Evaluating individuals with extreme phenotypes of HIV-1 contributes towards better healthcare management of all HIV-1 positive individuals

Roen, A.

Evaluating individuals with extreme phenotypes of HIV-1 contributes towards better healthcare management of all HIV-1 positive individuals

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Table of Contents

Abstract

Human Immunodeficiency Virus (HIV)-1 disease progression is variable within patients where some remain asymptomatic for long periods (elite controllers (EC)) while others rapidly progress to disease (rapid progressors (RP)), representing the 'extreme phenotypes'. There is substantial heterogeneity in how these phenotypes are defined, and we examined the relative merit of published definitions using data on HIV-1 seroconverters. We propose standard definitions for future research of these rare groups: ECs – maintain consecutive HIV-RNA < 50 copies/ml for at least 6 months, RPs – at least one CD4 cell count < 100 cells/mm^3 within one year of HIV-1 seroconversion.

Evidence shows that less than 1% of individuals are EC and the majority should start treatment to maximise quality and length of life. We supported the 'when to start' evidence by demonstrating a 62% reduced risk of serious Aquired Immunodeficiency Syndrome (AIDS), non-AIDS events or death for those immediately initiating combination antiretroviral therapy (cART) (vs not immediately initiating) among those with high CD4 cell counts (>500cells/mm^3) and high HIV-RNA (>100,000 copies/ml). We contributed towards the 'what cART to start' question; among individuals initiating boosted protease inhibitor, atazanavir might be preferable compared to lopinavir, with 30% lower mortality risk, and 9% lower virological failure risk, which could lead to lower transmitted drug resistance (TDR). We found TDR was significantly decreasing throughout Europe, but remains prevalent (8.5% in 2012); therefore, genetic testing among newly diagnosed remains justifiable.

For most, starting treatment is a lifelong commitment; however, some report on post treatment control (PTC) upon cART cessation. We found individuals having viral blips on

cART had shorter time to viral rebound upon stopping treatment, but most do not become PTC and initiate lifelong cART. We investigated three cART associated toxicities, namely hypersensitivity reactions (HSR) due to abacavir (ABC) utilization, AIDS-defining neurological conditions related to cART, and immune reconstitution inflammatory syndrome (IRIS) shortly after cART initiation. We found that HSR from abacavir utilization is low (Incidence Rate (IR)=1.67/100 person-years follow-up), cART with high central nervous system penetration scores increase HIV-1 dementia risk, and apart from mycobacterial infections, unmasking IRIS may not be a cART complication in high-income countries.

List of Tables

List of Figures

List of accompanying material - publications submitted for this Doctor of Philosophy in order of their appearance

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Authors declaration

I declare that all the material contained in this thesis is my own work.

Signed: Ashley Dyan Olson Roen 27 April 2020

List of abbreviations

3TC	lamivudine
ABC	abacavir
AIDS	aquired immunodeficiency syndrome
ATV/r	atazanavir
AZT	azidothymidine
BHIVA	British HIV Association
bPI	boosted protease inhibitor
cART	combination antiretroviral therapy
CASCADE	Concerted action on seroconversion to AIDS and death in Europe
CHIP	Centre of Excellence for Health, Immunity and Infections
CI	confidence interval
CMV	cytomegalovirus
CNS	central nervous system
CPE	central nervous system penetration effectiveness
CREME	Centre for Clinical Research, Epidemiology, Modelling and Evaluation
CRFs	Circulating recombinant forms
CROI	Conference on Retroviruses and Opportunistic Infections
DRV/r	darunavir
e.g.	example
EACS	European Clinical AIDS Society
EC	elite controllers
EVF	efavirenz
FDA	Food and Drug Administration
FTC	emtricitabine
GWAS	genome wide association studies
HCV	hepatitis C virus
HIC	HIV controllers
HICDEP	HIV Cohorts Data Exchange Protocol
HIV	human immunodeficiency virus
HR	hazard ratio
HSR	hypersensitivity reactions
HSV2	herpes simplex virus
IAS	International AIDS Society
IDU	injection drug use
INSTI	integrase strand transfer inhibitor
IQR	interquartile range
IR	incidence rate
IRIS	immune reconstitution inflammatory syndrome
KS	kaposi's scarcoma
LAV	Lymphadenopathy-Associated Virus
LPV/r	lopinavir
LTNP	long term non-progressor
MAC	mycobacterium avium complex
mRNA	messenger RNA
MSM	men who have sex with men
MSW	sex between men and women
NC	noncontroller
NCI	National Cancer Institute
NHL	Non-Hodgkin lymphoma
NNRTI	non-nucleoside reverse transcriptase inhibitors
NP	nonprogressor

NRTI	nucleoside reverse transcriptase inhibitor
OR	odds ratio
OTH	other
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
PHI	primary HIV infection
PI	Protease Inhibitor
PML	progressive multifocal leukoencephalopathy
PrEP	pre-exposure prophylaxis
PRISMA	referred Reporting Items for Systematic Reviews and Meta-Analysis
PTC	post treatment controllers
RAMs	resistance associated mutations
RCT	randomized controlled trial
REDCap	Research Electronic Data Capture
RP	rapid progressors
RPV	rilpivirine
RR	relative risk
SC	seroconversion
SNPs	single nucleotide polymorphisms
SP	slow progressor
TDF	tenofovir
TDR	transmitted drug resistance
UNAIDS	Joint United Nations Program on HIV/AIDS
VC	viremic controller
VCY	viremia copy-years
WHO	World Health Orginization

1 Introduction

1.1 History of HIV

Human Immunodeficiency Virus (HIV)-1 originated in chimpanzees in Central and Western Africa, and it is believed that its emergence in humans began in the early 1920's in Kinshasa, or what is now the Democratic Republic of Congo[1]. The first officially reported cases of acquired immunodeficiency syndrome (AIDS), however, were not observed until 1981 in the United States, where a string of cases of Pneumocystis carinii pneumonia (PCP) and Kaposi's sarcoma (KS) diagnosed in healthy young homosexual men was reported[2, 3]. Both PCP and KS are rare diseases that until 1981, only occurred in immunosuppressed individuals; it was therefore recognized that these men had a common immunological deficit. Originally, it was thought that AIDS was related to the homosexual lifestyle[4, 5], but this idea was dismissed when AIDS cases appeared in injection drug users, blood transfusion and organ donation recipients[6, 7], and in female partners of bisexual men[8, 9].

In May of 1983, a group of French doctors at the Pasteur Institute lead by Dr. Luc Montaginer discovered a new retro-virus named Lymphadenopathy-Associated Virus (LAV) from a single patient with AIDS which was thought to be the cause of AIDS[10]. In 1984, Dr. Rober Gallo's team at the National Cancer Institute (NCI) in Maryland isolated a retrovirus called HTLV-III present in 48 individuals with AIDS and in none of the 115 individuals with no known risk for AIDS[11]. These two viruses were shown to be identical and were termed HIV-1[12]. A second HIV strain was discovered in 1985 in Senegal[13] originating in West African Sooty mangabeys[14, 15]. This virus was isolated in 1986 and subsequently termed HIV-2[16].

1.2 The HIV-1 Life Cycle

HIV-1 is an enveloped, RNA virus, a member of the Retroviridae family; and infects and replicates within a host for survival. HIV-1 infects and destroys CD4 T lymphocytes, an integral part of the cell mediated immune response that is required to combat viral pathogens. The virus binds to CD4 receptors on the host cell along with chemokine receptors (CCR5 or CXCR4) allowing for viral attachment and fusion which is followed by uncoating of the viral capsid upon entry into the cell, and allowing the viral RNA to enter the cytoplasm of the cell where reverse transcriptase is used to convert viral RNA into DNA[17]. Using viral integrase, the newly synthesised DNA becomes integrated into the host cell genome and transcription into messenger RNA (mRNA) occurs. mRNA is translated into viral proteins by the host cell which in turn create a provirus[18]. The provirus can remain active without replicating for many years, and presents as a cell type or anatomical site of latently-infected T-cells termed "viral reservoirs".[19] These cells are not detected by the immune system or modern treatment, meaning HIV-1 almost always returns when treatment is stopped[20-22], except in rare reports of individuals who control viral replication upon stopping treatment, or post treatment controllers (PTC)[23-30]. When latently infected CD4 cells are activated, transcription begins, and the provirus creates HIV virons capable of infecting other cells, Figure 1.1. Each infected cell can create up to 10,000 new HIV virions over its life span, with up to 10^{10} HIV-1 virons created daily within an untreated individual[31, 32]. This high rate of viral replication coupled with reverse transcriptase that lacks 3'-5' exonuclease proof reading activity makes HIV-1 prone to mutational errors, contributing to the genetic diversity of the virus.

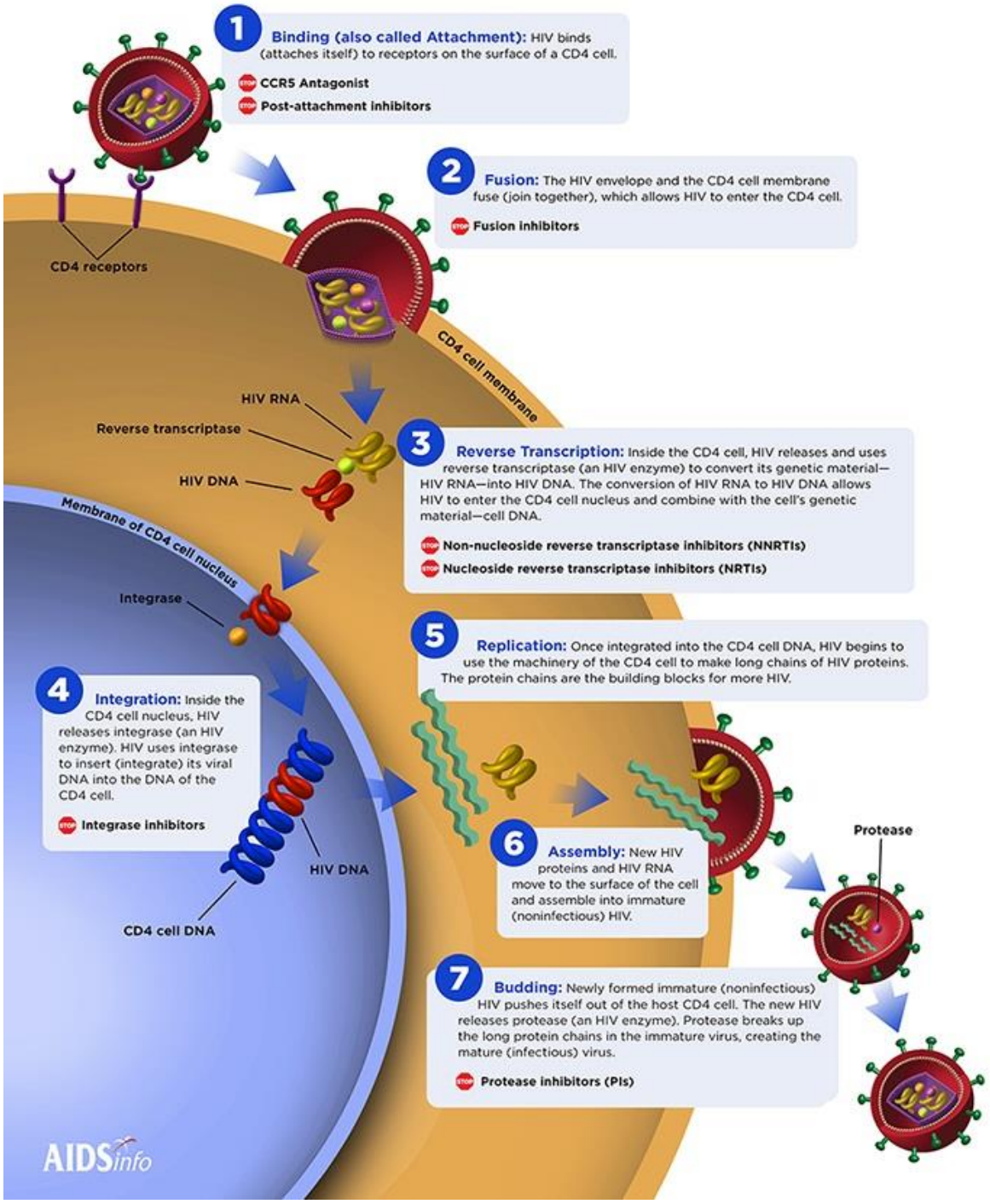


Figure 1.1: The life cycle of HIV-1 and antiretroviral therapy target areas adapted from AIDSinfo and the US Department of Health and Human Services, 2019[33].

1.3 The Global HIV Pandemic

Since the beginning of the HIV pandemic, the Joint United Nations Program on HIV/AIDS (UNAIDS) estimates that 74.9 million individuals globally have acquired HIV, 32 million of whom have since died of AIDS related illnesses (data to the end of 2018)[34]. There are currently an estimated 37.9 million individuals living with HIV, approximately 1-2 million of which are infected with HIV-2[34, 35]. HIV-2 is 55% genetically different, less transmissible, progresses slower than HIV-1[36], and is predominately found in West Africa or countries with direct economic links to West Africa. HIV-1 is responsible for most (94-97%) of the AIDS pandemic with a global prevalence of 0.8%[34, 37]. There is substantial heterogeneity in the prevalence of HIV-1 between countries and regions worldwide, with the centre of the pandemic located in Africa, where an estimated 4.2% of the adult population (and two thirds of the worldwide pandemic) is living with HIV-1[37]. This is in contrast to a much lower prevalence in Europe (0.4%) and the Americas (0.5%)[37], Figure 1.2.

It is impossible to ascertain exactly how many individuals have acquired HIV-1, as statistics do not encompass details of undiagnosed, recently diagnosed, or post-mortem diagnosed cases. This uncertainty is reflected in the wide confidence intervals for prevalence estimates of HIV status (79% (67%-92%)) provided by UNAIDS[34] using modelling techniques developed by Avenir Health[38] to produce annual global HIV and AIDS statistics. It is acknowledged that inconsistencies exist in the data used to inform these statistics (which is country specific and varies based on the countries rates of HIV transmission and overall prevalence).

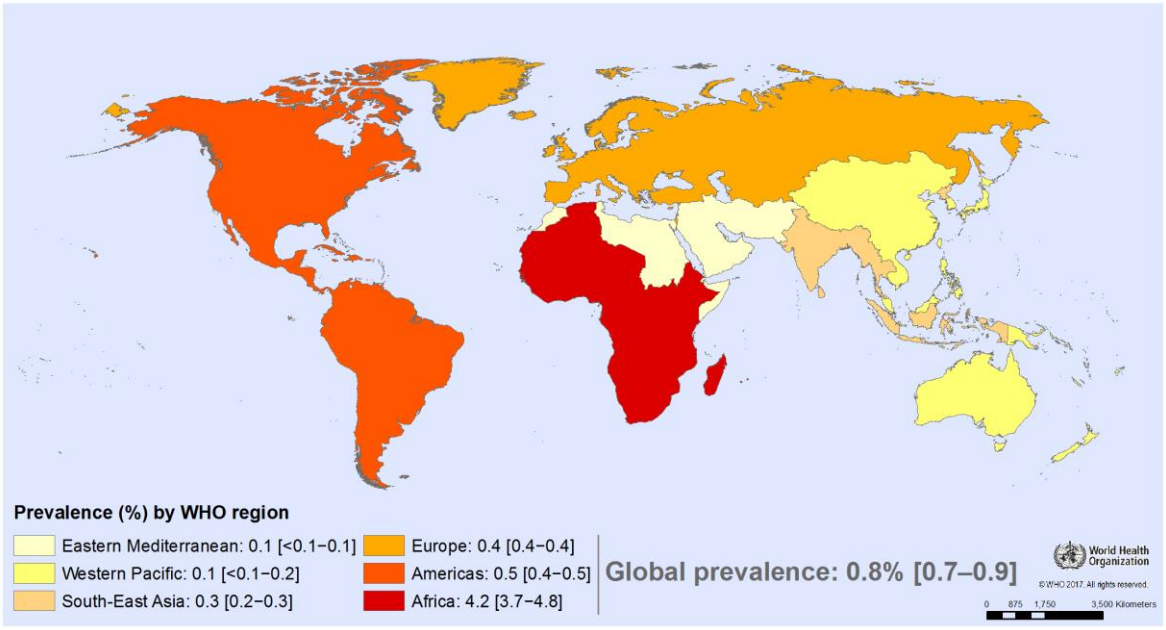


Figure 1.2: Global Prevalence of HIV among adults aged 15-49 according to the World Health Organization, published 2017 [37].

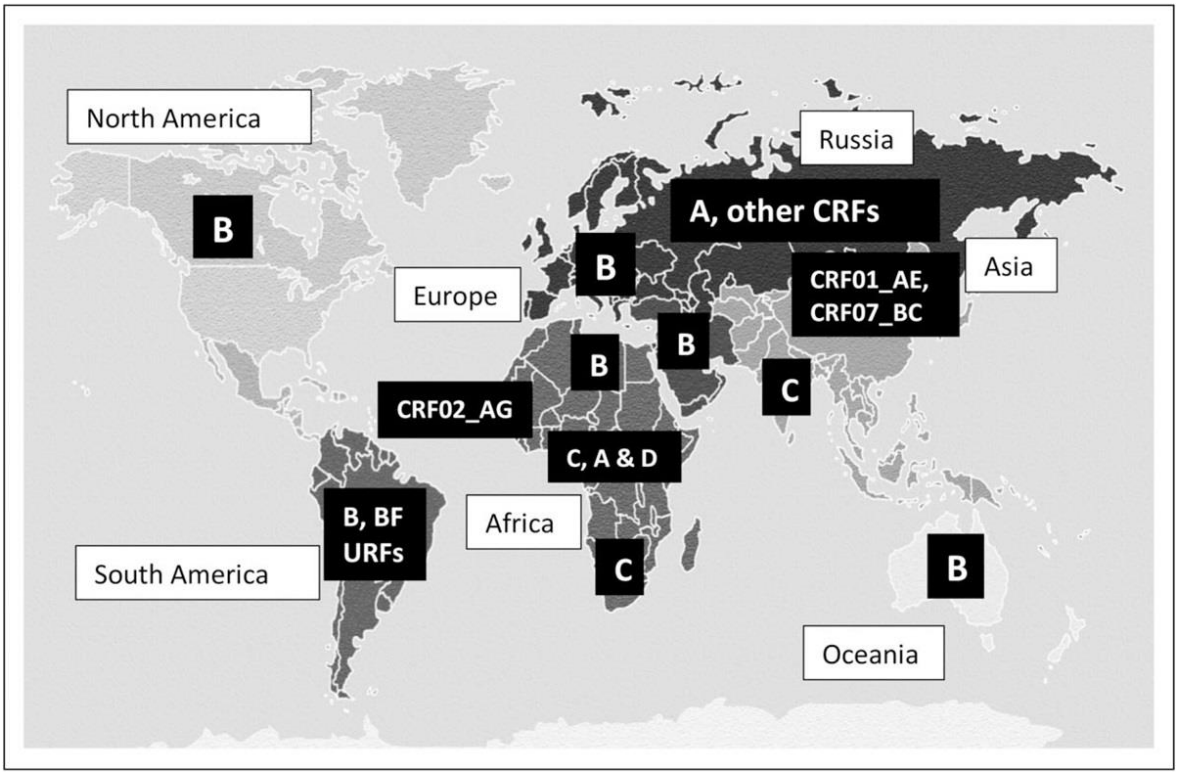


Figure 1.3: Global distribution of the major HIV-1 subtypes by Bbosa et al, Current Opinion in HIV and AIDS, 2019[39].

Coupled with geographic diversity in prevalence, HIV-1 has high genetic variability with at least ten genetically distinct subtypes: A, B, C (the three most common), D, F, G, H J, K, L,

and numerous hybrid combinations of subtypes known as circulating recombinant forms (CRFs). Although each subtype is found in all parts of the world, distribution is geographically uneven,Figure 1.3. Subtype A for example comprises approximately 12% of the global prevalence of HIV-1 and is predominant in eastern Africa and eastern Europe. Subtype C accounts for nearly 50% of the global HIV-1 pandemic and is most common in Africa and India, while subtype B accounts for around 10% of global HIV-1 infections found predominantly in Western Europe, the Americas and Australia[39, 40]. Although subtype B represents a small proportion of global infections, its predominance in resource rich countries has contributed to the an observed focus of drug development being based on its virology[41, 42].

1.4 Natural Course of HIV disease and progression

HIV-1 causes immunodeficiency marked by depletion of CD4-T lymphocytes, and in the absence of treatment, leads to opportunistic infections, AIDS, and death. After HIV-1 infection, there is a transient rise in HIV-1 viral load and a drop in CD4-T lymphocytes until HIV-1 antibodies are formed. Subsequently, HIV-1 viral load drops to a somewhat steady state nadir, termed viral set point, and CD4-T cell counts rise[43-48]. From this point, the average time to AIDS before combinational therapy became available (1996) was 8-10 years[49-51], Figure 1.4.

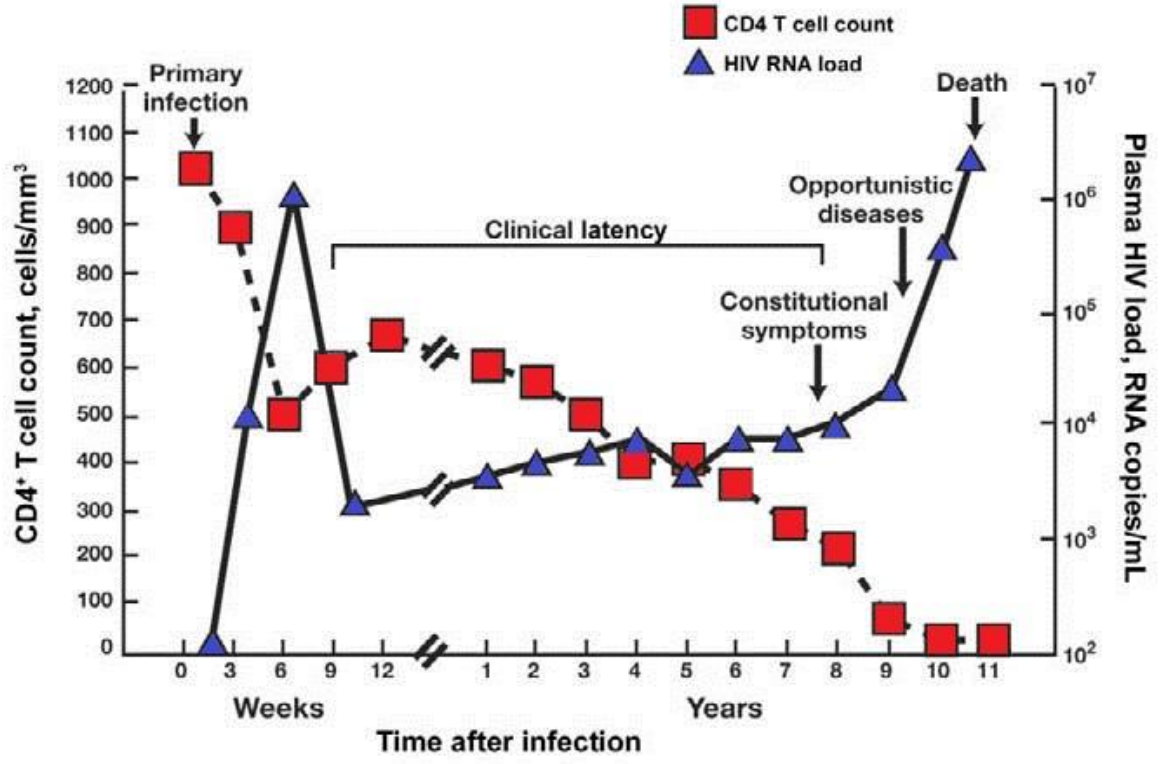


Figure 1.4: Natural course of HIV disease and progression in a typical individual adapted from Anthony S. Fauci, Clinical Infectious Diseases, 2007 [51].

1.5 PhD rationale and research objectives: extreme HIV-1 phenotypes and beyond

Viral pathogenesis is a dynamic process and varies widely, with some individuals progressing to AIDS less than a year after HIV-1 seroconversion, while others remain asymptomatic in the absence of treatment for more than 20 years[52, 53]. These two ends of the clinical spectrum represent the extreme ranges of HIV-1, termed 'extreme phenotypes'. Evaluating these sufficiently rare subsets of individuals with extreme HIV-1 phenotypes, is useful in advancing knowledge of the biology and pathogenesis of disease as well as viral control and progression of HIV-1 infection[54-57]. HIV-1 positive individuals who are able to naturally control the virus, termed elite controllers (EC), provide a natural model for disease control and understanding the biological mechanisms of this phenomenon and could provide insight into novel therapeutic targets and vaccine development[57, 58]. HIV-1 positive

individuals who quickly progress to disease, termed rapid progressors (RP), conversely could provide insight as to why some individuals develop AIDS, or die soon after HIV-1 seroconversion, improving capacity to mitigate risk for individuals who acquire HIV-1 in the future. Although extreme phenotypes of HIV-1 have been intensely studied, there remains heterogeneity in how these extremes are defined in scientific research, making it difficult to ascertain the biological mechanisms underlying these phenotypes[59]. In this PhD, we investigate how extreme phenotypes of HIV-1 have been previously defined in the literature and, using the most common terms as a guide, provide a framework for developing consensus definitions. Building on this framework, we use data on HIV-1 seroconverters to assess the relative merit of the most commonly used definitions in the literature and suggest definitions for HIV-1 ECs and RP to be used in future research.

Since the discovery of the virus in the 1980's, advances in clinical care has transformed HIV diagnosis from a terminal illness to a chronic, treatable condition[60]. However, 6 years after the first AIDS cases were reported, there was still no HIV-1 treatment. The pharmecutical company Burroughs and Wellcome were well known for their antivral drugs and put forth azidothymidine (AZT) to be tested by for it's anti HIV properties by the NCI[61]. In 1985, the NCI had shown AZT was potent against HIV invivo and after quick phase I and phase II randomized controlled trials, the United States Food and Drug Administration (FDA) approved AZT for use against HIV/AIDS on March 20, 1987, the quickest drug development in modern history[62, 63]. However, AZT was not as successful as originally hoped. There were reports of terrible side effects, where individuals felt worse on AZT compared to off it. Additionally, rapid mutation of HIV was observed in some cases resulting in AZT resistance and the onset of AIDS[64]. It was not until 1996, almost 10 years after AZT came to market, that combination antiretroviral therapy (cART) was introduced. This expanded

monotherapy to a minimum of two active drugs from two different classes, where each class of drug interferes with different stages of the HIV-1 life cycle and infection process, Figure 1.1. Modern cART is more effective than monotherapy and has continually evolved with increasing genetic barriers to resistance, becoming more tolerable with fewer side effects, and reduced pill burden[65, 66]. There are currently 32 FDA approved HIV-1 drugs among 6 classes, Figure 1.5. cART has greatly reduced morbidity and mortality rates [67] and now life expectancy for HIV-1 positive individuals on cART is similar to that of the general population, where HIV-1 positive Europeans starting cART at 20 years have an average life expectancy of around 68 years old[68]. cART, however is not a cure, a likely consequence of an inaccessible reservoir of latently infected cells. HIV positive individuals wanting the best prognosis, might need to make lifestyle adjustments (e.g. quitting smoking and injection drug use, maintaining regular exercise and eating a balanced diet) and commit and adhere lifelong medication to main control of the virus. Adherence is a major component of successful cART, and it is estimated that if adherence is less than 95%, viral breakthrough is likely to occur[69]. There are some reports of individuals controlling viral replication after stopping cART[23-30], in essence, creating HIV-1 ECs, but nearly all individuals experience viral rebound soon after cART cessation. A core question expanded on inthis PhD considers how we can use cART to prevent HIV-1 positive individuals from rapidly progressing to disease.

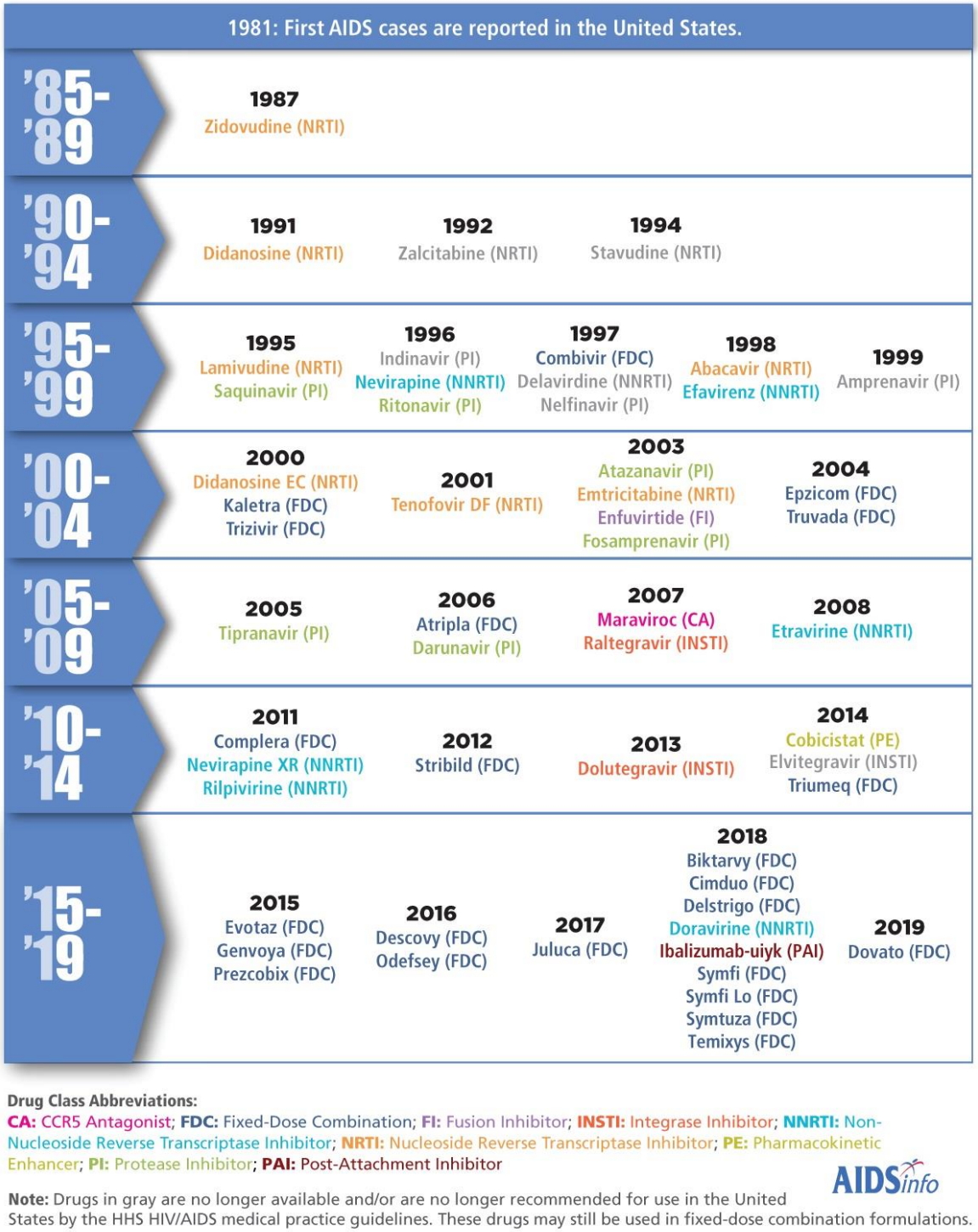


Figure 1.5: Timeline of FDA approval of HIV Medicine from 1987-2019s, adapted from AIDSinfo, for the National Institue of Health[70].

Although modern treatments are highly effective at suppressing viral replication, the exact timing of cART initiation has not always been clear with varying national and international guidelines. Several studies, including one we present here, suggest immediate cART

initiation upon HIV diagnosis irrespective of CD4 cell count, as this reduces the risk of developing serious illness or death by up to 57% [71-73]. Although immediate treatment is advised, challenges of cART remain, for example, the lifelong pill burden, the risk of accumulated toxicities, risk of drug resistance with non-adherence, efficacy in non-B subtypes (as most of the drug development studies are in subtype B[74]), and financial pressures limiting access to care, particularly in resource-limited settings where the majority of HIV-1 positive individuals are not typically infected with subtype B HIV-1.

In this PhD, we address these major questions in the natural history of HIV-1, and provide suggestions on how to optomize treatment, targeting post-treatment control, and reducing rapid disease progression of HIV-1 positive individuals. The data used to answer these questions is predominately from Europe, therefore acknowledgement that the majority of HIV-1 cases are subtype B is noted from the outset.

2 Methods

The data used for this PhD was sourced from three main cohort collaborations, namely CASCADE[75], EuroSIDA[76], and HIV-CAUSAL[77]. Each collaboration has unique aims and combine data from hundreds of clinics, allowing questions to be investigated with more statistical power than from individual studies or clinics alone. For this reason, all three collaborations utilize a common protocol for data exchange called HIV Cohorts Data Exchange Protocol (HICDEP)[78]. HICDEP provides a standard format for HIV datasets and currently incorporates 32 data tables with codes for specific variables. Exact details on all the tables and codes are available on the HICDEP website https://hicdep.org/. Individual cohorts map their data to the HICDEP protocol which creates a standard format for merging, allowing big cohorts to easily and repeatedly formed by combining individuals identified in hundreds of sources. The first HICDEP protocol was formed in 2003 and has continually evolved with changes to clinical care of HIV positive individuals, for example, as new HIV drugs are developed, new codes are added to the HIV drug table. A list of the HICDEP tables each cohort routinely collects is provided in Table 2.1, and the associated HICDEP data dictionaries are available in Appendix 3. Details of each cohort coordinating centers are included in Appendix 2.

2.1 CASCADE

2.1.1 Study description

Concerted action on seroconversion to AIDS and death in Europe (CASCADE) is a HIV-1 cohort collaboration focusing on individuals with well estimated dates of HIV-1 seroconversion. The study was established in 1997 and has expanded to include data from over 30,000 HIV-1 positive seroconverters drawn from 29 cohorts representing over 300

clinics across Europe (95%), Canada (1%), Australia (1%) and Sub-Saharan Africa (3%). The main aim is to collect routine clinical HIV data on newly infected and previously enrolled individuals, covering the entire duration of HIV-1 infection. Although the collaboration was formed in 1997, data was retrospectively collected, and some individuals seroconverted as early as 1980, with the most recent data update preformed in 2015. CASCADE data therefore uniquely provides the opportunity to study events occurring during and around HIV-1 seroconversion, the natural history of HIV-1 from the early 1980's until 2015, convering the pre-treatment, monotherapy and modern cART eras, and differences in survival over time and through the advent of cART and into modern HIV-1 treatments. One of the main premises that formed the collaboration is that through pooling data, issues can be addressed that require more statistical power than any single study alone, for example studying rare groups of individuals such as HIV-1 ECs or HIV-1 RPs.

2.1.2 Data collection

Seroconverters are enrolled into individual cohorts where data is collected from routine clinical practice, and therefore all individuals are typically followed up for the duration of their life. Date of seroconversion is estimated by three standard methods, most commonly as the midpoint between the last documented HIV-1 negative and the first positive HIV-1 antibody test dates with an interval of less than three years between the two test dates (85%). For the remainder, date of seroconversion was estimated through laboratory evidence of seroconversion (polymerase chain reaction (PCR) positivity in the absence of HIV-1 antibodies or antigen positivity on Western blot) (13%), or as the date of a seroconversion illness (2%) with both an earlier documented negative and a later positive HIV-1 test not more than three years apart. The midpoint method and seroconversion illness method for estimating HIV-1 seroconversion are comparable as both methods require HIV-1 negative and positive test dates to be no further than three years apart.

Laboratory evidence of seroconversion is much more specific, and could indicate a different type of individual, for example someone that is experiencing seroconversion illness, or someone who is presenting at a clinic for other co-infections. Participating cohorts were asked to send data incorporated in 10 HICDEP tables, which included descriptive background and demographic data (tblBAS, see Appendix 3, page 191) for complete variable list), laboratory measurements of CD4 cell counts (tblLAB_CD4, Appendix 3, page 198) and HIV-RNA measurements (tblLAB_RNA, Appendix 3, page 204) which were performed by various in-house methods, antiretroviral treatment (tblART, Appendix 3, page 189) as well as other medications (tblMED, Appendix 3, page 208), virological data of all negative and positive HIV-1, Hepatitis C and B results (tblLAB_VIRO, Appendix 3, page 205), nucleotide sequence data and resistance tests (tblLAB_RES, tblLAB_RES_LVL_1, Appendix 3, page 199), and death and last follow-up (tblLTFU, Appendix 3, page 206), Table 2.1. Anonymized data for the CASCADE collaboration were collected and stored at the Medical Research Council Clinical Trials Unit at University College London in London, United Kingdom.

2.2 EuroSIDA

2.2.1 Study Description

The EuroSIDA study is a prospective observational cohort study founded in 1994 and houses data on approximately 23,000 HIV-1 positive individuals accessing care in over 100 hospitals in 35 European countries, and uniquely in all European Regions, as well as Israel and Argentina. EuroSIDA's original objective was to follow the general population of HIV positive individuals living in Europe while assessing clinical progression and impact of cART on HIV prognosis, and over the two decades the objectives have broadened to also include monitoring adverse events on cART, longitudinal changes and regional differences in HIV

care and across Europe, and uptake of Hepatitis C (HCV) therapy among HIV/HCV co-infected individuals.

2.2.2 Data Collection

A principal investigator leading each clinic was responsible for enrolling patients, collecting and reporting data, maintaining ethical approval, and obtaining informed consent from all individuals. EuroSIDA collects data from routine clinical visits once annually using the electronic case report system REDCap[79] and in the HICDEP data format. The data collected is similar to CASCADE with information on baseline patient clinical characteristics and demographics, standard HIV laboratory measurements of CD4 cell counts and HIV-RNA measurements, antiretroviral treatment information, AIDS and non-AIDS defining illness and date of death, Table 2.1. EuroSIDA additionally focuses on specific diseases and events, including laboratory information on renal function, liver function, cardiovascular health and non-AIDS defining cancers. EuroSIDA also requests plasma samples from all patients every 6 months which are intermittently shipped to the central repository at the coordinating centre in Copenhagen. The EuroSIDA coordinating centre stores anonymized data at the Centre of Excellence for Health, Immunity and Infections (CHIP) in Copenhagen, Denmark and collaborated with the statistical centre at the Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CREME) at University College London.

