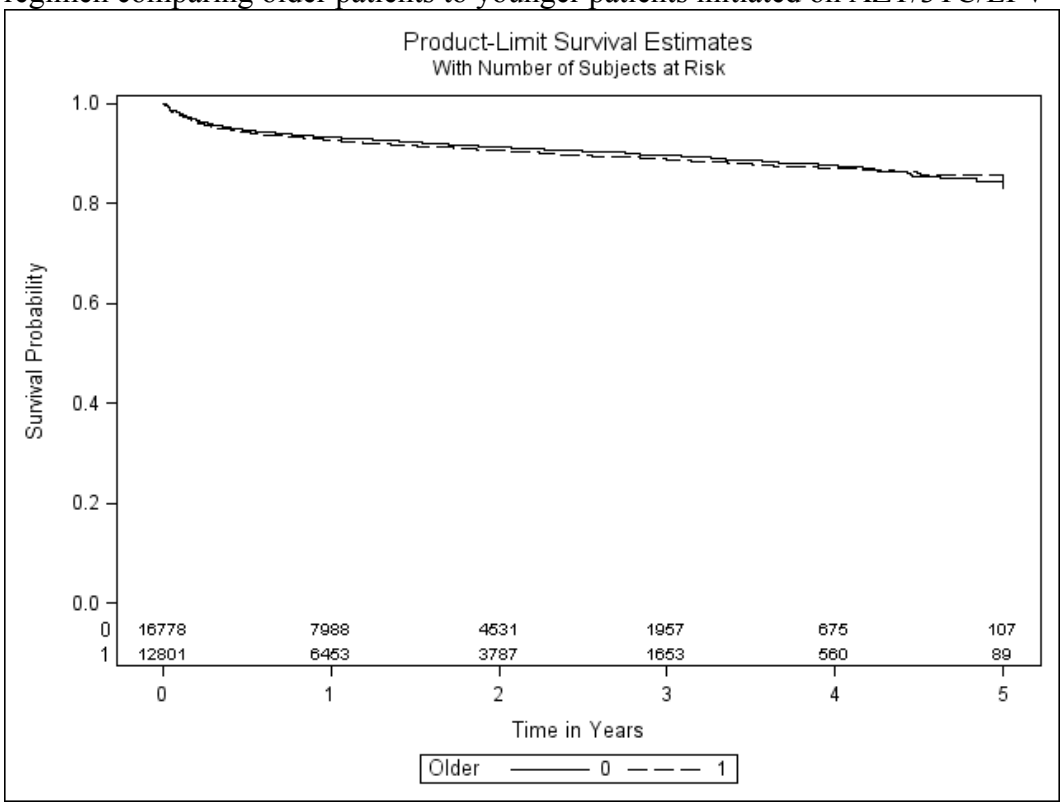
Characteristic	Older n (%) n=12,803	Younger n (%) n=16,788	p-value*
Sex			<0.0001
Female	6,717 (52)	10,816 (64)	
Male	6,086 (48)	5,972 (36)	
CD4 cells/mm^3			<0.0001
0 - 50	1,605 (13)	2,301 (14)	
51 – 100	1,372 (11)	1,688 (10)	
101 – 200	2,624 (21)	3,006 (18)	
201 – 350	3,110 (24)	3,941 (23)	
>350	818 (6)	1,465 (9)	
Missing	3,274 (26)	4,387 (26)	
Hepatotoxicity			0.13
None	2,983 (23)	4,014 (24)	
Grade 1	329 (3)	446 (3)	
Grade 2	19 (<1)	37 (<1)	
Grade 3	6 (<1)	20 (<1)	
Grade 4	6 (<1)	6 (<1)	
Missing	9,460 (74)	12,265 (73)	

Characteristic	Older n (%) n=12,803	Younger n (%) n=16,788	p-value*
Table 26 continued			
Creatinine clearance			<0.0001
Normal	870 (7)	1,278 (8)	
30 – 49 mL/min	412 (3)	471 (3)	
15 – 29 mL/min	85 (1)	69 (<1)	
5 – 14 mL/min	11 (<1)	12 (<1)	
<5 mL/min	0 (0)	1 (0)	
High	312 (2)	529 (3)	
Missing	11,113 (87)	14,428 (86)	
Hemoglobin			0.0001
Normal	3,762 (29)	4,593 (27)	
Grade 1	798 (6)	1,032 (6)	
Grade 2	216 (2)	314 (2)	
Grade 3	80 (1)	151 (1)	
Grade 4	90 (1)	153 (1)	
Missing	7,857 (61)	10,545 (63)	
BMI			0.02
Normal	2,753 (22)	3,887 (23)	
Underweight	1,380 (11)	1,766 (11)	
Overweight	472 (4)	636 (4)	
Obese	171 (1)	217 (1)	
Missing	8,027 (63)	10,282 (61)	
WHO stage			<0.0001
I	2,573 (20)	4,187 (25)	
II	3,079 (24)	4,005 (24)	
III	5,024 (39)	5,916 (35)	
IV	1,651 (13)	2,050 (12)	
Missing	476 (4)	630 (4)	
Functional status			<0.0001
Working	11,079 (87)	14,706 (88)	
Ambulatory	1,360 (11)	1,530 (9)	
Bedridden	120 (1)	193 (1)	
Missing	244 (2)	359 (2)	
Tuberculosis			0.0003
No	12,029 (93)	15,596 (93)	
Yes	774 (6)	1,192 (7)	
Cryptococcal disease			0.04
No	12,759 (99)	16,703 (99)	
Yes	44 (<1)	85 (<1)	
Pneumocystis *jiroveci* pneumonia			0.84
No	12,764 (99)	16,739 (99)	
Yes	39 (<1)	49 (<1)	
Other opportunistic infection			0.16
No	11,362 (89)	14,985 (89)	
Yes	1,441 (11)	1,803 (11)	

*p-value from chi-square test of proportions

At four years after initiation of cART, older patients had approximately a 14 % probability of switching from AZT/3TC/EFV due to toxicity or a significant side-effect compared to a little under a 13 % probability for younger patients (p=0.01) (Figure 28).

Figure 28. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on AZT/3TC/EFV



The crude association between age group and the hazard of regimen change due to toxicity or side-effect was 1.08 (95% CI 1.00 – 1.18). There were a total of 941 (7%) regimen changes due to toxicity or side-effect among the older group and 1,103 (6%) among the younger group. After controlling for covariates either known to be associated with AZT or EFV associated toxicity and side-effects, or that improved model fit, older patients progressed to regimen change for toxicity or side-effect 1.10 (95% CI 1.01 – 1.20) times as fast as younger patients (Table 27). Covariates that did not improve model

fit or change the estimate of the association of age and rate of regimen change, such as year of cART initiation, were not included in the multivariable model.