2.3 HIV-CAUSAL

2.3.1 Study description

HIV-CAUSAL collaboration is a HIV-1 cohort collaboration, but with a different focus than CASCADE and EuroSIDA. This collaboration is formed from prospective cohort studies in Europe and the United States, based on standard clinical data collected within national health care systems with universal access to care. The consortia aims to address methodological problems around comparative effectiveness and safety research in HIV, the

most notable of which is how to appropriately handle time-dependent confounding in statistical models. Methods that have been pioneered by HIV-CAUSAL and included in this PhD are inverse probability weighting of marginal structural model to appropriately adjust for time dependent confounding[80]. Sample programs are freely available on the HIV-CAUSAL website: https://www.hsph.harvard.edu/causal/software/.

2.3.2 Data Collection

Recorded data include patient specific characteristics and demographic data, laboratory measurements of CD4 cell counts and plasma HIV-RNA measurements, antiretroviral treatment information, AIDS and non-AIDS defining illnesses and death dates, Table 2.1. Anonymized data were sent and stored at the HIV-CAUSAL Coordinating Center at Harvard University in Boston, USA.

2.4 Ethics

All cohorts in each collaboration received approval from their individual ethics review boards to pool anonymized data for analyses and dissemination.

2.5 Data Validation

All cohorts underwent unique verification of collected data using cohort specific quality assurance programs. These included, but are not limited to, the data checks recommended on the HICDEP website, data validity checks on dates of HIV acquisition, HIV treatment and other medications, laboratory measurements, and AIDS and other clinical events, death and loss to follow-up.

Table 2.1: HICDEP Data tables collected in CASCADE, EuroSIDA and HIV-CAUSAL.

	Appendix, page	CASCADE	EuroSIDA	HIV-CAUSAL
tblART – Antiretroviral treatment	3, 189	X	X	X
tblBAS – Basic clinical, background and demographic information	3, 191	X	X	X
tblCENTER – Center information	3, 193		X	
tblCEP – Clinical events and procedures	3, 194		X	X
tblDIS – CDC-C and WHO Stage Diseases	3, 195	X	X	X
tblLAB – Laboratory values	3, 196		X	X
tblLAB_BP – blood pressure	3, 197		X	X
tblLAB_CD4 – CD4 cell count	3, 198	X	X	X
tblLAB_RES – Resistance testing	3, 199	X	X	X
tblLAB_RES_LVL_1 - Nucleotide sequences (PRO, RT, GP41, GP120,)	3, 201	X	X	X
tblLAB_RES_LVL_2 – Mutations	3, 202		X	X
tblLAB_RES_LVL_3 – Resistance test result	3, 203		X	X
tblLAB_RNA – HIV-RNA values	3, 204	X	X	X
tblLAB_VIRO - viro-/serology	3, 205	X	X	X
tblLTFU – Death and drop-out	3, 206	X	X	X
tblMED – Other medication	3, 208	X	X	X
tblSAMPLES – Blood samples	3, 209		X	
tblVIS – Basic follow-up/visit related data	3, 201		X	X

3 Extreme Phenotypes of HIV-1

3.1 Natural process of HIV-1 disease progression

HIV is typically characterised by a period of viral replication and CD4 decline leading to AIDS and death in the absence of cART[81], Secion 1.4, Figure 1.4. There are also variations in these markers between individuals over time[82, 83], attributed to differences in the infecting virus (e.g. subtype, or an attenuated virus) and host genetic characteristic leading to variations in disease progression, where some remain clinically asymptomatic for long periods, while others progress rapidly to disease[52, 53]. These two ends of the clinical spectrum represent the extreme ranges of HIV, termed 'extreme phenotypes' are defined using host biomarkers, the two most common are assessing host immune function using CD4 cell counts, or host viral control measuring circulating plasma HIV-RNA (viraemia). Viral genetics also play a part in disease progression, for example, different subtypes within HIV-1 have been shown to have varying disease progression rates[84, 85].

3.2 Extreme ends of HIV-1 disease progression

Evaluating sufficiently rare subsets of individuals among a relatively homogenous population with extreme HIV-1 phenotypes, is useful in advancing knowledge of the biology and pathogenesis of HIV-1 disease control and progression[54-57]. Genome wide association studies (GWAS) have identified common variants associated with HIV-1 viral control and HIV-1 disease progression, yet these studies do not capture all the variation in the genome and do not explain a large part of the "heritability" of extreme phenotypes, or the fraction of variability in a population explained by host genetics[86]. It is possible that co-existance of several variants with smaller effects, or rare variants with large effects could explain some of this missing heritability[87]. Rare variants are poorly detected by GWAS, as conventional

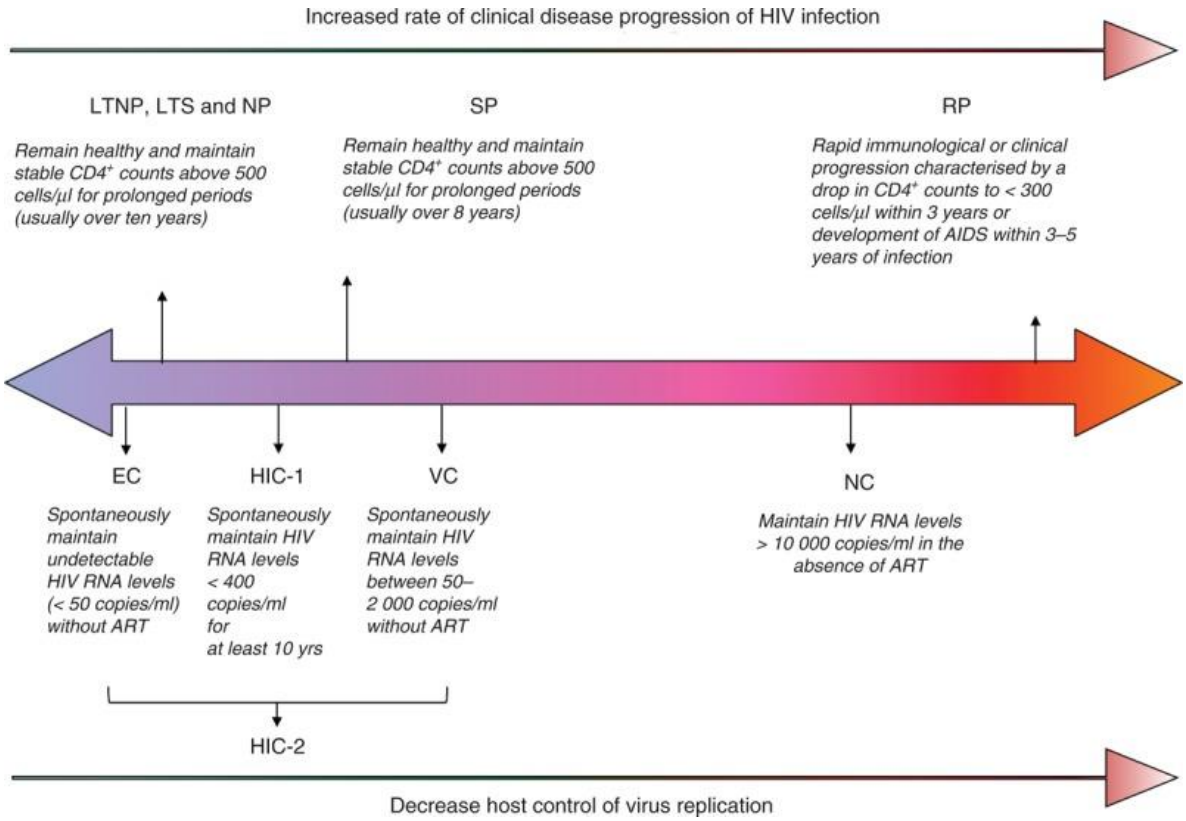
Sanger sequencing detects variants present in 20% of the population. Sequencing on the genetic variants that alter protein sequences, or whole genome sequencing, has been proposed to explain an additional proportion of HIV-RNA variability[88, 89]. Using exome sequencing and sampling individuals with extreme phenotypes can help to identify rare variants, as they are more likely found in the extreme traits[90].

The long term non-progressor (LTNP) phenotype was initially described to characterize individuals with slow clinical disease progression, defined by stable CD4 cell counts over a period of 10 years or more[53, 91]. Simultaneously, RP also became an area of research interest focused on poor immune response and rapid CD4 cell count depletion. With the introduction of HIV-RNA viral load assays in the mid-1990's, research shifted to focus on the mechanisms which lead to host control of viral replication, and the EC phenotype became an area of intense study[59]. HIV-1 EC provide a natural model for disease control and understanding the biological mechanisms of this phenomenon could provide insight into novel therapeutic targets and vaccine development[57, 58]. On the opposite spectrum, HIV-1 RP provide an opportunity to investigate why individuals progress to disease; and could provide insight into ways we can better manage HIV treatment, HIV monitoring, and improve overall health to prevent disease progression.

Heterogeneity in criteria used to define extreme phenotypes can introduce inconsistencies in results and make it difficult to identify the biological mechanisms underlying these phenotypes[59]. Despite a depth of research into these groups, definitions used are inconsistent therefore it is important to systematically review the literature to find and assess the relative merit of common links between definitions and provide a framework for developing consensus definitions.

3.3 Systematic review of HIV-1 extreme phenotypes definitions

We undertook a systematic review of the literature in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[92] guidelines and searched for terms relating to virological and clinical progression of HIV-1. We reviewed 501 articles published between January 1st, 2000 and 15th March 2012 and found a total of 714 extreme phenotype definitions, 600 definitions for 26 terms used to describe slow progression/viral control extremes of HIV infection, and 114 definitions with eight terms used to define RP or lack of viral control[93]. Substantial variation was evident among definitions in the literature, with a large proportion unique for progression (50-54%) and viral control (43-59%) related terms. This heterogeneity could represent important biological endophenotypes and select individuals with varying clinical outcomes[59, 94-96], suggesting the need for harmonized definitions. The three most common terms used for extreme phenotypes were LTNP, EC and RP, and their associated definitions were 1. LTNP: asymptomatic and ART-naive for 10 years during follow-up with all CD4 cell counts above 500 cells/mm^3 during this period, 2. EC: spontaneously maintaining HIV-RNA below 50 copies/ml without cART, and 3. RP: HIV-1 infected with CD4 cell counts < 300 cells/mm^3 within three years after the last HIV-1 seronegative test. We also identified common components in existing definitions, Figure 3.1, where LTNPs were broadly defined by maintaining normal CD4 cell counts and remaining healthy for 10 years, viral controller phenotypes were broadly characterised by regulating HIV-RNA without cART, and RPs were characterised by a drop in CD4 cell counts or the development of AIDS within 3-5 years. This could provide a framework for developing consensus definitions of HIV-1 extreme phenotypes.



HIC-1 and HIC-2 refer to the two most commonly used definitions for HIV controllers. ART, antiretroviral therapy; EC, elite controller; HIC, HIV controller; LTNP, long-term nonprogressor; LTS, long-term survivor; NC, noncontroller; NP, nonprogressor; RP, rapid progressor; SP, slow progressor; VC, viremic controller.

Figure 3.1: Characteristics of phenotypes by different terms in the literature from Gurdasani et al, AIDS, 2014[93].

3.4 Evaluation of HIV-1 elite controllers within a large seroconverter cohort collaboration

HIV-1 EC, classified by maintaining control of HIV-RNA, was one of the most common phenotypes identified in the literature review, likely because these individuals lead the way for the development of new treatment strategies [57, 58]. Following our literature review, it was unclear if any currently used definitions best identify this rare phenotype. We sought to assess the relative merit of common definitions of viral control using the CASCADE data on 25,692[1] HIV-1 seroconverters[97] (study details in Section 2.1). We evaluated 10 commonly used definitions (Table 3.1) and estimated the proportions of individuals maintaining elite control during cART naïve follow-up time and evaluated disease

[1] Patient population defined in Appendix 1, page 93-95.

progression comparing EC to non-EC. We also examined HIV-1 RNA copies/ml and CD4 cell counts cells/mm^3 among EC during periods of EC and total cART naïve follow-up. Most definitions classified a sufficiently rare population (~1%) as EC's, but definitions that require consecutive undetectable HIV-RNA measurements (HIV-RNA < 50 or 75 copies/ml) for at least six months were most sensitive to classifying individuals with the slowest disease progression, lowest HIV-RNA and highest CD4 cell counts during EC periods and total cART naïve follow-up, Table 3.2, Table 3.3. We suggested this definition be used for future EC research.

Table 3.1: 10 definitions of elite control from the literature applied to the CASCADE dataset; all require individuals to be AIDS-free and ART-naïve from Olson et al, PLOSone, 2014[97].

Definition	
A	HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml [98]
B	HIV-positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml [57]
C	HIV-positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml [99]
D	HIV-positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml [100]
E	HIV-positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months [101]
F	HIV-positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml [59]
G	HIV-positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml [102]
H	HIV-positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml [103]
I	HIV-positive ≥10 years, with all measured HIV-RNA <50 copies/ml [104]
J	HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml [105]

Table 3.2: Estimated hazard ratios comparing non-elite and unknown to elite controllers (EC) for time from estimated HIV seroconversion to a composite endpoint of AIDS, Death, ART, or CD4 <350 cells/mm³ restricting entry to the risk set at 10 years post seroconversion using the CASCADE dataset applied to 10 definitions of EC found in the literature from Olson et al, PLOSone, 2014[97].

Def.	EC evaluated (experiencing composite endpoint) n (n)††	HR for time to composite endpoint† (95% CI)	% (IQR*) ART-naïve follow-up time classified as EC
A[ғ]	46 (4)	12·5 (4·7, 33·6)	100 (78-100)
B[ғ]	53 (11)	4·6 (2·5, 8·3)‡	100 (78-100)
C[ғ]	86 (18)	4·8 (3·0, 7·7)‡	99 (72-100)
D[ғ]	134 (35)	4·0 (2·8, 5·7)‡	97 (71-100)
E[ғ]	36 (2)	19·0 (4·7, 76·4)	100 (78-100)
F[ғ]	26 (5)	15·3 (3·8, 61·3)	92 (66-100)
G[ғ]	60 (9)	7·5 (3·9, 14·5)‡	100 (86-100)
H[ғ]	56 (22)	2·9 (1·9, 4·4)‡	100 (75-100)
I[ғ]	4 (1)	3·4 (0·5, 24·0)	100 (100-100)
J[ғ]	35 (3)	13·2 (4·2, 41·3)	100 (98-100)

*IQR: Interquartile range

††Number of ECs making it to 10 years follow up without experiencing composite endpoint and number subsequently experiencing composite endpoint.

†Hazard ratios comparing ECs to Non-ECs (including those with unknown EC status) allowing for late entry at 10 years. For each definition, p-values were obtained from unadjusted log-rank test for time to composite endpoint and were all highly significant $p < 0{\cdot}001$

‡ Statistically different HRs compared to definition E, F, and A from 1000 bootstrap replicates. No definitions were statistically different from definition J at $\alpha = 0{\cdot}05$.

[ғ] Definitions listed in Table 3.1

Table 3.3: HIV-RNA and CD4 values during elite control (EC), and throughout ART-naïve follow-up using the CASCADE dataset applied to 10 definitions of EC found in the literature from Olson et al, PLOSone, 2014[97].

Def.	During Elite Control				During ART-naïve follow-up			
	HIV-RNA value*		CD4 Value*		HIV-RNA value*		CD4 Value*	
A[ғ]	50	(35, 276)	675	(454, 877)	66	(35, 495)	654	(441, 840)
B[ғ]	425	(35, 11641)	573	(409, 792)	1043	(89, 13000)	548	(404, 751)
C[ғ]	354	(50, 8700)	596	(427, 796)	660	(75, 11066)	567	(415, 764)
D[ғ]	903	(287, 1863)	615	(478, 789)	1274	(370, 3304)	590	(451, 756)
E[ғ]	50	(35, 81)	699	(528, 922)	50	(35, 165)	681	(527, 909)
F[ғ]	50	(35, 50)	839	(654, 1070)	50	(35, 77)	796	(629, 1020)
G[ғ]	113	(49, 1197)	644	(439, 824)	176	(50, 2160)	625	(438, 806)
H[ғ]	76	(35, 283)	697	(541, 879)	89	(35, 356)	687	(530, 879)
I[ғ]	35	(1, 35)	583	(575, 905)	35	(1, 35)	583	(575, 905)
J[ғ]	50	(35, 127)	783	(628, 970)	50	(35, 169)	740	(583, 970)

Note- all values are median (IQR)

*units are HIV-RNA copies/ml and CD4 cells/mm^3

[ғ] Definitions listed in Table 3.1

Beyond HIV-RNA control, HIV-1 positive individuals progress to disease at varied rates measured by CD4 cell counts. Individuals with slow and fast CD4 cell count progression, are termed long-term non-progressors and rapid progressors, respectively.

3.5 Characterisation of HIV-1 long term non-progressors (LTNP) within a large seroconverter cohort collaboration

We sought to describe the characteristics of LTNP, and to identify factors associated with the loss of LTNP status using CASCADE data on HIV-1 seroconverters[106]. Using the most common definition of LTNP from the literature review, where individuals were required to be HIV-1 positive for more than 10 years, cART naïve and AIDS free with CD4 cell counts > 500 cells/mm^3, we found 283 of 4979[2] eligible individuals included in our analysis achieved LTNP status. Most individuals subsequently lost LTNP status (n=202/283), and this was associated with lower CD4 cell counts at 10 years after seroconversion (Hazard ratio (HR) 95% confidence interval (CI) for loss of LTNP = 0.39 (0.24, 0.62) for CD4 = 900 vs. CD4 = 600 cells/mm^3). Excluding CD4 cell counts in the model, higher HIV-1 RNA at 10 years was associated with loss of LTNP status (HR for loss of LTNP = 1.38 (0.91, 2.13) for $\log_{10}$ HIV-RNA copies/ml = 4 vs. $\log_{10}$ HIV-RNA copies/ml = 3), Figure 3.2. LTNP is rare and most individuals eventually lose this status (in our study 202/283 lost LTNP) likely due to slow CD4 cell decline and slow increases in plasma HIV-1 RNA.

[2] Patient population defined in Appendix 1, page 101-104

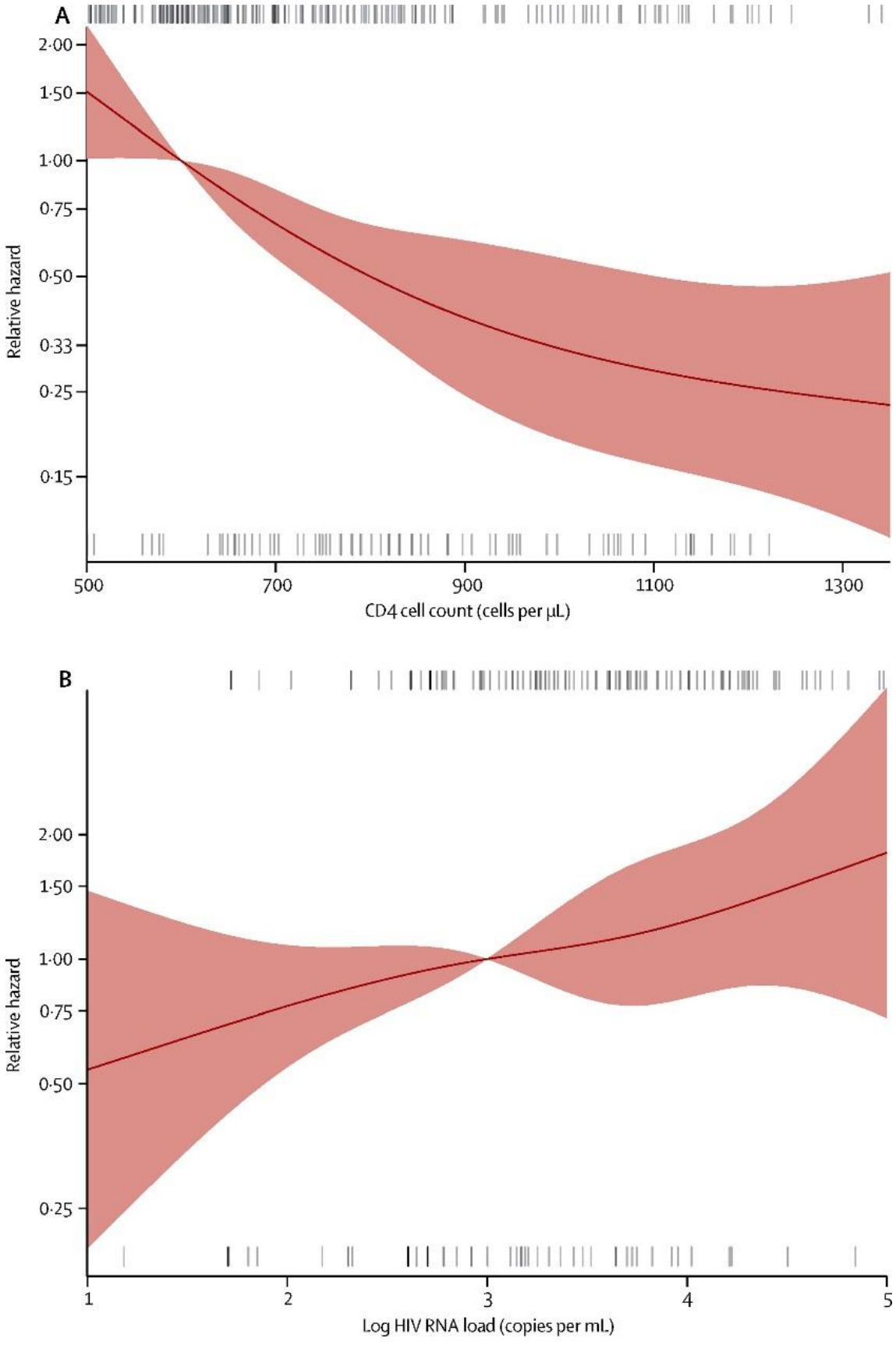


Figure 3.2: Relative hazard of CD4 cell count (A) and HIV RNA load (B) at 10 years after seroconversion with loss of LTNP from van der Helm et al, Lancet HIV, 2014[106].

Reasons why some individuals are able to maintain viral suppression or slow disease progression has not been fully elucidated, although several factors could be involved, including being infected with an attenuated virus[107-109]. Several case reports show some LTNPs have HIV-1 viruses that have single nuclear polymorphisms, or large deletions in the nef, vpr, vif or rev genes[107]. Other studies have shown, however, that EC and LTNP are infected with a pathogenic virus, suggesting that host factors play a large part in controlling viral replication and maintaining high CD4 cell counts[110]. Host genetic factors from GWAS have uncovered CCR5Δ32, HLA-B57 polymorphisms, and single nucleotide polymorphisms (SNPs) in class I and III MHC sub regions to be associated with EC and LTNP status[107, 111, 112]. Notably, the only two known cures of HIV-1 were Timothy Brown i.e. the Berlin patient[113], and the London patient[114], which resulted from treating cancer with stem cell transplants using donors with the CCR5Δ32 mutation. However, these polymorphisms do not explain 100% of the variability in viral load and CD4 cell counts. These studies do not assess the full spectrum of functional variants within the host coding regions which could explain further variability in viral load and CD4 cell count maintenance[88, 89]. Using definitions identified in our previous manuscripts, our collaborators sequenced the exome of 1327 eligible individuals (including n= 85 EC and n= 98 RP from the CASCADE cohort), and performed whole exome sequencing to identify rare variants associated with viral control[115]. Unfortunately, they found no strong exonic variants with large effect sizes contributing to the control of HIV infection. More recently, however, a study published by Nissen et al. (2018) sequenced the whole exome of seven LTNP and identified several rare variants in genes involved with HIV entry and inward trafficking, HIV transcription, cell homeostasis sensing and inflammation[116, 117]. Interestingly, this study found no significant variants associated with EC status. This suggests there are different pathogenesis and biological

mechanisms distinguishing viral control and immune preservation phenotypes, which warrants further investigation.

3.6 Evaluation of HIV-1 rapid progressors within a large seroconverter cohort collaboration

Rapid progression is an extreme phenotype which can contribute to our understanding of early risk factors leading to disease progression. Studying these individuals could help guide clinical monitoring and aid in understanding when and what cART to initiate. However, they are less frequently studied then slow progressor and viral control counterparts, as seen by the number of articles and definitions relating to RP uncovered in our literature review (51 unique definitions for RP compared to 209 unique definitions for LTNP and EC)[93]. The optimal rate and CD4 cell count threshold for identifying this phenotype was unclear, so we sought to define the RP phenotype using CASCADE data on HIV seroconverters in the pre-cART era[118] [3]. We found that low CD4 cell counts (CD4 <350 cells/mm^3) during the first year after an HIV-1 positive test was not uncommon, where 2.8% of individuals experienced a CD4 cell count <100 cells/mm^3 and 10% experienced at least one CD4 cell count measurement <231 cells/mm^3. The risk of AIDS defining illness or death was substantially higher for those experiencing at least one CD4 cell count <100 cells/mm^3 in the first year after HIV-1 diagnosis compared to individuals with CD4 cell counts at higher levels, Figure 3.3. We concluded that individuals with at least one CD4 cell count <100 cells/mm^3 identified a rare group (2.8%) at the highest risk of disease progression and should be the basis for defining the RP phenotype. This suggests the importance of CD4 monitoring near HIV seroconversion, as it can play a part in identifying those at highest risk of progression and in need of immediate cART and potentially more frequent monitoring, aligning with

[3] Patient population defined in Appendix 1, page 109-111.

current British HIV Association (BHIVA) guidelines[119] which suggest more frequent monitoring among asymptomatic patients with lower CD4 cell counts who refuse cART.

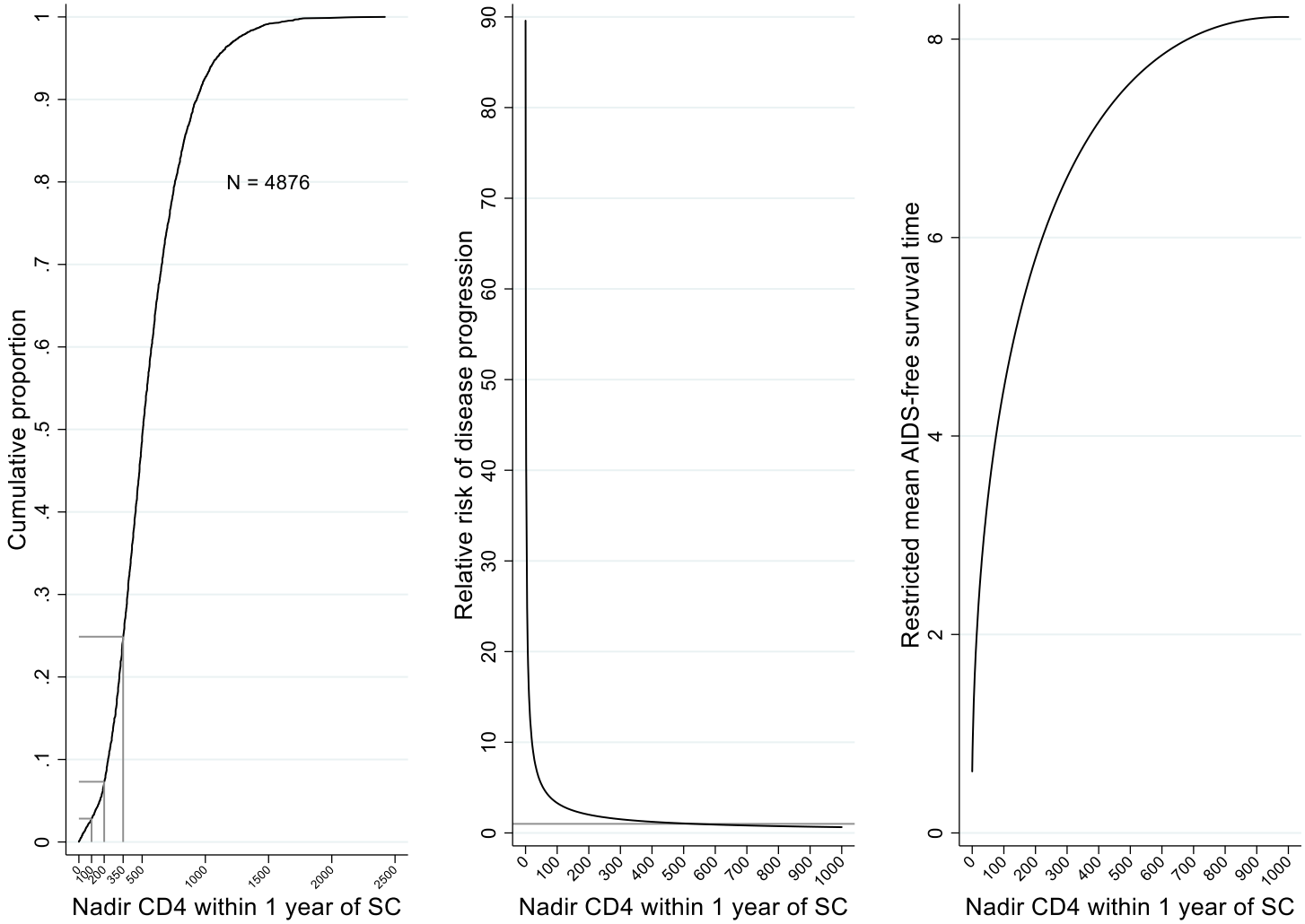


Figure 3.3: Cumulative proportions of nadir CD4 cell count (left hand panel), relative risk of AIDS/death compared with individuals whose CD4 counts remained at 500 cells per cubic millimeter (center panel), and mean AIDS-free survival time at 10 years follow-up (right hand panel) for individuals in CASCADE experiencing specific nadir levels within 1 year of SC during that period: all individuals seroconverted in the pre-cART era from Olson et al, JAIDS, 2014[118].

4 Treatment Optimization

Exact timing of cART initiation has not always been clear with varying national and international guidelines. Until 2015, global cART guidelines varied widely and changed over time, Table 4.1. The guidelines are for the majority of individuals who do progress to disease and require treatment to prevent AIDS and death, where their engagement in treatment is a key component its success. It is worth noting that these are just guidelines, and cART needs to be optimized within the individual. For example, the guidelines do not make specific recommenations for HIV-1 EC, who may, infact, not need treatment as they maintain favourable CD4 cell counts and control viral replication (HIV-RNA <50 copies/ml).

Table 4.1: 2010 and 2015 cART initiation recommendations from the International AIDS Society (IAS), World Health Orginixatin (WHO), European Clinical AIDS Society (EACS) and BHIVA guidelines

Governing Body	2010 cART recommendations	2015 cART recommendations
IAS	Initiate when CD4 <500 cells/mm^3	Immediate cART
WHO	Initiate when CD4 ≤ 350	Initiate when CD4 <500 cells/mm^3
EACS	Initiate when CD4 ≤ 350 cells/mm^3	Initiate when CD4 <350 cells/mm^3, considered when CD4 350-500 cells/mm^3
BHIVA	Initiate when CD4 ≤ 350	Initiate before CD4 <350 cells/mm^3

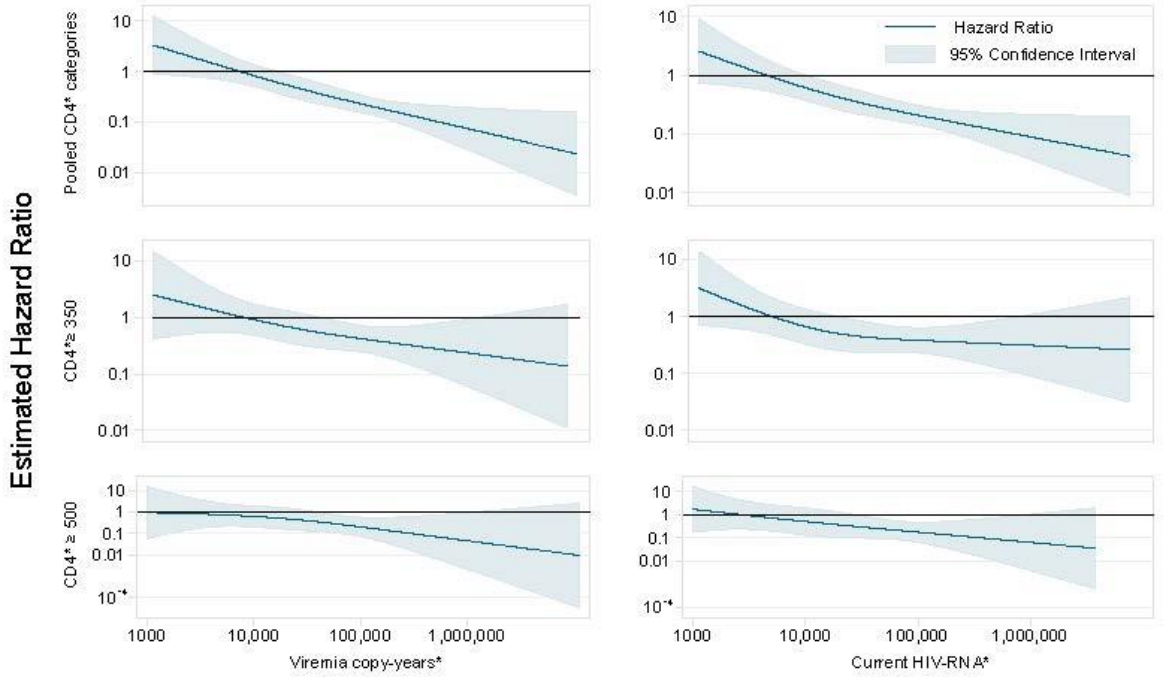
Since 2015, the START[71] and TEMPRANO[72] trials showed 57% and 44% reduction in death or severe illness among individuals immediately initiating cART, respectively, these trials might not, however, be representative of clinical practice. In 2015, data from HIV-CAUSAL highlighted a modest clinical benefit among individuals immediately initiating treatment: relative risk (RR) of death 1.02 (1.01, 1.02) compared to 1.06 (1.04, 1.08) for waiting to initiate when CD4 <500 cells/mm^3 and CD4 < 350 cells/mm^3 vs immediate initiation of cART, respectively[73].

4.1 The utility of HIV-RNA and HIV viremia copy/years in deciding when to start cART

Most of the clinical evidence used to determine when to start cART is based on CD4 cell counts except where indications prompt advice for immediate treatment (e.g. pregnancy or some co-infections), but viral load measurements have an important role in the monitoring and staging of HIV-1 positive individuals. HIV-RNA measurements have been used to tailor first line cART regimens, or determine when someone is failing their current cART regimen[120], and can also assess HIV transmission risk[121, 122]. Importantly, HIV-RNA measurements could also help inform when to start treatment among those who do not want to initiate cART immediately and have high CD4 cell counts. It is unlikely that randomized evidence will be available to answer this question for two main reasons: first, evidence suggests all individuals with HIV-1 should immediately initiate cART[71, 72] and second, randomized controlled trials (RCT) are extremely costly, so funding would be unlikely when two independent RCTs have provided good evidence linked to immediate cART initiation. In this case, we must rely on observational data to inform whether HIV-RNA should play a role in treatment initiation and optimization. We sought to investigate the effect of initiation or deferring cART by varying levels of current HIV-RNA on HIV-1 disease progression. Although there is utility in obtaining a single HIV-RNA measurement, this fails to capture cumulative exposure to HIV-1 throughout the entire disease period. Viremia copy-years (VCY) is a measure of cumulative HIV-RNA exposure and could provide additional information beyond a single snapshot of HIV-RNA. VCY has been shown to predict AIDS and death in individuals on and off cART[123-125] even after adjusting for viral load and CD4 cell count measurements. It is therefore important to determine whether cART initiation before the accrual of VCY could reduce morbidity and mortality, even among those with high CD4 cell counts. We also investigated the effect of initiating or deferring

cART by varying levels of VCY[126]. We used CASCADE data on 9,353[4] HIV-1 seroconverters in the cART era, which incorporated serial HIV-RNA and CD4 cell count measurements on each individual throughout the entire HIV-1 clinical follow-up. Uniquely, we were able to estimate total cumulative viral exposure as well as current HIV-RNA values. It has previously been shown that there is a highly protective effect of immediate treatment initiation for individuals with baseline CD4 > 500 cells/mm^3, with 57% reductions in the risk of serious AIDS or non AIDS events or death (HR = 0.43 (0.30, 0.62) compared to non initiators)[71], and we specifically investigated whether or not individuals with CD4 ≥ 500 cells/mm^3 but with high VCY or HIV-RNA would benefit from cART initiation. We found a 62% reduction in the risk of serious AIDS, non-AIDS events or death among those initiating cART (compared to non-initiators) with CD4 >500 cells/mm^3 and the highest current HIV-RNA (HIV-RNA > 100,000 copies/ml [HR = 0.38 (0.19, 0.77)]). We also investigated the utility of VCY and found similar results with a benefit of cART initiation among individuals with CD4 >500 cells/mm^3 and high cumulative exposure to HIV-RNA; VCY >100,000 copy-years/ml [HR = 0.41 (0.19, 0.87)], Figure 4.1. Our results support the findings from the START[71] and TEMPRANO[72] trials and suggest that the benefit of immediate cART initiation is likely to be the greatest in those with high HIV-RNA burden – our results can be used to inform clinical guidelines on when to start cART.

[4] Patient population defined in Appendix 1, page 116-119.



*Units of measurement are as follows: CD4 cell count – cells/mm^3; Viremia copy-years – copy-years/ml; current HIV-RNA – copies/ml

Figure 4.1: The effect of initiating compared with deferring cART on time to AIDS/death by VCY and CD4 cell count modelled continuously with 3 knot splines using the CASCADE data set from Olson et al, JAIDS, 2016[126].

4.2 What boosted protease inhibitor achieves the best immunologic, virologic and clinical outcomes?

Another component of treatment optimization is identifying suitable cART regimens. More than 32 antiretroviral drugs[70] are currently available with different efficacy[127], toxicity[128, 129], genetic barriers to resistance[130], central nervous system (CNS) penetration[131], and pill burden[132]. Randomized evidence has alluded to preferred drug regimens which, optimize viral suppression with low toxicity, and a majority of national and international guidelines now recommend first line cART regimens containing an integrase strand transfer inhibitor (INSTI) alongside two nucleoside reverse transcriptase inhibitor (NRTI) backbones[133-136]. boosted protease inhibitors (bPI) are also recommended as a part of first line therapy alongside two NRTI backbones, but bPIs can cause adverse metabolic events such as dyslipidaemia. New evidence suggests that INSTIs are as effective as bPIs, but more

tolerable, resulting in fewer treatment discontinuations[137, 138]. bPIs still have utility however, especially given their high genetic barrier to resistance[130]. For this reason, they are recommended for individuals who have poor adherence or if immediate cART initiation is necessary before resistance results are available[134]. Two of the most widely used bPIs are lopinavir and atazanavir, but limited evidence exists as to which one reduces adverse clinical outcomes. The available RCT evidence comparing them only investigated viral suppression and did not focus on other important endpoints such as AIDS or death[139-141]. The aim of the second part of our study was therefore to compliment available RCTs by providing new evidence on clinical events (AIDS and death) among those initiating lopinavir or atazanavir boosted ritonavir as a part of their first line cART regimen using HIV-CAUSAL data, in which 6668 and 4301[5] individuals started lopinavir and atazanavir regimens, respectively[142]. We used logistic regression models to estimate the hazard ratio of time from treatment initiation to AIDS, AIDS or death, and virological failure within 12-months while maintaining treatment. We found evidence to support atazanavir bPIs are superior than lopinavir bPIs, with 30% and 33% reduced hazard rate of death or AIDS defining illness or death, and a 9% reduced hazard rate of virologic failure at 12-months (HR = 0.70 (0.53, 0.91); 0.67 (0.55, 0.82); 0.91 (0.84, 0.99), respectively), Figure 4.2. Our results can improve current bPI guidelines, as we have shown individuals prescribed atazanavir have reduced risk of clinical events compared to those prescribed lopinavir. Therefore, among newly diagnosed individuals without access to resistance results, or among individuals who are less adherent to medication, atazanavir might be a preferable bPI as our estimates showed lower mortality risk, lower incidence of AIDS defining illness, and lower risk of virologic failure compared to lopinavir.

[5] Patient population defined in Appendix 1, page 125-127.

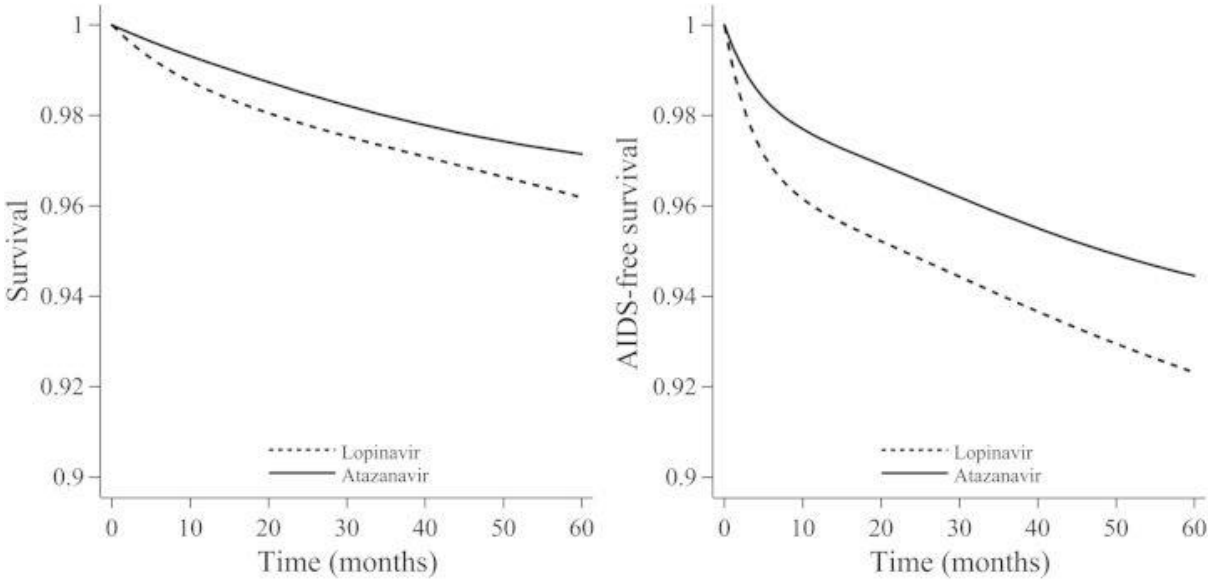


Figure 4.2: Survival (left) and AIDS-free survival (right) for atazanavir vs lopinavir, HIV-CAUSAL Collaboration, 2004–2013. The curves are standardized by the baseline covariates of sex, age, race, geographic origin, mode of acquisition, CD4 cell count, HIV RNA, calendar year, and years since HIV diagnosis from Cain et al, Clinical Infectious Diseases, 2015[142].

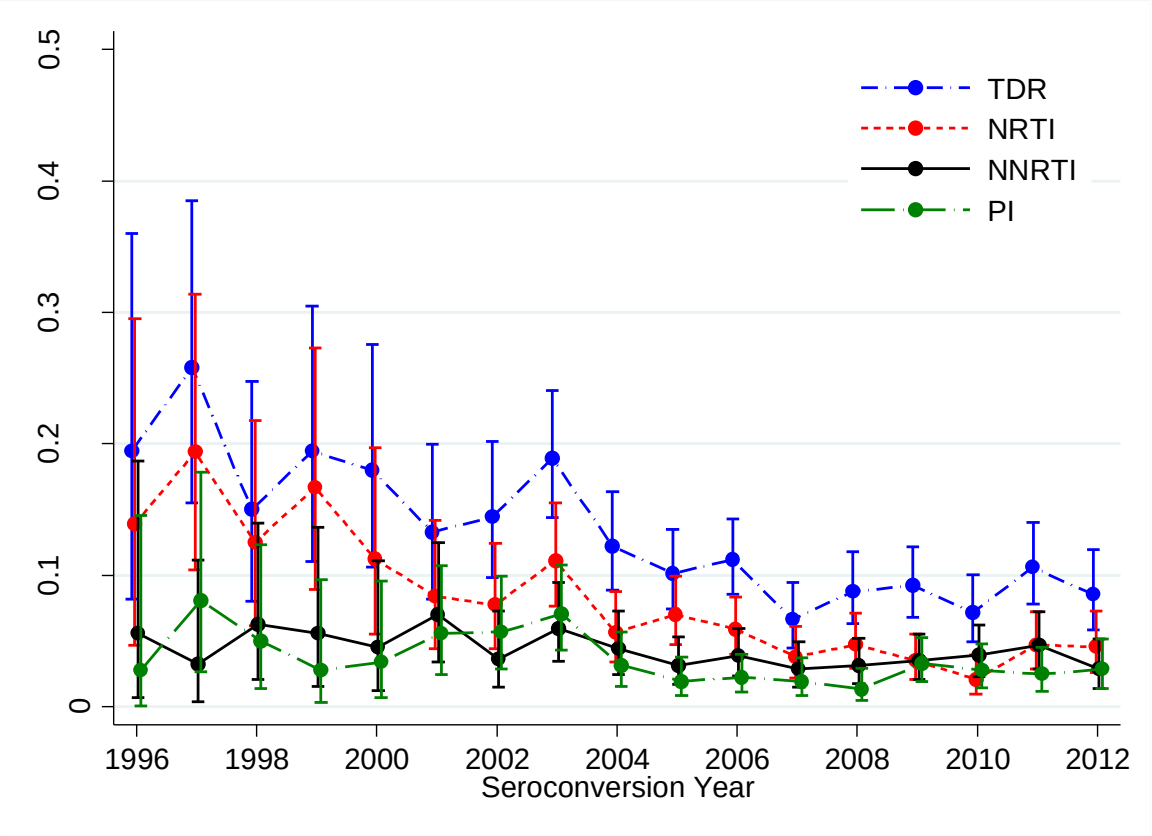
4.3 Impact of transmitted drug resistance on cART initiation in a large seroconverter cohort collaboration

Another component of cART optimization is the impact of HIV-1 drug resistance on viral suppression and its implications for available treatment choices. Poor adherence to cART can lead to the development of viral mutations[69, 143-147] which are associated with HIV drug resistance and subsequent treatment failure. Not only is cART failure linked to adverse health outcomes[148-150], but individuals failing treatment who develop HIV drug resistance can transmit resistant strains to others[121, 151-157]. First line optimal treatment regimens are limited once an individual develops drug resistance, and although second line therapy successfully suppresses HIV-RNA, it can be less tolerable and have increased toxicity risk. An important first step is to monitor transmitted drug resistance (TDR) trends so we can assess how to prevent further spread of TDR. CASCADE data on HIV-1 seroconverters

provides a unique opportunity to investigate TDR. We analysed 4717[6] individuals in CASCADE seroconverting in the cART era (1996-2012) with HIV-1 genotypic resistance data available within 12 months of testing positive for HIV-1[158]. Individuals were categorized as having a TDR associated mutation if their virus contained one or more of the mutations mentioned in the Surveillance Drug Resistance Mutations list defined by the WHO[159]. Drug susceptibility was inferred from the viral genotypic resistance data that was submitted to the Stanford HIV database algorithm. We identified mutations associated with drugs in the first line recommendations according to categories A and B of EACS guidelines[160]. Using logistic regression, we examined the association between TDR and year of HIV-1 seroconversion adjusting for confounding factors. We observed a significant decline (p-trend < 0.001) in the prevalence of TDR to any drug class during our study period 1996–2012; odds ratio (OR) 0.92 (95% CI; 0.90, 0.95) per year. Estimated TDR started at 19.4% (8.2, 36.0) in 1996 (n = 36) and fell to 8.5% (5.9, 11.9) in 2012 (n = 352), Figure 4.3. The same decreasing trend over time was observed for transmitted NRTI resistance, OR = 0.89 (0.86, 0.91) per year, non-nucleoside reverse transcriptase inhibitors (NNRTI) resistance, OR = 0.96 (0.93, 1.00) per year, and protease inhibitor resistance, OR = 0.93 (0.89, 0.97) per year. Of the individuals in our study, 296 (6.3%) had transmitted drug resistant mutations associated with high-level resistance according to the Stanford HIV database algorithm (Stanford score higher than three), and 190 (4%) individuals had a mutation associated with any drug recommended by EACS as part of first-line treatment (abacavir, lamivudine, dolutegravir, tenofovir, emtricitabine, elvitegravir, rilpivirine, raltegravir, ritonavir, darunavir, efavirenz, atazanavir, lopinavir). 2.62% of individuals had a mutation associated with high level resistance to efavirenz, markedly higher than any other drug, Figure 4.4.

[6] Patient populatin defined in Appendix 1, page 132-135.

Although the rate of transmitted drug-resistant HIV-1 has decreased since 1996, a fair proportion of newly infected individuals (8.5%) are being diagnosed with drug-resistant strains. Resistance testing remains cost effective for baseline resistance above 1%[161], therefore preforming resistance tests for newly diagnosed individuals remains justifiable.



*Abbreviations: TDR – transmitted drug resistance; NRTI - nucleoside reverse transcriptase inhibitors; NNRTI - non-nucleoside reverse transcriptase inhibitors; PI - protease inhibitors

ᵗstatistically significant decline (p<0.01 for TDR, NRTI and PI) in the prevelance of transmitted drug resistance over time using linear mixed models

Figure 4.3: Temporal trends in transmitted drug resistance over time for individuals with at least one ART naïve nucleotide sequence within one year of testing positive for HIV: CASCADE data of HIV seroconverters from Olson et al, AIDS, 2018[158].

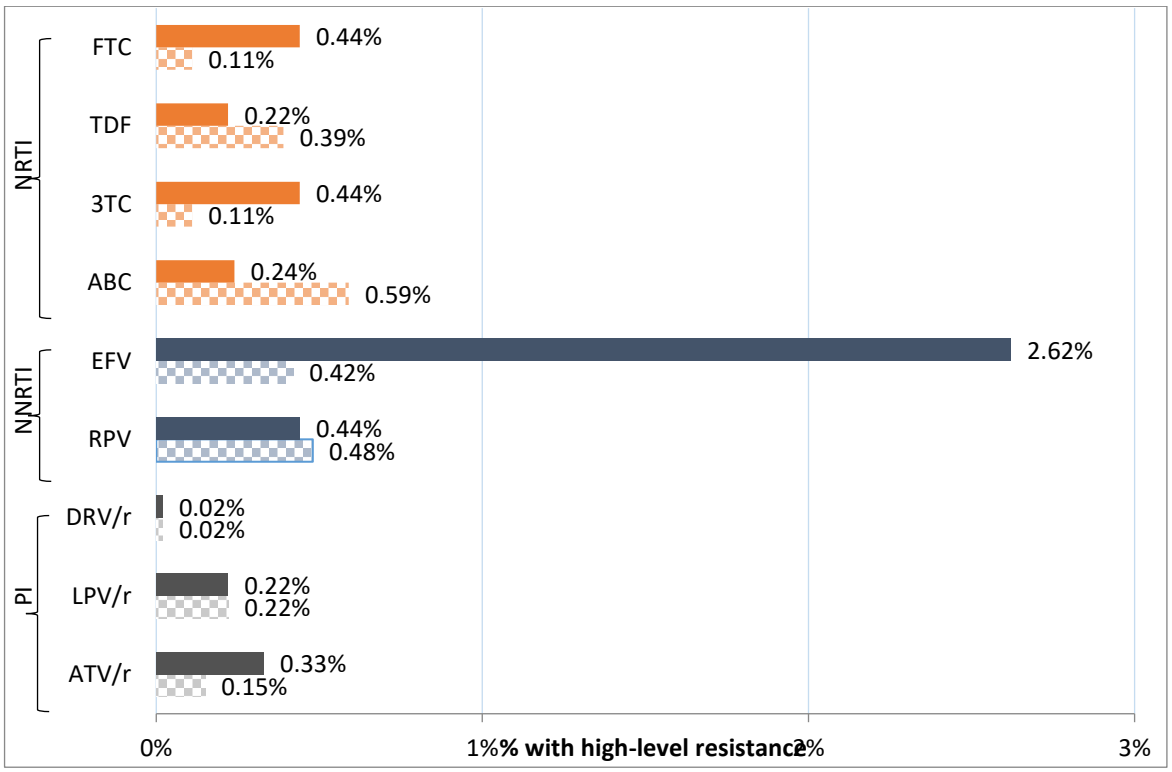


Figure 4.4: High level resistance (Stanford scores >3; solid bars indicate a score of 5, checked bars indicate a score of 4) associated with first-line antiretroviral drugs recommended by the European AIDS clinical Society for individuals with at least one cART naive nucleotide sequence within 1 year of testing positive for HIV: CASCADE data of HIV seroconverters. ABC, abacavir; ATV/r, atazanavir; DRV/r, darunavir; EVF, efavirenz; FTC, emtricitabine; LPV/r, lopinavir; NRTIs, Nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; RPV, rilpivirine; TDF, tenofovir; 3TC, lamivudine adapted from Olson et al, AIDS, 2018[158].

5 Outcomes on treatment

With the advent of cART in the late 1990's, HIV became a treatable and chronic condition that results in lifelong treatment and potential lifestyle adjustments such as regular exercise, balanced nutrition and ceasing smoking to reduce the risk of falling ill[162]. There are some rare reports of individuals who can control viral replication after stopping cART[24, 26, 27], but viral rebound and cART reinitiation at a later point in time is typical. cART is not perfect, and one size does not fit all; but recommended first line therapies are effective at suppressing viral replication. Treatment associated toxicities can develop either immediately or over time however, and this needs careful monitoring, and sometimes treatment changes due to adverse reactions, development of drug resistance, or new co-infections for example.

5.1 Post treatment control of viral replication through cART

Not only can cART reduce morbidity and mortality among HIV-1 positive individuals, but for a small proportion of individuals on cART, plasma viremia remains undetectable after cART cessation; in essence, creating HIV-1 ECs. This phenotype is termed post treatment control (PTC) and is more common among individuals initiating cART in primary HIV Infection (PHI)[163] where the viral reservoir is small[26, 164] and when individuals are less likely to have immune dysfunction compared to chronic infection[165]. Studies reporting on PTC estimate around 0-26%[23-30, 166, 167] of those interrupting treatment achieve PTC status. This is arguably the best possible outcome among those initiating treatment. Like EC's, the biological mechanism underlying PTC is not fully elucidated. We know predictors of PTC from the ANRS VISCONTI Study, the SPARTAC trial, the CHAMP study, and studies in primates, which include small viral reservoir before cART, cART initiation in PHI, and long duration of therapy before treatment interruption (e.g. PTCs were treated for a median

36.5 months in the ANRS VISCONTI study)[27, 163, 168-170]. Transient periods of detectable viremia, or "blips", while on cART initiated in PHI could play a role in predicting individuals who will not achieve PTC status, as they could indicate lack of cART adherence or lifestyle adjustments, poor drug absorbption, a larger viral reservoir, emergence of drug resistance, or intermediate immune activation. It is well documented that blips while on cART in chronic HIV infection is associated with subsequent virological failure[171-174], so perhaps this is true of blips while in cART in PHI. As treatment interruptions have been associated with increased morbidity[22, 175, 176] and should be avoided in individuals who are not likely to achieve PTC, our aim was to identify if blips among those initiating cART in PHI predict the probability of PTC status among individuals who subsequently stopped cART. Using CASCADE data on HIV-1 seroconverters, we included individuals who initiated cART within 6 months of HIV-1 seroconversion[166] [7]. Using Cox models, we examined the association between time from cART cessation to loss of viral control (two consecutive viral load measurements over 1000 copies/mL) and the magnitude and frequency of blips while on cART adjusting for other variables of interest including time on cART, time between HIV-1 seroconversion to cART initiation, viral load at seroconversion, CD4 cell count at cART initiation, CD4 cell count at cART cessation, and cART class. We also adjusted for confounders: cART initiation year, age at HIV-1 seroconversion, sex, and HIV-1 transmission risk group. Of 228 stopping treatment, only 22 (10%) achieved PTC status. We found that time to loss of viral control was associated with a longer time interval between HIV seroconversion and cART initiation and viral blips on cART HR = 1.16 per month (1.04, 1.28); 1.71 per blip (0.94, 3.10), respectively, but longer time on cART was associated with longer durations of viral control, HR = 0.84 (0.76, 0.92) per month increase, Table 5.1. We were

[7] Patient population defined in Appendix 1, page 141-143.

the first study to provide evidence that blips on cART initiated in PHI are associated with viral rebound among individuals who eventually interrupt cART[166]. These results should be reviewed in future HIV-1 cure studies where planned treatment interruptions are, by definition, necessary. Individuals who have blipped on cART should be carefully considered for treatment interruption as viral rebound is more likely once cART is stopped compared to those that have not had a viral load blip while on cART that was initiated in PHI.

Table 5.1: Multivariable analysis of the factors associated with virologic rebound among those stopping cART initiated within 6 months of HIV seroconversion using the CASCADE dataset from Fidler et al, AIDS, 2017[166].

	HR (95% CI)	p-value
Time on cART ⨍	0.84 (0.76, 0.92)	<0.001
Time from SC to cART ǂ	1.16 (1.04, 1.28)	0.006
# blips >400 copies/ml	1.71 (0.94, 3.10)	0.077
# mean HIV-RNA measurements/year	1.10 (1.02, 1.17)	0.005
HIV-RNA at SC ⨏	1.15 (0.98, 1.35)	0.086
cART initiation year	0.91 (0.84, 0.98)	0.016
Time from cART to viral suppression ⅂ ǂ	0.99 (0.97, 1.02)	0.93
CD4 at cART initiation Ω	0.99 (0.90, 1.08)	0.75
CD4 at cART cessation Ω	1.10 (1.01, 1.20)	0.035
ART class		0.33
NNRTI	1	
PI	0.92 (0.57, 1.48)	
3 N	1.32 (0.74, 2.36)	
3 Class	0.24 (0.03, 1.79)	
Integrase Inhibitor	0.77 (0.10, 6.12)	
SC age	1.00 (0.98, 1.02)	0.77
Sex		0.49
Male	1	
Female	0.75 (0.33, 1.69)	
HIV Risk Group		0.28
MSM	1	
MSW	0.84 (0.44,1.58)	
IDU	0.53 (0.14, 2.03)	
OTH	0.23 (0.03, 1.73)	

⨍ Per 6 month increase; ǂ Per month increase; ⨏Per log_{10} increase; Ω: per 100 cell increase; ⅂ HIV-RNA < 50 copies/ml
Abbreviations are: cART – combination antiretroviral therapy; SC – seroconversion; MSM – men who have sex with men; MSW – sex between men and women; IDU – injection drug use; OTH – other; NNRTI – non-nucleoside reverse-transcriptase inhibitors; PI – protease inhibitor; 3N – three nucleoside reverse transcriptase inhibitors ; 3 class – drugs from 3 or more classes.

For the vast majority of HIV positive individuals, cART is not a functional cure, likely due to an inaccessible reservoir of latently infected cells[20-22], a treatment interruption would therefore result in viral rebound. Numerous treatment interruption studies have been conducted[177], the landmark SMART trial was the largest, randomizing 5472 individuals to continuous cART or episodic CD4 guided cART, stopping treatment when CD4 surpassed 350 cells/mm^3. This trial was stopped early, as it showed a 2.6-fold increased risk of an opportunistic infection or death among those not reveicing continuous cART[22]. Therefore, guidelines do not recommend treatment interruptions as it is associated with increased morbidity[22, 175, 176]. The previous chapter highlighted randomized controlled trials (START[71] and TEMPRANO[72]) coupled with our observational evidence to suggest immediate cART reduces morbidity and mortality rates. Another benefit of cART is its role in HIV prevention. Several studies have confirmed that there is little to no risk of HIV transmission among individuals with undetectable viral load[121, 151-156, 178], and it has also been shown that pre-exposure prophylaxis (PrEP) in HIV negative individuals with tenofovir-emtricitabine (Truvada®) reduces the risk of HIV infection by up to 90%[179] . This implies that time spent off cART among HIV positive individuals or undertaking treatment interruptions is non-advantageous as it can result in transmission. The key to reducing morbidity and mortality risk, and to reduce transmission of HIV to others, is timely cART initiation. However, cART does not come without disadvantages, for instance, there remains an ongoing risk of drug toxicity among individuals on cART, as well as pill burden can contribute to lack of adherence, causing viral breakthrough which can lead to the development of resistance associated mutations (RAMs).

Individuals therefore need to be monitored once treatment is commenced. Infrequent monitoring could lead to delays in detecting treatment toxicity, viral breakthrough, AIDS or

other opportunistic infections, and delay timely cART re-configuration. On the other hand, frequent monitoring causes financial pressures on health care systems and can be unrealistic in low income countries such as sub-Saharan Africa where most HIV positive people reside. One of my collaborations with HIV-CAUSAL added to the body of evidence on when to monitor and suggested that less frequent monitoring (9-12 months compared to every 3 months) has little impact on clinical outcomes at 18 months among individuals on cART achieving undetectable HIV-1 RNA viral load, however, monitoring every 9-12 months increases the risk of virologic failure compared with monitoring every three months[180].

5.2 cART associated Toxicities

With lifelong treatment being the standard of care for HIV positive individuals, there is an ongoing need to monitor immediate or accumulated toxicity, where people rapidly progress to disease or, in more serious cases, die. Reflecting on the recent upscale of cART and increasing use of antiretrovirals to prevent HIV transmission, it is ever more important to monitor toxicities, especially those that could result in long term disability or death and vary depending on treatment type. Three toxicities we investigated were hypersensitivity reactions due to abacavir (ABC) utilization, AIDS-defining neurological conditions related to cART penetrating the blood brain barrier, and immune reconstitution inflammatory syndrome (IRIS) shortly after cART initiation.

5.2.1 Abacavir associated hypersensitivity reactions

The first toxicity investigated was hypersensitivity which can occur with abacavir utilization. ABC is a nucleoside reverse transcriptase inhibitor which remains a commonly used drug recommended as a part of first- and second-line therapy[133, 181]. In the absence of genetic screening, 5-8% of HIV positive individuals who initiate ABC experience hypersensitivity

reactions (HSR)[182, 183], a multi-organ clinical syndrome which varies in severity[184] and in rare cases, results in death[185, 186]. There is an increased risk of ABC related HSR among those who test positive for the HLA-B*5701 allele, and it is recommended that all individuals initiating ABC be screened for this allele to prevent avoidable complications[181]. It is therefore important that ABC-associated HSR are continually monitored and additional associated risk factors are investigated. Using EuroSIDA data on 10,076[8] HIV-1 positive individuals receiving cART between 2009 and 2016, we calculated the proportion of individuals receiving cART containing ABC and the incidence rate of HSR reactions among this group[187]. Poisson regression was used to identify factors associated with ABC discontinuation due to HSR. During our study period, 34% received ABC cART and 113 discontinued within 6 weeks of ABC initiation, 13 because of reported HSR (incidence rate (IR) = 1.67 (0.97, 2.87) per 100-person years follow-up), Table 5.2. We found no significant factors associated with ABC discontinuation due to reported HSR or any toxicity. These results are particularly important for those who initiate ABC as second line therapy as drug options become scarce. Some individuals switch to ABC because they have become resistant to other drugs, or switch to ABC from tenofovir due to decreasing renal function[188], as ABC has no reported adverse effects on renal function[189, 190].

[8] Patient population defined in Appendix 1, page 149-151.

Table 5.2: Reasons and incidence rates for ABC discontinuation by reason for stopping treatment in the EuroSIDA cohort from 1/1/2009 to 4/1/2016. Individuals were censored at 6 weeks after ABC initiation, ABC discontinuation or death, whichever came first from Roen et al HIV Medicine, 2017[187].

Reason for stopping treatment as reported to EuroSIDA	Failures	Rate	95% CI
Any Reason	113	14.51	(12.07, 17.45)
HSR or any toxicity	35	4.49	(3.23, 6.26)
Any toxicity	22	2.82	(1.86, 4.29)
Unknown	21	2.70	(1.76, 4.14)
Patient's wish/decision	20	2.57	(1.66, 3.98)
Other causes	17	2.18	(1.36, 3.51)
Physician's decision	16	2.05	(1.26, 3.35)
Toxicity – GI tract	16	2.05	(1.26, 3.35)
HSR	13	1.67	(0.97, 2.87)
Toxicity – Liver	2	0.26	(0.06, 1.03)
Toxicity, predominantly CNS	2	0.26	(0.06, 1.03)
Toxicity, predominantly kidneys	2	0.26	(0.06, 1.03)
Treatment Failure	1	0.13	(0.02, 0.91)
Concern of cardiovascular disease, including dyslipidaemia	1	0.13	(0.02, 0.91)
Other Toxicity	1	0.13	(0.02, 0.91)
Non-compliance	1	0.13	(0.02, 0.91)

*Total person years follow-up = 778; Rate = per 100 person years

5.2.2 cART utilization and neurological AIDS

The second toxicity investigated is not a consequence of one particular drug, but rather how cART as a whole impacts neurological AIDS defining illness. Neurological AIDS defining conditions have decreased since the advent of cART in the late 1990's, but cART related neurotoxicity remains a concern. This depends on the concentration of drugs in the CNS which requires drug penetration through the blood brain barrier, a function not provided by all drugs. Higher concentrations of cART in the CNS likely decreases HIV-1 RNA in cerebrospinal fluid[191], but could also be neurotoxic[192]. The CNS Penetration Effectiveness (CPE) ranking system assesses a drugs penetration into the CNS and has been shown to be associated with HIV-1 RNA detected in cerebrospinal fluid[191, 193-195], but it is unclear how CPE rank relates to clinical outcomes[194, 196-199]. Our aim was to examine the association between CPE scores and four neuroAIDS conditions, namely, HIV dementia, toxoplasmosis,

cryptococcal meningitis, or progressive multifocal leukoencephalopathy. Using HIV-CAUSAL data on 61,938[9] individuals starting cART, we classified individuals into low, medium or high CPE scores and estimated the hazard ratio for each neuroAIDS condition using the low CPE score as the reference group[200]. We found a significant association between high CPE score and HIV dementia, HR = 1.01 (0.73, 1.39) and 1.74 (1.15, 2.65) for medium and high CPE scores compared to low CPE scores, respectively,Table 5.3. We found no evidence for an association between CPE scores and toxoplasmosis, HR = 0.80 (0.56, 1.15) and 0.90 (0.50, 1.62), cryptococcal meningitis, HR = 1.08 (0.73, 1.62) and 1.13 (0.61, 2.11) or progressive multifocal leukoencephalopathy, HR = 1.08 (0.73, 1.58) and 1.32 (0.71, 2.47).

[9] Patient population defined in Appendix 1, page 158-161.

Table 5.3: Hazard ratios for CPE score by neuroAIDS condition in the HIV-CAUSAL Collaboration dataset 1998–2013 from Caniglia et al, Neurology, 2014[200].

CPE Score	Person years	No. of events	Unadjusted hazard ratio	95% CI	Adjusted hazard ratio[a]	95% CI
HIV Dementia						
Low	140,962	127	1	--	1	--
Medium	86,799	72	0.97	(0.72,1.30)	1.01	(0.73, 1.39)
High	32,097	36	1.55	(1.06, 2.26)	1.74	(1.15, 2.65)
Opportunistic Infections[b]						
Low	140,553	245	1	--	1	--
Medium	86,455	134	1.09	(0.88, 1.34)	0.99	(0.80, 1.22)
High	31,985	49	1.18	(0.87, 1.62)	1.08	(0.77, 1.52)
Toxoplasmosis						
Low	140,983	106	1	--	1	--
Medium	86,807	45	0.86	(0.60, 1.22)	0.80	(0.56, 1.15)
High	32,099	18	0.94	(0.57, 1.57)	0.90	(0.50, 1.62)
Cryptococcal meningitis						
Low	141,098	64	1	--	1	--
Medium	86,818	48	1.35	(0.92, 1.98)	1.08	(0.73, 1.62)
High	32,121	16	1.43	(0.83, 2.48)	1.13	(0.61, 2.11)
Progressive multifocal leukoencephalopathy						
Low	141,109	81	1	--	1	--
Medium	86,849	43	1.12	(0.77, 1.64)	1.08	(0.73, 1.58)
High	32,116	17	1.36	(0.80, 2.33)	1.32	(0.71, 2.47)

Abbreviations: CI - confidence interval; CPE - CNS Penetration Effectiveness.

[a] Adjusted for cohort, month of follow-up, baseline CD4 cell count, baseline HIV RNA level, sex, acquisition group, calendar year, age, geographic origin, race, years since HIV infection, and type of drug regimen, as well as time-varying CD4 cell count, RNA level, time since last measurement, and AIDS. Stabilized inverse probability weights were used to account for censoring due to infrequent follow-up.

[b] Includes toxoplasmosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy.

5.2.3 Opportunistic infections and AIDS malignancies shortly after initiating cART

The last complication of cART we investigated also is a consequence of introducing multiple drugs, where some patients initiating cART experience inflammatory reactions shortly after treatment initiation, termed IRIS[201-203]. IRIS can trigger new opportunistic infections, worsen existing opportunistic infections and may be associated with excess morbidity[204]. It has also been linked to a multitude of conditions such as mycobacterial and other infections[203, 205-212], cancer[213, 214], rheumatoid arthritis[215], and sarcoidosis[216]. The HIV-CAUSAL collaboration reported on increased incidence of tuberculosis by 36% shortly after cART initiation among older individuals or those with CD4 cell counts < 50 cells/mm^3 [212], which is suggestive of IRIS. Our intent was to extend this study to include other AIDS

defining events suggested to be associated with IRIS, namely, tuberculosis, mycobacterium avium complex (MAC), cytomegalovirus (CMV) retinitis, progressive multifocal leukoencephalopathy (PML), herpes simplex virus (HSV2), KS, non-Hodgkin lymphoma (NHL), cryptococcosis, and candidiasis[217]. Cox proportional hazards models were used to estimate the hazard ratios for time to AIDS defining event for no cART versus cART for less than 3 months and cART for 3 or more months, adjusting for potential confounders of CD4 cell count, HIV-RNA, sex, HIV transmission risk group, calendar year, geographical origin, time since HIV-1 diagnosis, and cohort. There were 96,562[10] eligible individuals for this study and the incidence rate for each AIDS defining condition was 2.3 for tuberculosis, 0.4 for MAC, 0.3 for CMV retinitis, 0.3 for PML, 1.6 for HSV, 1.9 for KS, 1.2 for NHL, 0.4 for cryptococcosis, and 1.4 for candidiasis. Compared with no cART, Hazard ratio for each AIDS defining for cART < 3 months was 1.21 (0.90–1.63) for tuberculosis, 2.61 (1.05–6.49) for MAC, 1.17 (0.34–4.08) for CMV retinitis, 1.18 (0.62–2.26) for PML, 1.21 (0.83–1.75) for HSV, 1.18 (0.87–1.58) for KS, 1.56 (0.82–2.95) for NHL, 1.11 (0.56–2.18) for cryptococcosis and 0.77 (0.40–1.49) for candidiasis, Table 5.4. Compared to no cART, there was a reduced risk in all AIDS defining events among individuals on cART for more than three months.

[10] Patient population defined in Appendix 1, page 166-169.

Table 5.4: Hazard ratios of AIDS-defining events by time since initiation of combined antiretroviral therapy, HIV-CAUSAL Collaboration 1996–2013 from Lodi et al, AIDS, 2014[217].

Event	Time since cART initiation	N cases	Person-years	Incidence rates per 1000 person years	Hazard Ratio (95% CI)
Tuberculosis	No cART	422	143523.33	2.9	1
	<3 months	97	9259	10.5	1.21 (0.90–1.63)
	≥3 months	379	236095.92	1.6	0.36 (0.26–0.49)
Mycobacterium Avium Complex	No cART	46	143936.5	0.3	1
	<3 months	37	9306.83	4	2.61 (1.05–6.49)
	≥3 months	80	238799.67	0.3	0.31 (0.16–0.59)
CMV Retinitis	No cART	35	143938.83	0.2	1
	<3 months	12	9308.42	1.3	1.17 (0.34,4.08)
	≥3 months	58	238917.67	0.2	0.13 (0.04–0.39)
PML	No cART	38	143944	0.3	1
	<3 months	19	9307.42	2	1.18 (0.62–2.26)
	≥3 months	56	238960.75	0.2	0.21 (0.06–0.71)
Herpes Simplex Virus	No cART	254	143476.42	1.8	1
	<3 months	42	9282	4.5	1.21 (0.83–1.75)
	≥3 months	324	236713.5	1.4	0.69 (0.51–0.92)
KS	No cART	404	143755.17	2.8	1
	<3 months	95	9250.67	10.3	1.18 (0.87–1.58)
	≥3 months	249	236065.5	1.1	0.14 (0.10–0.21)
Non Hodgkin Lymphoma	No cART	198	143875.92	1.4	1
	<3 months	38	9288.17	4.1	1.56 (0.82–2.95)
	≥3 months	252	237871.5	1.1	0.40 (0.27–0.58)
Cryptococcosis	No cART	60	143924.67	0.4	1
	<3 months	21	9305.67	2.3	1.11 (0.56,2.18)
	≥3 months	58	238860.92	0.2	0.06 (0.02–0.19)
Candidiasis	No cART	275	143745.33	1.9	1
	<3 months	36	9275.67	3.9	0.77 (0.40–1.49)
	≥3 months	224	237213.75	0.9	0.13 (0.09–0.20)

cART Combined antiretroviral therapy; CMV cytomegalovirus; KS Kaposi's Sarcoma; MAC Mycobacterium avium complex; PML Progressive multifocal leukoencephalopathy.

cART has greatly reduced morbidity and mortality for HIV positive individuals, and reduced transmission risk to others rationalising current guidelines for immediate treatment of all HIV positive individuals[136]. There are, however, treatment associated toxicities which need clinical monitoring, and in some instances require adjustments in medication. cART is a lifelong commitment, and any deviations in treatment adherence can lead to the

emergence of mutations that are associated with HIV drug resistance. Although cART has greatly improved the prognosis and quality of life of HIV positive individuals, adverse outcomes while on treatment persist and there is a greater need for careful monitoring and continued research as life expectancy increases.

6 Discussion

6.1 Main findings of the PhD

We identified substantial heterogeneity in how extreme phenotypes of HIV-1 are defined in the literature which could lead to difficulties identifying the biological mechanisms underlying these phenotypes. We propose standardized definitions which can be used for future research of these rare groups[118]. Once standardized definitions are adopted, the same biological and clinical phenotpyes will be studied allowing for better interpretation across studies. This is particularily important for HIV-1 elite controllers who can be used as a model for cure.

Reflective to the very small proportion of individuals who can control viral replication (we estimate <1%[97]), our focus moved to determine 'when' and 'what' cART regimens should be initiated. We added to the existing randomized[71, 72] and observational evidence[73] suggesting immediate cART for all HIV-1 positive individuals, irrespective of CD4-T cell counts. We demonstrated a 62% reduced risk of serious AIDS, non AIDS events or death for those immediately initiating cART (vs not immediately initiating) among those with high CD4 cell counts (>500 cells/mm^3) and high HIV-RNA (>100,000 copies/ml)[126]. Despite the move towards immediate cART[119, 133, 134, 136], some individuals still chose not to initiate treatment. Our estimates can inform when individuals off cART should initiate (e.g individuals with high HIV-RNA values >100,000 copies/ml and high CD4 cell counts > 500 cells/mm^3 could benefit from cART) and our study design compares that with the challenges of initiating lifelong therapy such as adherence and adverse side effects.

Beyond the 'when to start' question, we also contributed towards the 'what cART regimen to start' question. We demonstrated that if one is to initiate a bPI, atazanavir might be

preferable compared to lopinavir as it has a 30% lower mortality risk, 33% lower incidence of AIDS and death and 9% lower risk of virological failure compared to lopinavir[142]. Our estimates can help to optimize bPIs as a part of first line therapy by showing improved survival, reducing virologic failure, and preventing rapid disease preogression among those taking atazanivir compared to lopinavir. Of course, cART is personal, and some individuals may not want to take atazanavir, for example due to drug drug interactions, but our extimates can serve as a guide for clinicians to recommend the best line of care. We also found both atazanavir and lopinavir had low levels of TDR in Europe (0.33% and 0.22%, respectively), but the most common transmitted mutation associated with high level resistance was to the drug efavirenz (2.62%)[158]. Our results show TDR is significantly decreasing throughout Europe, yet it still prevalent (8.5% in 2012[158]) and genetic testing for resistant strains among newly diagnosed remains cost-effective and justifiable[161].