Table 27. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing older to younger patients initiated on AZT/3TC/EFV (n=29,591)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age			
20 – 39	1	-	-
≥ 40	1.10	1.01 – 1.21	0.03
Sex			
Female	1	-	-
Male	0.80	0.73 – 0.87	<0.0001
CD4 cells/mm^3			
0 - 50	1	-	-
51 – 100	1.07	0.91 – 1.26	0.41
101 – 200	0.84	0.73 – 0.98	0.03
201 – 350	0.78	0.67 – 0.90	0.001
>350	0.72	0.59 – 0.88	0.002
Missing	0.74	0.64 – 0.86	<0.0001
Hepatotoxicity			
None	1	-	-
Grade 1	1.11	0.85 – 1.45	0.45
Grade 2	1.67	0.74 – 3.74	0.21
Grade 3	-	-	-
Grade 4	-	-	-
Missing	1.42	1.26 – 1.60	<0.0001
Hemoglobin			
Normal	1	-	-
Grade 1	1.28	1.07 – 1.52	0.006
Grade 2	1.67	1.27 – 2.19	0.0002
Grade 3	2.92	2.12 – 3.98	<0.0001
Grade 4	1.92	1.33 – 2.78	0.0005
Missing	0.96	0.86 – 1.07	0.44
WHO stage			
I	1	-	-
II	1.21	1.06 – 1.39	0.005
III	1.29	1.14 – 1.46	<0.0001
IV	1.59	1.35 – 1.86	<0.0001
Missing	0.68	0.48 – 0.96	0.03
Functional status			
Working	1	-	-
Ambulatory	1.03	0.89 – 1.19	0.67
Bedridden	0.66	0.39 – 1.13	0.13
Missing	1.02	0.66 – 1.60	0.91
Other opportunistic infection			
No	1	-	-
Yes	1.12	0.98 – 1.28	0.07

In order to assess the impact of adherence on the association between age group and time to regimen change we ran two models, one with adherence as a covariate and one without, and compared the estimate of effect for the association between age and hazard of regimen change. The effect estimate for age was the same in both models (data not shown). We additionally assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found sex not be an effect measure modifier of the association between age and regimen change on the hazard ratio scale. We therefore included sex as a binary covariate in the adjusted model.

Sensitivity analysis

Of the patients initiated on AZT/3TC/EFV, 1,005 (8%) older patients and 1,060 (6%) younger patients died before changing regimen. Additionally 903 older patients (7%) and 1,692 (10%) younger patients were lost to follow up before experiencing a regimen change. After imputing a switch event at the last visit for each of the patients previously censored due to death or loss to follow up, older patients progressed to regimen change 0.83 (95% CI 0.78 – 0.88) times as fast as younger patients.

K. Time to first regimen change among patients initiated on TDF/3TC/NVP

Of the study patients initiating cART during the study period, 22,466 had a first regimen of TDF/3TC/NVP (Table 28). Of these 5,323 (24%) were age 40 or older and 17,143 (86%) were age 20 to 39. The median age among patients in the older group was 45.9 (IQR 42 – 51) and 30.3 (IQR 26 – 34) among patients in the younger group. There was a significantly lower proportion of female patients among the older age group compared to the younger age group (66% vs. 86% respectively). Older and younger patients had similar distributions of baseline covariates including CD4 cell count strata, liver functioning, creatinine clearance, hemoglobin, BMI, WHO stage, WAB stage, active TB, active cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections.

Table 28. Baseline characteristics by age group among patients initiated on TDF/3TC/NVP (n=22,466)

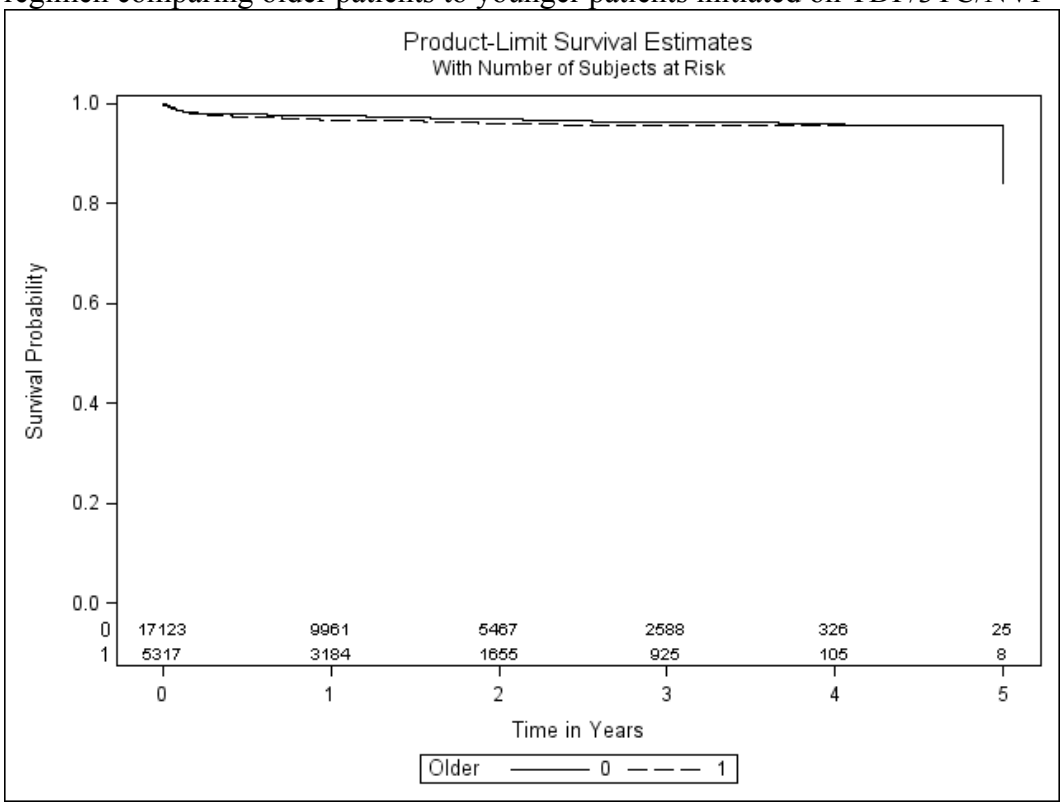
Characteristic	Older n (%) n=5,323	Younger n (%) n=17,143	p-value*
Sex			<0.0001
Female	3,494 (66)	14,726 (86)	
Male	1,829 (34)	2,417 (14)	
CD4 cells/mm^3			0.0001
0 - 50	401 (7)	1,491 (9)	
51 – 100	459 (9)	1,601 (9)	
101 – 200	1,107 (21)	3,742 (22)	
201 – 350	1,628 (30)	5,094 (30)	
>350	312 (6)	1,102 (6)	
Missing	1,416 (27)	4,113 (24)	
Hepatotoxicity			<0.0001
None	2,669 (50)	9,220 (54)	
Grade 1	153 (3)	494 (3)	
Grade 2	7 (<1)	34 (<1)	
Grade 3	3 (<1)	17 (<1)	
Grade 4	6 (<1)	10 (<1)	
Missing	2,485 (47)	7,368 (43)	
Creatinine clearance			<0.0001
Normal	1,343 (25)	3,757 (22)	
30 – 49 mL/min	379 (7)	1,058 (6)	
15 – 29 mL/min	66 (1)	86 (0.5)	
5 – 14 mL/min	19 (<1)	29 (<1)	
<5 mL/min	1 (<1)	2 (<1)	
High	219 (4)	756 (4)	
Missing	3,296 (62)	11,458 (67)	
BMI			<0.0001
Normal	2,110 (40)	5,948 (35)	
Underweight	807 (15)	2,397 (14)	
Overweight	396 (7)	1,077 (6)	
Obese	156 (3)	339 (2)	
Missing	1,854 (35)	7,382 (43)	
Functional status			<0.0001
I	2,564 (48)	9,218 (54)	
II	1,499 (28)	4,325 (25)	
III	863 (16)	2,400 (14)	
IV	186 (4)	548 (3)	
Missing	211 (4)	652 (4)	
WAB stage			0.70
Working	4,946 (93)	15,847 (93)	
Ambulatory	178 (3)	617 (4)	
Bedridden	14 (<1)	52 (<1)	
Missing	185 (4)	627 (3)	
Tuberculosis			0.80
Yes	155 (3)	488 (3)	
No	5,168 (97)	16,655 (97)	
Cryptococcal disease			0.47
Yes	14 (<1)	36 (<1)	
No	5,309 (99)	17,107 (99)	

Characteristic	Older n (%) n=5,323	Younger n (%) n=17,143	p-value*
Table 28 continued			
Pneumocystis *jiroveci* pneumonia			0.42
Yes	4 (<1)	20 (<1)	
No	5,319 (99)	17,123 (99)	
Other opportunistic Infection			0.61
Yes	596 (11)	1,877 (11)	
No	4,727 (89)	15,266 (89)	

*p-value from chi-square test of proportions

At four years after initiation of cART, both older and younger patients had approximately a 5 % probability of switching from TDF/3TC/NVP due to toxicity or a significant side-effect with older patients have a slightly shorter time to event (p=0.05) (Figure 29).

Figure 29. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on TDF/3TC/NVP



The crude association between age group and the hazard of regimen change due to toxicity or side-effect was 1.21 (95% CI 1.01 – 1.44). There were a total of 175 (3%) regimen changes due to toxicity or side-effect among the older group and 453 (3%) among the younger group. After controlling for covariates either known to be associated with AZT or EFV associated toxicity and side-effects, or that improved model fit, older patients progressed to regimen change for toxicity or side-effect 1.08 (95% CI 0.90 – 1.30) times as fast as younger patients (Table 29). Covariates that did not improve model fit or change the estimate of the association of age and rate of regimen change, such as year of cART initiation, were not included in the multivariable model.

Table 29. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing patients 40 years of age and older to patients under 40 years of age initiated on TDF/3TC/NVP (n=22,466)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age group			
20 – 39	1	-	-
≥ 40	1.13	0.95 – 1.37	0.16
Sex			
Female	1	-	-
Male	1.16	0.96 – 1.42	0.13
CD4 cells/mm^3			
0 – 50	1	-	-
51 – 100	0.60	0.41 – 0.88	0.01
101 – 200	0.73	0.54 – 0.97	0.01
201 – 350	0.66	0.49 – 0.87	0.004
>350	0.74	0.50 – 1.12	0.16
Missing	0.90	0.68 – 1.20	0.49
WHO stage			
I	1	-	-
II	1.49	1.24 – 1.80	<0.0001
III	1.24	0.98 – 1.57	0.08
IV	1.86	1.29 – 2.69	0.001
Missing	1.20	0.80 – 1.80	0.37
Tuberculosis			
No	1	-	-
Yes	1.46	1.00 – 2.13	0.05
Cryptococcal disease			
No	1	-	-
Yes	1.61	0.58 – 4.43	0.36
Other Opportunistic Infection			
No	1	-	-
Yes	1.54	1.24 – 1.91	<0.0001

In order to assess the impact of adherence on the association between age group and time to regimen change we ran two models, one with a variable for adherence and one without, and then compared the hazard ratios for the effect of age on the rate of regimen change between the two models. The hazard ratios for time to regimen change comparing older to younger patients were the same in both models (data not shown). We additionally assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found sex not to be an effect measure

modifier of the association between age and regimen change on the hazard ratio scale. We therefore included sex as a binary variable in the adjusted model.

Sensitivity analysis

Patients who died or were lost to follow up before experiencing a switch event are likely to represent informative censoring. That is the reason for their having missing outcome data is likely related informatively to both the exposure of interest (age) and the outcome (regimen change due to toxicity or side-effect). Of the patients initiated on TDF/3TC/NVP, 437 (8%) older patients and 1,147 (7%) younger patients died before a regimen change occurred. Additionally 253 older patients (5%) and 1,271 (7%) younger patients were lost to follow up before changing regimen. After imputing a regimen change at the last visit for each of the patients previously censored due to death or loss to follow up prior to experiencing a regimen change, older patients progressed to regimen change at 0.88 times (95% CI 0.82 – 0.96) the rate of younger patients.

L. Time to first regimen change among patients initiated on TDF/3TC/EFV

Of the study patients initiating cART during the study period, 37,163 had a first regimen of TDF/3TC/EFV (Table 30). Of these 14,906 (40%) were age 40 or older and 22,257 (60%) were age 20 to 39. The median age among patients in the older group was 46.7 (IQR 43 – 52) and 32.1 (IQR 28 – 36) among patients in the younger group. There was a moderately lower proportion of female patients among the older age group compared to the younger age group (50% vs. 58% respectively). Older and younger patients had similar distributions of baseline covariates including CD4 cell count strata, liver functioning, creatinine clearance, hemoglobin, BMI, WHO stage, WAB stage, active TB, active cryptococcal disease, active pneumocystis *jiroveci* pneumonia, and other active opportunistic infections (Table 30).

Table 30. Baseline characteristics by age group among patients initiated on TDF/3TC/EFV (n=37,163)

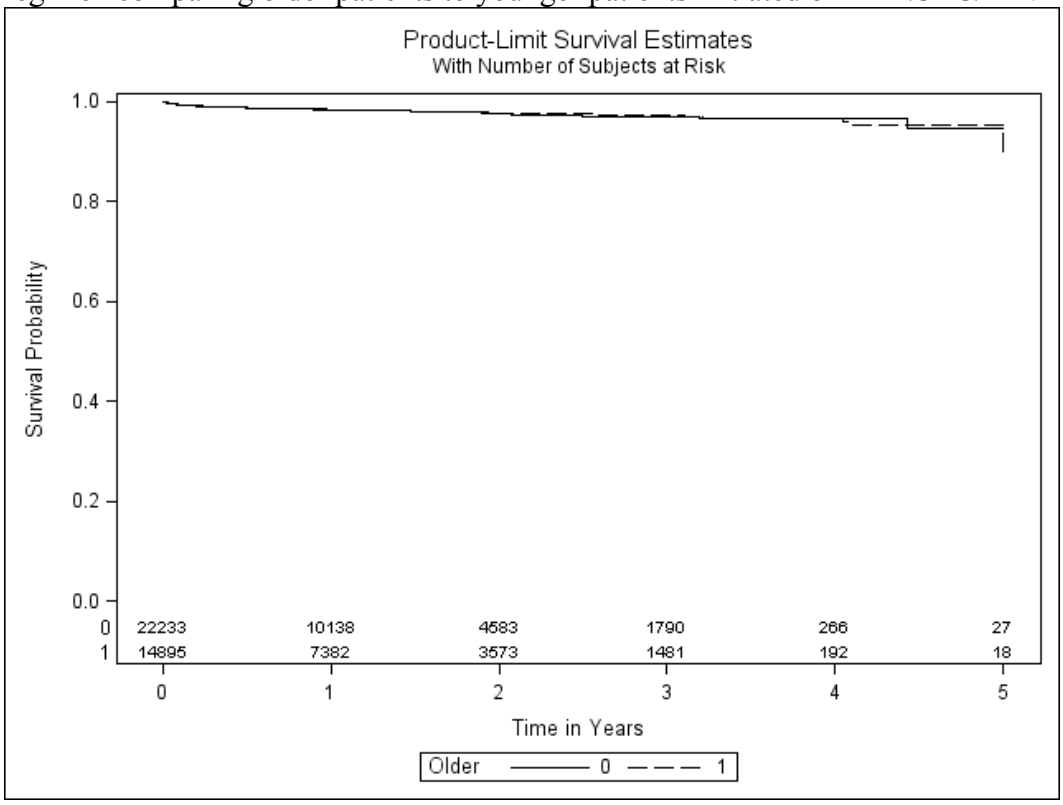
Characteristic	Older n (%) n=14,906	Younger n (%) n=22,257	p-value*
Sex			<0.0001
Female	7,514 (50)	12,849 (58)	
Male	7,392 (50)	9,408 (42)	
CD4 cells/mm^3			<0.0001
0 - 50	1,297 (9)	2,409 (11)	
51 – 100	1,469 (10)	2,098 (9)	
101 – 200	2,925 (19)	3,696 (17)	
201 – 350	4,339 (29)	6,496 (29)	
>350	1,206 (8)	2,184 (10)	
Missing	3,670 (25)	5,374 (24)	
Hepatotoxicity			0.0001
None	7,500 (50)	11,420 (51)	
Grade 1	458 (3)	855 (4)	
Grade 2	45 (<1)	68 (<1)	
Grade 3	18 (<1)	23 (<1)	
Grade 4	8 (<1)	22 (<1)	
Missing	6,877 (46)	9,869 (44)	
Creatinine clearance			<0.0001
Normal	2,317 (21)	5,288 (24)	
30 – 49 mL/min	1,054 (7)	1,302 (6)	
15 – 29 mL/min	180 (1)	126 (1)	
5 – 14 mL/min	23 (<1)	27 (<1)	
<5 mL/min	4 (<1)	6 (<1)	
High	850 (6)	1,227 (6)	
Missing	9,628 (65)	14,281 (64)	
BMI			<0.0001
Normal	5,182 (35)	7,784 (35)	
Underweight	2,268 (15)	3,522 (16)	
Overweight	1,037 (7)	1,368 (6)	
Obese	400 (3)	469 (2)	
Missing	6,019 (40)	9,114 (41)	
WHO stage			0.0003
I	6,702 (45)	10,373 (47)	
II	3,686 (25)	5,095 (23)	
III	3,120 (21)	4,613 (21)	
IV	927 (6)	1,398 (6)	
Missing	471 (3)	778 (3)	
Functional status			0.04
Working	13,764 (92)	20,406 (92)	
Ambulatory	663 (4)	1,102 (4)	
Bedridden	75 (<1)	92 (<1)	
Missing	404 (3)	657 (3)	
Tuberculosis			0.002
No	13,581 (91)	20,060 (90)	
Yes	1,325 (9)	2,197 (10)	
Cryptococcal disease			0.95
No	14,855 (99)	22,180 (99)	
Yes	51 (<1)	77 (<1)	