Most individuals who start treatment will need to continue throughout their lifetime which can results in toxicities that need continual monitoring. However, very early cART initiation can potentially lower the viral reservoir to levels found among ECs[218, 219], with some reports of post treatment control upon stopping cART[23-30]. In this study, we found that individuals with viral blips on cART initiated in PHI had shorter time to viral rebound upon stopping treatment[166], implying there should be caution about stopping treatment among those experiencing blips during early cART. We also found a longer duration on cART initiated in PHI is associated with a greater chance of PCT[166], suggesting this could be a pathway to achieving cure. Among the majority of HIV-1 positive individuals who do not become PTC and initiate life-long cART, a multitude of treatment associated-toxicities have been reported. We investigated three in this PhD: hypersensitivity reactions due to ABC utilization, AIDS-defining neurological conditions related to cART penetrating the blood

brain barrier, and immune reconstitution inflammatory syndrome (IRIS) shortly after cART initiation. We found that hypersensitivity due to abacavir utilization is low (IR = 1.67 (0.97, 2.87) per 100 person years follow-up[187]), cART therapies with a high CNS penetration score increase the risk of HIV dementia but not other neurological AIDS conditions[200], and apart from mycobacterial infections, unmasking IRIS does not appear to be a complication of cART in high-income countries[217].

cART has greatly improved the quality and longevity of life for those diagnosed with HIV-1. With 32 FDA approved HIV drugs on the market however, Figure 1.5, personal optimization of cART is essential. Some of the major influences for an individual's cART regimens include drug tolerability, absorbtion and interactions alongside consideration of adherence and pill burden issues. cART is complex and can take time to get the right combination for a given person; it is also a life-long commitment which requires good adherence. Suboptimal cART adherence rates less than 95%[69], can result in viral breakthrough which can lead to the development of drug resistance and the possibility of transmitting resistant strains of HIV-1 to others. Not only does TDR result in limited treatment options for newly diagnosed individuals acquiring TDR strains, resistance testing is expensive to implement ($30-$400/test)[161, 220], especially in resource-limited settings where 25 times more new infections are reported each year compared to Western Europe[221]. Some suggest reducing pill burden increases adherence and thus reducing viral breakthrough, but arguably the best solution would be to find a functional cure without the need for ongoing treatment and its multitude of side effects. Given that natural control of viral replication is rare (<1%), the next best thing would be to make individuals become ECs through therapy or other means. The two known functional cures of HIV, namely the Berlin and the London patient, were achieved through stem cell transplants – a very risky and life-threatening procedure

which was appropriate in these cases only due to their secondary cancer diagnoses. These treatments are not scalable solutions. Our research contributes towards understanding the mechanisms of viral control as well as tailoring therapy and monitoring disease to improve treatment outcomes with the end goal of promoting the EC and PTC phenotypes. Our research findings can be applied to resource poor areas such as sub-Saharan Africa, the epicentre of the HIV-1/AIDS pandemic.

6.2 Limitations

There are some limitations that should be noted.

6.2.1 Cohort data limitations

Cohort data was used for these analyses which comes from clinical practice, where individuals undergoing regular patient care and health outcomes were observed. Extrapolation of results to less routinely controlled patients therefore may not be practical. Collaborations used were uniquely large, achieved through pooling data throughout Europe, North America, and Australia using HICDEP data formatting. It is important to note these data are more heterogeneous than in randomized clinical trials where strict study visit timelines and standardized data collection and analytic processes.

6.2.2 Variability in laboratory measurements

Details of laboratory data collection is cohort specific and not always consistent across cohorts and over time. Laboratory measurements of HIV-RNA measurements, CD4 cell counts, HIV sequencing, and other virology assessments, were performed in the country of care using a variety of in house and commercial methods and assays. The data on assays used were requested to be documented in each LAB file, where available, and our models tried to account for the varying techniques used.

6.2.2.1 Variability of HIV-RNA viral load

There are a number of HIV-RNA assays that have been available throughout our study period, with current standard HIV-RNA assays show good agreement in viral load measurements[222-225]; yet not to the same extent at the lower limit of detection (20, or 40 copies/ml)[222, 226], which could lead to misclassification of EC and LTNP. In addition, the lower limits of detection for HIV-RNA have dramatically changed over the study period, starting at 1000 copies/ml when the assays first became available, and are now as low as 20 copies/ml. This makes comparing temporal trends of viral suppression challenging, which too has particular importance for HIV-1 ECs and LTNPs, whose suppressed HIV-RNA values, by definition, are essential to their categorization. We have tried to account for this by looking at different thresholds for undetectable HIV-RNA in our search for an appropriate definition. Given that the lower limit for available assays has been less than 1000 copies/ml for at least 10 years justifies our suggested definitions.

6.2.2.2 Variability of CD4 cell counts

Like HIV-RNA assays, the numerous methods of analysing CD4 cell counts are variable. This could lead to RP misclassification and, until 2015, impact how individuals were assessed for cART eligibility. Until 2000, the recommended methods for assessing total CD4 T-cell count involved multiple platforms (hematology instruments and a flow cytometry) and three independently derived values, namely whole blood cell count, percent of lymphocytes and percentage of CD4 cells. This method was highly variable as measurement error for each step was compounded when deriving the total CD4 cell counts. In the late 1990's, a single platform approach was introduced which allowed CD4 cell counts to be derived directly from the flow cytometric analysis, removing the multiplicative effect of errors that could occur when using dual platforms[227]. It has been shown that the differences between dual and single platform analysis is relatively small, 8%[228], and the variation is smaller as CD4 cell counts decrease, with the bias at CD4 < 350 cells/mm^3 ranging from -35.2 to +13.1

cells/mm^3 and bias at CD4 > 350 cells/mm^3 ranging from -70.7 to +47 cells/mm^3 [229]. Even though the CD4 cell counts were analysed in multiple laboratories utilizing different methods, the errors between methods are relatively small and likely have a negligible impact on our findings throughout our study period.

6.3 Future work: expanding our study beyond the predominately HIV-1 B subtype

Data used for this PhD was predominately from Europe, the Americas and Australia where a majority of the population is male with HIV-1 subtype B infections. Globally, subtype B only accounts for around 10% of HIV-1 infections[40], yet is the most studied HIV-1 subtype[230] because it is the most common in the richest countries of the world where much of the research funding and drug development is based[41, 42]. There are reports of different virulence and disease progression rates between subtypes[231-234] which would imply extreme phenotypes also vary between HIV-1 subtypes. For example, a recent study in African women with subtype C infection (globally the most dominant strain of HIV-1) by Garrett et al. presented at the 2019 Conference on Retroviruses and Opportunistic Infections (CROI) found a higher proportion (35%) of individuals with a CD4 cell count <350 cells/mm^3 within the first year of infection[235] which is slightly higher than our mainly subtype B setting with an estimated 25% experiencing a CD4 <350 cells/mm^3 within one year[118]. It has also been shown that subtype C has slower progression than subtype A or D[232], the latter of which has the fastest rates of progression[84, 231, 233, 234]. This heterogeneity in progression rates among other subtypes, suggests that RP varies among HIV-1 subtype and could be worse among non-subtype B populations prompting further research in defining extreme phenotypes among individual subtypes and in resource-limited settings. There is some work looking at predictors of RP in subtype C, but this does not assess the merit of definitions, rather using what appears to be definitions created for subtype B[85].

This further promotes the need of immediate cART initiation, even in resource-limited settings, where the majority of the HIV-1 pandemic is located and non-B subtypes prevail.

In addition to varying extreme phenotypes among subtypes, it is possible there are differences in response to cART and toxicity development between subtypes. Many studies analysing virologic response to cART have found no significant differences when comparing subtype B to all other subtypes[236-241], however, all non-B subtypes tend to be grouped together. There are a handful of studies comparing specific subtypes; Geretti et al showed faster viral suppression in subtype A and C compared to B (HR = 1.35 (1.04, 1.74) and 1.16 (1.01, 1.33), respectively) [242]; Easterbrook et al showed a higher virological rebound (HIV-RNA > 400 copies/ml at 6 months 70% vs 45%) after cART cessation for subtype D compared to B and for subtype A compared to B (35% vs 45%)[84]. However, Paraskevis et al found individuals with subtype A had improved virological response compared to subtype B (HR = 1.35 (1.08, 1.68))[243]; and Touloumi et al found little differences in virologic and immunologic response to cART between subtypes, but subtype CRF01_AE and A achieving HIV-RNA <500 copies/ml earlier than subtype B (HR = 1.37 (1.01, 1.86) and 1.29 (0.96, 1.72), respectively) [244]. These studies all had small sample sizes for non-B subtypes and yet they highlight that there are subtle differences in response to treatment. In the 'test and treat' era, more research is needed comparing the efficacy in viral suppression, sustained viral suppression, viral rebound, and CD4 T-cell responses between all subtypes and specific drugs.

6.4 Importance of cohort studies in research

Although there are limitations with cohort data, HIV-1 cohorts play an important role in understanding HIV-1 pathogenesis and disease progression. Cohort studies take over answering questions where clinical Phase III trials stop by informing on the pragmatic

effectiveness of interventions at a population level. They also enable evaluation of long-term outcomes, or rare events and toxicities that are beyond the scope of RCTs. For example, we have investigated the utilization of ABC and the incidence of HSR, a very rare outcome that requires large populations with longitudinal structured data to monitor. Without cohorts like EuroSIDA, hypersensitivity reactions among those prescribed ABC in Europe would be difficult to monitor. Cohorts are also uniquely able to monitor the trends of the HIV-1 epidemic, for example, as we have done by looking at the temporal trends of TDR in Europe.

Finally, cohorts help to identify and target future problems as HIV treatment and clinical care evolves. For example, a newly identified side effect of Integrase strand transfer inhibitors (INSTIs) is a significant amount of weight gain, and cohorts have been at the forefront of identifying this problem[245-247]. The infrastructure of cohorts allows us to identify emerging problems, quantify the problem and assess interventions.

6.5 Final Remarks

We have suggested definitions for extreme phenotpyes of HIV-1 in a predominately subtype B cohort which can be used in future research aiming to understand the biological mechanisms of HIV-1 elite control and rapid progression. We also have contributed to the 'when to start' and 'what to start' questions among individuals with HIV-1 and contributed towards understanding toxicities developed after cART initiation, Table 6.1.

Table 6.1: Main Findings of the PhD

Chapter	Question	Key Findings
10	• What is the heterogenity in extreme phenotypes in published literature? • What are the best definitions for EC and RP moving forward?	• Substantial heterogeneity in slow progressor definitions, 600 definitions, 26 terms used. EC and LTNP were most common terms used in literature • Substantial heterogeneity in fast progressor definitions, 114 definitions, 8 terms used, RP most common term used in literature • Consecutive undetectable HIV-RNA measurements (HIV-RNA < 50 or 75 copies/m) for at least six months were mose sensitive in classifying individuals with the slowest disease progression, lowest HIV-RNA and highest CD4 cell counts during EC periods and total cART naïve follow-up • Individuals with at least one CD4 cell count <100 cells/mm3 identified a rare group (2.8%) at the highest risk of disease progression
11	• What is the utility of HIV-RNA and HIV-RNA copy-years in deciding when to start cART? • What first line bPI acheives the best immunologic, virologic and clinical outcomes? • What are the TDR trends in Europe and how does this impacts treatment options?	• 62% reduction in the risk of serious AIDS, non-AIDS events or death among those initiating cART (compared to non-initiators) with CD4 >500 cells/mm^3 and the highest current HIV-RNA (HIV-RNA > 100,000 copies/ml [HR = 0.38 (0.19, 0.77)]) • Atazanavir is superior to lopinavir with 30% and 33% reduced HR of death and AIDS or death, respectively, and 9% reduction in the HR of virologic failure at 12-months • TDR is decreasing in Europe (19.4% in 1996 reduced to 8.5% in 2012), but resistance testing among newly diagnosed remains justifiable
12	• Can we create PTCs and do viral blips on cART predict PTC? • Are HSR common among those utilizing ABC? • Are cART regimens with high CPE scores neurotoxic? • Do individuals experience IRIS after cART	• PTC is rare: 22/228 (10%) stopping cART acheived PTC status. Blips and longer time interval between SC and cART were associated with loss of viral control HR = 1.16 per month (1.04, 1.28); 1.71 per blip (0.94, 3.10), respectively • A longer time on cART was associated with a longer time to loss of viral control HR = 0.84 (0.76, 0.92) • HSR among ABC utilizers is rare (IR = 1.67 (0.97, 2.87) per 100 person-years follow-up) • cART regimens with high CPE scores are associated with HIV dementia HR = 1.74 (1.15, 2.65), but not other neurologicalAIDS conditions • Apart from MAC, HR = 2.61 (1.05, 6.49), unmasking IRIS does not appear to be a complication of cART in high income settings

Abbreviations are as follows: EC – elite controllers; LRNP – long term non progressors RP – rapid progressors; bPI – boosted protease inhibitor; TDR – transmitted drug resistance; PTC – post treatment controller; cART – combination antiretroviral therapy; HSR – hypersensitivity reaction; ABC – abacavir; CPE clinical penetrative effectiveness; IRIS – immune reconstitution inflammatory syndrome; MAC - Mycobacterium avium complex

Appendix 1 – published manuscripts

EDITORIAL REVIEW

A systematic review of definitions of extreme phenotypes of HIV control and progression

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The study of individuals at opposite ends of the HIV clinical spectrum can provide invaluable insights into HIV biology. Heterogeneity in criteria used to define these individuals can introduce inconsistencies in results from research and make it difficult to identify biological mechanisms underlying these phenotypes. In this systematic review, we formally quantified the heterogeneity in definitions used for terms referring to extreme phenotypes in the literature, and identified common definitions and components used to describe these phenotypes. We assessed 714 definitions of HIV extreme phenotypes in 501 eligible studies published between 1 January 2000 and 15 March 2012, and identified substantial variation among these. This heterogeneity in definitions may represent important differences in biological endophenotypes and clinical progression profiles of individuals selected by these, suggesting the need for harmonized definitions. In this context, we were able to identify common components in existing definitions that may provide a framework for developing consensus definitions for these phenotypes in HIV infection.

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Keywords: definitions, elite controllers, extreme-trait designs, HIV, HIV controllers, long-term nonprogressors, phenotypes, slow progressors, systematic review, viremic controllers

Introduction

Individuals with HIV infection show variable rates of disease progression and viral control. Whereas some subgroups of individuals control infection very well and remain asymptomatic for several years, others show rapid immunological and clinical progression. A number of terms have been used to describe individuals at these extremes of the clinical spectrum, including 'long-term nonprogressors' (LTNPs) [1–5], 'elite controllers' [6,7],

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'slow progressors' [8–10], 'HIV controllers' (HICs) [11], 'viremic controllers' [1], 'noncontrollers' [12], and 'rapid progressors' [13–15]. These terms represent extremes within the virological and clinico-immunological range of disease, with LTNPs and rapid progressors lying on opposite extremes of the clinico-immunological distribution, and elite controllers and noncontrollers lying on opposite ends of the spectrum of viral control. The study of these individuals has provided valuable insights into the biology and pathogenesis of disease control and progression [5,16,17]. Indeed, elite controllers have been regarded as a natural model for disease control, and understanding the underlying biological mechanisms of this phenomenon could provide novel therapeutic targets [17,18].

Although these groups have been the focus of intense study, there is no consistency in how they have been defined. Studies suggest that different definitions may select for groups with varying clinical outcomes, and represent different biological endophenotypes [1,14,19]. This variability in definitions also has important implications for the design of future biological research in HIV and for the interpretation of results from existing literature. Recommendations for consensus definitions are needed. To examine variability in these definitions, we conducted a systematic review of the literature. Here, we describe heterogeneity in the definitions used, and identify common definitions that may provide a framework for developing consensus definitions for extreme HIV clinical phenotypes.

Methods

Search strategy

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20]. We used a combination of MeSH and non-MeSH terms representing extremes of virological and clinical progression in HIV in PubMed, and reviewed abstracts for all articles available between 1 January 2000 and 15 March 2012 (Fig. 1). Terms representing extremes of disease progression and control were included in the search strategy, as shown in Fig. 1. A total of 1639 abstracts were reviewed in order to shortlist relevant publications (Fig. 1, Supplementary Data 1, http://links.lww.com/QAD/A411). We further reviewed the full-text articles if the abstract or title mentioned an extreme phenotype term for disease progression or control in HIV infection and pertained to HIV infection in human adults. Extreme phenotypes in both HIV-1 and HIV-2 infection were considered for the purposes of this review. Articles were excluded if there was no mention of disease progression or control in the abstract or title, or if extreme phenotype definitions applied to children (<18 years of age) or to studies in animals. Articles were also reviewed if it was unclear whether they met the inclusion or exclusion criteria for the analysis.

On reviewing 1639 abstracts, we identified 730 articles for full-text review, and 501 studies were included in the final analysis (Fig. 1, Supplementary Data 2, http://links.lww.com/QAD/A412). Full-text articles were reviewed for terms referring to extreme phenotypes in HIV infection, and definitions were entered into a database. We listed terms that described extremes of the clinical spectrum in HIV infection through this review. These included words and phrases used to describe extreme groups in each article, such as 'LTNPs', 'elite controllers', 'slow progressors', 'viremic controllers', 'rapid progressors', or 'noncontrollers' (Table 1). These phrases will hereby be referred to as 'terms', and represent variously defined phenotypes of HIV control and progression. The set of clinical and immunological criteria used to describe the terms in each study are referred to as 'definitions' (Table 2). The data obtained using the search strategy were independently reviewed by two investigators (DG and LI) to identify articles for inclusion and to assess observer bias. Data obtained by the two investigators were then synthesized and collated. Any discrepancies in results were resolved by a consensus discussion. The database was examined for any duplicate definitions and these were deleted.

Data retrieval

Definitions, as described above, were collated on an electronic database. Definitions were only included if they incorporated at least one quantitative element and pertained to extreme phenotypes in the context of the natural course of HIV infection. Purely conceptual definitions of phenotypes without any quantitative element and definitions pertaining to extremes of viral or immunological control following antiretroviral treatment were not included in the analysis. However, definitions were included if they referred to extremes in the natural progression of HIV infection or viral control, even if they did not explicitly specify individuals being antiretroviral therapy (ART)-naive, as long as definitions did not pertain specifically to treatment-related viral control/disease progression phenotypes. Articles reviewing HIV phenotypes, listing several definitions, were not included. Studies describing case series with no defining criteria were not included in the analysis (Fig. 1, Supplementary Data 2, http://links.lww.com/QAD/A412).

When more than one definition was applied to a term, we listed this as two separate definitions in the database. Conversely, if more than one term was used to describe a group of individuals, definitions were listed under all terms used to refer to the individuals in the study. Therefore, the number of definitions may be different from the number of studies listed, as more than one term may appear in a single study and/or more than one definition may apply to a single term in a study. For

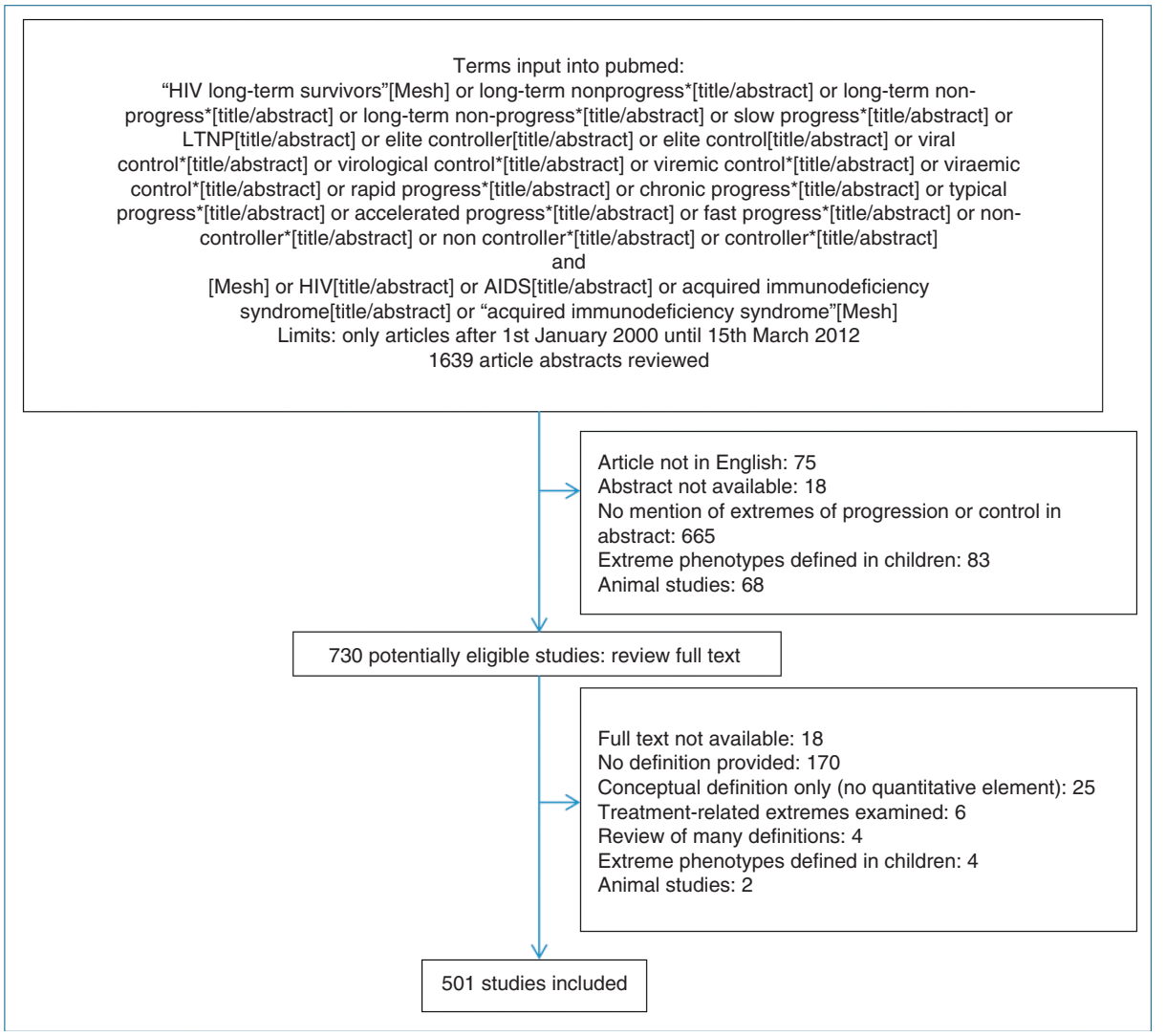

Fig. 1. Search strategy.

example, in one study, the terms 'LTNPs' and 'slow progressors' were used synonymously, and were defined as HIV-infected individuals who maintain $CD4^+$ cell counts above 500/μl for at least 10 years after seroconversion or suppress viral replication to levels of HIV-1-RNA below 300 copies/ml and maintain $CD4^+$ cell counts of at least 1000/μl for at least 6 years [21]. In this case, maintenance of $CD4^+$ cell counts above 500 cells/μl for more than 10 years following seroconversion, and suppression of viral loads to below 300 copies/ml with maintenance of $CD4^+$ cell counts above 1000 cells/μl for 6 years, were considered as separate independent definitions of long-term nonprogression/slow progression. In addition, both definitions were listed under the terms LTNPs and slow progressors separately, as both terms were used to describe this group. Thus, although these definitions pertained to one study, they were included as four separate data points in the review, two for LTNPs and two for slow progressors. Lists of collated definitions for all terms can be found in Supplementary Data 3 and 4, http://links.lww.com/QAD/A414, http://links.lww.com/QAD/A413.

Data synthesis

Of 1639 papers examined, 501 articles included definitions of terms used to describe extremes of disease progression or viral control. We listed all terms applying to definitions of extreme groups in the clinical/virological spectrum of HIV, and examined definitions within these groups. For the purposes of listing definitions, terms representing similar extreme groups were collapsed as shown in Table 1 and Fig. 2. For example, the term 'slow progressors' encompasses the terms 'slow progressors' and 'long-term slow progressors', and the term 'elite controllers' encompasses the terms 'elite controllers' and 'elite suppressors'. Table 1 describes these individual terms, their frequency, and the terms collapsed under generic term labels.

To facilitate comparison of definitions, and explore heterogeneity among definitions and terms, we collapsed

Table 1. Frequency of occurrence of terms used to describe extreme phenotypes in HIV in the literature.

Slow progression	No.	Viral control	No.	Slow progression- viral control hybrid	No.	Rapid progressor	No.	Noncontroller	No.
Long-term nonprogressor (LTNP)[a]	264	Controller (C)[d]	8	Long-term nonprogressor-elite controller (LTNP-EC)[g]	5	Fast progressor (FP)[h]	11	High viral load individual	1
Clinical LTNP (CLTNP)[a]	1	HIV controller (HIC)[d]	46	Elite-LTNP (E-LTNP)[g]	1	Rapid progressor (RP)[h]	79	Medium-high viral load individual	2
Slow progressor (SP)[b]	70	Elite controller (EC)[e]	103	Long-term nonprogressor-viral controller (LTNP-VC)	1	Super fast progressor (SFP)	1	Noncontroller (NC)	16
Long-term slow progressor (LTSP)[b]	1	Elite suppressor (ES)[e]	14	Long-term nonprogressor-controller (LTNP-C)	1	Accelerated progressor (AP)	1	Viremic individual (VI)	3
Long-term survivor (LTS)	20	Natural viral suppressor (NVS)[f]	4	Nonprogressor-elite controller (NP-EC)	1				
Long-term asymptomatic (LTA)	4	Viral suppressor (VS)[f]	1	Viremic nonprogressor (VNP)	2				
Nonprogressor (NP)[c]	12	Low viral load individual (LVLI)	2	Viremic noncontrollers (VNC)	1				
Clinical nonprogressor (CNP)[c]	1	Relative controller (RC)	2						
Slow progressor with robust replication (SP-RR)	1	Viremic controller (VC)	32						
		Aviremic individual (AVI)	2						

[a]Collated under the term 'long-term nonprogressors'.
[b]Collated under the term 'slow progressors'.
[c]Collated under the term 'nonprogressor'.
[d]Collated under the term 'HIV controller'.
[e]Collated under the term 'elite controller'.
[f]Collated under the term 'natural virus suppressor'.
[g]Collated under the term 'elite-LTNP'.
[h]Collated under the term 'rapid progressor'.

Table 2. Glossary of descriptors used in review.

	Definitions
Terms	Words or phrases describing extremes of the clinico-immunological and virological spectrum in HIV infection, e.g., 'elite controllers'.
Definitions	The set of clinical and immunological criteria used to describe the terms in each study. For example, elite controllers are HIV-infected individuals who suppress plasma HIV-RNA levels to <50 copies/ml without antiretroviral treatment.
Component	Broad categories of common clinical and immunological criteria used in definitions. For example, 'HIV-RNA level' is a component of definitions for elite controllers
Component threshold/ category	Thresholds or categories for the specific component in each definition. For example, a HIV-RNA threshold of <50 copies/ml in definitions of elite controllers

definitions that contained common components and component thresholds for clinical and immunological criteria under each term (Table 3). These broad components were identified by reviewing all definitions for each term. The term 'component' here refers to categories of common clinical and immunological criteria used in definitions, and 'component threshold/ categories' refers to the thresholds or categories used for these components in each definition (Table 2). For example, key components identified for definitions of LTNPs were duration of follow-up, $CD4^+$ cell count threshold, HIV-RNA thresholds, $CD4^+$ cell slopes and clinical criteria, such as asymptomatic or AIDS-free follow-up (Table 3). To identify unique definitions for each term, we then collapsed definitions based on each distinct combination of components and component thresholds or categories, with a view to grouping broadly similar definitions. We only collapsed definitions for terms for which we had identified more than 10 definitions in the literature. When different duration

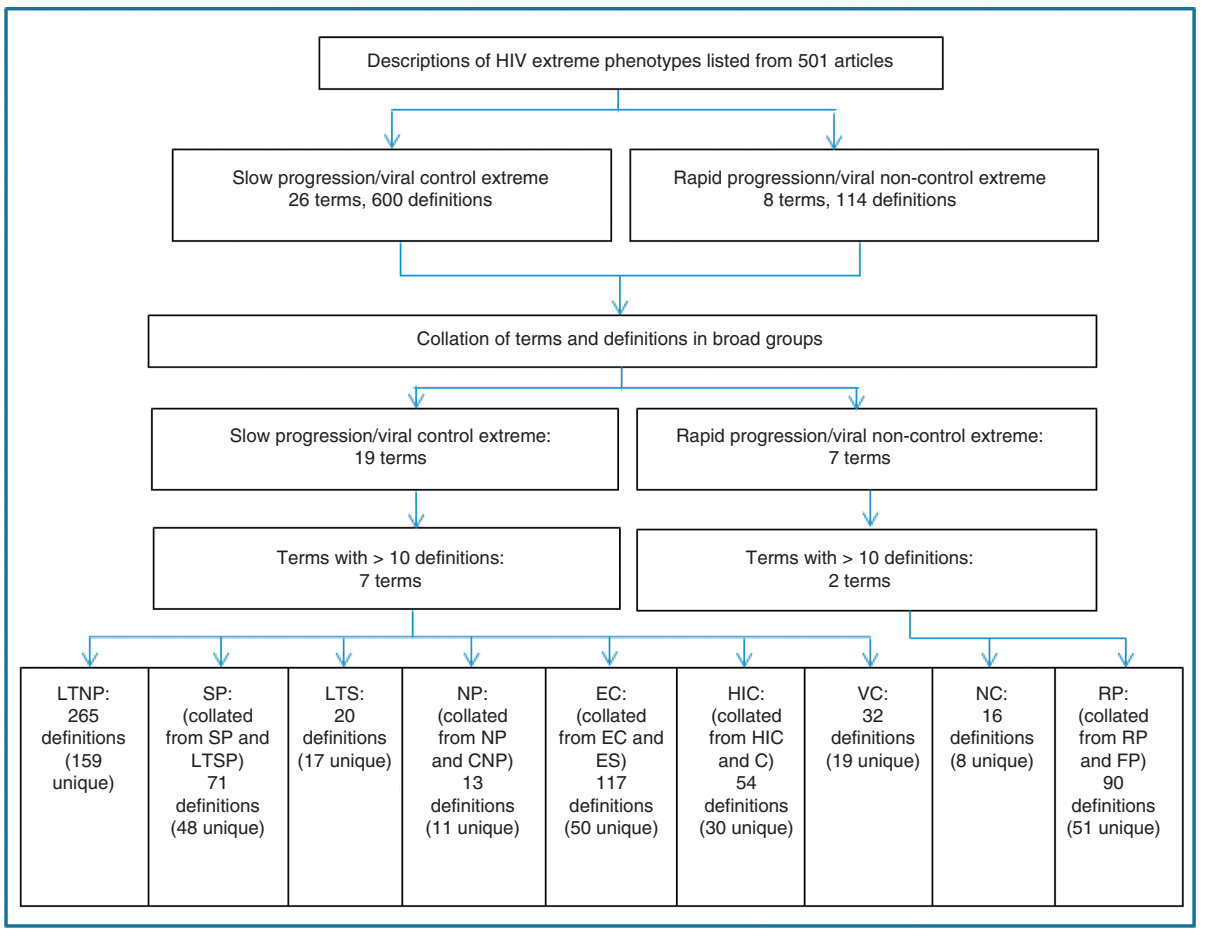

Fig. 2. Data synthesis: a process for collapsing and categorizing individual terms and definitions. C, controller; CNP, clinical nonprogressor; EC, elite controller; ES, elite suppressor; FP, fast progressor; HIC, HIV controller; LTNP, long-term nonprogressor; LTS, long-term survivor; LTSP, long-term slow progressor; NC, noncontroller; NP, nonprogressor; RP, rapid progressor; SP, slow progressor; VC, viremic controller.

Table 3. Broad components used to collapse definitions of progression and control HIV phenotypes.

Components of slow progression/viral control phenotype definitions (terms – LTNP, SP, LTS, NP, EC, HIC, and VC)		Components of rapid progression/viral noncontrol phenotype definitions (terms RP and NC)	
Components	Component threshold/category	Component	Component threshold/category
Duration of follow-up	Minimum duration of follow-up in years	Duration of follow-up	Minimum duration of follow-up in years
$CD4^+$ cell count	$CD4^+$ cell count threshold in count/μl	$CD4^+$ cell endpoint	$CD4^+$ cell count threshold in count/μl
HIV-RNA level	Plasma HIV-RNA threshold in copies/ml	HIV-RNA level	Plasma HIV-RNA threshold in copies/ml
Clinical symptoms	Asymptomatic/AIDS-free/OI free (yes/no)	$CD4^+$ cell slope	Numeric threshold of decline in cells/μl per year
$CD4^+$ cell slope	Numeric threshold of decline in cells/μl per year or qualitative (e.g., 'stable' $CD4^+$ cell levels)	AIDS endpoint	AIDS endpoint present in definition (yes/no)
Viral blips	Threshold for occasional spikes in HIV-RNA levels allowed	ART endpoint	ART endpoint present in definition (yes/no)
		Death endpoint	Death endpoint present in definition (yes/no)
		Seroconversion status	Seroconversion status known (yes/no/unspecified)

ART, antiretroviral therapy; EC, elite controllers; HIC, HIV controllers; LTNP, long-term nonprogressors; LTS, long-term survivors; NC, noncontrollers; NP, nonprogressors; OI, opportunistic infection; RP, rapid progressors; SP, slow progressors; VC, viremic controllers.

thresholds were applied to different components in a definition (e.g., duration of asymptomatic follow-up, duration of $CD4^+$ cell level below a threshold), only the greatest duration was considered, as this would be the minimum duration of follow-up needed to meet the criteria for a given definition. When multiple HIV-RNA assays were used, the assay with the highest threshold for lower limit of detection was considered.

After collapsing, definitions with a distinct combination of components and component thresholds/categories were identified as being unique. The proportion of unique definitions was calculated for each term. This proportion reflects the heterogeneity of definitions in literature. We ranked definitions identified in this way by frequency of occurrence, and listed the most common definitions for each term. The salient features of each definition were listed based on common components identified across definitions, to describe the most common components used to define terms referring to HIV extreme phenotypes in the literature. We also compared the frequency of component thresholds used in definitions of different terms, in order to assess the overlap of components and component thresholds/categories between definitions of different terms.

Results

On reviewing 501 articles, 600 definitions were listed for 26 terms used to describe slow progression/viral control extremes in HIV infection and 114 definitions for eight terms used to define fast progressor/viral noncontrol extremes in HIV infection (Fig. 2). The various terms used to describe these extremes in the literature are outlined in Table 1. Following collapsing of terms under broad groups, 19 terms for slow progression/viral control phenotypes and seven terms for rapid progression/noncontrol phenotypes were examined (Table 1, Fig. 2). Of the 26 terms listed, only nine terms that included more than 10 definitions each, were considered for further analysis (Fig. 2). The most common terms used in studies of slow progression extremes were 'LTNP' (265 instances), followed by 'slow progressor' (71 instances; Table 1), 'long-term survivor (LTS)' (20 instances), and 'nonprogressors' (13 instances). Common terms used to describe the extreme of viral control were 'elite controllers/elite suppressors' (117 instances), 'viremic controller' (32 instances), and 'HIC/controller' (54 instances; Table 1). Fewer terms were identified for the rapid progression extremes in HIV infection, with 90 instances of 'rapid progressors/fast progressors' (Table 1). For the extreme of noncontrol of HIV, 'noncontroller' was the commonest term used, with 16 definitions appearing in the literature.

We also examined the pattern of term usage by time of publication. We observed a greater diversity of terms used to describe viral control and noncontrol phenotypes in the period 2006–2012 as compared to the literature published between 2000 and 2005 (Fig. 3). Notably, terms pertaining to viral control phenotypes, such as 'elite controller', 'HIC', 'viremic controller', and 'noncontroller' seem to be used almost exclusively from 2006 onward, indicating the more recent interest in viral control-related phenotypes as compared with clinical phenotypes of nonprogression or rapid progression in the literature.

Redundancy of definitions in the literature

The total number of definitions and the number of unique definitions identified for each term are presented in Table 4. Heterogeneity in definitions was high, with a large proportion of definitions being unique for progression (54–85%) and viral control (43–59%)-related terms. Unique definitions for all terms can be found in

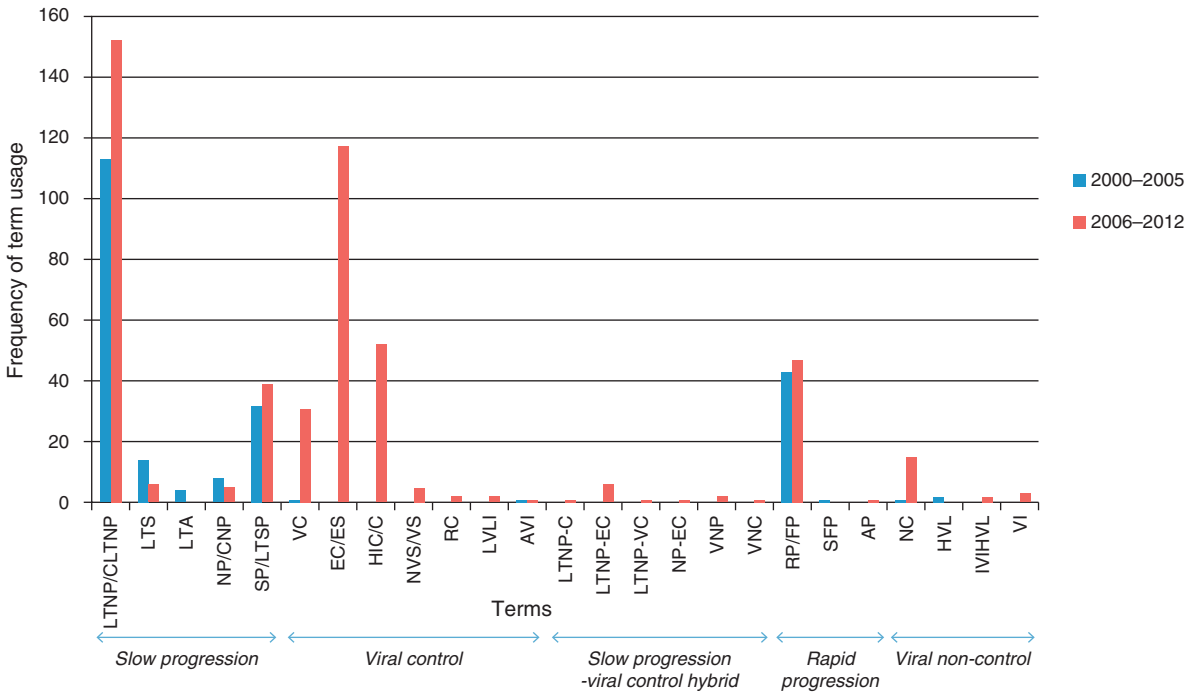

Fig. 3. Frequency of term usage by calendar period. AP, accelerated progressor; AVI, aviremic individual; C, controller; CLTNP, clinical long-term nonprogressor; CNP, clinical nonprogressor; EC, elite controller; ES, elite suppressor; FP, fast progressor; HIC, HIV controller; HVL, high viral load individual; LTA, long-term asymptomatic; LTNP, long-term nonprogressor; LTNP-C, long-term nonprogressor controller; LTNP-EC, long-term nonprogressor-elite controller; LTNP-VC, long-term nonprogressor-viremic controller; LTS, long-term survivor; LTSP, long-term slow progressor; LVLI, low viral load individual; MHVL, medium-high viral load individual; NC, noncontroller; NP, nonprogressor; NVS, natural viral suppressor; RC, relative controller; RP, rapid progressor; SFP, super fast progressor; SP, slow progressor; VC, viremic controller; VI, viremic individual; VNC, viremic noncontroller; VNP, viremic nonprogressor; VS, viral suppressor.