Characteristic	Older n (%) n=14,906	Younger n (%) n=22,257	p-value*
Table 30 continued			
Pneumocystis *jiroveci* pneumonia			0.53
No	14,631 (98)	21,866 (98)	
Yes	275 (2)	391 (2)	
Other opportunistic infection			0.27
No	13,132 (88)	19,691 (88)	
Yes	1,774 (12)	2,566 (12)	

*p-value from chi-square test of proportions

At four years after initiation of cART, both older and younger patients had approximately a 5 % probability of switching from TDF/3TC/EFV due to toxicity or a significant with older patients have a slightly shorter time to event (p=0.81) (Figure 30).

Figure 30. Kaplan-Meier curve showing five year probability of remaining on first regimen comparing older patients to younger patients initiated on TDF/3TC/EFV



The crude association between age group and the hazard of regimen change due to toxicity or side-effect was 0.98 (95% CI 0.83 – 1.16). There were a total of 246 (2%) regimen changes due to toxicity or side-effect among the older group and 361 (2%) among the younger group. After controlling for covariates either known to be associated with AZT or EFV associated toxicity and side-effects, or that improved model fit, older patients progressed to regimen change for toxicity or side-effect 0.99 (95% CI 0.84 – 1.16) times as fast as younger patients (Table 31). Covariates that did not improve model fit or change the estimate of the association of age and rate of regimen change, such as year of cART intiation, were not included in the multivariable model.

Table 31. Adjusted hazard ratios (HR) for time to regimen change due to toxicity or side-effect comparing patients 40 years of age and older to patients under 40 years of age initiated on TDF/3TC/EFV (n=37,163)

Baseline Characteristic	Adjusted HR	95% CI	p-value
Age group			
20 – 39	1	-	-
≥ 40	0.99	0.84 – 1.16	0.88
Sex			
Female	1	-	-
Male	0.93	0.78 – 1.09	0.35
CD4 cells/mm^3			
0 - 50	1	-	-
51 – 100	0.66	0.46 – 0.94	0.02
101 – 200	0.67	0.49 – 0.91	0.01
201 – 350	0.67	0.50 – 0.89	0.005
>350	0.96	0.69 – 1.35	0.82
Missing	0.85	0.64 – 1.12	0.22
WHO stage			
I	1	-	-
II	1.49	1.21 – 1.83	0.0001
III	1.65	1.33 – 2.04	<0.0001
IV	1.49	1.06 – 2.10	0.02
Missing	1.12	0.71 – 1.77	0.62
Tuberculosis			
No	1	-	-
Yes	1.09	0.83 – 1.42	0.53
Cryptococcal disease			
No	1	-	-
Yes	2.07	0.85 – 5.05	0.10
Other opportunistic infection			
No	1	-	-
Yes	1.19	0.95 – 1.51	0.13

In order to assess the impact of adherence on the association between age group and time to regimen change we ran two multivariable models, one including a variable for adherence and one without. We then compared the estimate of effect for the association between age and the hazard of regimen change between the two models. The hazard ratios for time to regimen change comparing older to younger patients were the same in both models (data not shown). We additionally assessed whether sex modified the association between age and time to regimen change on the hazard ratio scale and found

sex to not be an effect measure modifier (data not shown). We therefore controlled for it as a binary variable in the adjusted multivariable model.

Sensitivity analysis

Patients who died or were lost to follow up before experiencing a switch event are likely to represent informative censoring. That is, the reason for their having missing outcome data is likely related informatively to both the exposure of interest (age) and the outcome (regimen change due to toxicity or side-effect). Of the patients initiated on TDF/3TC/EFV, 1,108 (7%) older patients and 1,472 (7%) younger patients died before a regimen change occurred. Additionally 736 older patients (5%) and 1,412 (6%) younger patients were lost to follow up before changing regimens. After imputing a regimen change at the last visit for each of the patients previously censored due to death or loss to follow up, older patients progressed to regimen change 0.92 (0.88 – 0.98) times as fast as younger patients.

CHAPTER V. DISCUSSION

Our study demonstrated that HIV-infected adults age 40 and older have consistently lower mean CD4 cell counts over time compared to those younger than 40. We further demonstrated the importance of evaluating the effect of age on mean CD4 cell counts within strata of baseline CD4 cell count. While the differences between the age groups in mean CD4 cell count were similar in the three lowest strata and did not reach a level of clinical importance, larger differences between the age groups which may be clinically relevant were observed among patients whose baseline CD4 cell count was between 200 and 350 cells/mm^3 and above 350 cells/mm^3. We also demonstrated that the variability of CD4 cell counts was lower among older adults than in younger adults in almost all time periods and across all baseline CD4 cell count strata. By modeling the variance we additionally showed that a substantial proportion of patients, in both the older and younger group, are not achieving high levels of immunologic improvement after starting cART.

We found that older HIV-infected adults progressed to regimen change associated with toxicity or side-effect at a higher rate than younger adults within D4T and AZT containing regimens. We did not find a difference between the age groups in the hazard for a regimen change in regimens containing TDF.

Most studies reporting on age associated outcomes of cART in sub-Saharan Africa have compared HIV-infected adults who are 50 and older to those under 50 (Bakanda et al., 2011; Balestre et al., 2012; Eduardo et al.; Maskew et al., 2012; J. Negin, van Lettow, et

al., 2011). Yet, a study on the prevalence of HIV among older adults from nine countries in sub-Saharan Africa found, as we did, a large group in the 40-50 year old age group with only a small proportion over 50 (Joel Negin & Cumming, 2010). In the United States and Europe most researchers classify HIV-infected individuals as older at age 50 and over, compared to 65 for HIV-uninfected individuals. We thought it plausible that due to higher levels of immune activation, differences in nutrition, exposure to infections, and other socioeconomic factors unique to resource limited settings which may impact the immune system over time, differences in treatment outcomes would be observed at a younger age than in higher income nations.

Age and immune system reconstitution over time: Our study found that adults 40 years of age and older had consistently lower mean CD4 cell counts over time following the initiation of cART compared to younger adults. While we found similar differences in mean CD4 cell counts comparing older to younger adults as previous studies, we have additionally demonstrated that the differences in CD4 cell counts following the initiation of cART can be observed using the age 40 cutoff. Most previous studies have shown lower immune response when comparing adults age 50 and older to those younger than 40. This approach ignores potential differences in immune system response to treatment in patients aged 40 to 49, who constitute a large proportion of patients in treatment (Althoff et al., 2010; Balestre et al., 2012; Cohen Stuart et al., 2002; Eduardo et al.; Florence et al., 2003; Goetz et al., 2001; Greig et al., 2012; Viard et al., 2001). The pattern of CD4 cell response following the initiation of cART that we observed,

particularly among patients starting treatment with more seriously compromised immune systems, is supported by previous studies. The methodological differences in our study compared to the previous studies, including the stratification by baseline CD4 cell count, using 40 years of age as the cut-off for comparison (rather than comparing 50 and older to under 40), the length of follow-up, modeling the variance over time, and sensitivity analysis to assess the impact of informative missingness, provide a more robust picture of the association between age and immune response in these settings.

Most previous studies focused on one to two years after cART initiation, with the longest follow-up being three years, while our study included five years of post-cART CD4 cell count data. Previous studies that focused on immune response in the first six months of treatment (Maskew et al., 2012; P. C. Mutevedzi et al., 2011) found no difference in mean CD4 cell count comparing older to younger adults. We also found no difference in mean CD4 cell count in the first six months of therapy, which supports the need to look beyond the short term response to cART when investigating differences by age. The studies that were conducted in resource-rich countries where baseline clinical factors are quite different from those in our setting (K. A. Gebo, 2008; Greenbaum et al., 2008; Tumbarello et al., 2004).

In addition to consistently lower mean CD4 cell counts over time, we also found that the size of those differences between older and younger adults varied by strata of baseline CD4 cell count. There were smaller differences in mean CD4 cell count in the lower strata of baseline CD4 cell count (0 to 50, 51 to 100, and 101 to 200), and larger

differences in the highest two strata (201 to 350 and > 350). All previous studies have either controlled for baseline CD4 strata in adjusted models or included CD4 cell count as a continuous variable. When a patient starts cART in these settings is often related to their CD4 cell count. It is important to know how immune system reconstitution differs not only by age but also by baseline CD4 cell count since the amount of immune system reconstitution patients will experience is directly linked to their CD4 cell count when they begin treatment (Moore & Keruly, 2007).

A few studies did not find an effect of age on immune reconstitution after treatment initiation (Greenbaum et al., 2008; Tumbarello et al., 2004). One of these (Tumbarello et al., 2004) compared 162 patients age 40 and under to 81 patients age 50 and over. While the study demonstrated differences in mean CD4 cell counts as large as 100 cells, the baseline CD4 cell count among the older group was significantly lower than among the younger group (111 cells/mm^3 vs 184 cells/mm^3). The authors concluded no effect of age on CD4 cell gain based on the absolute number of cells gained each six months after initiating therapy. It has been previously demonstrated that patients initiating HIV treatment at lower CD4 cell counts can have immune reconstitution, relative to their baseline level, similar to those with higher CD4 cell counts. However, they fail to achieve similar levels of immune reconstitution in terms of absolute total CD4 cell count (Moore & Keruly, 2007). Even if the authors had structured their study to evaluate the difference in mean CD4 cell count over time comparing older to younger patients at the same level of immune suppression at treatment start, they would have lacked the power to detect a clinically relevant difference due to the small sample size. The other study

(Greenbaum et al., 2008) had a high rate of loss to follow-up (only eight older and 57 younger patients remaining by 36 months) and did not examine the potential effects of loss to follow-up.

While we found statistically significant differences at most time points following the initiation of cART for all strata, it was only in the highest stratum of baseline CD4 cell count (> 350 cells/mm^3) that the difference between age groups was what we, *a priori*, defined as a difference in immune reconstitution large enough to keep older patients below the CD4 cell count threshold at which they would be at risk for certain adverse outcomes, such as TB reactivation (Bartlett et al., 2013). This knowledge will be particularly important when treatment guidelines expand eligibility for cART in resource limited setting to HIV-infected adults with a CD4 cell count greater than 350 cells/mm^3. Clinicians should anticipate a less robust immune response in patients age 40 and older at the time they initiate therapy leading to lower pathogen-specific immune response in this age group.

Further, we were able to demonstrate that the variability of the CD4 cell count was lower in the older group as compared to the younger group. While the older group demonstrated a moderate increase in variability over time similar to that of the younger group, the older patients still had lower variability over time than the younger group. It may be that the larger variability in the younger group means that cART affects immune system reconstitution to a greater extent for some proportion of the group and to a lesser extent for others, whereas less variability may indicate that cART elicits a more

homogenous response in the older group. The variance we modeled is a direct estimate of the distribution of CD4 cell counts in the older and younger group, taking into consideration other covariates. We have demonstrated that while the mean response over time gives the impression of immune system improvement in both the older and younger groups, a substantial proportion of patients are going to have CD4 cell counts much lower than the mean over time. Given the size of the SD under the approximate normal assumption, a large proportion (approximately 54%) of patients in both groups, are not achieving high levels of immune reconstitution.

Most studies focus only on the mean response and do not consider the breath of response to treatment comparing one group to another. This is the first study to our knowledge to model the variance of CD4 cell count over time by age group and by baseline CD4 cell count strata. In the absence of virologic testing, which is not commonly available in resource limited settings, CD4 cell counts after cART is started are used to monitor the effectiveness of cART. The studies that have thus far informed the expectations that clinicians have regarding immune system reconstitution comparing older to younger patients have not addressed the variability that can be expected in each group. Many clinicians rely on a single criterion, based on the mean response to treatment across all adult age groups, when evaluating whether an individual patient's response to therapy is robust enough to indicate that therapy is working. It is not uncommon for patients who do not reach this criterion CD4 cell count to have their regimens changed for immunologic failure. These criteria have not previously considered age. Understanding

that older adults may have lower CD4 cell counts and less variation over time compared to younger patients may help prevent unnecessary regimen changes.

One concern in evaluating changes in CD4 counts over time is the potential effect those who may be informatively missing could have on their estimates of association. Previous studies investigating the effect of age on CD4 cell response reported higher proportions of loss to follow up and mortality than we observed in our study; however, none report the results of a sensitivity analysis, although Eduardo *et al.*, did comment on the problem.. From our closed cohort sensitivity analysis, we concluded that when patients who are lost to follow up are excluded, the differences observed in the open cohort persist, and are in fact slightly larger. While those missing follow-up CD4 cell counts due to mortality or loss to follow up may differ in CD4 response from those who remained active, it is unlikely that the differences would be differ substantially by age category and therefore are unlikely to explain the observed difference in mean CD4 cell count over time comparing older to younger adults. In fact, ignoring the effect of informative missingness may attenuate the true difference and may explain the moderate differences observed in other studies that did not stratify by baseline CD4 cell count.

Age and regimen change due to toxicity or side-effect: Previous studies that have investigated first line regimen durability have not investigated age differences overall or by treatment regimen (Amoroso et al., 2012; Takuva, Evans, Zuma, Okello, & Louwagie, 2013; van Oosterhout et al., 2012). In this study, access to a large cohort of patients

receiving cART provided sufficient numbers of older and younger patients on each of the primary treatment regimens to investigate the effect of age on the time to change due to a toxicity or side-effect within strata of the first prescribed cART regimen.