Supplementary Tables 1–9, http://links.lww.com/QAD/A415.

Description of extreme phenotypes in the literature

Long-term nonprogressors

Of 265 definitions of LTNPs, 159 were unique when combinations of duration of follow-up, $CD4^+$ cell thresholds, $CD4^+$ cell slopes, clinical symptoms, and viral load components were considered. There was substantial variation in components and component thresholds (Fig. 4, Supplementary Table 10, http://links.lww.com/QAD/A415). Duration of follow-up varied between 1 and 25 years among definitions, with 10 years being the most common duration of follow-up required (Fig. 4). Although $CD4^+$ cell thresholds were a prominent feature of LTNP definitions, with 74% of all definitions including a $CD4^+$ cell threshold criterion, thresholds showed marked variation across definitions with a range between 300 and 1000 cells/μl. The most

Table 4. Proportion of unique definitions within each term.

Phenotype	Terms	Total number of definitions	Unique definitions (%)
Slow progression	LTNP	265	159 (60%)
	SP	71	48 (69%)
	LTS	20	17 (85%)
	NP	13	11 (85%)
Viral control	EC	117	50 (43%)
	HIC	54	30 (56%)
	VC	32	19 (59%)
Rapid progression	RP	90	51 (54%)
Viral noncontrol	NC	16	8 (50%)

EC, elite controller; HIC, HIV controller; LTNP, long-term nonprogressor; LTS, long-term survivor; NC, noncontroller; NP, nonprogressor; RP, rapid progressor; SP, slow progressor; VC, viremic controller.

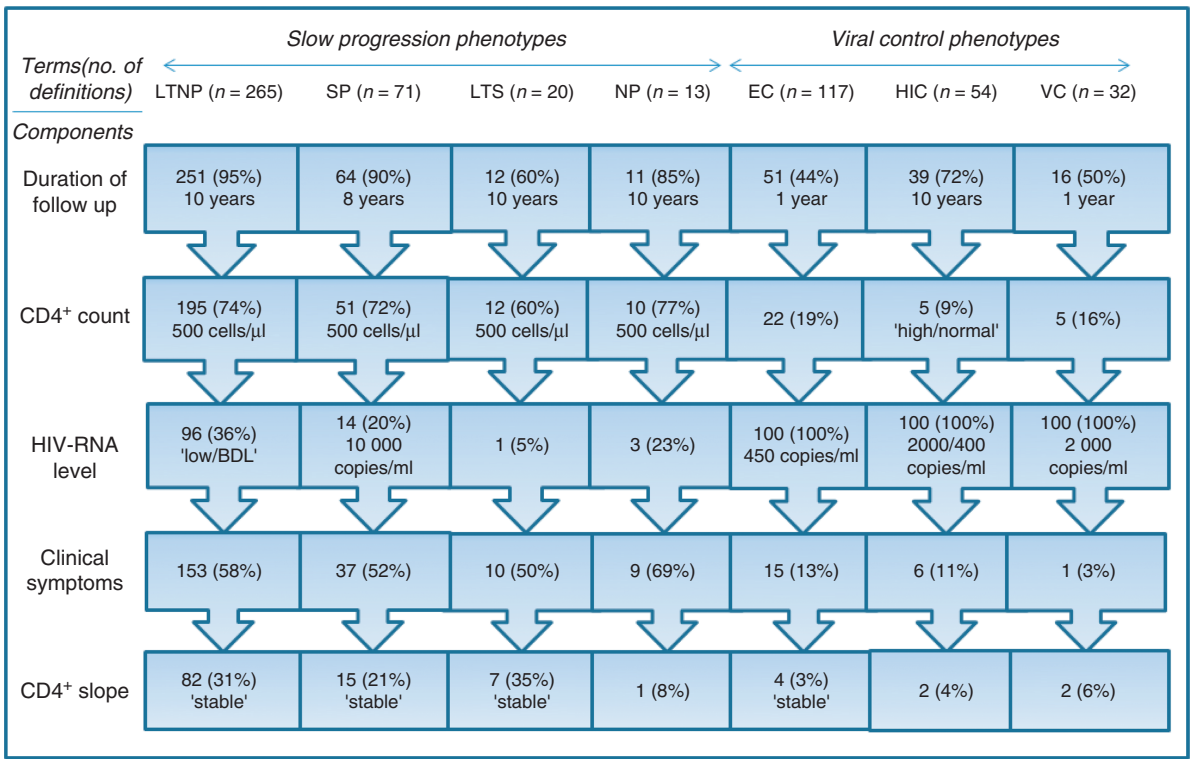

Fig. 4. Predominant components and common thresholds used in definitions of slow progression/viral control terms in HIV infection. The figure depicts the number of definitions (with proportions in parentheses) for slow progressor/viral control terms that include specific components and component thresholds as part of the definition. The most frequent component threshold applied for each of these also represented, where a single common threshold could be identified. BDL, below detection limit; EC, elite controller; HIC, HIV controller; LTNP, long-term nonprogressor; LTS, long-term survivor; NP, nonprogressor; SP, slow progressor; VC, viremic controller.

frequent $CD4^+$ cell threshold was 500 cells/μl (Fig. 4, Supplementary Table 10, http://links.lww.com/QAD/A415). A component including a HIV-RNA threshold was less common, with only 36% of definitions including a viral load criterion. The absence of clinical symptoms also appeared to be a prominent criterion, with around 58% of definitions including a criterion of being asymptomatic, without opportunistic infection or AIDS-free. $CD4^+$ cell slopes were also common features of definitions, with 31% of definitions including a criterion for stability of $CD4^+$ cell counts (Fig. 4).

Slow progressors

Of 71 definitions identified for slow progressors, 69% were unique with respect to the components described (Table 4). As with LTNP definitions, duration of follow-up was an important component, with 90% of definitions including a criterion for minimum duration of follow-up (Fig. 4). There was marked variation in duration thresholds, ranging from 10 months to 16 years, the most frequently appearing threshold being 8 years of follow-up (Fig. 4, Supplementary Table 10, http://links.lww.com/QAD/A415). In general, the duration of follow-up needed to define slow progressors was lower than that for LTNPs (Supplementary Table 10, http://links.lww.com/QAD/A415). $CD4^+$ cell thresholds and the absence of clinical symptoms were also important components, with 72 and 52% of definitions including these, respectively (Fig. 4). As with LTNPs, a $CD4^+$ cell threshold of 500 cells/μl was most common, with thresholds ranging from 200 to 1000 cells/μl (Supplementary Table 10, http://links.lww.com/QAD/A415). The frequency of various component thresholds can be found in Supplementary Table 10, http://links.lww.com/QAD/A415. $CD4^+$ cell slope and HIV-RNA thresholds were less common for these definitions, with only 21 and 20% of definitions including each of these components, respectively (Fig. 4).

Long-term survivors

Of 20 definitions listed for LTSs, 17 were unique (Table 4). As expected, duration of follow-up was a prominent component with 10 years being the most frequent threshold (Supplementary Table 10, http://links.lww.com/QAD/A415). $CD4^+$ cell thresholds were also prominent components, with 12 definitions including a threshold, the commonest being 500 cells/μl (Table 4). Clinical criteria of symptom/AIDS-free follow-up were also common with 50% of definitions including this component (Table 4, Supplementary

Table 10, http://links.lww.com/QAD/A415). HIV-RNA levels and $CD4^+$ cell slopes appeared to be less prominent components in these definitions.

Nonprogressors

A total of 13 definitions were identified for nonprogressors, of which 11 were unique (Table 4). Duration of follow-up and $CD4^+$ cell threshold components were prominent in this group, with 10 years and 500 cells/μl being the most common thresholds, respectively. HIV-RNA levels, clinical criteria, and $CD4^+$ cell slopes only appeared in a minority of definitions (Fig. 4).

Elite controllers

A total of 117 definitions were identified for elite controllers, of which 50 were unique (Table 4). As expected from the terminology, HIV-RNA thresholds appeared in all definitions listed, with thresholds ranging from 40 to 500 copies/ml (Fig. 4, Supplementary Table 10, http://links.lww.com/QAD/A415). The most frequent HIV-RNA threshold used was 50 copies/ml. Only five definitions included a criterion for occasional blips in viral load (Fig. 4). Duration of follow-up also appeared to be important with 44% of definitions including a minimum duration of follow-up criterion. Duration thresholds varied from 6 months to 16 years, with a threshold of 1 year being most frequent (Supplementary Table 10, http://links.lww.com/QAD/A415). $CD4^+$ cell thresholds appeared only in 19% of definitions, in contrast with LTNPs and slow progressors, wherein 74 and 72% of definitions included this component.

HIV controllers

A total of 54 definitions for HICs were identified, of which 56% were unique (Table 4). All definitions included a HIV-RNA threshold (Fig. 4). HIV-RNA thresholds varied from 40 to 10 000 copies/ml, with 400 and 2000 copies/ml both being common thresholds applied (Supplementary Table 10, http://links.lww.com/QAD/A415). Duration of follow-up was also an important component of these definitions, with 72% of definitions including a cut-off for the minimum duration of follow-up required (Fig. 4). Thresholds of 10 years and 1 year appeared to be most common for these definitions, which can be seen as a product of the two most common definitions of this term (Supplementary Table 10, http://links.lww.com/QAD/A415, Table 5).

Viremic controllers

Of 32 definitions applied to viremic controllers, the majority (59%) were unique, suggesting marked variability in definitions used (Table 4). As with elite controllers and HICs, all definitions included a HIV-RNA threshold (Fig. 4). Thresholds were generally higher in comparison with elite controller definitions and varied between 500 and 15 000 copies/ml, with a threshold of 2000 copies/ml being most common (22/32 definitions; Supplementary Table 10, http://links.lww.com/QAD/A415). $CD4^+$ cell thresholds appeared as components in five of 30 definitions, and there was marked variability in thresholds used (Supplementary Table 10, http://links.lww.com/QAD/A415). As with elite controllers and HICs, clinical criteria only appeared in a minority of definitions.

Rapid progressors

Of 90 definitions identified for the terms 'rapid progressor' or 'fast progressor', 51 definitions were unique based on combinations of components considered (Table 4). $CD4^+$ cell thresholds and AIDS endpoints appeared to be the most common components of definitions, with 56% of definitions including a $CD4^+$ cell endpoint, and 48% of definitions including an AIDS endpoint (Fig. 5). Among $CD4^+$ cell endpoints, a threshold of 300 cells/μl was the most frequent (Fig. 5,

Table 5. Common definitions identified for common terms used to describe extremes in HIV infection.

Term	Commonest definition[a]	Frequency
LTNP	Asymptomatic and ART-naive for 10 years during follow-up with all $CD4^+$ cell counts above 500 cells/μl during this period	15/265
SP	Seropositive asymptomatic individuals infected for 8 or more years with a $CD4^+$ T-cell count above 500 cells/μl in the absence of ART.	16/71
EC	Spontaneously maintain viral loads below 50 copies/ml without ART	33/117
HIC	HIV-infected patients who had been seropositive for >10 years and had received no ART for whom >90% of the HIV-RNA measurements were <400 copies/ml	8/54
	Alternate definition: HIV-infected individuals with at least three measurements of plasma HIV-RNA <2000 copies/ml over at least a 12-month period in the absence of ART	7/54
VC	Infected with HIV and maintaining viral loads of <2000 RNA copies/ml without ART	4/32
RP	HIV infected with $CD4^+$ T-cell counts of <300 cells/μl within 3 years after the last HIV-seronegative test	17/90
NC	HIV-infected individuals with plasma HIV-RNA >10 000 copies/ml without ART	6/16

ART, antiretroviral therapy; EC, elite controller; HIC, HIV controller; LTNP, long-term nonprogressor; NC, noncontroller; RP, rapid progressor; SP, slow progressor; VC, viremic controller.
[a]Single dominant definitions could not be identified for long-term survivors and nonprogressors and are, therefore, not presented here.

Supplementary Table 11, http://links.lww.com/QAD/A415). Time to end-point was a prominent component of definitions, with 92% of definitions including a duration component, the most frequent threshold being 3 years (Fig. 5, Supplementary Table 11, http://links.lww.com/QAD/A415). Known date of seroconversion appeared to be a prominent component, with 80% of definitions including this (Fig. 5). However, many definitions did not specify time since seroconversion, including either time from diagnosis or only $CD4^+$ cell slope-based criteria. HIV-RNA thresholds were rare (6%) among these definitions (Fig. 5). Death and ART initiation were also used as endpoints in a small number of definitions (3% each).

Noncontrollers

There were only 16 definitions of noncontrollers listed, of which eight were found to be unique based on combinations of components (Table 4). All definitions included a HIV-RNA component, with 10 000 copies/ml being the most common cut-off used (Fig. 5). $CD4^+$ cell endpoints were also used in two definitions (Fig. 5, Supplementary Table 11, http://links.lww.com/QAD/A415), but no clinical endpoints appeared in any definition.

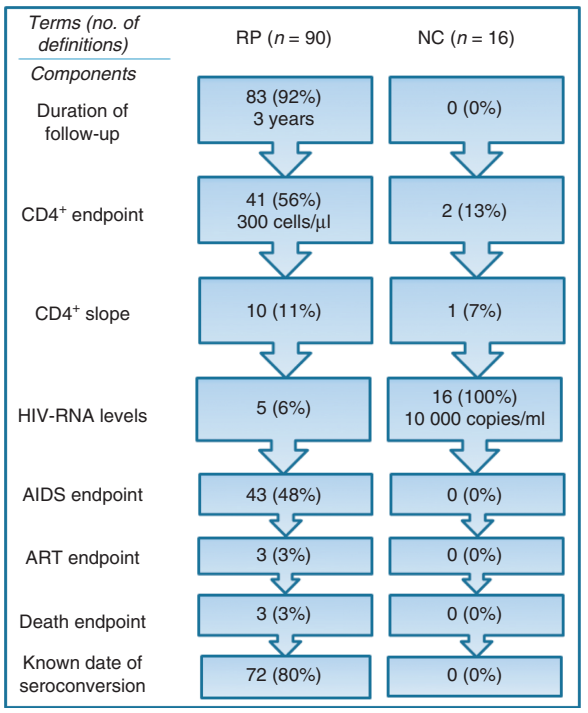

Fig. 5. Predominant components and common thresholds used in definitions of rapid progression/noncontroller terms in HIV infection. The figure depicts the number of definitions (with proportions in parentheses) for rapid progressor/noncontroller terms that include specific components and component thresholds. The most frequent component threshold applied for each of these also represented, wherein a single common threshold could be identified. NC, noncontroller; RP, rapid progressor.

Common definitions of HIV phenotypes

The most frequently occurring definitions for each term are listed in Table 5. Single dominant definitions that were clearly much more common than others could be identified for most terms, except HICs for whom two common definitions were identified (Table 5). Although common definitions are clearly identified for each term, it can be seen that these still represent the minority of all definitions listed (Table 5). It is also clear that, although there are marked differences in the components of definitions for each term, in most cases specific component thresholds can be identified for each term that are far more common than others (Figs. 4 and 5). Using the most common components and component threshold/category within components to derive common definitions produced identical results to those produced by grouping individual definitions (Figs. 4 and 5 and Table 5). For example, the most common definition for LTNP was an HIV-infected individual who is asymptomatic and ART-naive for 10 years during follow-up with all $CD4^+$ cell counts above 500 cells/μl during this period, which combines the most common components and component thresholds/categories listed for definitions of this term (Fig. 4).

Overlap between definitions

There was substantial overlap between components across terms, with 36% of LTNP definitions including HIV-RNA threshold criteria and 19% of elite controller and 16% of viremic controller definitions including $CD4^+$ cell threshold components (Fig. 4). There was marked overlap between components and thresholds/categories used across all slow progression terms, with substantial overlap between components of LTNPs, slow progressors and nonprogressors, and between viremic controller and HICs (Supplementary Table 10, http://links.lww.com/QAD/A415).

Broad phenotypes represented by different terms

On the basis of our review, we sought to characterize the broad HIV phenotypes represented by different terms in the literature. On considering components and component thresholds/categories of definitions for slow progression-related terms, the clinical phenotypes

represented by LTNPs, LTSs, and nonprogressors were broadly similar, and represented individuals who maintained normal $CD4^+$ cell counts, and remained healthy at least for 10 years of observed follow-up. In general, slow progressors represented a less stringent phenotype, and thresholds for duration of follow-up required tended to be lower (Supplementary Table 10, http://links.lww.com/QAD/A415). The relative representation of viral control phenotypes could be broadly characterized, with elite controllers representing the most extreme phenotype of viral control, and viremic controllers representing higher levels of viremia (Fig. 6). For HICs, two broad phenotypes seemed to predominate: one appeared to be similar to elite controllers, but with control of viremia to below 400 copies/ml over at least 10 years, and the second encompassing elite controller and viremic controller phenotypes (Fig. 6).

Discussion

In this systematic review, assessing 714 definitions of HIV extreme phenotypes in 501 eligible studies, we identified substantial variation among definitions used to describe extreme phenotypes in HIV infection. This heterogeneity in definitions may represent important differences in biological endophenotypes [14] and clinical progression profiles [1,22] of individuals selected by these, suggesting the need for harmonized definitions. In this context, we were able to identify common components in existing definitions that may provide a framework for developing consensus definitions for HIV extreme phenotypes.

Although recent studies have focused on extreme phenotypes in HIV infection as natural models of viral control and the extremes of disease progression in HIV, little is known about the impact of heterogeneity in definitions on clinical and biological phenotypes captured. This heterogeneity has implications for the design of studies exploring HIV biology and for the interpretation of existing research. Although several studies have referred to this marked variation in definitions, and the need for standardized phenotype definitions [2,22], the full extent of variability in the literature has never been formally quantified. To our knowledge, this is the first study that has attempted to address this in a systematic manner. Formal evaluation of the impact of varying definitions on clinical outcomes and characterizing biological endophenotypes is essential to develop

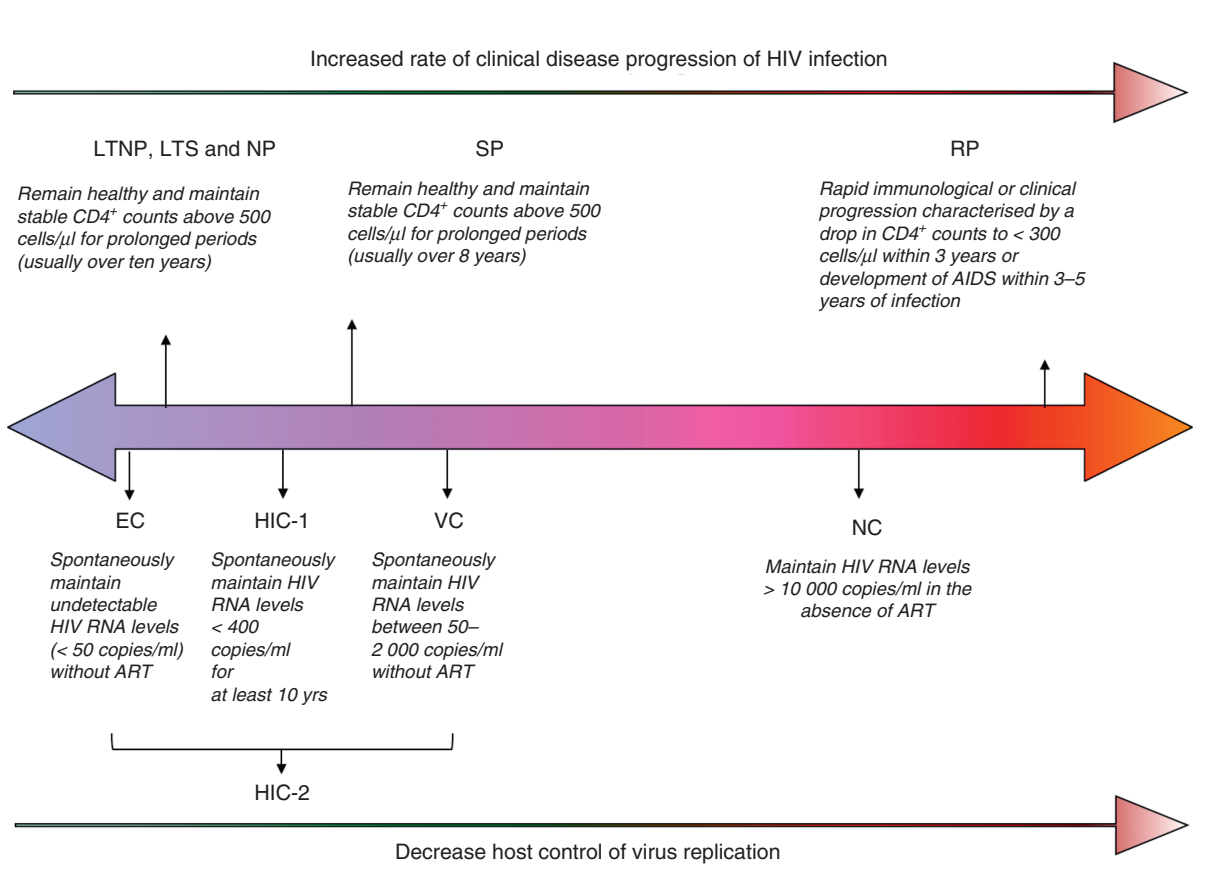

Fig. 6. Relative characteristics of phenotypes referred to by different terms in the literature. HIC-1 and HIC-2 refer to the two most commonly used definitions for HIV controllers. ART, antiretroviral therapy; EC, elite controller; HIC, HIV controller; LTNP, long-term nonprogressor; LTS, long-term survivor; NC, noncontroller; NP, nonprogressor; RP, rapid progressor; SP, slow progressor; VC, viremic controller.

harmonized definitions that will represent clinical and biological phenotypes of interest.

Understanding the impact of variability in phenotype definitions on clinical outcomes in HIV is important, as the literature suggests that small variations in phenotype definitions can substantially impact the trajectory of disease progression in patients selected. In one study, varying the duration of follow-up threshold for LTNPs resulted in the selection of groups with markedly different survival times [1]. A recent study showed that allowing for at least one nadir $CD4^+$ cell count below 500 cells/μl among LTNPs can lead to a significant reduction in the time to disease progression compared with individuals who maintain all $CD4^+$ cell counts above this threshold [22]. This is consistent with research within the French Hospital Database that showed that a positive $CD4^+$ cell slope was a more selective criterion than a longer duration of HIV infection (10 years instead of 8 years), for selecting patients who were asymptomatic and ART-naive several years after being infected by HIV [2]. Similar findings have been demonstrated with viral control phenotypes; individuals with viral loads less than 50 copies/ml (elite controllers) have markedly improved AIDS-free survival compared with individuals with viral loads between 50 and 2000 copies/ml [1]. Prevalence of phenotypes represented can also vary markedly with small changes in definitions. For example, in the French Hospital Database, increasing the $CD4^+$ cell threshold in LTNPs from 500 to 600 cells/μl changed the prevalence of the phenotype from 22 to 11% in the cohort, and addition of a criterion for positive $CD4^+$ cell slope further reduced the prevalence to 2.8% [2]. This is of particular relevance to studies that aim to recruit individuals with extreme phenotypes for further characterization of mechanisms of immune-virological control.

Variation in extreme phenotype definitions may also impact on the underlying biological endophenotype being examined. Our study suggests that there is marked overlap between components of definitions referring to different terms in the literature, which makes it difficult to delineate phenotypes represented by different terms. It is important to distinguish these terms in the literature, as different phenotypes may capture different underlying biology. Indeed, it has been shown that protective and high-risk alleles known to be associated with disease control and progression in HIV infection, show a graded change in frequency along the clinical spectrum of disease [14]. The limited overlap between LTNP and EC phenotypes in some studies, with only 8–32% of LTNPs meeting criteria for elite control [2,22,23], suggests that slow progression and viral control phenotypes are only modestly correlated, and may potentially represent distinct biological phenotypes. A recent genome wide association study further substantiated this with the discovery of a new locus associated with LTNP, when individuals who were EC (HIV-RNA levels <100 copies/ml) were excluded from the cohort [19], suggesting that the determinants of viral control and slow progression phenotypes may be distinct.

Major strengths of our study include the comprehensive search strategy applied and the large number of articles reviewed. As the correlation between the studies short-listed for review by the two reviewers was high (>95%), there is unlikely to be substantial observer bias in the review process. We acknowledge that our review of definitions also has several limitations. Our search strategy was only restricted to one search engine, to published articles, and to articles available after the 1 January 2000, which may have limited the sensitivity of the search. Additionally, we did not examine definitions by differences in HIV subtypes and clades, and extreme phenotypes represented by definitions in these groups may differ. In spite of these limitations, we believe that our review is a fair representation of the heterogeneity in definitions observed in the literature, and the lower sensitivity of the review would only underestimate existing heterogeneity among definitions. Moreover, to our knowledge, this is the first attempt to formally characterize the variability in definitions of these terms in literature, and identify common components used to define these terms.

Given the possible differences in biological and clinical phenotypes captured by different definitions, it is important to standardize case definitions of these phenotypes for consistency in methods and ease of interpretation across studies. Although the various studies described have provided clues to the clinical and biological correlates of different definitions, the literature examining this is limited and further research specifically addressing variation in these phenotypes with varying definitions of phenotypes is essential to develop a framework for consensus definitions.

Several attributes of definitions must be considered when formulating consensus definitions. First, phenotype definitions should capture a truly extreme phenotype, as sampling from extremes can be a powerful way to examine HIV biology. This approach has been shown to be effective [19,24,25] in identifying genetic variants associated with HIV control and progression. Second, definitions should represent biologically relevant endo-phenotypes, so that underlying biology associated with these can be examined efficiently. Further research specifically examining the heritability and underlying biology of different phenotypes is needed in order to establish which phenotypes are likely to be most biologically relevant. Third, the phenotype definition should include components that are clinically relevant and adequately stable to predict long-term clinical outcomes. It is also important that the components described can be easily assessed and data for these can be readily extracted from existing cohorts. This would require systematic

assessment of clinical outcomes of commonly used definitions in large-scale consortia, which have adequate numbers of these rare individuals, and appropriate data on seroconversion and detailed clinical outcomes. While ascertaining the most useful definitions for extreme HIV phenotypes is challenging, our study shows that in spite of the large amount of heterogeneity observed in definitions, common components and thresholds used in definitions can be identified for most terms, indicating that there are common threads that have been used to define these groups in the literature, which could provide the framework for consensus definitions. Further work specifically examining the biological characteristics and differences in clinical progression, among these groups of individuals is needed in order to inform the utility of different definitions in HIV research.

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Conflicts of interest

There are no conflicts of interest.

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An Evaluation of HIV Elite Controller Definitions within a Large Seroconverter Cohort Collaboration

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Abstract

Background: Understanding the mechanisms underlying viral control is highly relevant to vaccine studies and elite control (EC) of HIV infection. Although numerous definitions of EC exist, it is not clear which, if any, best identify this rare phenotype.

Methods: We assessed a number of EC definitions used in the literature using CASCADE data of 25,692 HIV seroconverters. We estimated proportions maintaining EC of total ART-naïve follow-up time, and disease progression, comparing to non-EC. We also examined HIV-RNA and CD4 values and CD4 slope during EC and beyond (while ART naïve).

Results: Most definitions classify ~1% as ECs with median HIV-RNA 43–903 copies/ml and median CD4>500 cells/mm^3. Beyond EC status, median HIV-RNA levels remained low, although often detectable, and CD4 values high but with strong evidence of decline for all definitions. Median % ART-naïve time as EC was ≥92% although overlap between definitions was low. EC definitions with consecutive HIV-RNA measurements <75 copies/ml with follow-up≥ six months, or with 90% of measurements <400 copies/ml over ≥10 year follow-up preformed best overall. Individuals thus defined were less likely to progress to endpoint (hazard ratios ranged from 12.5–19.0 for non-ECs compared to ECs).

Conclusions: ECs are rare, less likely to progress to clinical disease, but may eventually lose control. We suggest definitions requiring individuals to have consecutive undetectable HIV-RNA measurements for ≥ six months or otherwise with >90% of measurements <400 copies/ml over ≥10 years be used to define this phenotype.

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Introduction

HIV is typically characterised by a period of viral replication and CD4 cell decline leading to AIDS and death in the absence of antiretroviral therapy (ART). [1] Differences in the evolution of both markers over time, however, result in large variations in disease progression among HIV-positive individuals. [2,3] The long-term non-progressor (LTNP) phenotype was initially described to characterise individuals who experienced slow disease progression and stable CD4 counts over a number of years. [4,5] With the introduction of HIV-RNA assays in the mid-1990s, research shifted to focus on mechanisms which lead to control of viral replication [6].

A small proportion of individuals have been described who are able to suppress viral replication to undetectable levels for extended periods of time without use of ART, delaying the onset of AIDS. [7,8] Many terms are used in the literature for such individuals, with the most common being elite controllers (EC). [8] Mechanisms of EC remain unclear, although it is now believed that host response, including CD4 and CD8 T cell-specific immune response, [9,10] as well as HLA Class I alleles, [11] are likely to be the main mechanisms of control, rather than infection by defective virus, as initially postulated. [12] Whatever the mechanisms, a study of this group of individuals gives potential for the development of new treatment strategies, can guide research on HIV vaccines,[13–16] and provide models for a functional cure of HIV [17,18].

Table 1. 10 definitions of elite control from the literature applied to the CASCADE dataset; all require individuals to be AIDS-free and ART-naïve.

Definition	
A	HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml [22]
B	HIV-positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml [16]
C	HIV-positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml [15]
D	HIV-positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml [21]
E	HIV-positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months [8]
F	HIV-positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml [6]
G	HIV-positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml [19]
H	HIV-positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml [23]
I	HIV-positive ≥10 years, with all measured HIV-RNA <50 copies/ml [20]
J	HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml [7]

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Since the publication of initial definitions of EC, as was the case with the long-term non-progressor (LTNP) phenotype, many more definitions have been proposed; presumably to arrive at one definition which best defines true EC. There are currently numerous definitions, each of which differs by the follow-up time required and the number and threshold of undetectable HIV-RNA measurements.[6–8,15,16,19–23] It is not known which, if any, best characterise this rare phenotype, however. This is important to ensure that any difference between elite controllers and non-controllers can be attributed to the phenotype itself. An assessment of the relative merits of each definition has never been undertaken or a comparison between them preformed.

The CASCADE (Concerted Action on SeroConversion on AIDS and Death in Europe) Collaboration, of HIV-positive individuals followed-up since HIV seroconversion, offers a unique opportunity to assess the ability of these definitions to capture EC. Using data from CASCADE, we aimed to evaluate a number of commonly-used definitions to estimate prevalence of EC and associated factors, proportion of total follow-up time spent as elite, and to describe CD4 and HIV-RNA values during EC and beyond the EC period.

The work provides the basis for choosing a definition appropriate to the objectives of future research on this rare phenotype.

Materials and Methods

Study Population

We used pooled data from the CASCADE September 2011 data release in EuroCoord (www.EuroCoord.net), which consists of 25,629 seroconverters from 28 cohorts across Europe, Canada, Australia and sub-Saharan Africa. [24] Date of seroconversion is estimated by various methods, most commonly as the midpoint between the last documented HIV negative and the first positive HIV antibody test dates with an interval of <3 years between the two test dates (85%). For the remainder, date of seroconversion was estimated through laboratory evidence of seroconversion (PCR positivity in the absence of HIV antibodies or antigen positivity with fewer than four bands on Western blot) (13%), or as the date of a seroconversion illness (2%) with both an earlier documented negative and a later positive HIV test not more than 3 years apart. Anonymized data for the CASCADE collaboration are collected and stored at the Medical Research Council Clinical Trials Unit at University College London. Access is available to bona fide researchers through submission of a proposal to the Steering Committee which is reviewed by CASCADE investigators.

Elite Control Definitions

We undertook a systematic review of the literature, which is described elsewhere [25]. Briefly, we searched for terms previously used to describe control of HIV infection including "Long term non-progressors", "LTNP", "elite controller", "elite control", "viral controller" and "viral control" and evaluated 10 EC definitions (Table 1).[6–8,15,16,19–23] The list of definitions included in this paper is not intended to be exhaustive; rather it is representative of the spectrum by which the elite control phenotype is defined in the literature. Three definitions were most commonly used, all requiring HIV positive individuals to meet the following criteria while ART-naive and AIDS-free: 1) Definition E, used by the International HIV Controllers Consortium, of individuals who maintain HIV-RNA levels below 75 copies/mL for at least 1 year, [8] 2) Definition F, an adaptation of definition E allowing no previous HIV-RNA levels >1000 copies/ml, [6] and 3) Definition J, initially proposed by the ANRS, of individuals known to be HIV positive for ≥10 years with ≥2 HIV-RNA measurements, ≥90% of which were required to be <400 copies/ml [7].

Statistical Methods

We identified three groups of individuals for each definition: those who fulfilled it, those who did not, and those whose EC status could not be determined, e.g. because insufficient follow-up or ART-naive HIV-RNA measurements were not available. For each definition we estimated the proportion of EC excluding individuals whose EC status was unknown from the denominator. Because there were large numbers with unknown status, we also estimated proportion of EC by assuming them to be non-EC and including them in the denominator, thus providing minimum proportion estimates.

For each definition we estimated the proportion of time they remained as EC by considering all available ART-naive follow-up. To estimate the effect of EC status on disease progression we restricted entry to the risk set at 10 years post seroconversion, as this was the longest duration of follow-up required by all definitions considered, and used multivariable time dependent Cox proportional hazards models to estimate the hazard ratio for a

composite endpoint of AIDS, death (all cause), ART initiation or CD4<350 cells/mm^3 comparing non-ECs and unknowns to ECs. We formally tested differences in hazard ratios between the definitions by using 1000 bootstrap replicates. Definition I was excluded from the bootstrap analysis as there were few follow-up measurements among a small number of elite controllers providing unstable estimates.

We described median and interquartile ranges (IQR) of HIV-RNA and CD4 levels, based on median individual values, while classified as EC and during total ART-naïve follow-up time. CD4 slopes were estimated using a linear mixed model on the square root scale, while classified as an elite controller, and also during total ART-naïve follow-up time.

Results

Data from 28 cohorts of 25,692 individuals formed the base from which sub-populations of EC and non-EC were drawn according to each definition. Median (IQR) year of HIV seroconversion was 1999 (1992, 2005) and median age at seroconversion 31 years (25, 37). HIV risk groups were MSM (55%), MSW (26%) or IDU (14%), and the majority were male (78%).

Proportion Classified as EC and Patient Characteristics

The proportion classified as EC by each definition was 0.15–7.70% and did not necessarily reflect the length of follow-up required by the definition (**Table 2**). While variations in age, sex and risk group were observed for each definition, no consistent differences were observed across definitions.

The number of individuals fulfilling two definitions was generally low with 33% of individuals overlapping by <30% with another definition (**Table 3**).

Total Time Spent as EC

The risk of composite endpoint was consistently significantly higher for non-EC/unknown compared to ECs for all definitions, with hazard ratios ranging from 2.9–19.0 and being greatest for definitions A, E, F and J (**Table 4**). 1000 bootstrap replicates confirmed the superiority of A, E and F ($\alpha = 0.05$) above definitions B, C, D, G and H, the former 3 definitions not being statistically different from each other. Definition J was not statistically superior to any other definition.

Considering all available ART-naïve follow-up from seroconversion, the proportion of time spent as EC, according to each definition, was remarkably high with median follow-up≥92% for all definitions, although 25% of EC, according to definitions C, D and F, spent ≤72% of their ART-naïve follow-up time as EC (**Table 4**). Figure 1 illustrates total ART-naive follow-up for all individuals classified as EC by definitions A, E, F, and J.

HIV-RNA and CD4 Values during EC Status

Median HIV-RNA during the time of EC was generally low for all definitions varying from 35–903 copies/ml and median CD4 levels high at >500 cells/mm^3 (**Table 5**). There was strong evidence of CD4 loss during this EC period, however, for at least 5 of the 10 definitions considered. For the remaining definitions,

Table 2. Number of elite controllers (EC), their proportion, and demographic characteristics applying the CASCADE dataset to 10 definitions of EC found in the literature.