We found significantly higher rates of regimen change due to toxicity or side-effect among patients 40 years of age and older compared to patients age 20 to 39 initiated on D4T/3TC/NVP (34% versus 25%, 1.6 as high) and D4T/3TC/EFV (36% versus 28%, 1.4 times as high). While D4T containing regimens are no longer part of the first line regimens recommended by the WHO and many PEPFAR supported countries (WHO, 2013a), it was a predominant component of the majority of the regimens prescribed in the first half of PEPFAR (OGAC, 2004; WHO, 2006, 2013b) and is still used in many countries (van Oosterhout et al., 2012). As the distribution of age among HIV-infected patients continues to shift towards older age groups, it will be important for clinicians to consider the increased rate of regimen change from D4T containing regimens among older adults in their decision of which drugs to prescribe. Most countries that still use D4T despite changes in guidelines do so for financial reasons (van Oosterhout et al., 2012); however, the fiscal savings of starting with a D4T containing regimen may be short lived for older adults, and may be costlier in the long run due to the higher cost of second line regimens, particularly for the patients who develop toxicity and side-effects.

We also found significantly higher rates of regimen change due to toxicity or side-effect among older compared to younger patients initiated on AZT/3TC/NVP (12% versus 10%, 1.2 times as high), and moderately higher rates of regimen change for older compared to

younger adults initiated on AZT/3TC/EFV (14% versus 13%, 1.1 times as high). We did not find significantly higher rates of regimen change due to toxicity or side-effects among patients 40 years of age and older compared to patients age 20 to 39 initiated on TDF/3TC/NVP (5% versus 4%, 1.08 times as high) or TDF/3TC/EFV (5% versus 5%, 0.99 times as high).

There was no difference between older and younger patients in the rate of regimen change among patients initiated on TDF/3TC/NVP or TDF/3TC/EFV. This could be particularly important for clinicians to be aware of since TDF containing regimens are now the most commonly recommended first line regimen.

While we originally postulated that adherence may mediate the relationship between age and time to regimen change due to toxicity or side-effect, we found no evidence to support that hypothesis. This is most likely due to very similar distributions of adherence between older and younger patients.

Outcomes among older adults are an increasingly important concern in the field of HIV treatment. Treatment success and new infections among older adults increase both the prevalence of HIV among older adults and the overall proportion of HIV-infected adults who are older. This study has addressed the question about whether older patients exhibit a reduced immune response following the initiation of cART and how large that

difference is based on their CD4 cell count at baseline. This study has also demonstrated that older individuals progress to regimen change due to toxicity and side-effects faster than younger individuals within regimens known to have high rates of toxicity such as D4T and AZT. Additionally, it does not appear that this higher rate of progression is explained by better adherence among older patients.

Further research examining the difference by age in time to regimen change between treatment regimens will be important to inform clinicians as to whether certain regimens are more tolerable to older adults. The demonstration of lower CD4 cell counts over time across all strata of baseline CD4 cell count as well as large variability in both groups is important for the long term evaluation of treatment success. Further studies are warranted to determine at what baseline CD4 cell count level older patients would need to begin treatment to achieve the same pathogen-specific immune reconstitution thresholds as younger patients.

Strengths

Our study includes the largest number of HIV-infected adults initiated on cART in sub-Saharan Africa age 40 and older, or age 50 and older, reported to date. Having a large sample of patients aged 40 and older allowed us to precisely estimate differences in treatment outcomes comparing older to younger groups. Our study is also the first to specifically investigate cART treatment outcomes comparing patients age 40 and older to

patients age 20 to 39. Furthermore, this study represents the clinical settings that are most relevant to the potential audience for these findings.

We had a large sample of patients in both the older and younger groups, which enabled us to perform the analysis of time to regimen change within each primary regimen stratum. We included all patients in our analyses and additionally performed sensitivity analyses to assess the impact of death and loss to follow up. In the published literature on CD4 response, toxicity, and side-effects, most cohorts have been limited to patients who survived at least three months after the initiation of therapy (Balestre et al., 2012). However, more than half of the mortality after the initiation of cART in sub-Saharan Africa occurs within the first three months of therapy (M. W. Brinkhof et al., 2008; Etard et al., 2006; Herbst et al., 2009; Mermin et al., 2008). This restriction could have led to an underestimation of the rate of adverse outcomes and an overestimation of the amount of CD4 gain seen in patients after initiating cART. Additionally, almost all of the existing studies focus on 12-month outcomes whereas we present response to treatment over five years. By having a cohort with five years of follow up, rather than focusing on the first six to 12 months of treatment, we were able to reconcile the findings from previous studies. Finally, by excluding patients with previous exposure to any ART including single dose-nevirapine (which can impact baseline clinical characteristics and effect treatment success) we have investigated a treatment naïve cohort. Using a treatment naïve cohort allows us to attribute the associations we observed following the initiation of cART to age and cART alone as opposed to any benefits or harms from

previous treatment that could affect the outcomes of interest. This is also the largest five year adult cohort reported to date with the largest proportion of HIV-infected adults age 40 and older.

Limitations

Our study has several limitations. The data used in these analyses were collected for the purpose of providing routine HIV-associated care and treatment, not for the purpose of studying patient outcomes after treatment. Therefore, missing data, misclassification, and data entry errors may be present in this data set to a greater extent than in a study designed to prospectively study these outcomes. Additionally, we did not have access to important confounding variables, such as duration of HIV infection or viral suppression status. Therefore, there is the potential for unmeasured confounding which may partially explain the relationships we observed and any causal inference should be tempered with this knowledge. Adherence to HIV medication, particularly early adherence, has been shown to be strongly associated with viral suppression. A study conducted in HIV treatment clinics supported by Médicins Sans Frontières across Asia and Africa showed that adherence to clinic appointments was strongly associated with increased viral suppression(Bastard et al., 2012). We investigated adherence in our study sample and found the distributions of adherence to be high in both age groups. Previous program evaluations through annual random samples of at least 10% of patients in this cohort revealed viral suppression proportions of 90% or higher over the five years of this study which confirms the high level of adherence we observed. While we did not have a

precise measure of the duration of a patient's HIV infection, duration of HIV infection and CD4 cell count decline are strongly associated (Pantaleo et al., 1993). Therefore, by performing these analyses stratified by CD4 cell count at baseline, we should be comparing older to younger patients with similar durations of infection. By investigating CD4 cell count after the initiation of treatment within strata of baseline CD4 cell count, the distribution of covariates that may affect the association of age with the outcomes more homogeneous than if we had not stratified.

The amount of missing data for baseline laboratory values made it challenging to efficiently control for underlying comorbidities at cART initiation. As laboratory tests to measure hemoglobin, liver functioning, and kidney functioning are clinically rather than protocol driven, it is likely that those without laboratory values for these variables did not have clinical symptoms that warranted testing and may resemble those with normal laboratory values. It is therefore possible that those classified as having a missing value were misclassified. However, this misclassification was independent of age group as demonstrated by the equivalent proportions of patients in both age groups with missing laboratory values at baseline. When we analyzed the data as a dichotomous variable, combining the missing values with the normal values and all of the non-normal together, the primary estimate of effect remained stable. Given that the proportion of missing values did not differ by age group, any misclassification would bias our estimate of effect towards the null.

Limitations related to the generalizability of our findings should be considered when interpreting the results of our study. The patients included in these analyses were not randomly sampled from the entire population of people living with HIV in each of these countries, but instead are those who chose to present to the clinics supported by AIDSRelief. Reasons for this self-selection to receive HIV/AIDS care and treatment may have included proximity of the clinic to the patient's residence, the fact that many of the facilities are faith-based, and the fact that the services and treatment were provided free of charge. This could result in our study population being more likely to live in a rural area and more likely to be poor compared to the entire population of HIV-infected adults in each country. While these differences from the general HIV-infected population should not be overlooked, we would expect patients who seek treatment in governmental facilities and urban areas to demonstrate the same relationship between age and the outcomes of interest as observed in this study. We also found that patients initiated on cART at age 40 or older were significantly more likely to be male as compared to younger adults who were predominantly female. This most likely explains the differences in the distribution of initial cART regimens between the older and younger groups due to sex based prescribing practices (e.g., women are more often prescribed NVP and men EFV). These findings are consistent with other reported studies and closely reflect the general distribution of HIV infection by age and sex in sub-Saharan Africa (Eduardo et al.; J. Negin, van Lettow, et al., 2011) and we found that regimen did not impact the association between age and mean CD4 cell count over time and that sex did not modify the relationship between age and either study outcome..

Because the data were obtained from medical records, there is the potential for misclassification of study variables and outcomes. Due to the challenges in diagnosing the OIs upon which WHO staging is based, WHO stage has the largest potential for misclassification but it is unlikely to be differential by age. Subject status (e.g., lost to follow up) is also prone to misclassification in resource limited settings which could affect the estimated association between age and the outcomes of interest. It has been demonstrated in the literature that when patients who have not been seen in the clinic for three or more months are tracked, older patients are more likely to have died but younger patients, are more likely to have dropped out of care and treatment (M. W. G. Brinkhof, Pujades-Rodriguez, & Egger, 2009). Differential misclassification of the outcomes of interest could have occurred if the intensity and frequency of patient monitoring differed by age.

Selection bias due to death and loss to follow-up may also represent an important limitation to this study. If deaths or losses to follow-up were associated with both age and the outcomes of interest, bias in the estimates of association could have been introduced. Our findings from the sensitivity analysis for the D4T/3TC/NVP regimen were robust to violations of the missing at random assumption while our findings for D4T/3TC/EFV and for the AZT and TDF containing regimens were not. The sensitivity analysis we performed was intended as a "worst-case" approach, where all patients who died or were lost to follow-up were considered to have had a regimen change. This scenario is probably too extreme to conclude that the observed results were biased by

death and loss to follow up. However, the model of care and treatment that was implemented in the clinics from which this study sample arises included a strong community based follow-up and retention component. Data from the entire eight years of the AIDSRelief program demonstrated cumulative mortality under 10% and loss to follow-up under eight% (unpublished final report of program outcomes to the CDC). Our study sample also had very low cumulative mortality and loss to follow up (nine % and eight %, respectively).

Significance

Our study has demonstrated that adults age 40 and older have lower CD4 cell counts over time, in all strata of baseline CD4 cell count, compared to younger patients. We have also demonstrated that there is less variability in immune response among older as compared to younger patients. No other study to date has modeled the variance of CD4 cell count over time in addition to the mean. The large variance in both groups indicates that a substantial proportion of patients are not achieving high levels of immune response to cART. This finding should be considered in the evaluations of the success of cART in these settings and will hopefully encourage others to create a fuller picture of immune response to cART by including variance when they study treatment outcomes. The methodological differences in our study compared to the previous studies, including the stratification by baseline CD4 cell count, using 40 years of age as the cutoff for comparison (rather than comparing 50 and older to under 40), the length of follow up, modeling the variance over time, and sensitivity analysis to assess the impact of

informative missingness, have provided a more complete picture of the association between age and immune response in these settings. The findings from our study indicate that future research should investigate at what CD4 cell count older individuals need to initiate cART in order to achieve the same level of immune reconstitution as younger patients.

We have additionally demonstrated that older adults change D4T and AZT containing regimens due to toxicity and side-effects at a significantly higher rate than younger patients, indicating the need for closer monitoring of older adults. As laboratory monitoring for cART associated toxicity is clinically rather than protocol driven, this study demonstrates that clinicians may need to request laboratory monitoring for older patients earlier than they would for younger patients. Additionally, they may need to check for clinical symptoms of toxicity and side-effect earlier and more often than for their younger patients, particularly for older individuals on D4T and AZT containing regimens. Instituting closer monitoring of older patients could be critical for successful long term care and treatment as the proportion of older adults with HIV increases in these settings.

While D4T containing regimens are no longer part of the first line regimens recommended by the WHO and many PEPFAR supported countries (WHO, 2013a), it was a predominant component of the majority of the regimens prescribed in the first half of PEPFAR (OGAC, 2004; WHO, 2006, 2013b) and is still used in many countries (van

Oosterhout et al., 2012). As the distribution of age among HIV-infected patients continues to shift towards older age groups, it will be important for clinicians to consider the increased rate of regimen change from D4T containing regimens among older adults in their decision of which drugs to prescribe. Future research on regimen change due to toxicity and side-effects should focus on which regimens are more durable for older patients.

A recent study using data from four PEPFAR supported countries demonstrated significant increases in the proportion of older adults newly initiating cART from 2006 to 2010 (Eduardo et al.). It is estimated that approximately one out of every eight people living with HIV in sub-Saharan Africa is at least 50 years of age (Joel Negin & Cumming, 2010). As life expectancy continues to increase among people on cART (Mills et al., 2011), and as older adults continue to become infected, the number of older adults living with HIV will continue to grow. It will be critical for future research to take this into account and increase the focus on HIV treatment outcomes in older adults, particularly in regions such as sub-Saharan Africa which are disproportionally impacted by the epidemic.

Appendix 1.

REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR ADULTS AND ADOLESCENTS (WHO, 2013a)

TABLE 1. REVISED WHO CLINICAL STAGING OF HIV/AIDS FOR ADULTS AND ADOLESCENTS

Primary HIV infection
Asymptomatic
Acute retroviral syndrome

Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy (PGL)

Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulcerations
Papular pruritic eruptions
Seborrhoeic dermatitis
Fungal nail infections of fingers

Clinical stage 3

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations
Severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (intermittent or constant for longer than one month)
Oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (National Center for HIV/AIDS) diagnosed in last two years
Severe presumed bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary
Unexplained anaemia (< 8 g/dl), and or neutropenia (<500/mm^3) and or thrombocytopenia (<50 000/ mm3) for more than one month

All clinical events or conditions referred to are described in the Annexes. The UN defines adolescents as persons aged 10−19 years but, in the present document, the category of adults and adolescents comprises people aged 15 years and over for surveillance purposes.

Clinical stage 4

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe or radiological bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's
duration)
Oesophageal candidiasis
Extrapulmonary TB
Kaposi's sarcoma
Central nervous system (CNS) toxoplasmosis
HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary:
Extrapulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacteria infection
Progressive multifocal leukoencephalopathy (PML)
Candida of trachea, bronchi or lungs
Cryptosporidiosis
Isosporiasis
Visceral herpes simplex infection
Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
Recurrent non-typhoidal salmonella septicaemia
Lymphoma (cerebral or B cell non-Hodgkin)
Invasive cervical carcinoma
Visceral leishmaniasis

Appendix 2.

WHO functional status measurement (WHO, 2006)

1. Functional status defined as:

a) Working = able to perform usual work in or out of the house, harvest, go to school or, for children, normal activities or playing

b) Ambulatory = able to perform activities of daily living but not able to work or play

c) Bedridden = not able to perform activities of daily living

Appendix 3.