Def.	*EC (n)*	*Non-EC (n)*	*Unknown*[†] *(n)*	*EC Proportion Best Estimate*[‡] *n (%)*	*EC Proportion Minimum Estimate*[¶] *n (%)*	*Seroconversion Age (Median)*		*Male (%)*		*MSM*[¶] *(%)*		*IDU*[¶] *(%)*		*MSW*[¶] *(%)*	
						ECs	*Non ECs*	*ECs*	*Non ECs*	*ECs*	*Non ECs*	*ECs*	*Non ECs*	*ECs*	*Non ECs*
A[F]	282	20951	4396	1.33	1.10	32	31	74	78	53	57	11	11	32	27
B[F]	495	19568	5566	2.47	1.93	32	31	79	78	59	56	15	11	23	28
C[F]	827	19236	5566	4.12	3.23	32	31	74	78	54	56	11	11	31	27
D[F]	1416	16964	7249	7.70	5.52	30	31	67	79	49	58	11	10	36	27
E[F]	174	17160	8295	1.00	0.68	33	31	75	78	52	57	13	10	30	28
F[F]	95	17239	8295	0.55	0.37	32	31	63	78	37	57	18	10	38	28
G[F]	392	16891	8346	2.27	1.53	32	31	76	77	55	56	12	11	30	28
H[F]	146	10899	14584	1.32	0.57	31	30	74	77	47	55	18	13	29	27
I[F]	10	6694	18925	0.15	0.04	26	29	80	77	30	53	60	18	0	25
J[F]	47	6554	19028	0.71	0.18	31	29	74	77	34	53	28	17	30	25

[†]Individuals in the cohort without adequate follow-up or number of HIV-RNA measurements to classify them as EC or non-EC.
[‡]Based on number of seroconverters whose EC status could be determined.
[¶]HIV risk groups: MSM: Men who have sex with men; IDU: Injection drug users; MSW: Heterosexual contact.
[¶]Assuming all individuals with unknown EC status are non-EC.
[F]**A**: HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml; **B**: HIV- positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml, **C**: HIV- positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml, **D**: HIV- positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml, **E**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months **F**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml, **G**:HIV- positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml, **H**: HIV- positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml, **I**: HIV- positive ≥10 years, with all measured HIV-RNA <50 copies/ml, **J**: HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml.
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Table 3. Two-way overlap of 10 definitions of elite control found in the literature applied to the CASCADE dataset.

Def.	*A, n (%)*	*B, n (%)*	*C, n (%)*	*D, n (%)*	*E, n (%)*	*F, n (%)*	*G, n (%)*	*H, n (%)*	*I, n (%)*	*J, n (%)*	*Total*
A[F]	–	195 (39)	279 (34)	275 (19)	174 (100)	95 (100)	250 (64)	113 (77)	4 (40)	35 (74)	282
B[F]	195 (69)	–	495 (60)	341 (24)	119 (68)	45 (47)	286 (73)	94 (64)	10 (100)	36 (77)	495
C[F]	279 (99)	495 (100)	–	542 (38)	174 (100)	95 (100)	392 (100)	133 (91)	10 (100)	42 (89)	827
D[F]	275 (98)	341 (69)	542 (66)	–	174 (100)	95 (100)	354 (90)	146 (100)	4 (40)	41 (87)	1416
E[F]	174 (62)	119 (24)	174 (21)	174 (12)	–	95 (100)	165 (42)	95 (65)	3 (30)	31 (66)	174
F[F]	95 (34)	45 (9)	95 (11)	95 (7)	95 (55)	–	91 (23)	53 (36)	3 (30)	25 (53)	95
G[F]	250 (89)	286 (58)	392 (47)	354 (25)	165 (95)	91 (96)	–	125 (86)	6 (60)	41 (87)	392
H[F]	113 (40)	94 (19)	133 (16)	146 (10)	95 (55)	53 (56)	125 (32)	–	3 (30)	29 (62)	146
I[F]	4 (1)	10 (2)	10 (1)	4 (0)	3 (2)	3 (3)	6 (2)	3 (2)	–	6 (13)	10
J[F]	35 (12)	36 (7)	42 (5)	41 (3)	31 (18)	25 (26)	41 (10)	29 (20)	6 (60)	–	47
Total	282	495	827	1416	174	95	392	146	10	47	

Example: 95 seroconverters were classified as EC by definition F of whom 25 (26%) were classified as EC by definition J. Conversely, of 47 seroconverters classified as EC by definition J, 25 (53%) were classified as EC by definition F.
[F]**A**: HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml; **B**: HIV- positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml, **C**: HIV- positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml, **D**: HIV- positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml, **E**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months **F**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml, **G**:HIV- positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml, **H**: HIV- positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml, **I**: HIV- positive ≥10 years, with all measured HIV-RNA <50 copies/ml, **J**: HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml.
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slopes were either level (no strong evidence of CD4 loss) or otherwise with a statistically significant positive slope. Such positive slopes are likely due to short follow-up, chance, or possibly informative censoring, as follow-up is censored for those with a negative slope once ART is initiated [26]. Median HIV-RNA, CD4 values and CD4 slopes during EC status excluding counts within 6 months of seroconversion showed similar results (data not shown).

HIV-RNA and CD4 Values during Total ART-naïve Follow-up

As expected, throughout available ART-naïve follow-up, median HIV-RNA values were generally higher, and CD4 counts lower than those considering only the time spent as EC. Nevertheless, median HIV-RNA throughout ART-naïve follow-up was low, <200 copies/ml for most definitions, and median CD4 values were >500 cells/mm^3 for all definitions (**Table 5**).

Table 4. Estimated hazard ratios comparing non-elite controllers (EC) and unknown to EC for time from estimated HIV seroconversion to a composite endpoint of AIDS, Death, ART, or CD4<350 cells/mm^3 restricting entry to the risk set at 10 years post seroconversion using the CASCADE dataset applied to 10 definitions of EC found in the literature.

Def.	*EC evaluated (experiencing composite endpoint) n (n)*[††]	*HR for time to composite endpoint*[†] *(95% CI)*	*% (IQR) ART-naïve follow-up time classified as EC*
A[F]	46 (4)	12.5 (4.7, 33.6)	100 (78–100)
B[F]	53 (11)	4.6 (2.5, 8.3)‡	100 (78–100)
C[F]	86 (18)	4.8 (3.0, 7.7)‡	99 (72–100)
D[F]	134 (35)	4.0 (2.8, 5.7)‡	97 (71–100)
E[F]	36 (2)	19.0 (4.7, 76.4)	100 (78–100)
F[F]	26 (5)	15.3 (3.8, 61.3)	92 (66–100)
G[F]	60 (9)	7.5 (3.9, 14.5)‡	100 (86–100)
H[F]	56 (22)	2.9 (1.9, 4.4)‡	100 (75–100)
I[F]	4 (1)	3.4 (0.5, 24.0)	100 (100–100)
J[F]	35 (3)	13.2 (4.2, 41.3)	100 (98–100)

[††]Number of Elites making it to 10 years follow up without experiencing composite endpoint and number subsequently experiencing composite endpoint.
[†]Hazard ratios comparing ECs to Non-ECs (including those with unknown EC status) allowing for late entry at 10 years. For each definition, p-values were obtained from unadjusted log-rank test for time to composite endpoint and were all highly significant $p<0.001$.
‡Statistically different HRs compared to definition E, F, and A from 1000 bootstrap replicates. No definitions were statistically different from definition J at $\alpha = 0.05$.
[F]**A**: HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml; **B**: HIV- positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml, **C**: HIV- positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml, **D**: HIV- positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml, **E**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months **F**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml, **G**:HIV- positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml, **H**: HIV- positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml, **I**: HIV- positive ≥10 years, with all measured HIV-RNA <50 copies/ml, **J**: HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml.
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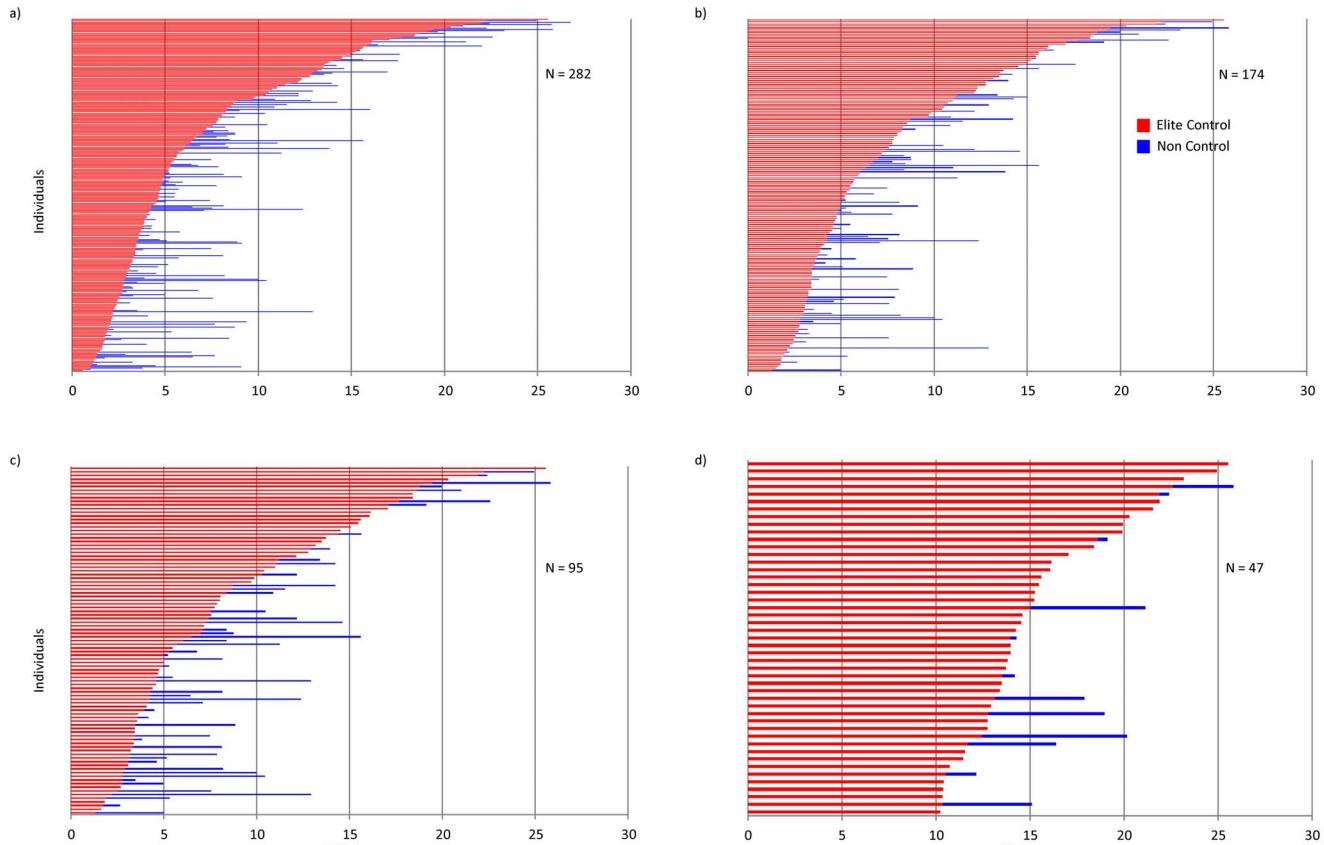

Figure 1. Total ART-naïve follow-up time spent as an elite controller for 4 of the best performing definitions: a) A ǂ, b) E ǂ, c) F ǂ,and d) J ǂ using the CSCADE dataset. ǂA: HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml; **E:** HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months **F:** HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml, J: HIV- positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml.
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Of note, however, CD4 slopes during total ART-naïve follow-up were significantly negative ($\alpha = 0.05$) for all but one definition. HIV-RNA and CD4 values and CD4 slopes showed consistent results when CD4 values within 6 months of seroconversion were excluded (data not shown).

Discussion

Using the large size of the CASCADE dataset we were able to provide reliable estimates of the proportion likely to be elite controllers in an HIV-positive population. Our findings confirm that, by whichever definition, elite control is a rare phenotype likely to comprise around 1% of individuals. This is in line with estimates reported by others [7,8,23] although it should be noted that the choice of denominator may distort the proportion (for example, considering all individuals regardless of their length of follow-up or HIV infection duration will tend to under-estimate this proportion of ECs). Interestingly, we also find evidence that ECs may eventually lose control of viraemia.

Definitions A, E, F and J which require low consecutive or a high proportion of low HIV-RNA measurements are best at capturing individuals with the slowest disease progression. When restricting the dataset to those with 10 years of follow-up, definitions A, E and F and J demonstrated the lowest hazard of AIDS, Death, ART or CD4<350 cells/mm^3 compared to definitions with single measurements or higher levels of viremia. Definition J, with the longest follow-up of 10 years was not significantly different from all other definitions, although this is likely due to low numbers of individuals classified by this definition.

The proportion classified as EC varied according to each definition, with definition D, requiring an HIV-RNA threshold of <2000 copies/ml, classifying the greatest proportion as EC. This definition performed particularly poorly overall with the highest median HIV-RNA and fastest CD4 cell loss while classified as EC and during ART naïve follow-up. Given that the lower limit for available assays has been less than 1000 copies/ml for at least 10 years, inclusion of 2000 copies/ml limit is justifiably termed "viral controllers" rather than EC. The requirement of only one HIV-RNA measurement below a certain threshold (B and C) also resulted in relatively high proportions of EC (2.47% and 4.12%, respectively), agreeing with studies previously reporting proportions of individuals with ≥1 HIV-RNA undetectable [27,28], and suggesting that one undetectable measurement is insufficient in defining EC status. HIV-RNA values were relatively high during EC period for both definitions with the upper quartile experiencing HIV-RNA values >8000 copies/ml. Similarly, even while classified as EC, CD4 cell counts were significantly declining. Thus, the use of at least two HIV-RNA counts results in more

Table 5. HIV-RNA and CD4 values and estimated CD4 slope during elite control (EC), and throughout ART-naïve follow-up using the CASCADE dataset applied to 10 definitions of EC found in the literature.

Def.	*During Elite Control*			*During ART-naïve follow-up*		
	HIV-RNA value	*CD4 Value*	*CD4 slope† (95% CI)*	*HIV-RNA value*	*CD4 Value*	*CD4 slope† (95% CI)*
A[F]	50 (35, 276)	675 (454, 877)	0.04 (0.01, 0.08)	66 (35, 495)	654 (441, 840)	−0.09 (−0.12, −0.06)‡
B[F]	425 (35, 11641)	573 (409, 792)	−0.16 (−0.19, −0.12)‡	1043 (89, 13000)	548 (404, 751)	−0.28 (−0.30, −0.25)‡
C[F]	354 (50, 8700)	596 (427, 796)	−0.18 (−0.21, −0.15)‡	660 (75, 11066)	567 (415, 764)	−0.31 (−0.34, −0.29)‡
D[F]	903 (287, 1863)	615 (478, 789)	−0.27 (−0.29, −0.25)‡	1274 (370, 3304)	590 (451, 756)	−0.43 (−0.45, −0.41)‡
E[F]	50 (35, 81)	699 (528, 922)	0.06 (0.01, 0.10)	50 (35, 165)	681 (527, 909)	−0.06 (−0.10, −0.02)
F[F]	50 (35, 50)	839 (654, 1070)	0.05 (−0.00, 0.11)	50 (35, 77)	796 (629, 1020)	−0.08 (−0.13, −0.03)
G[F]	113 (49, 1197)	644 (439, 824)	−0.06 (−0.09, −0.03)‡	176 (50, 2160)	625 (438, 806)	−0.15 (−0.18, −0.13)‡
H[F]	76 (35, 283)	697 (541, 879)	−0.09 (−0.12, −0.05)‡	89 (35, 356)	687 (530, 879)	−0.23 (−0.26, −0.20)‡
I[F]	35 (1, 35)	583 (575, 905)	−0.07 (−0.24, 0.09)	35 (1, 35)	583 (575, 905)	−0.05 (−0.21, 0.11)
J[F]	50 (35, 127)	783 (628, 970)	−0.03 (−0.09, 0.02)	50 (35, 169)	740 (583, 970)	−0.11 (−0.16, −0.06)‡

Note- all values unless otherwise stated are median (IQR).

†CD4 slope modelled on the square root scale with linear mixed models, specific p-values for CD4 slope and median number of CD4 measurements are presented in Table S1.

‡CD4 slope highly significant $p<0.001$.

[F] Number of total CD4 and HIV-RNA measurements **A**: HIV-positive ≥6 months, with ≥2 consecutive HIV-RNA <75 copies/ml; **B**: HIV- positive ≥1 year, with ≥1 HIV-RNA <50 copies/ml, **C**: HIV- positive ≥1 year, with ≥1 HIV-RNA <75 copies/ml, **D**: HIV- positive ≥1 year, with ≥3 HIV-RNA <2000 copies/ml, **E**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months **F**: HIV- positive ≥1 year, with ≥3 consecutive HIV-RNA <75 copies/ml spanning ≥12 months with no previous blips ≥1000 copies/ml, **G**:HIV- positive ≥2 years, with ≥2 HIV-RNA <75 copies/ml, **H**: HIV- positive ≥5 years, with ≥5 consecutive HIV-RNA <500 copies/ml, **I**: HIV- positive ≥10 years, with all measured HIV-RNA <50 copies/ml, **J**: HIV-positive ≥10 years, with ≥90% of HIV-RNA (≥2 HIV-RNA ever) <400 copies/ml.

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robust measures of stable viraemia and thus captures individuals with lower risk of disease progression.

In contrast, definitions I, J, E and F had the lowest proportion classified as EC varying between 0.15–1.00%. While this is expected for definitions that required ten years of follow-up (I and J), definition E and F, requiring only one year follow-up seemed to capture an equally rare group. The proportion of EC according to definition F was much lower than for definition E, indicating that inclusion of a criterion and threshold for viral blips, as defined by spikes in viral replication and subsequently maintaining control, does impact the group of individuals captured by the definition. This is consistent with studies suggesting that blips are not uncommon among elite controllers. [7,29] In addition to selecting for a rare group, definitions E, F and J also selected groups with the lowest risk for the composite outcome, suggesting that these definitions capture a rare and extreme group on the clinical spectrum of HIV infection. Unsurprisingly, definition I led to the classification of the smallest proportion (0.15%) of EC. This is the most stringent definition as all HIV-RNA measurements needed to have been quantified by assays with a lower limit of detection <50 copies/ml for ≥10 years. The denominator from which this population has been drawn is, by definition, limited to the most recent period when routinely used assays had such low detection limits. Median HIV-RNA and CD4 values for those classified as EC by this definition are based on few measurements and are, therefore, unreliable.

Interestingly, a greater duration of follow-up did not necessarily lead to a more clinically-extreme group of individuals being identified, as definition E and J performed similarly, in spite of E requiring only 1 year of follow-up. There were 16 individuals classified by J but not by E. Six of these 16 were known to have naïve HIV-RNAs between 75 and 400 copies/ml, while HIV-RNA for the remaining 10 individuals were measured using assays with a 400 copies/ml lower detection threshold. Of 143 individuals classified as EC by E but not by J, 113 had <10 years of naïve follow-up. It may, therefore, be that overlap could be greater had all individuals been measured for the same duration with similar assays. In addition, median CD4 counts, HIV-RNA levels and CD4 slopes during the ART-naïve period were also similar for these definitions. This observation may have important implications for the design of future studies, as it seems to suggest that stringent definitions requiring only one year of follow-up, with consecutive undetectable HIV-RNA measurements, can identify an extreme group comparable to that identified by definitions requiring much longer follow-up and higher HIV-RNA threshold. There is, therefore, potential to sampling of participants in such studies from a much wider cohort of individuals.

It is important to note that, despite the fairly low levels of viraemia in individuals classified as EC over extended periods, there was strong evidence of CD4 cell loss, the exception being those classified as EC by definition I, which was based on relatively few measurements. Whether true LTNP status exists remains unknown [30,31]. Our findings lead us to conclude, however, that this is unlikely among elite controllers.

Our study has several strengths. First, the large size of our cohort allows us to make reliable comparisons of different definitions of such a rare group of individuals. Second, the availability of seroconversion information allows for a meaningful assessment of time to clinical outcomes to be made. Finally, and most importantly, until now, examination of CD4 and HIV-RNA changes in studies has been restricted to the period in which HIV-positive individuals fulfil the respective definition. [29,32,33] Due to the detailed information available to us, we were able to study evolution of both these markers over an extended period of follow-up, beyond the duration of EC as defined.

The main limitation to this study is for each definition, the number of individuals with unknown EC classification varied which could have introduced bias in proportion estimates. This is most evident in definition J, requiring >2 HIV-RNA measurements and at least 10 years of follow-up with >19,000 individuals

with either insufficient follow-up or number of HIV-RNA measurements. To examine the impact of missing data on this, for each definition we classified individuals with inadequate information (insufficient number of HIV-RNA measurements or follow-up requirements specified by the definition) as EC and then as non-EC. The proportion of EC; however, may theoretically range from 0.04, if all unknowns are classified as non-EC, to 74%, if all unknowns are classified as EC (data not shown) indicating the difficulties with estimating the true proportion of this group in the presence of missing data. In spite of these possible limitations, our study highlights important differences captured by different EC definitions.

In conclusion, identification of a rare and extreme group may be possible even with definitions requiring a relatively short period of follow-up. We have shown that definitions requiring 6 months or more of follow-up with consecutive measurements requiring HIV-RNA $\leq$75 copies/ml preform just as well as definitions requiring $\geq$10 years follow-up with HIV-RNA measured using assays with a higher detection limit. Although Definition E preforms best overall in terms of percent classified, time to composite endpoint, percent of naïve follow-up time spent as EC, HIV-RNA, and CD4 decline, definition A (2 consecutive HIV-RNA $<$75 copies/ml over 6 months), F (similar to E, but not allowing for blips above 1000 copies/ml) and J (10 years of follow-up with 90% HIV-RNA $<$400 copies/ml) also have their merits. It is unlikely, however, that elite control is an indefinite state, and that the few HIV-positive individuals who spontaneously control HIV replication may eventually need treatment or develop AIDS given the on-going, albeit slow, CD4 cell loss. However, ECs are much less likely to progress to clinical disease compared with non-ECs, and a better understanding of the mechanisms that lead to such control over extended periods may lead to new therapeutic strategies or the development of HIV vaccines.

Supporting Information

Table S1 Number of HIV-RNA and CD4 measurements during elite control and ART naïve follow-up, time from SC to first HIV-RNA and number of HIV-RNA measurements within 6 months of HIV positive test date using the CASCADE dataset from 10 definitions found in the literature. Note- all values unless otherwise stated are median (IQR) †CD4 slope modelled on the square root scale with linear mixed models [F] **A**: HIV-positive $\geq$6 months, with $\geq$2 consecutive HIV-RNA $<$75 copies/ml; **B**: HIV- positive $\geq$1 year, with $\geq$1 HIV-RNA $<$50 copies/ml, **C**: HIV- positive $\geq$1 year, with $\geq$1 HIV-RNA $<$75 copies/ml, **D**: HIV- positive $\geq$1 year, with $\geq$3 HIV-RNA $<$2000 copies/ml, **E**: HIV- positive $\geq$1 year, with $\geq$3 consecutive HIV-RNA $<$75 copies/ml spanning $\geq$12 months **F**: HIV- positive $\geq$1 year, with $\geq$3 consecutive HIV-RNA $<$75 copies/ml spanning $\geq$12 months with no previous blips $\geq$1000 copies/ml, **G**:HIV- positive $\geq$2 years, with $\geq$2 HIV-RNA $<$75 copies/ml, **H**: HIV- positive $\geq$5 years, with $\geq$5 consecutive HIV-RNA $<$500 copies/ml, **I**: HIV- positive $\geq$10 years, with all measured HIV-RNA $<$50 copies/ml, **J**: HIV-positive $\geq$10 years, with $\geq$90% of HIV-RNA ($\geq$2 HIV-RNA ever) $<$400 copies/ml. (DOCX)

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Conceived and designed the experiments: AO LM RT DG AB MS KP. Performed the experiments: AO AB KP LM. Analyzed the data: AO AB RT. Wrote the paper: AO LM MP RT DG MG MC PA AB MS KP.

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Characterisation of long-term non-progression of HIV-1 infection after seroconversion: a cohort study

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Summary

Background Some individuals remain AIDS-free with a high and stable CD4 cell count without antiretroviral therapy (ART) for many years. We estimated long-term progression-free survival after HIV seroconversion and aimed to identify factors associated with loss of long-term non-progression (LTNP) status.

Methods For this cohort study, we used data for individuals with well-estimated dates of HIV-1 seroconversion from the CASCADE Collaboration a network of 28 HIV seroconverter cohort studies in Europe, Australia, Canada, and sub-Saharan Africa. The first cohort began enrolling patients in 1979, and for this analysis we used data pooled in May 1, 2011. We defined non-progression as being HIV-positive without AIDS, ART-naive, and with CD4 counts of 500 cells per μL or higher. We defined LTNP as non-progression during the first 10 years after seroconversion. We used longitudinal methods to characterise LTNP.

Findings Of the 4979 HIV seroconverters in our dataset, 3708 (75%) were men. Median time to progression was 2·07 years (95% CI 1·96–2·17), giving estimated progression-free survivals of 18·4% (17·2–19·6) 5 years, 4·0% (3·6–4·5) 10 years, and 1·4% (0·9–1·5) 15 years after seroconversion. The rate of progression did not change beyond 10 years after seroconversion (0·28 [95%CI 0·26–0·31] per person-year at 10 years after seroconversion, 0·24 [0·19–0·29] per person-year at 15 years, and 0·18 [0·10–0·33] per person-year at 20 years). At 10 years since HIV seroconversion, 283 individuals had LTNP, of whom 202 subsequently lost this status (median time to loss of status 2·49 years [2·05–2·92]). In univariable analyses, loss of LTNP status was associated with CD4 cell count at 10 years after seroconversion (p<0·0001) and HIV RNA load at 10 years after seroconversion (p=0·005), but not age (p=0·544), mode of infection (p=0·621), sex (p=0·676), or calendar year of seroconversion (p=0·397). In the multivariable analyses, loss of LTNP status was associated with lower CD4 counts at 10 years after seroconversion (p<0·0001). After exclusion of CD4 cell counts from the model, higher HIV RNA load at 10 years after seroconversion was independently associated with loss of LTNP status (p=0·009).

Interpretation Progression-free survival is rare. Most individuals with LTNP eventually lose immunological and clinical control of HIV infection eventually.

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See Comment page e8

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Introduction

Before the advent of combination antiretroviral treatment (ART) in 1996, median time from primary HIV infection to the development of AIDS ranged from 5 years to 11 years.[1] With widespread use of combination ART, this period has lengthened substantially. Some individuals remain AIDS-free with a high and stable CD4 cell count without ART for many years.[2] In the mid-1990s, much research focus was on studying such individuals with long-term non-progression (LTNP). Since viral load measurements became available,[3] this interest has shifted to individuals who were able to naturally suppress the virus (known as HIV-controllers or elite controllers).[4] Findings from basic science studies of biological samples from these patients might yield important information about the correlates of control of infection—this information could be beneficial for the development of therapeutic vaccines.[5]

Studies of LTNP are difficult to compare because of heterogeneity in the definitions of non-progression, study design, and lengths of follow-up.[6–10] Moreover, these studies are often limited by small sample size and missing information about date of seroconversion. Additionally, LTNP has generally been established cross-sectionally at a fixed time (eg, at 8 years after infection).[6,7] Some researchers have suggested some people with LTNP have slow progression:[11] these people eventually have disease progression rather than being a distinct subpopulation able to naturally control the development of HIV infection.[12,13] Moreover, how rare or common non-progression is beyond 10 years after infection is unknown, as is whether individuals with LTNP have no signs of HIV disease progression with continued follow-up.

The CASCADE Collaboration is, to our knowledge, one of the largest groups of HIV-positive individuals worldwide with known dates of HIV seroconversion, of diverse risk groups, and with long (>10 years) follow-up. As such, the study provides a unique opportunity to study LTNP. We therefore examined the probability of progression-free

	Total study population (N=4979)	Individuals with LTNP (N=283)
Total follow-up (person years)*	12 478·2	2742·3
Median age at HIV seroconversion (years)	28·4 (24·1–34·0)	26·7 (22·9–31·1)
Mean (SD) age at HIV seroconversion (years)	29·8 (8·2)	28·0 (7·5)
Route of HIV infection		
Sex between men	2371 (48%)	115 (41%)
Sex between men and women	1088 (22%)	65 (23%)
Injection drug use	1035 (21%)	74 (26%)
Contaminated factor VIII given to men with haemophilia	74 (2%)	4 (1%)
Mixed route or other†	312 (6%)	21 (7%)
Unknown	99 (2%)	4 (1%)
Sex		
Male	3708 (75%)	204 (72%)
Ethnic origin		
Same country where enrolment took place‡	3084 (62%)	182 (64%)
Western Europe, different to country of enrolment	631 (13%)	50 (18%)
Outside western Europe, different to country of enrolment	250 (5%)	13 (5%)
Unknown	1014 (20%)	38 (13%)
Median calendar year of HIV seroconversion	1992 (1989–1996)	1990 (1987–1993)
Hepatitis C virus infection ever		
No	1202 (24%)	55 (19%)
Yes	1162 (23%)	87 (31%)
Unknown	2615 (53%)	141 (50%)
CD4 cell count characteristics§		
Total median number of measurements since seroconversion	22 (10–38)	19 (11–28)
Median number of measurements during follow-up	2 (1–5)	9 (4–18)
Median time in years between measurements during follow-up	0·40 (0·25–0·56)	0·47 (0·30–0·64)
Median first count after HIV seroconversion (cells per μL)	700 (590–875)	840 (690–1078)
Median lowest count in the first 10 years after HIV seroconversion (cells per μL)	315 (180–468)	629 (567–740)
Median maximum count in the first 10 years after HIV seroconversion (cells per μL)	881 (713–1115)	1090 (825–1342)
Median count at 10 years after HIV seroconversion (or latest before)	..	756 (627–971)
HIV RNA load characteristics§ ¶		
Median number of measurements in first 10 years after HIV seroconversion	12 (5–20)	6 (2–13)
Median first load (log_{10} copies per μL)	4·04 (3·30–4·65)	3·20 (2·70–3·84)
Median lowest load in the first 10 years after HIV seroconversion (log_{10} copies per μL)	2·30 (1·69–3·14)	2·76 (2·26–3·36)
Median maximum load in the first 10 years after HIV seroconversion (log_{10} copies per μL)	4·77 (4·15–5·29)	3·79 (3·25–4·36)
Median load at 10 years after HIV seroconversion or latest before (log_{10} copies per μL)	..	3·43 (2·70–4·13)

Data are n (%) or median (IQR) unless otherwise stated. LTNP=long-term non-progression. *Since cohort enrolment or first CD4 cell count measurement 6 months or more after seroconversion (whichever was later) until event or censoring. †Mixed route (ie, sex between men and injection drug use; injection drug use and heterosexual contact) or other (eg, nosocomial infection). ‡Several European countries and Canada. §Excludes CD4 cell count measurements and HIV RNA measurements in the first 6 months after HIV seroconversion. ¶Missing values: 1040 for total study population and 87 for individuals with long-term non-progression.

Table 1: **Baseline characteristics**

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survival, the rate of loss of non-progression status, and epidemiological characteristics of those with LTNP at 10 years after HIV seroconversion. We also assessed the probability of an individual retaining an LTNP status and the factors associated with loss of LTNP status after 10 years of HIV infection.

Methods

Study population

Concerted action on seroconversion to AIDS and death in Europe (CASCADE) is a collaboration within EuroCoord), a network of 28 HIV-1 seroconverter cohort studies in Europe, Australia, Canada, and sub-Saharan Africa. All collaborating cohorts received approval from their regulatory or national ethics review boards (appendix). Details of CASCADE are described elsewhere.[14] Briefly, CASCADE data comprise 25 629 HIV-positive individuals who had their seroconversion date estimated by the midpoint between a last negative and first positive test separated by a maximum of 3 years (n=21 670 [85%]), the date of laboratory evidence of seroconversion (n=3231 [13%]), the date of seroconversion illness together with negative and positive tests separated by a maximum of 3 years (n=522 [2%]), or the most likely date that infected factor VIII concentrate infusion for men with haemophilia was given (n=206 [1%]; we used a back-calculation method to estimate the time the infected blood product was used). The first cohort began enrolling patients in 1979, and for this analysis we used data pooled in May 1, 2011.

For these analyses, we included individuals aged 15 years or older at seroconversion who had at least two CD4 cell count measurements 6 months or more after seroconversion, of which at least one was in the first 10 years after seroconversion. The estimated date of seroconversion had to be at least 10 years before the administrative censoring date of each individual cohort to allow for potential follow-up beyond 10 years.

Definition of long-term non-progression

We defined non-progression as being HIV-positive and AIDS-free, ART-naive, and never having a CD4 count below 500 cells per μL. We defined the end of non-progression status as ART initiation, development of an AIDS event, or first measurement of a CD4 count below 500 cells per μL, whichever occurred first. Because CD4 counts might drop sharply soon after seroconversion and subsequently rebound,[2] we excluded those measured in the first 6 months after HIV seroconversion. We defined LTNP as non-progression during the first 10 years after seroconversion. AIDS diagnosis was based on the Centers for Disease Control revised case definition.[15]

Statistical analyses

We calculated follow-up from HIV seroconversion until the date of event or censoring. Individuals were included in the risk set from the later date of cohort enrolment or first CD4 cell count measurement 6 months or more after seroconversion. We censored follow-up at the date when individuals were last assessed for CD4 cell count or ART,

whichever occurred first. We used Kaplan-Meier methods to estimate the probability of progression-free survival in all individuals and in those who were free of progression at 10 years after seroconversion. To investigate trends in the rate of loss of non-progression status, we estimated hazard rates over time since seroconversion with Poisson regression. Restricted cubic splines were used to allow for smoothly varying trends of the hazard over time. We used a Cox proportional hazards model to identify determinants associated with loss of LTNP status beyond 10 years. Factors considered were age at seroconversion, mode of infection, sex, calendar year of seroconversion, baseline CD4 cell count (first measurement between 6 months and 3 years after HIV seroconversion), lowest CD4 cell count in the first 10 years after seroconversion, CD4 cell count at 10 years from HIV seroconversion (or the latest in the preceding 3 years), baseline HIV RNA load (first measurement between 6 months and 3 years after HIV seroconversion), and HIV RNA load at 10 years of HIV seroconversion (or the latest in the preceding 3 years). We used restricted cubic splines to model the effect of CD4 cell count and HIV RNA. We imputed missing HIV RNA values and values below the detection limit using multiple imputation techniques (appendix). We imputed missing mode of infection (four individuals) at random using the distribution of the corresponding variable. All p values were based on null hypotheses against two-sided alternatives. We regarded p values less than 0·05 as statistically significant.

The robustness of the estimated progression-free survival and retention of LTNP status was checked with sensitivity analyses with respect to the role of CD4 count below 500 cells per μL in the definition of non-progression. We did three separate sensitivity analyses. First, we estimated the time when the CD4 count would have dropped below 500 cells per μL on the basis of individual CD4 slopes (which were based on CD4 counts measured before ART initiation only). We obtained these estimates by fitting a linear regression model for each individual with time as the only covariate. We applied a square-root transformation to the CD4 cell count to better normalise the marker distribution as previously described.[16] We applied left truncation by including individuals in the risk set from their first CD4 measurement onwards. Therefore, individuals were excluded from the analysis if the estimated CD4 value was below 500 cells per μL at the moment of the first measurement. Second, with the same method, we required at least four CD4 cell counts to estimate the individual slopes instead of two. Third, we defined progression as two consecutive CD4 counts below 500 cells per μL rather than one. Therefore, individuals had to have at least three CD4 cell counts. We censored follow-up on individuals who did not have two consecutive CD4 counts below 500 cells per μL at the date of the penultimate count. When an individual had two consecutive CD4 counts below 500 cells per μL, we estimated the time to crossing below the 500 cells per μL threshold by interpolation

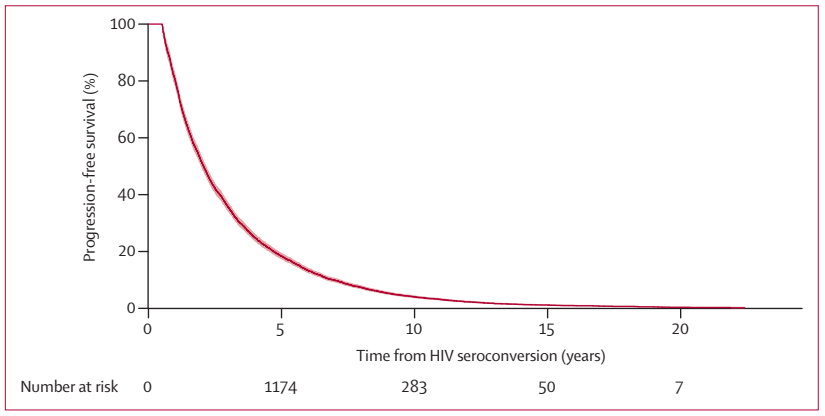


Figure 1: **Kaplan-Meier estimate of the probability of HIV progression-free survival in HIV-positive individuals (n=4979) with a known interval of HIV-seroconversion.**
The shaded area is the 95% CI.

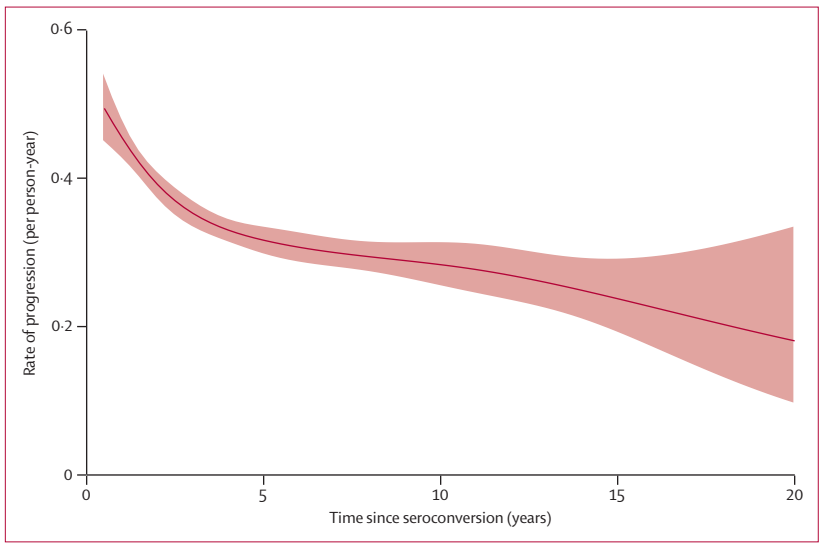


Figure 2: **Rate of progression by year since HIV seroconversion in HIV-infected individuals (n=4979)**
The shaded area is the 95% CI.