Aim 1a.Minimal sufficient adjustment sets for estimating the total effect of Age at cART Start on Mean CD4 count:

- {1st_Treatment_Regimen, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}
- {Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}

Adjustment for direct effect

Minimal sufficient adjustment sets for estimating the direct effect of Age at cART Start on Mean CD4 count:

- {1st_Treatment_Regimen, Adherence, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}
- {Adherence, Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}

Testable implications

The model implies the following conditional independences:

- Age at cART Start ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Age at cART Start ⊥ Facility_Type | Sex
- Age at cART Start ⊥ Country | Facility_Type
- Age at cART Start ⊥ Country | Sex
- Age at cART Start ⊥ Year_of_cART_Start
- Mean CD4 count ⊥ Baseline_BMI | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Mean CD4 count ⊥ Facility_Type | 1st_Treatment_Regimen, Baseline_BMI, Baseline_OIs, Sex
- Mean CD4 count ⊥ Facility_Type | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Mean CD4 count ⊥ Country | 1st_Treatment_Regimen, Baseline_BMI, Baseline_OIs, Sex
- Mean CD4 count ⊥ Country | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Mean CD4 count ⊥ Baseline_WAB_Stage | Age at cART Start, Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Mean CD4 count ⊥ Baseline_WAB_Stage | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex

- Mean CD4 count ⊥ Year_of_cART_Start | 1st_Treatment_Regimen, Baseline_BMI, Baseline_OIs, Sex
- Mean CD4 count ⊥ Year_of_cART_Start | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Sex ⊥ Baseline_OIs | Baseline_CD4_count
- Sex ⊥ Baseline_WHO_Stage | Baseline_CD4_count
- Sex ⊥ Adherence | Age at cART Start
- Sex ⊥ Country | Facility_Type
- Sex ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- Sex ⊥ Year_of_cART_Start
- Baseline_CD4_count ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Baseline_CD4_count ⊥ Adherence | Age at cART Start
- Baseline_CD4_count ⊥ Facility_Type | Sex
- Baseline_CD4_count ⊥ Country | Facility_Type
- Baseline_CD4_count ⊥ Country | Sex
- Baseline_CD4_count ⊥ Year_of_cART_Start
- Baseline_OIs ⊥ Adherence | Age at cART Start
- Baseline_OIs ⊥ Facility_Type | Sex
- Baseline_OIs ⊥ Facility_Type | Baseline_CD4_count
- Baseline_OIs ⊥ Country | Facility_Type
- Baseline_OIs ⊥ Country | Sex
- Baseline_OIs ⊥ Country | Baseline_CD4_count
- Baseline_OIs ⊥ Year_of_cART_Start
- Baseline_WHO_Stage ⊥ Baseline_BMI | Baseline_CD4_count, Baseline_OIs
- Baseline_WHO_Stage ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Baseline_WHO_Stage ⊥ 1st_Treatment_Regimen | Baseline_CD4_count, Baseline_OIs
- Baseline_WHO_Stage ⊥ Adherence | Age at cART Start
- Baseline_WHO_Stage ⊥ Facility_Type | Sex
- Baseline_WHO_Stage ⊥ Facility_Type | Baseline_CD4_count
- Baseline_WHO_Stage ⊥ Country | Facility_Type
- Baseline_WHO_Stage ⊥ Country | Sex
- Baseline_WHO_Stage ⊥ Country | Baseline_CD4_count
- Baseline_WHO_Stage ⊥ Baseline_WAB_Stage | Baseline_CD4_count, Baseline_OIs
- Baseline_WHO_Stage ⊥ Year_of_cART_Start
- Baseline_BMI ⊥ Adherence | Age at cART Start
- Baseline_BMI ⊥ Facility_Type | Sex
- Baseline_BMI ⊥ Country | Facility_Type
- Baseline_BMI ⊥ Country | Sex
- Baseline_BMI ⊥ Year_of_cART_Start
- 1st_Treatment_Regimen ⊥ Adherence | Age at cART Start

- 1st_Treatment_Regimen ⊥ Adherence | Baseline_BMI, Baseline_OIs, Sex
- 1st_Treatment_Regimen ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- 1st_Treatment_Regimen ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_OIs, Sex
- Adherence ⊥ Facility_Type | Sex
- Adherence ⊥ Facility_Type | Age at cART Start
- Adherence ⊥ Country | Facility_Type
- Adherence ⊥ Country | Sex
- Adherence ⊥ Country | Age at cART Start
- Adherence ⊥ Baseline_WAB_Stage | Age at cART Start
- Adherence ⊥ Year_of_cART_Start
- Facility_Type ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- Facility_Type ⊥ Baseline_WAB_Stage | Sex
- Facility_Type ⊥ Year_of_cART_Start
- Country ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- Country ⊥ Baseline_WAB_Stage | Sex
- Country ⊥ Baseline_WAB_Stage | Facility_Type
- Country ⊥ Year_of_cART_Start
- Baseline_WAB_Stage ⊥ Year_of_cART_Start

Aim 2. Minimal sufficient adjustment sets for estimating the total effect of Age at cART Start on 1st Regimen Change:

- {1st_Treatment_Regimen, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}
- {Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}

Adjustment for direct effect

Minimal sufficient adjustment sets for estimating the direct effect of Age at cART Start on 1st Regimen Change:

- {1st_Treatment_Regimen, Adherence, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}
- {Adherence, Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex}

Testable implications

The model implies the following conditional independences:

- Age at cART Start ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Sex ⊥ Baseline_OIs | Baseline_CD4_count
- Sex ⊥ Baseline_WHO_Stage | Baseline_CD4_count
- Sex ⊥ Adherence | Age at cART Start
- Sex ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- Baseline_CD4_count ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Baseline_CD4_count ⊥ Adherence | Age at cART Start
- Baseline_OIs ⊥ Adherence | Age at cART Start
- Baseline_WHO_Stage ⊥ Baseline_BMI | Baseline_CD4_count, Baseline_OIs
- Baseline_WHO_Stage ⊥ 1st_Treatment_Regimen | Baseline_BMI, Baseline_OIs, Sex
- Baseline_WHO_Stage ⊥ 1st_Treatment_Regimen | Baseline_CD4_count, Baseline_OIs
- Baseline_WHO_Stage ⊥ Adherence | Age at cART Start
- Baseline_WHO_Stage ⊥ Baseline_WAB_Stage | Baseline_CD4_count, Baseline_OIs
- Baseline_BMI ⊥ Adherence | Age at cART Start
- Baseline_BMI ⊥ 1st Regimen Change | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- 1st_Treatment_Regimen ⊥ Adherence | Age at cART Start
- 1st_Treatment_Regimen ⊥ Adherence | Baseline_BMI, Baseline_OIs, Sex

- 1st_Treatment_Regimen ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_CD4_count, Baseline_OIs
- 1st_Treatment_Regimen ⊥ Baseline_WAB_Stage | Baseline_BMI, Baseline_OIs, Sex
- Adherence ⊥ Baseline_WAB_Stage | Age at cART Start
- Baseline_WAB_Stage ⊥ 1st Regimen Change | 1st_Treatment_Regimen, Age at cART Start, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex
- Baseline_WAB_Stage ⊥ 1st Regimen Change | Age at cART Start, Baseline_BMI, Baseline_CD4_count, Baseline_OIs, Baseline_WHO_Stage, Sex

Appendix 4.

Mean difference in CD4 cell count comparing older to younger patients, by strata of baseline CD4 cell count from time of cART start to 60 months

Older vs younger	0 – 50 cells/mm^3	51 – 100 cells/mm^3	101 – 200 cells/mm^3	201 – 350 cells/mm^3	> 350 cells/mm^3
Baseline	2	0	0	-1	-9
6 months	-7	-15	-18	-33	-54
12 months	-15	-15	-24	-34	-62
18 months	-25	-22	-30	-37	-56
24 months	-25	-28	-29	-41	-42
30 months	-26	-33	-27	-36	-48
36 months	-29	-34	-29	-40	-57
42 months	-33	-22	-16	-33	-24
48 months	-23	-16	-29	-26	-17
54 months	-25	-24	-28	-38	-64
60 months	-7	-5	-37	-14	-85

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