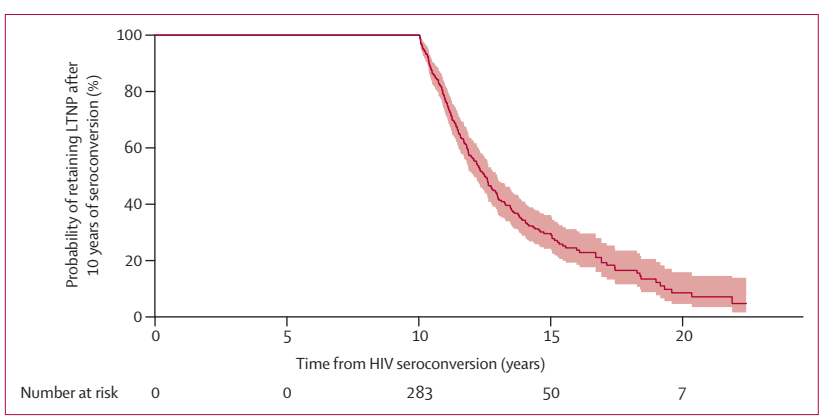


Figure 3: **Kaplan-Meier estimate of the probability of retaining LTNP status after 10 years of HIV-seroconversion**
The shaded area is the 95% CI. LTNP=long-term non-progression.

See Online for appendix

between the first count and the previous one, which, by definition, was 500 cells per μL or higher.

We used SPSS (version 19.0), Stata (version 11.2), and R (version 3.0.1)[17] for the analyses.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Most participants were men and the most frequent route of infection was sex between men, followed by sex

	HR (95 % CI)	p value	Adjusted HR (95 % CI)*	p value	Adjusted HR (95 % CI)†	p value
Age at seroconversion (per 10-year increase)	0·94 (0·77–1·15)	0·544	1·07 (0·83–1·38)	0·588	1·06 (0·83–1·37)	0·622
Age at seroconversion (years)		0·319				
15–24	1					
25–29	0·90 (0·65–1·25)					
30–34	0·66 (0·42–1·04)					
≥35	0·86 (0·55–1·34)					
Mode of infection‡		0·621		0·851		
Sex between men or sex between a man and a woman	1		1		1	0·959
Injection-drug use or receipt of contaminated factor VIII concentrate in people with haemophilia	1·08 (0·80–1·44)		1·04 (0·70–1·54)		0·99 (0·67–1·45)	
Sex		0·676				
Male	1		1	0·197	1	
Female	1·07 (0·78–1·46)		1·29 (0·87–1·22)		1·22 (0·83–1·79)	0·316
Calendar year of HIV seroconversion (per year increase)	0·98 (0·95–1·02)	0·397	0·82 (0·49–1·38)	0·449	0·97 (0·92–1·02)	0·254
Calendar year of HIV seroconversion		0·719				
<1989	1					
1989–1992	0·86 (0·62–1·20)					
1993–1996	0·83 (0·58–1·19)					
≥1997	0·80 (0·35–1·81)					
Baseline CD4 count (cells per μL)§		0·290				
600	1					
900	0·80 (0·52–1·23)					
Lowest CD4 count in the first 10 years after seroconversion (cells per μL)§		0·084				
600	1					
900	0·63 (0·40–0·99)					
CD4 count at 10 years of HIV seroconversion (cells per μL)§		<0·0001		<0·0001		
600	1		1			
900	0·36 (0·23–0·55)		0·39 (0·24–0·62)			
Baseline HIV RNA (log_{10} copies per μL) ‡ §		0·090				
3	1					
4	1·27 (0·85–1·91)					
HIV RNA at 10 years of HIV seroconversion (log_{10} copies per μL) ‡ §		0·005		0·120		0·009
3	1		1		1	
4	1·40 (0·92–2·13)		1·24 (0·81–1·86)		1·38 (0·91–2·13)	

LTNP=long-term non-progression. HR=hazard ratio. *Adjusted for all factors for which adjusted HRs are shown; owing to the strong correlation between the measurements of baseline CD4 count, lowest CD4 count, and CD4 count at 10 years since infection, these three covariates were not included together in the multivariable model; baseline HIV RNA and HIV RNA at 10 years since infection were not included together in the same model. †Adjusted for all factors for which adjusted HRs are shown; CD4 cell count is not included in the model because it is included in the definition of progression; owing to the strong correlation between the measurements baseline HIV RNA and HIV RNA at 10 years since infection these two covariates were not included together in the same model. ‡Missing mode of HIV infection (n=4) and missing HIV RNA values (n=87) were imputed. §HR estimated with restricted cubic splines.

Table 2: **Cox proportional hazard analyses of factors associated with of loss of LTNP status in 283 individuals with known dates of HIV-seroconversion identified as having LTNP at 10 years after HIV seroconversion**

between men and women, injection drug use, and receipt of contaminated factor VIII concentrate in people with haemophilia (table 1). The median year of HIV seroconversion was 1992 and all included individuals were from high-income countries, except for three individuals who were included in cohorts in Africa (table 1).

4246 individuals progressed within 10 years of HIV infection: 3253 with CD4 counts below 500 cells per µL, 729 started ART, 81 had AIDS event, and 183 had a combination of these signs of progression. The median time to progression was 2·07 years (95% CI 1·96–2·17). The estimated progression-free survival probability at a given time since seroconversion was 18·4% (17·2–19·6) at 5 years, 4·0% (3·6–4·5) at 10 years, 1·4% (0·9–1·5) at 15 years, and 0·3% (0·2–0·6) at 20 years (figure 1).

The rate of progression over time since HIV seroconversion decreased sharply in the first years after seroconversion from 0·45 (0·43–0·48) per person-year in the first year to 0·32 (0·30–0·33) per person-year at 5 years after seroconversion (figure 2). Thereafter, the rate continued to decrease, but slowly, to 0·24 (0·19–0·29) per person-year at 15 years and 0·18 (0·10–0·33) per person-year at 20 years after seroconversion.

After 10 years of HIV infection, 283 individuals were classified as having LTNP (table 1). Of these people with LTNP, 202 lost this status after 10 years of HIV seroconversion: 150 because of decrease in CD4 cell count to below 500 cells per µL, 28 started ART, seven had an AIDS event, and 17 had a combination of these signs of progression. Median time to loss of LTNP status was 2·49 years (95% CI 2·05–2·92; figure 3). The estimated progression-free survival in those with LTNP was 29·6% (23·8–35·6) at 15 years from seroconversion and 8·6% (4·3–14·8) at 20 years from seroconversion.

In univariable analysis the loss of LTNP status was associated with lower CD4 cell count and higher HIV RNA load at 10 years after HIV seroconversion (table 2). In multivariable analysis, the loss of LTNP status was independently associated with a lower CD4 count at 10 years after seroconversion (table 2). The adjusted hazard of loss of LTNP status decreased with increasing CD4 cell count (p<0·0001; figure 4). Although not statistically significant, the adjusted hazard of loss of LTNP status seemed to increase with increasing HIV RNA at 10 years after HIV seroconversion (p=0·12; figure 4). We detected no associations between loss of LTNP status and age at HIV seroconversion, route of infection, sex, or calendar year of seroconversion. After exclusion of CD4 cell count from the model because it was part of the definition of non-progression, we noted that a higher HIV RNA at 10 years after HIV seroconversion was independently associated with loss of LTNP status (p=0·009; table 2). When we included baseline CD4 cell counts and HIV RNA in the model instead of the values measured at 10 years after seroconversion, we saw no association between loss of LTNP status and HIV RNA (p=0·18) or CD4 cell count (p=0·72).

The CD4 cell count and HIV RNA trajectories for the seven individuals who still qualified as having LTNP at 20 years after HIV seroconversion were heterogeneous with respect to demographic characteristics; six of them were male, all routes of infection were present, age at HIV seroconversion ranged between 19 years and 33 years, and they were enrolled into six different cohorts.

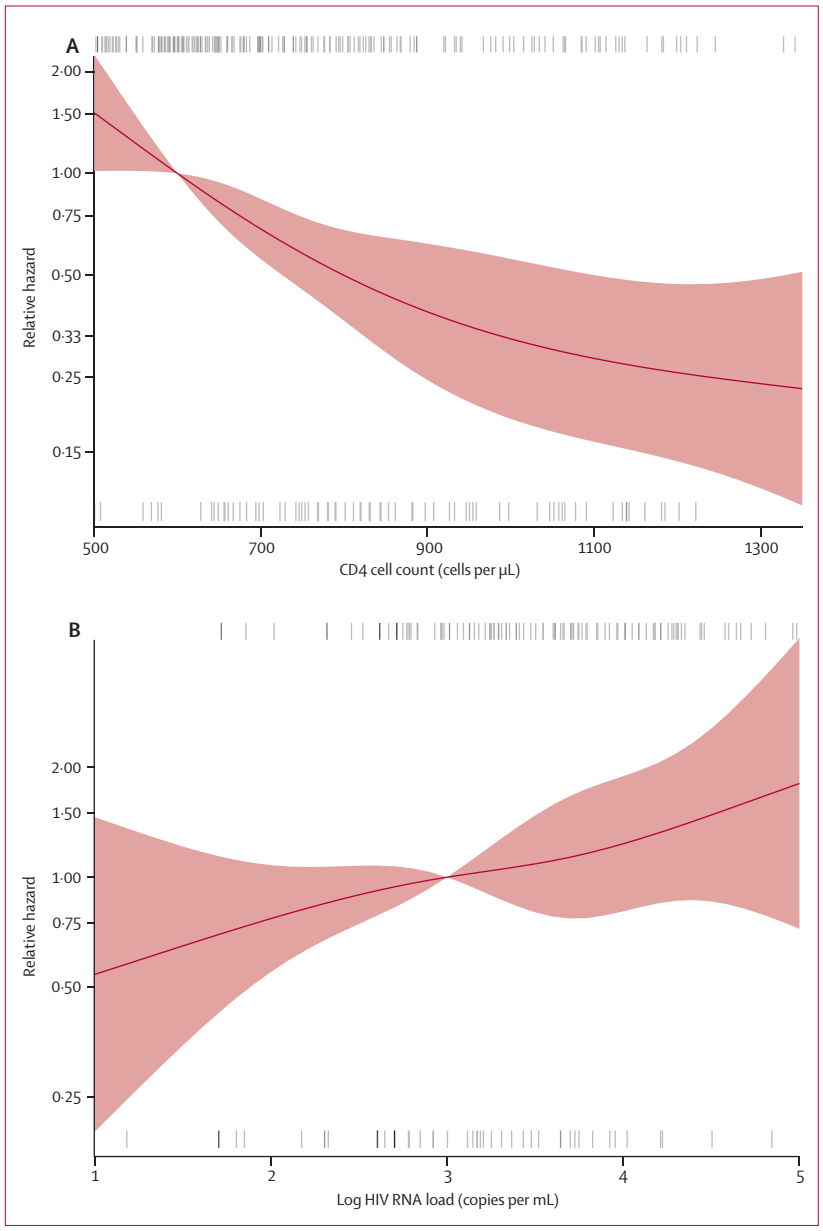
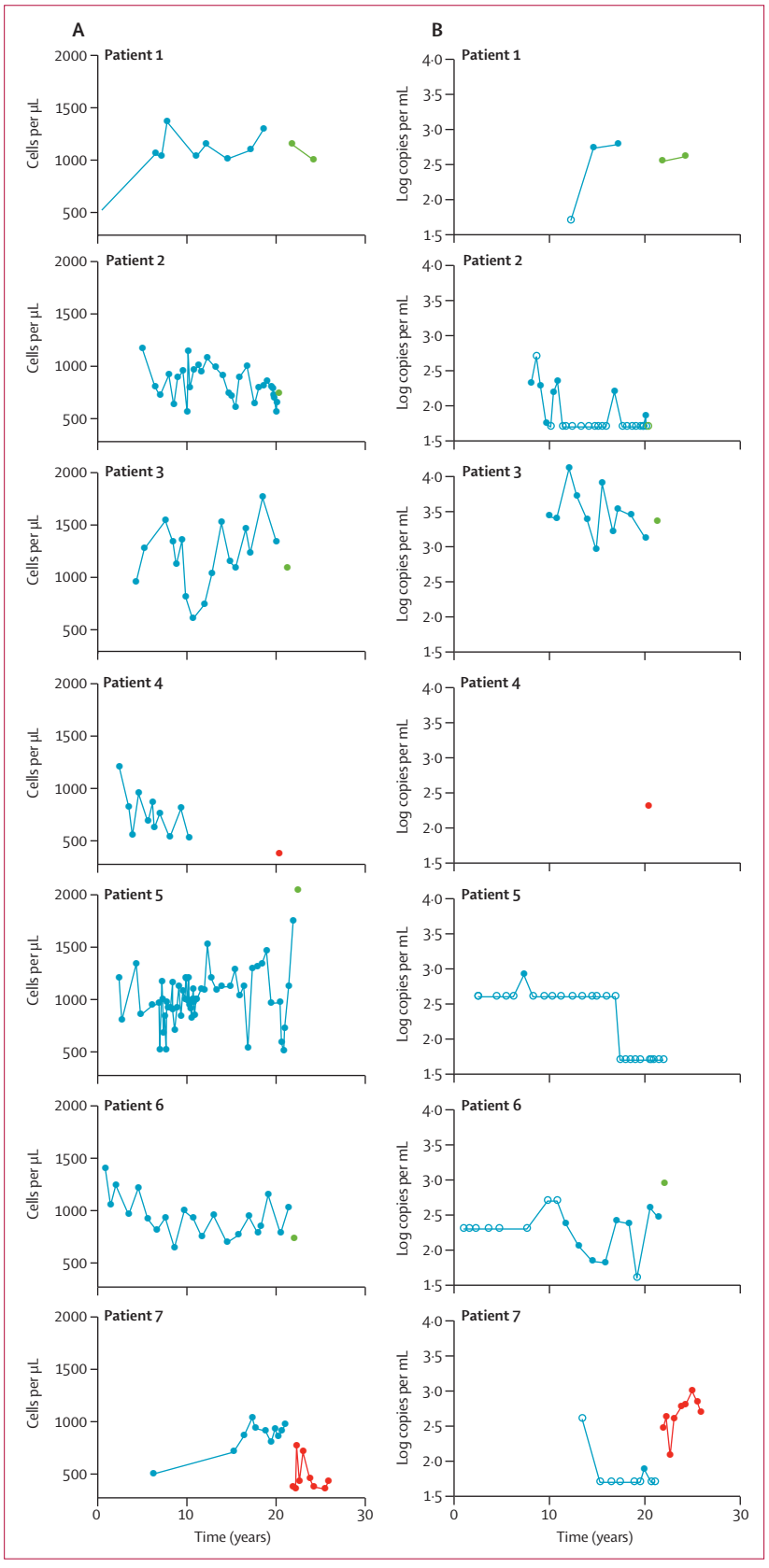


Figure 4: **Association of CD4 cell count (A) and HIV RNA load (B) at 10 years after seroconversion with loss of LTNP**
Data are for 283 HIV-positive individuals with a known interval of HIV-seroconversion who had long-term non-progression (LTNP) at 10 years after HIV seroconversion. The shaded area is the 95% CI. (A) Adjusted hazard of CD4 cell count with reference 600 cells per µL together with rug plot of CD4 cell counts for people with event (top) and without event (bottom). (B) Adjusted hazard of log HIV RNA load with reference 3 log HIV RNA load together with rug plot of RNA values for those with event (top) and without event (bottom).



Two of the individuals subsequently lost LTNP status (individuals 4 and 7; figure 5). Individual 1 had an increase in viral load, which might indicate progression. The other four individuals did not have signs of clinical or immunological progression and their viral load remained stable (figure 5).

In the first and third sensitivity analyses, the median duration to progression and the median progression-free survival times after LTNP were very similar (data not shown). In the second sensitivity analyses, the median progression-free survival time increased slightly to 2·87 years (2·69–3·00). The estimated progression-free survival at a given time from seroconversion was slightly higher at 26·3% (24·7–27·8) at 5 years, 6·7% (6·0–7·5) at 10 years, and 2·5% (2·0–3·0) at 15 years. Median progression-free survival time after LTNP was similar to that seen in the main analysis (data not shown).

Discussion In our large cohort study we found that LTNP is rare, with progression-free survival decreasing rapidly, being about 18% 5 years after seroconversion and about 4% 10 years after seroconversion. To the best of our knowledge, our study is the first to report median time from seroconversion to loss of non-progression, which was 2·07 years (IQR 1·14–3·99). By contrast with our findings, cross-sectional estimates of LTNP from other studies have ranged from 0·2% to 22·3%, depending on the definition used (panel).[6,7,18,19] Estimation of the prevalence of individuals with LTNP, especially when the date of HIV seroconversion is unknown, might result in the exclusion of people with more rapid progression and, therefore, might lead to an overestimation of the prevalence. In our study, the rate of progression decreased rapidly over the first few years after seroconversion but remained constant beyond 5 years. Although our data lend support to previous suggestions that individuals with LTNP are more likely to represent the end of the tail from a distribution than a distinct subpopulation, we cannot exclude the possibility that some individuals will never have disease progression.[8,11,12]

Individuals with LTNP at 10 years after seroconversion were heterogeneous in terms of their demographic characteristics and viral load, as were those who remained free of progression for 20 or more years. To identify underlying mechanisms of LTNP, stringent and uniform definition criteria are important because a small change in definition might have a large effect on the apparent outcome.[6,20] The criteria we used to define LTNP were stringent with respect to follow-up and CD4 cell counts.[6,7]

Figure 5: **CD4 cell counts (A) and HIV RNA loads (B) in seven individuals who remained progression-free for 20 or more years after HIV seroconversion**
(A) Blue dots are values recorded during follow-up, green dots are values recorded after an individual's follow-up was censored, and red dots are values recorded after an event had taken place (CD4 count <500 cells per µL).
(B) Solid dots are values above the detection limit; circles are values below the detection limit of the HIV RNA test.

The results of retention of LTNP status did not change in the sensitivity analyses, and all analyses substantiated the finding that LTNP is uncommon.

Although the reason for the slow—or absent—progression in people with LTNP is unclear, several factors are likely involved,[21,22] including infection with an attenuated virus.[23,24] However, findings from previous studies also suggest that non-progressors are infected with a pathogenic virus, lending support to the idea that host, rather than viral, factors play a large part in the absence of disease progression.[22] Host genetic factors such as CCR5Δ32 deletion and heterozygous HLA-B57 alleles have been described.[21] A genome-wide association study showed five single-nucleotide polymorphisms in class I and III MHC subregions that were associated with LTNP.[5] Few studies have been done of the immunological variables in people with LTNP, and researchers who did a review of available studies recommended the study of T-cell subsets with proinflammatory and anti-inflammatory properties such as Th17 and regulatory T-cells and their role in the preservation of normal CD4 cell counts in those with LTNP.[22] Another review showed the effect of heritability of HIV on viral load.[25] Phylogenetic analysis might, therefore, be of interest to identify any role of heritability of the virus on LTNP.

Only two studies have assessed on the loss of LTNP status by use of data from HIV seroconverters. Findings from a study from San Francisco showed a median time to loss of LTNP status after 10 years of HIV infection of 14 years (95%CI 13·0–14·7), slightly longer than our estimate of 12·5 years (12·1–12·9).[2] The second study, done in France, showed a time to loss of LTNP status similar to ours, but was estimated after 8 years rather than 10 years since HIV infection.[26] Older age at HIV seroconversion is associated with more rapid progression to AIDS and death,[1] as was shown in our study, with age associated with loss of non-progression in the total study population (data not shown). Our findings suggest that once someone had been free of progression for 10 years or longer, age is no longer significantly associated with progression—a finding also seen in the French study.[26]

The identification of people with LTNP might become challenging in the future if the trend towards earlier initiation of combination ART continues; although the individual benefits of earlier ART remain debatable, treatment guidelines now recognise the possibility that the initiation of ART at a very early time during HIV infection to prevent HIV transmission (so-called treatment as prevention) could have public health benefits.[27,28] Progression-free survival in our study was low, indicating that, if earlier start of ART is implemented, for most this earlier start will be a few years, which might be short in comparison with the many years of treatment to follow.

An overlap between the LTNP group and HIV-controllers and elite-controllers has been reported.[18,29] In our study, 30 (21%) of the identified people with LTNP also met the criteria for HIV control, as described in a previous study from CASCADE Collaboration that identified 140 HIV controllers (data not shown).[30]

Limitations of our study included the fact that all individuals, apart from three, were included in high-income countries and that we were not able to estimate ethnicity-specific progression-free survival. Therefore, our results might not be generalisable to other countries. However, both white and non-white individuals were identified as LTNP, suggesting that the possibility to have LTNP is not restricted to one ethnic group. Also, coding imperfection might have occurred and we cannot rule out residual and unmeasured confounding.

Although lifetime natural control of HIV is unlikely, further studies of host immunity and genetics using biological samples of the few individuals with durable control might help in the development of therapeutic vaccines.

Panel: Research in context

Systematic review

We searched PubMed using the search term "HIV" with "long-term non-progression", "long-term non-progressor", "LTNP", "long-term survivor", and "survivor". Most retrieved studies of long-term non-progression (LTNP) were from the mid-1990s. When viral load measurements became availabe, most studies were in HIV controllers and elite controllers. The few, more recent (in the past 5 years), studies of LTNP were included in a 2013 review[8] that concluded that prevalence estimates differ widely because they partly depend on the required period of follow-up. Also, very few individuals defined as LTNP have been followed up beyond 8 years of seroconversion and all of those who were followed up remained without any evidence of disease progression. Most studies report prevalence estimates of LTNP. We identified no study that reported median time from HIV seroconversion to loss of non-progression status.

Interpretation

Findings from our large study of long-term non-progression in HIV seroconverters with more than a decade of follow-up showed that progression-free survival after seroconversion is low (median time to loss of status 2·07 years and 18% at 5 years after infection). This finding is of interest in this era with ongoing debate over the benefits of early initiation of antiretroviral treatment. Most individuals with LTNP will lose immunological and clinical control of HIV infection eventually. Progression-free survival is a rare but real occurrence. Studies of host and viral factors of these individuals with LTNP might yield important information about the correlates of control of infection.

Contributors

JvdH obtained data, contributed to the design of the study, drafted the paper, and did the statistical analysis, analysis, and interpretation of data. RG obtained data, contributed to the design of the study, did statistical analysis, analysis, and interpretation of data, critical revised the paper, and supervised analysis. SL obtained data, revised the paper, and did the statistical analysis, analysis, and interpretation of data. LM, HS, BGB, AdAM, AO, GT, and CS contributed to the obtaining of data, analysis and interpretation of the data, and revision of the paper. KP obtained data, coordinated the data pooling, analysis, and interpretation of data, revised the paper, and obtained funding. MP obtained data, contributed to study concept and design, did the analysis and interpretation of data, provided important input for the drafting and

revision of the paper, and supervised the study. All authors approved the final version of the paper.

Declarations of interest
We declare no competing interests.

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OPEN

Evaluation of Rapid Progressors in HIV Infection as an Extreme Phenotype

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Design: Rapid CD4 cell loss represents an HIV phenotype used to identify causal variants of accelerated disease progression. The optimal rate and threshold for identifying this extreme phenotype in recently infected individuals is unclear.

Methods: Using a cohort of patients with known dates of HIV-1 seroconversion (SC), CASCADE (Concerted Action on SeroConversion on AIDS and Death in Europe), we identified proportions experiencing nadir CD4 cell levels within 1 year of SC, and assessed their mean AIDS-free survival time at 10-year follow-up and hazard of AIDS/death, compared with those whose CD4 remained >500 cells per cubic millimeter. Follow-up was censored at December 31, 1996 to avoid bias due to combination antiretroviral therapy initiation.

Results: Of 4876 individuals, 2.8%, 7.3%, and 24.9% experienced ≥1 CD4 <100, 200, and 350 cells per cubic millimeter, respectively, within 1 year of SC. Minimum CD4 levels of 30, 166, 231, and 506 cells per cubic millimeter were experienced during this period by 1%, 5%, 10%, and 50% of individuals, respectively. Mean (95% confidence interval) AIDS-free survival at 10 years follow-up was 2.9 (2.3 to 3.6), 5.5 (5.0 to 6.1), 6.7 (6.5 to 7.0), 7.4 (7.2 to 7.6), and 8.1 (7.9 to 8.3), for those with minimum counts ≤100, 100–200, 200–350, 350–500, >500 cells per cubic millimeter, respectively. Using counts of >500 cells per cubic millimeter as reference, the hazard ratios (95% confidence interval) of AIDS/death were 15.0 (11.9 to 18.9), 3.6 (2.9 to 4.5), 2.1 (1.8 to 2.4), and 1.5 (1.3 to 1.7), respectively. The hazard ratio increased to 37.5 (26.5 to 53.1) when a minimum CD4 count <100 was confirmed within 1 year of SC.

Conclusion: At least 1 CD4 ≤100 cells per cubic millimeter within the first year of SC identifies a rare group of individuals at high risk of disease progression and could form the basis for defining the rapid progressor phenotype.

Key Words: HIV, rare phenotype, disease progression, genetics

(*J Acquir Immune Defic Syndr* 2014;67:15–21)

INTRODUCTION

Rapid HIV disease progression is an extreme HIV phenotype, although there is little consensus on a definition. Differences in HIV disease progression can be assessed by variability in biomarkers related to HIV disease, the 2 most common of which being CD4 and HIV-RNA. Variability in HIV-RNA, specifically low-circulating HIV-RNA, has defined those with slow disease progression, termed long-term nonprogressor or elite controller phenotypes, which are of particular importance to vaccine studies.[1–10] Rapid progression, however, is equally important as it also contributes to our understanding of early risk factors of disease progression. This may in turn help optimize the frequency of clinical monitoring and antiretroviral therapy initiation.

In addition to the well-documented relationship between slow disease progression and low HIV-RNA, there is also known variation in CD4 levels at or shortly after seroconversion (SC). A number of studies to date have defined rapid progression based on various levels of immunosuppression,[11–21] but it is not yet clear if this variability in CD4, 1 early measure or consecutive low CD4 measurements do, indeed, constitute rapid progression and how this rare phenotype should be defined.

The CASCADE (Concerted Action on SeroConversion on AIDS and Death in Europe) Collaboration, of HIV-positive individuals followed-up since HIV SC offers a unique opportunity to evaluate HIV rapid progression. Using data from CASCADE, we aim to document low CD4 near SC and examine HIV rapid disease progression. This work provides

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For a complete list of CASCADE Collaboration in EuroCoord, see Appendix 1.

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the basis for choosing a definition appropriate to the objectives of future research on this extreme phenotype.

METHODS

Study Population

We used data from the CASCADE 2011 data release in EuroCoord (www.EuroCoord.net), which consists of 25,629 seroconverters from 28 cohorts across Europe, Canada, Australia, and sub-Saharan Africa.[22] Date of HIV SC were estimated by various methods, most commonly as the midpoint between the last documented HIV-negative and the first positive HIV antibody test dates with an interval of <3 years between the 2 test dates (85%). For the remainder, date of SC was estimated through laboratory evidence of SC (polymerase chain reaction positivity in the absence of HIV antibodies or antigen positivity with fewer than 4 bands on Western blot) (13%), or as the date of a SC illness (2%) with both an earlier documented negative and a later positive HIV test not more than 3 years apart. All cohorts contributing data to CASCADE received approval from their individual ethics review boards.

Rapid Progression

Because of the inconsistency of definitions described in the literature,[11–21] with nadir CD4 cell counts ranging between 200 and 500 cells per cubic millimeter and follow-up ranging between 6 months and 8 years, we sought to identify those at highest risk of disease progression by severity of immunosuppression in early infection. We evaluated the frequency of low CD4 counts during the first year after SC and estimated the mean survival time at 10 years of follow-up and hazard of AIDS/death by nadir CD4 levels compared with individuals whose CD4 measurements remained above such levels during that period.

Statistical Methods

For all estimates, we considered only data from individuals who met the requirements for length of follow-up and minimum number of CD4 measurements. More specifically, only individuals with at least 1 CD4 count measured within the first year of SC could contribute information to assessing the risk associated with experiencing any specific nadir CD4 cell count within 1 year.

To provide estimates of the prevalence of low CD4 near SC, we calculated proportions experiencing various nadir CD4 levels within the first year of SC by mode of HIV transmission and age categories and plotted the cumulative proportion of individuals experiencing different nadir CD4 levels.

We used Cox proportional hazards models to estimate the relative hazard of AIDS/death among eligible individuals. As the nonproportionality assumption was not met in 3 of the 4 models, we used log-rank P values.[23] We estimated restricted mean AIDS-free survival times (the area under the length of AIDS-free survival curves) at 10 years of follow-up using clinically relevant CD4 categories (<100, 100–200, 200–350, 350–500, and >500 cells/mm^3) using pseudovalues, as described previously.[23,24] The follow-up time of 10 years was chosen as this is the median time to AIDS in the pre–combination antiretroviral therapy (cART) era for individuals infected between 25 and 35 years of age and was close to the last observed event time.[25] We also used fractional polynomials to explore the relationship of nadir CD4 measurements to the hazard of AIDS/death and restricted mean AIDS-free survival.[26] For all models, we adjusted for the following potential confounders: sex, mode of HIV transmission, age at SC, and year at SC. Age and year of SC were modeled using restricted cubic splines with 3 knots.[27] For all analyses, follow-up was censored at the earliest of AIDS or death date or on December 31, 1996 to avoid bias because of treatment initiation. AIDS was defined using the European case definition, which excludes CD4 <200 cells per cubic millimeter.[28]

In sensitivity analyses, we investigated the proportions experiencing nadir CD4 measurements within 6 months of SC

TABLE 1. Baseline Characteristics for (A) 4876 Individuals With ≥1 CD4 Cell Measurement(s) Within 1 year of SC Included in Analysis and (B) 6084 Individuals Not Included in the Analysis but Seroconverting in the Pre-cART Era Using the CASCADE Data Set

	A	B
Risk category, n (%)		
MSM	2564 (53)	2612 (43)
MSW	1026 (21)	1741 (29)
IDU	1085 (22)	1243 (21)
Other/unknown	201 (4)	486 (7)
Sex, n (%)		
Male	3798 (78)	4638 (76)
Female	1078 (22)	1446 (24)
SC year		
Median (IQR), yrs	1992 (1989–1994)	1991 (1988–1994)
SC age		
Median (IQR), yrs	29 (25–35)	28 (24–33)
<20, n (%)	239 (5)	454 (7)
≥20–30, n (%)	2481 (51)	3377 (56)
≥30–40, n (%)	1479 (30)	1613 (27)
≥40, n (%)	677 (14)	640 (11)
Geographical origin, n (%)		
Europe	3652 (78)	4765 (75)
Africa	117 (3)	170 (2)
Americas	50 (1)	77 (1)
Unknown/Other	1057 (18)	1072 (22)
Time from SC to nadir CD4: median (IQR), mo	7.4 (4.5–9.8)	—
Time from SC to first CD4: median (IQR), mo	5.2 (3.0–7.9)	—
Number of CD4 counts: median (IQR)	2 (1–2)	—
Follow-up time: median (IQR)	3.8 (1.9–6.1)	—

IDU, injection drug users; MSM, men who have sex with men; MSW, heterosexual contact.

and the hazard of AIDS/death observed by these minimum levels. We also investigated if confirmed CD4 measurements (ie, 2 counts) had an impact on the proportion, mean AIDS-free survival times, and hazard of AIDS/death within 6 months and 1 year of SC. Additionally, as the individuals in CASCADE are geographically diverse, we stratified all analysis by geographical origin. Analyses were conducted using Stata/IC 13.0.

RESULTS

Baseline Characteristics

Of 25,629 seroconverters, 20,753 were excluded for following reasons: 14,669 seroconverted after 1997, 6074 had no CD4 measurements within the first year of SC, 6 had an unknown AIDS date and 4 were <15 years of age at SC. Of the remaining 4876 individuals who were studied, 53% were men infected through sex between men (men who have sex with men), 21% through heterosexual contact, 22% through injection drug use, and the remainder were hemophiliacs or with unknown risk categories. The majority (78%) were male seroconverting at a median [interquartile range [IQR]) 29 (25–35) years old between 1982 and 1996. Median (IQR) time from SC to the lowest CD4 was 7.4 (4.5–9.8) months. Geographical origin was predominately European (78%) with few individuals from Africa (3%) and the Americas (1%) (Table 1). HIV subtype was missing for >80% of individuals in this analysis, but of those with known subtype, the data comprised mainly subtype B (>90%).

Baseline characteristics of the 6084 individuals seroconverting in the pre-cART era excluded from this analysis and the sensitivity analysis were similar to the 4876 individuals included in this analysis (Table 1) (see **Table S1, Supplemental Digital Content**, http://links.lww.com/QAI/A542).

CD4 Near SC

Median (IQR) initial CD4 count during the first year of SC was 550 (384–726). A total of 138 (2.8%), 356 (7.3%), and 1213 (24.9%) experienced at least 1 CD4 below 100, 200, and 350 cells per cubic millimeter, respectively, in the first year of SC (Fig. 1, Table 2). About 1%, 5%, 10%, and 50% of individuals experienced at least 1 CD4 <30, 166, 231, and 506 cells per cubic millimeter, respectively, within the first year of SC (Table 2). Higher CD4 cell levels were experienced by younger individuals and those infected through injection drug use (Table 2).

In sensitivity analyses, data were available from 2641, 2825, and 894 individuals with a confirmed CD4 within 1 year of SC, at least 1 CD4 within 6 months, and a confirmed CD4 within 6 months, respectively. Nadir CD4 percentiles remained qualitatively similar to those obtained from the main analysis (see **Table S2, Supplemental Digital Content**, http://links.lww.com/QAI/A542).

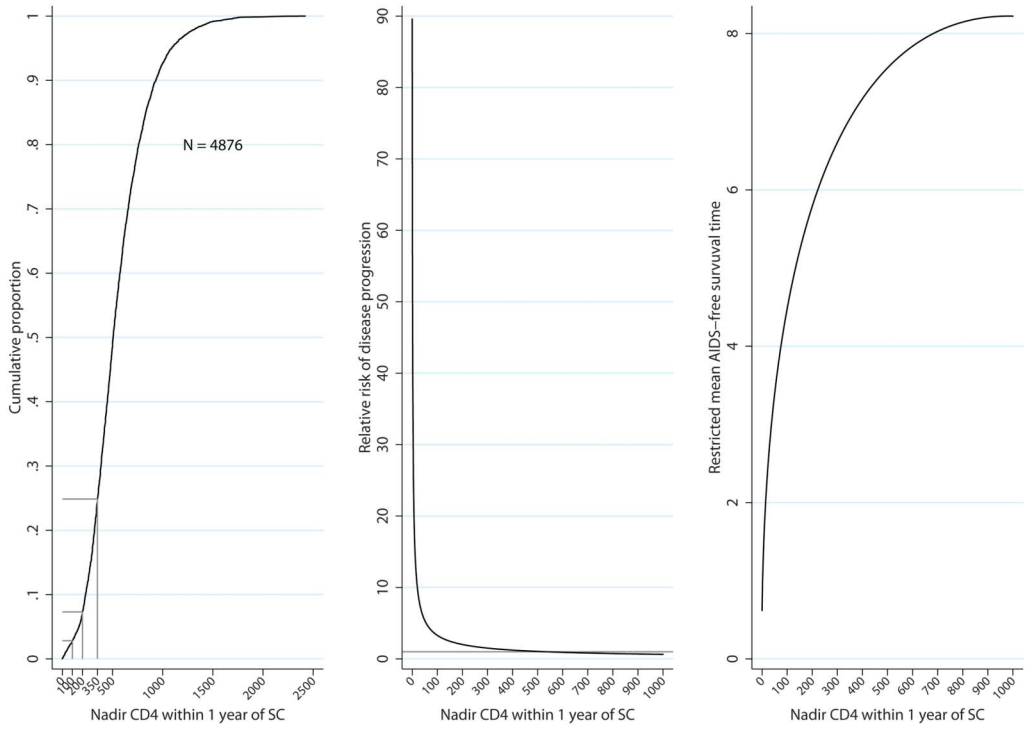

FIGURE 1. Cumulative proportions of nadir CD4 cell count (left hand panel), relative risk of AIDS/death compared with individuals whose CD4 counts remained at 500 cells per cubic millimeter (center panel), and mean AIDS-free survival time at 10 years follow-up (right hand panel) for individuals in CASCADE experiencing specific nadir levels within 1 year of SC during that period: all individuals seroconverted in the pre-cART era.

TABLE 2. Minimum CD4 Percentiles Within 1 year of SC by HIV Risk Group and Age Categories in the Pre-cART Era Using the CASCADE Data Set

	<20	≥20–30	≥30–40	≥40	Overall
1%					
MSM	165	49	27	30	35
IDU	244	53	22	185	40
MSW	154	19	27	23	27
OTH	—	65	0	24	19
Overall	156	49	24	24	30
5%					
MSM	220	168	156	124	161
IDU	318	210	141	240	200
MSW	257	168	135	95	156
OTH	—	136	48	64	112
Overall	257	180	148	118	166
10%					
MSM	270	237	223	213	224
IDU	338	269	220	339	265
MSW	300	255	220	190	225
OTH	—	240	182	192	200
Overall	307	250	220	204	231
25%					
MSM	342	352	340	315	340
IDU	439	425	343	364	405
MSW	440	370	350	268	350
OTH	—	404	289	328	318
Overall	389	378	340	311	352
50%					
MSM	450	500	487.5	441	484.5
IDU	597	600	510	419.5	583
MSW	560	519	491	430	501.5
OTH	—	546	500	474	502
Overall	530	535	491	440	506
75%					
MSM	638	676	650	614	654
IDU	778	825	766	725	815
MSW	840	732	722.5	603	751
OTH	667	726	800	612	721
Overall	717	736	680	612	705.5
90%					
MSM	—	875	842	830	856
IDU	947	1063	1045	1029	1057
MSW	997	979	970	824	950
OTH	842.5	977	1154	800	942
Overall	947	966	907	830	933
100%					
MSM	1250	1704	1875	1584	1875
IDU	1984	2105	1744	2420	2420
MSW	1453	2231	2156	1625	2231
OTH	—	1704	2068	1357	2068
Overall	1984	2231	2156	2420	2420

IDU, injection drug users; MSM, men who have sex with men; MSW, heterosexual contact; OTH, hemophiliacs or unknown.

Predicted Mean Survival Time at 10-Year Follow-Up

The AIDS-free survival expectancy at 10 years follow-up significantly increased as nadir CD4 count measured within the first year of SC increased (Fig. 1, Table 3). Compared with individuals experiencing nadir CD4 counts <100 cells per cubic millimeter within the first year of SC, those with nadir CD4 of 100–200, 200–350, 350–500, and >500 have an increased AIDS-free survival expectancy of 2.6 (1.7–3.4), 3.8 (3.1–4.45), 4.5 (3.8–5.2), and 5.2 (4.5–5.5) years, respectively, during the first 10 years of HIV infection.

In a sensitivity analysis, the AIDS-free survival expectancy was qualitatively similar to results in the main analysis, increasing as nadir CD4 count and confirmed minimum CD4 measurements increased within 1 year and the 6 months of SC (Table 3). Predicted mean survival at 10 years follow-up was qualitatively similar when stratifying by geographical origin (data not shown).

Risk of AIDS/Death by Nadir CD4

The risk of AIDS/death increased as nadir CD4 count measured in the first year decreased. For individuals experiencing at least 1 count <100 cells per cubic millimeter, there was a 15-fold increased risk of AIDS/death compared with those whose nadir CD4 count remained >500 cells per cubic millimeter. Hazard of AIDS/death was significantly higher for those with nadir counts <500 cells per cubic millimeter, (Fig. 1, Table 3).

In sensitivity analyses, the risk of AIDS/death was qualitatively similar to results in main analyses. As expected, however, the risk was greatly elevated for those experiencing confirmed counts, namely; hazard ratio (95% confidence interval) 15.0 (11.9 to 18.9) vs. 37.5 (26.5 to 53.1) for CD4 ≤100 cells per cubic millimeter and 3.6 (2.9 to 4.5) vs. 6.3 (4.5 to 8.8) for CD4 100–200 cells per cubic millimeter comparing a single minimum CD4 count with a confirmed CD4 count within 1 year of SC. This same pattern was observed when comparing nadir CD4 with a confirmed minimum CD4 count within 6 months of SC, (Table 3). There was a similar trend of higher risk of AIDS/death for lower CD4 cell counts when stratifying by geographical origin (data not shown).

DISCUSSION

Individuals experiencing 1 or more CD4 cell count ≤100 cells per cubic millimeter within the first year of SC provide a rare group (2.8%) of HIV-positive individuals at the highest risk of disease progression with remarkably short mean AIDS-free survival of 2.9 years. These results suggest that CD4 monitoring close to SC may play an important role in identifying those at highest risk of progression. In addition to this, individuals with at least 1 CD4 cell count ≤500 cells per cubic millimeter are at an increased risk of AIDS/death compared with individuals whose CD4 remain above 500 cells per cubic millimeter.

We have shown that low CD4 cell counts <100 cells per cubic millimeter near SC is rare, but low CD4 near SC can

TABLE 3. HR for Time to AIDS/Death by Nadir CD4 Measured Within 1 year of SC Using the CASCADE Data Set

	Within 6 Months of SC			Within 1 Year of SC		
CD4 Value, cells/mm^3	N (Fail)	HR (95% CI)*	Mean AIDS-Free Survival (95% CI)†	N (Fail)	HR (95% CI)*	Mean AIDS-Free Survival (95% CI)†
Nadir CD4						
≤100	58 (37)	12.8 (9.0 to 18.2)‡	3.1 (2.1 to 4.1)	138 (94)	15.0 (11.9 to 18.9)‡	2.9 (2.3 to 3.6)
100–200	97 (40)	2.9 (2.1 to 4.1)‡	5.6 (4.7 to 6.5)§	218 (91)	3.6 (2.9 to 4.5)‡	5.5 (5.0 to 6.1)§
200–350	409 (134)	2.3 (1.9 to 2.8)‡	6.3 (5.9 to 6.6)§	857 (267)	2.1 (1.8 to 2.4)‡	6.7 (6.5 to 7.0)§
350–500	607 (180)	1.6 (1.3 to 1.9)‡	7.0 (6.7 to 7.3)§	1185 (316)	1.5 (1.3 to 1.7)‡	7.4 (7.2 to 7.6)§
>500	1654 (359)	1‡	8.0 (7.8 to 8.1)§	2478 (501)	1‡	8.1 (7.9 to 8.3)§
Confirmed CD4						
≤100	14 (10)	49.3 (23.4 to 104.2)	2.1 (0.4 to 3.7)	64 (51)	37.5 (26.5 to 53.1)‡	1.8 (1.1 to 2.6)
100–200	33 (17)	7.4 (4.2 to 13.0)	3.6 (2.3 to 5.0)	92 (40)	6.3 (4.5 to 8.8)‡	4.9 (4.0 to 5.7)§
200–350	118 (36)	2.5 (1.7 to 3.7)	5.6 (4.9 to 6.4)§	342 (106)	2.4 (1.9 to 3.0)‡	6.3 (5.9 to 6.7)§
350–500	186 (61)	1.6 (1.2 to 2.3)	6.1 (5.5 to 6.7)§	577 (171)	1.8 (1.5 to 2.1)‡	6.8 (6.4 to 7.1)§
>500	543 (130)	1	7.3 (6.9 to 7.7)§	1566 (344)	1‡	7.9 (7.7 to 8.1)§

*Adjusted for sex, risk group, SC age, SC year, log-rank test $P < 0.001$.
†Mean AIDS-free survival at 10 years of follow-up.
‡Cox proportional hazards $P < 0.05$.
§Statistically greater than ≤100 cells per cubic millimeter category ($P < 0.001$).
CI, confidence interval; HR, hazard ratio.

have other research implications. Our definition can be useful for researching extreme phenotypes, particularly for genetic studies aiming to identify rare causal variants by looking at extreme ends of HIV disease progression.[29,30] In addition to genetic implications, low CD4 near SC can have impact on HIV incidence measurements. HIV incidence measures such as The Recent Incidence Testing Algorithm aim to identify individuals infected within 4–6 months of sampling but exclude individuals who have AIDS, on ART or are identified with low CD4 as these individuals have been shown to be misclassified as recently infected.[31] Our study suggests that up to 5% of the HIV-positive population tested in the first 6 months of SC will have a CD4 below 200 and thus would be misclassified as longstanding infection according to The Recent Incidence Testing Algorithm. These results suggest the need for an incidence estimate correction factor to account for low CD4 cell counts near SC.

Individuals experiencing confirmed low CD4 measurements <200 cells per cubic millimeter had more than a 2-fold increased risk of AIDS/death compared with minimum CD4 measurement alone. Although this may suggest that a confirmatory CD4 has a higher prognostic value of disease progression than a single CD4 alone, it is unusual for individuals to have a confirmed CD4 so close to SC, shown by our reduced numbers for this population. We were able to analyze repeated low CD4 measurements because these data were restricted to the pre-cART era; however, it is unlikely that in the cART era naive low confirmed CD4 measurements would be available, as all individuals with 1 CD4 <200 cells per cubic millimeter are recommended to be on treatment.

Subtype was missing for >80% of individuals in this analysis, and comprised mainly subtype B (90%), which compared with other HIV subtypes, has previously been shown to have different rates of CD4 cell levels near SC and CD4 rates of decline suggesting these results may not be generalizable to other HIV subtypes.[32] We stratified the analysis by geographical origin and the same trend of higher risk of disease progression with lower CD4 cell counts was observed, suggesting these results are generalizable in different global epidemics.

Our study has several strengths. First, the availability of SC estimation is essential to identifying individuals with rapid disease progression. Without laboratory evidence of SC, individuals entering care with low CD4 would be termed late presenters instead of rapid progressors.[33] Second, the availability of data in an era when ART was not used early in the course of disease allowed us to assess rapid progression without the interaction of ART on disease progression. In the cART era, individuals with CD4 <350 cells per cubic millimeter would be on cART and the impact of low CD4 <100 cells per cubic millimeter near SC would not be fully understood. Finally, the large sample size of our cohort allows us to compare between different possible combinations of this rare phenotype.

Our study has limitations. SC illness and HIV test intervals <31 days have been shown to be associated with faster disease progression,[34,35] suggesting our proportion and risk estimates could be overinflated because of the increased likelihood of individuals seeking care when experiencing SC illness, although the midpoint method of estimating SC was used for 85% of seroconverters. We were unable to test if rapid progressors are more likely to report SC illness, as this is unknown in >70% of the CASCADE data set. However, among 1481 individuals in our study with known SC illness status, > 50% of individuals reported of SC illness with CD4 count <350 cells per cubic millimeter, where <50% of individuals reported no SC illness in those with a CD4 count ≥350 cells per cubic millimeter (data not shown). Although our study only

investigates seroconverters, it has been shown that HIV progression, in particular CD4 decline, among seroconverters is similar to that of the general HIV-positive population suggesting our results are generalizable to the HIV-positive population.[36]

In conclusion, individuals with at least 1 CD4 ≤100 cells per cubic millimeter in the first year of SC are a rare and extreme group who are at a very high risk of rapid disease progression. Given that the HIV test intervals in this study are consistent with HIV testing guidelines,[37–40] our study allows clinicians to identify individuals at risk of progression at an early stage for whom immediate initiation of therapy may be indicated. This study has also helps to identify an extreme HIV phenotype that increases power to detect rare variants in causal viral and host genetics of rapid HIV disease progression. This may, in turn, lead to targeted treatments for individuals at the greatest risk of progression. We suggest future research use at least 1 CD4 ≤100 cells per cubic millimeter within 1 year of SC as a definition for rapid progression.

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APPENDIX 1

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Limiting Cumulative HIV Viremia Copy-Years by Early Treatment Reduces Risk of AIDS and Death

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Background: Viremia copy-years (VCY), a time-updated measure of cumulative HIV exposure, predicts AIDS/death; although its utility in deciding when to start combination antiretroviral therapy (cART) remains unclear. We aimed to assess the impact of initiating versus deferring cART on risk of AIDS/death by levels of VCY both independent of and within CD4 cell count strata ≥500 cells per cubic millimeter.

Methods: Using Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) data, we created a series of nested "trials" corresponding to consecutive months for individuals ≥16 years at seroconversion after 1995 who were cART-naive and AIDS-free. Pooling across all trials, time to AIDS/death by CD4, and VCY strata was compared in those initiating vs. deferring cART using Cox models adjusted for: country, sex, risk group, seroconversion year, age, time since last HIV-RNA, and current CD4, VCY, HIV-RNA, and mean number of previous CD4/HIV-RNA measurements/year.

Results: Of 9353 individuals, 5312 (57%) initiated cART and 486 (5%) acquired AIDS/died. Pooling CD4 strata, risk of AIDS/death associated with initiating vs. deferring cART reduced as VCY increased. In patients with high CD4 cell counts, ≥500 cells per cubic millimeter, there was a trend for a greater reduction for those initiating vs. deferring with increasing VCY (P = 0.09), with the largest benefit in the VCY ≥100,000 copy-years/mL group [hazard ratio (95% CI) = 0.41 (0.19 to 0.87)].

Conclusions: For individuals with CD4 ≥500 cells per cubic millimeter, limiting the cumulative HIV burden to <100,000 copy-years/mL through cART may reduce the risk of AIDS/death.

Key Words: viremia copy-years, seroconverters, when to start, cART initiation, CD4 cell count, HIV-RNA

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INTRODUCTION

Although CD4 cell counts are used routinely to monitor adults with HIV infection, viral loads also have an important role in the monitoring and staging of adults with HIV.[1,2] One or 2 values of an individual's viral load are often used to determine combination antiretroviral therapy (cART) failure, their risk of transmitting HIV to others, and to tailor first-line cART regimens.[3–5] However, assessment of an individual's viral load at a single point in time fails to capture cumulative exposure to HIV replication which may have been over a period of 10 years or more. Several investigators have proposed that a measure of cumulative viral burden might provide useful additional information and, in particular, a measurement of viremia copy-years (VCY) has been proposed.[6] VCY is akin to cigarette pack-years when assessing exposure to tobacco; A VCY of 1000 copy-years/mL is the equivalent to an individual having a viral load of 1000 copies per milliliter for an entire year or a viral load of 500 copies per milliliter for 2 years. The measurement of VCY has been shown to predict death and AIDS in both the absence[6] and presence[7,8] of cART, independently of the individual's most recent CD4 count and viral load. This independent association suggests that cumulative HIV burden is associated with an increased risk of development of clinical events through mechanisms other than immunodeficiency.

United States guidelines recommend immediate cART initiation, regardless of CD4 cell count[5,9] due to evidence that exposure to uncontrolled viremia is associated with an increased risk of death, AIDS, and non-AIDS events.[5,10–12] The START (Starting Antiretroviral Treatment Early Improves Outcomes for

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HIV-Infected Individuals) trial has recently reported that waiting to initiate cART until CD4 <350 cells per cubic millimeter increases the likelihood of serious illness or death compared with immediate initiation.[13] VCY serves as a measurement of cumulative exposure to HIV, and so it is important to determine whether VCY contributes to the likelihood of illness and death and whether cART initiation before the accrual of VCY could help optimize clinical and public health HIV outcomes.

Randomized trials are unlikely to be conducted to determine whether accrual of viremia VCY before cART initiation increases mortality because of the difficulty and expertise in enrolling participants soon after seroconversion, and because cART is now recommended in many asymptomatic populations. In addition, there is substantial potential for lead-time bias in analyses using VCY because of variability in the extent of HIV replication an individual will have been exposed to previous enrollment into care. One way to limit this bias is to restrict analyses to participants with serial viral load measurements since a known seroconversion date; such data are available from the Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) Collaboration, an international multicenter collaboration of data from persons with well-estimated dates of HIV seroconversion. Previous analyses of CASCADE data have shown a protective effect of initiating cART on AIDS/death at CD4 <500 cells per cubic millimeter [hazard ratio (HR) 0.59 (95% CI: 0.43 to 0.81) and HR 0.75 (0.49–1.14) in CD4 cell strata 200–349 and 350–499, respectively], but no evidence for a reduction in risk at CD4 ≥500 cells per cubic millimeter [HR 1.10 (0.67–1.79)].[14] Here we examine the effect of initiating or deferring cART at different levels of VCY on HIV disease progression. We investigate whether or not individuals with CD4 ≥500 cells per cubic millimeter but high VCY would benefit from starting cART.

METHODS

Study Population

Data from CASCADE in EuroCoord (www.EuroCoord.net) 2013 data update were used for this analysis.[15] Briefly, CASCADE is a cohort collaboration of 29 cohorts of individuals with well-estimated dates of HIV seroconversion from Europe (94%), Australia (2%), Canada (0.5%), and Sub-Saharan Africa (3%). Date of seroconversion is estimated as the midpoint between the last negative and first positive HIV antibody test results with a maximum of 3 years between the test dates (85%), laboratory evidence of acute seroconversion (real-time polymerase chain reaction positivity of incomplete Western blot) (13%), the date of seroconversion illness with a negative and positive test no more than 3 years apart (2%), or by a probability distribution to determine the most likely date of transmission for men with hemophilia infected with HIV after transfusion with clotting factor concentrates (<1% of the sample).

All cohorts contributing to CASCADE received ethical approval from their individual ethics review boards.

Adults (≥16 years old) seroconverting in the cART era (post 1995) were included provided they had at least 1 HIV-RNA measurement between 4 and 12 months after seroconversion. Two Sub-Saharan African cohorts were excluded from this analysis as their CD4 cell count and cART initiation patterns are different from those in industrialized country cohorts.[16]

Study Design

We created a series of sequential nested "trials" corresponding to consecutive months of follow-up beginning 4 months after seroconversion, where each month represents the baseline month for a new trial (Fig. 1). As described previously, this approach allows appropriate adjustment for time-dependent confounding.[17,18] We created new trials with all eligible individuals for each month between January 1996 and May 2013. Individuals were eligible for a trial if they were cART-naive before the baseline month, had a CD4 or HIV-RNA measurement 12 months before the baseline month, and were AIDS-free until the end of the baseline month. Time to AIDS/death was compared in those who initiated cART in each baseline month versus those who deferred, pooling across all trials.

AIDS events in the first year of seroconversion were not considered as disease progression outcomes, but rather as severe seroconversion illness. In addition, invasive candidiasis was not considered an outcome in this analysis as it is typically less severe and associated with longer survival compared with other AIDS-defining conditions.[19–22]

Viremia Measurements

If HIV-RNA could be continuously measured within an individual from seroconversion [with the viral load distribution at any time t called as $V(t)$], then VCY would be calculated as the area under the HIV-RNA curve, or the integral of HIV-RNA from seroconversion to time t = T.

$$\mathrm{VCY} = \int_{\mathrm{SC}}^{\mathrm{T}} \mathrm{V}(t)\mathrm{dt}.$$

However, in practice, we do not have continuously measured viral loads, but rather snapshots of HIV-RNA measurements for each individual at irregularly spaced intervals (usually approximately 3 monthly). The best approximation to the integral with the data available can be obtained through use of the trapezoidal rule, which is how we approximated VCY for the remainder of this analysis. At any given time point, J, say, VCY(J) is given by: $\mathrm{VCY(J)} = \sum_{\mathrm{j}=1}^{\mathrm{J}} \frac{t(\mathrm{j})-t(\mathrm{j}-1)*V(\mathrm{j})+\mathrm{V}(\mathrm{j}-1)}{2}$. We examined HIV-RNA data for implausible values and identified 3 individuals whose HIV-RNA dropped by factors of 4, 26, and 87 between consecutive measurements and without apparently starting cART. These are far greater drops than would be expected based on the known biological variation of HIV-RNA.[23,24] As all 3 individuals were recorded as having started cART in the following month, we assumed that the date of cART initiation had been incorrectly recorded, and reset the cART start dates for these individuals to 1 month before that recorded.

To estimate HIV exposure equally for all individuals, we removed HIV-RNA measurements taken in the first 3 months of seroconversion, as we were unlikely to capture the well-documented peak in viremia shortly after seroconversion[25] for

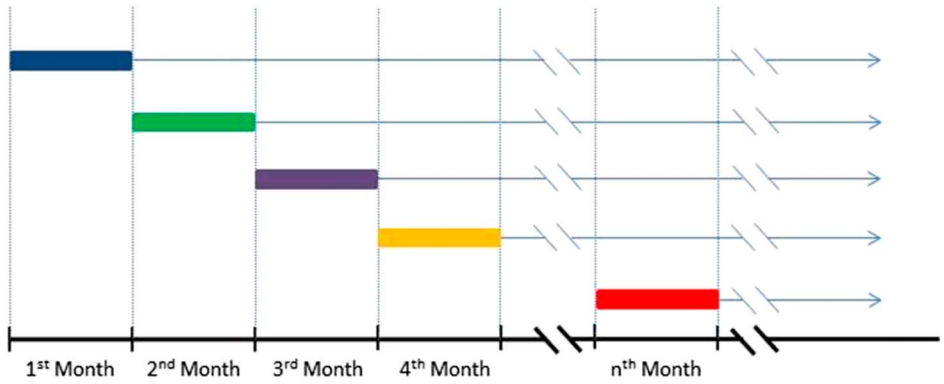

FIGURE 1. A diagram of the "trials" construction. Individuals are assessed for eligibility at the beginning of each month (respective trial baseline). Each eligible individual is classified as having initiated or deferred cART in the baseline month. Time is measured from the beginning of the following month until AIDS, death, or censoring for each eligible individual (excluding any with an outcome during the baseline month). Cox proportional hazards models are used to assess the effect of initiating compared with deferring cART on time to AIDS/death, pooled across all trials.

all individuals. In addition, we assumed that HIV-RNA measurements remained relatively stable over the period 4–12 months (consistent with findings of the viral load stabilizing after the initial peak in viremia), allowing us to make the assumption that an individual's first available HIV-RNA over the period 4–12 months was equal to their HIV-RNA at month 4.

Data Analysis

We describe baseline characteristics between those who initiated or deferred cART during the study period. We estimated the HRs for initiating versus deferring cART by levels of VCY (<10,000 copy-years/mL, ≥10,000–19,999 copy-years/mL, ≥20,000–49,999 copy-years/mL, ≥50,000–99,999 copy-years/mL, ≥100,000 copy-year/mL) pooled across and stratified by CD4 cell count strata (initiate at CD4 <350 cells/mm^3 compared with initiate at higher values, ≥350 cells/mm^3, and initiate at <500 cells/mm^3 compared with initiate at higher values, ≥500 cells/mm^3) using Cox proportional hazards models. We adjusted for trial-independent factors including country of care, sex, HIV transmission risk group, seroconversion year, and trial-dependent factors of current age, time since last HIV-RNA measurement, CD4, VCY, HIV-RNA, and mean number of previous CD4/HIV-RNA measurements per year. Trial-dependent factors were ascertained before the baseline month to ensure they were measured before the decision to initiate or defer cART in the current month. Continuous variables were modeled using restricted cubic splines, all with 3 knots with the exception of current CD4 which was modeled with 5 knots.[26] Most individuals contributed to more than 1 trial, so we used a robust variance estimator to account for within-person correlation. To investigate whether a threshold existed where cART initiation showed the most benefit, we fitted interactions between initiating cART and VCY as a continuous variable with a 3-knot spline.

Furthermore, we investigated whether there was a benefit of incorporating other measures of viremia into the decision about when to initiate cART, namely, current HIV-RNA (most recent measurement), average HIV-RNA (mean of all previous measurements), and maximum HIV-RNA (maximum of all previous measurements). To compare results between all HIV-RNA measurements with VCY, we used the same inclusion criteria for all analyses. We used the Akaike[27] information criteria (AIC), a measure of the relative quality of statistical models which evaluates trade-off between model complexity and goodness of fit, to determine which measure of viremia best fits the data.

RESULTS

Baseline Characteristics

The CASCADE 2013 update contains information on 30,006 individuals, of whom 21,082 seroconverted in the cART era, during or after 1996. Of those, we excluded 916 individuals from African cohorts and 10,813 individuals without at least 1 cART-naive HIV-RNA measurement within 4–12 months of seroconversion, leaving 9353 individuals in the analysis.

Among those seroconverting in the cART era (n = 21,082), men who have sex between men were slightly overrepresented in this analysis compared with those excluded (80% vs. 62%) and those who likely acquired HIV through sex between men and women were slightly underrepresented (9% vs. 29%). Date of seroconversion was later in those included in this analysis [November 2005 (July 2002–August 2008)] than in those excluded [July 2004 (July 2000–March 2008)] explained by availability of routine HIV-RNA measurements within the cohorts. All other baseline characteristics were similar among those included and excluded from this analysis (data not shown).

Of 9353 individuals, 5312 (57%) initiated cART, 326 (3%) acquired AIDS, and 160 (2%) died. Median [interquartile range (IQR) [25th–75th percentile] follow-up was 4.1 (1.8, 7.2) years. Most individuals were men (85%), and modes of HIV transmission included sex between men (71%), sex between men and women (21%), injection drug use (4%), and unknown (4%). Median (IQR) CD4 at cART initiation was 342 (265, 450) cells per cubic millimeter and did not vary by VCY category. Median (IQR) seroconversion age was 33 (27, 40) years between 1996 and 2013. Individuals contributed to a median (IQR) of 21 (13, 36) trials.

Individuals who initiated cART typically had much lower CD4 cell counts and higher HIV-RNA values than those deferring cART. Men were also more likely to defer in the lower viral copy-years strata (Table 1).

Viremia Copy-Years

Pooling across CD4 cell count strata, HRs for the effect of initiating cART compared with deferring on time to AIDS/death significantly decreased as VCY increased (P-trend $<$ 0.001). For example, at times when the VCY was in the range 10,000–20,000 copy-years/mL, there was only a modest 9% reduction in the hazard of AIDS/death associated with immediate initiation of cART compared with deferral [HR = 0.91 (95% CI: 0.57 to 1.46)], whereas at times when the VCY was >100,000 copy-years/mL, the estimated reduction in risk of AIDS/death associated with immediate versus deferred initiation was 56% [HR = 0.44 (95% CI: 0.35

TABLE 1. Baseline Characteristic for Individuals Who Initiated or Deferred cART by Levels of VCY

Characteristic	Initiated cART	Deferred cART
VCY < 10,000 copy-years/mL		
"Trial" observations, N	651	50,349
Follow-up, median (IQR) person-years	3.3 (1.4, 6.8)	4.2 (2.1, 7.0)
Male, N (%)	398 (61)	38,869 (77)
Seroconversion year	2005 (2000, 2009)	2004 (2001, 2007)
Seroconversion age	30 (26, 37)	33 (27, 39)
CD4 cell count median (IQR), mm^3	397 (291, 567)	637 (486, 826)
HIV-RNA copies/mL median (IQR) *	3.7 (3.2, 4.1)	3.4 (3.0, 3.8)
VCY median (IQR) *	3.6 (3.2, 3.8)	3.5 (3.0, 3.8)
VCY ≥ 10,000–19,999 copy-years/mL		
Trial observations, N	488	22,825
Follow-up, median (IQR) person-years	3.0 (1.4, 6.0)	4.0 (2.0, 6.6)
Male, N (%)	382 (78)	19,348 (85)
Seroconversion year	2006 (2002, 2009)	2005 (2002, 2007)
Seroconversion age	33 (26, 40)	32 (27, 39)
CD4 cell count median (IQR), mm^3	360 (282, 475)	553 (437, 707)
HIV-RNA copies/mL median (IQR) *	4.3 (3.9, 4.6)	4.1 (3.7, 4.4)
VCY median (IQR) *	4.2 (4.1, 4.2)	4.2 (4.1, 4.2)
VCY ≥ 20,000–49,999 copy-years/mL		
Trial observations, N	1026	39,675
Follow-up, median (IQR) person-years	3.3 (1.4, 6.6)	3.9 (1.9, 6.5)
Male, N (%)	853 (83)	34,804 (88)
Seroconversion year	2006 (2001, 2008)	2004 (2002, 2007)
Seroconversion age	34 (28, 41)	33 (27, 39)
CD4 cell count median (IQR), mm^3	355 (277, 466)	523 (417, 662)
HIV-RNA copies/mL median (IQR) *	4.6 (4.3, 4.9)	4.3 (3.9, 4.6)
VCY median (IQR) *	4.5 (4.4, 4.6)	4.5 (4.4, 4.6)
VCY ≥ 50,000–99,999 copy-years/mL		
Trial observations, N	950	30,925
Follow-up, median (IQR) person-years	3.1 (1.4, 5.7)	3.8 (1.8, 6.2)
Male, N (%)	837 (88)	27,657 (89)
Seroconversion year	2005 (2002, 2008)	2004 (2002, 2006)
Seroconversion age	33 (27, 41)	33 (27, 39)
CD4 cell count median (IQR), mm^3	340 (272, 440)	492 (393, 628)
HIV-RNA copies/mL median (IQR) *	4.8 (4.4, 5.1)	4.5 (4.2, 4.8)
VCY median (IQR) *	4.9 (4.8, 4.9)	4.8 (4.8, 4.9)
VCY ≥ 100,000 copy-years/mL		
Trial observations, N	2102	44,581
Follow-up, median (IQR) person-years	3.5 (1.6, 5.6)	3.9 (1.8, 6.2)
Male, N (%)	1928 (92)	41,702 (94)
Seroconversion year	2005 (2002, 2007)	2004 (2001, 2006)
Seroconversion age	35 (29, 42)	33 (28, 40)
CD4 cell count median (IQR), mm^3	320 (246, 411)	467 (370, 591)
HIV-RNA copies/mL median (IQR) *	5.2 (4.8, 5.5)	4.9 (4.5, 5.2)
VCY median (IQR) *	5.3 (5.2, 5.6)	5.3 (5.1, 5.5)

*Log_{10} copies per milliliter.
IQR, 25th and 75th percentiles.

to 0.55)], Table 2. Among individuals initiating with CD4 ≥350 cells per cubic millimeter, there was a modest trend ($P = 0.11$) toward a greater benefit of immediate initiation of cART (vs. deferral), although the results continued to suggest some benefit of earlier initiation among the group with VCY >100,000 copy-years/mL [HR = 0.68 (95% CI: 0.49 to 0.94)], Table 2. As expected among individuals initiating with CD4 <350 cells per cubic millimeter, immediate initiation was beneficial in all VCY categories (all HR < 1) (see **Table**, Supplemental Digital Content, http://links.lww.com/QAI/A817).

Modeling initiation of cART by VCY as a continuous variable showed the same trends as the categorical analysis, Figure 2. No obvious threshold of copy-years was found; however, pooling CD4 cell count categories, the upper bound of the 95% CI first fell below one when VCY passed 17,343 copies-years/mL, suggesting that among individuals with VCY values above this threshold, immediate initiation of cART may result in a reduction in the risk of AIDS/death. Stratifying by CD4 cell count, in those with CD4 ≥350 cells per cubic millimeter, the upper bound of the 95% CI fell below one when VCY surpassed 52,826 copy-years/mL, again suggesting that among individuals with high CD4 cell counts and VCY values above this threshold, immediate initiation of cART may result in a reduction in the risk of AIDS/death.

Using a CD4 count threshold of 500 cells per cubic millimeter showed similar results. For those with CD4 ≥500 cells per cubic millimeter, the greatest benefit of initiation was seen when VCY >100,000 copy-years/mL [HR = 0.41 (0.19, 0.87), P-trend = 0.09], Table 2. Modeling VCY continuously, the upper bound of the 95% CI in those with CD4 ≥500 cells per cubic millimeter fell below one when VCY surpassed 38,152 copy-years/mL. In those with a CD4 count <500 cells per cubic millimeter, there was an overall benefit of treatment initiation in VCY categories >10,000 copy-years/mL (see **Table**, Supplemental Digital Content, http://links.lww.com/QAI/A817).

Other Measures of Viremia

Pooling CD4 strata, the HRs for the effect of initiating cART on time to AIDS/death decreased as most recent HIV-RNA increased (P-trend < 0.001) with the largest benefit of initiation seen when current HIV-RNA exceeded 100,000 copies/mL [HR = 0.45 (0.36, 0.57)]. Among individuals with a CD4 count ≥350 cells per cubic millimeter, there was a modest trend (P-trend = 0.08) for an increased benefit of immediate initiation (vs. deferral) as the current HIV-RNA increased, with the largest benefit of immediate initiation seen if the current HIV-RNA was >100,000 copies/mL [HR = 0.65 (0.47, 0.89)], Table 2. Stratifying by CD4, there was a benefit of initiating versus deferring for all individuals with CD4 <350 cells per cubic millimeter regardless of current HIV-RNA level, as expected from the VCY analysis. The same trends were seen when modeling VCY and current HIV-RNA continuously, Figure 2, and when considering average and maximum viremia (data not shown).

Using a CD4 threshold of 500 cells per cubic millimeter, similar results were obtained for the average and maximum viremia (data not shown).

Pooling CD4 strata, model fit was best for VCY (minimum AIC, 230115) compared with current (increase in AIC = 238), average (increase in AIC = 124), and maximum HIV-RNA (increase in AIC = 163). Maximum HIV-RNA fits the model best in the CD4 <500 cells per cubic millimeter strata (minimum AIC, 1024345; increase in AIC = 32, 128, 15 for VCY, current, and average HIV-RNA, respectively, for

TABLE 2. The Effect of Initiation Compared With Deferring cART on Time to AIDS/Death by VCY Alone by CD4 Cell Count Strata (≥350, ≥500 Cells/mm³)

	All Patients			CD4 ≥ 350 Cells/mm³			CD4 ≥ 500 Cells/mm³		
	Events, N	HR (95% CI)	*P*, AIC	Events, N	HR (95% CI)	*P*, AIC	Events, N	HR (95% CI)	*P*
VCY, copy-years/mL									
<10,000	198	1.10 (0.74 to 1.63)	0.001*	181	1.04 (0.63 to 1.73)	0.51*	138	0.81 (0.36 to 1.80)	0.56*
10,000–20,000	202	0.91 (0.57 to 1.46)	<0.001†	175	0.79 (0.40 to 1.58)	0.11†	116	0.96 (0.37 to 2.52)	0.09†
20,000–50,000	260	0.69 (0.50 to 0.94)	230,115.30‡	227	0.88 (0.61 to 1.29)	186,803.80‡	166	0.70 (0.37 to 1.31)	113,258.00‡
50,000–100,000	242	0.56 (0.40 to 0.80)		206	0.60 (0.36 to 1.01)		146	0.45 (0.18 to 1.09)	
>100,000	225	0.44 (0.35 to 0.55)		182	0.68 (0.49 to 0.94)		117	0.41 (0.19 to 0.87)	
Current HIV-RNA, copies/mL									
<10,000	180	1.14 (0.81 to 1.61)	0.001*	167	1.37 (0.89 to 2.09)	0.03*	161	0.86 (0.46 to 1.61)§	0.40*
10,000–20,000	140	0.63 (0.36 to 1.08)	<0.001†	121	0.54 (0.22 to 1.33)	0.08†		—	0.08†
20,000–50,000	211	0.53 (0.37 to 0.76)	230,353.40‡	182	0.55 (0.34 to 0.89)	187,014.80‡	132	0.58 (0.28 to 1.23)	113,454.30‡
50,000–100,000	202	0.62 (0.45 to 0.86)		163	0.80 (0.57 to 1.25)		107	0.61 (0.28 to 1.31)	
>100,000	202	0.45 (0.36 to 0.57)		195	0.65 (0.47 to 0.89)		120	0.38 (0.19 to 0.77)	

*p-heterogeneity (df = 4).
†p-trend (df = 1).
‡AIC, Akaike information criterion.
§By chance, there were no failures among initiators in the CD4 ≥500 cells per cubic millimeter, VCY 10,000–20,000 category, so this category is <20,000 copies per milliliter.

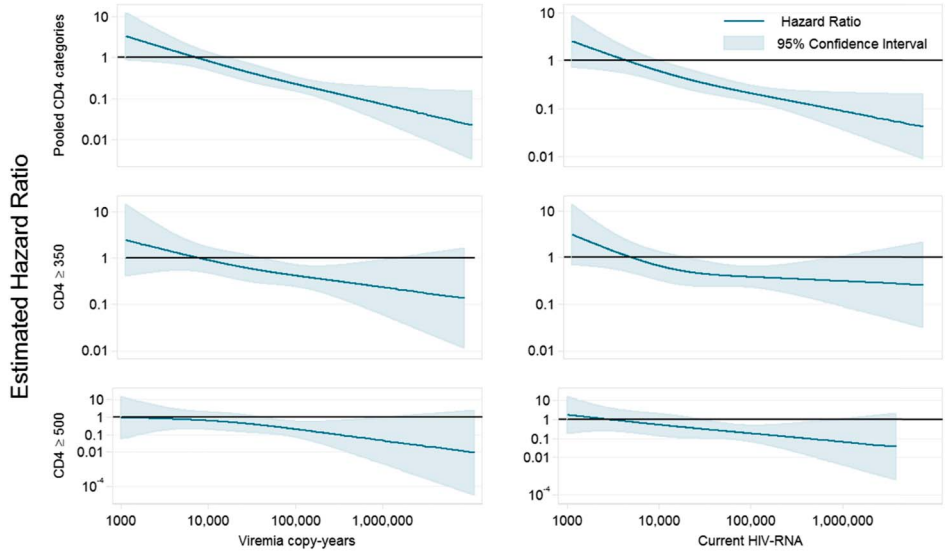

FIGURE 2. The effect of initiating compared with deferring cART on time to AIDS/death by VCY and CD4 cell count modeled continuously with 3 knot splines using the CASCADE data set.

copy-years, current, average, and maximum HIV-RNA, respectively). In the CD4 ≥500 cells per cubic millimeter strata, VCY gave the best model fit (minimum AIC = 113,258.00, increase in AIC = 196, 84, for current, average, and maximum HIV-RNA, respectively).

DISCUSSION

Pooling CD4 cell count strata, there is a benefit of initiating cART as the cumulative and absolute HIV-RNA increases, with benefits observed as the total VCY exceeds approximately 17,500 copy-years/mL. What is of clinical interest, however, is whether there is benefit of immediate cART initiation in individuals with healthy immune systems (CD4 ≥500 cells per cubic millimeter) and high levels of viremia. Among individuals with CD4 ≥500 cells per cubic millimeter, we found a modest benefit of earlier cART initiation for those with high cumulative and absolute HIV-RNA >100,000 copy-years/mL and copies per milliliter, associated with reducing risk of AIDS/death by 59% (13%–81%) and 62% (23%–81%). Our results support the recent evidence from the START trial[28] which found serious illness or death was reduced by 53% among those treated immediately vs. waiting to initiate until CD4 cell count dropped below 350 cells per cubic millimeter.[13]

All measures of viremia showed consistent and similar results with an increased benefit of cART initiation with increasing VCY. Among the pooled and separate CD4 cell count strata, there was not a single viremia measure that consistently showed best model fit using AIC. VCY fits best when pooling CD4 and in the CD4 ≥350 cells per cubic millimeter strata, whereas average viremia fits best in the CD4 <350 cells per cubic millimeter strata. Although VCY incorporates cumulative HIV burden, it requires frequent HIV-RNA measurements from the start of infection, which are not available in most HIV-positive individuals. Even if such measurements are available, cumulative viremia is difficult and time-consuming to calculate. Average and maximum HIV-RNA also require frequent measurements from seroconversion, so too are not relevant for most HIV-positive individuals. Current HIV-RNA, however, is a measure that is easily obtained from all HIV-positive individuals and is therefore of greatest clinical relevance.

Although observational studies are not designed to inform the "when to start" question, we provide evidence that cART initiation is beneficial when CD4 cell counts fall below 350 cells per cubic millimeter, supporting other observational studies.[14,29–31] The START trial has recently reported a modest absolute risk reduction of AIDS, other serious illnesses, and death for cART initiation at CD4 cell counts above 500 cells per cubic millimeter[11] compared with deferring initiation to CD4 below 350 cells per cubic millimeter.[13] Our analysis, using data before guidelines recommending immediate cART initiation, suggests that benefit is likely to be greatest in those with highest viremia burden and adds to the body of evidence which informs clinical guidelines.[32]

We reflected the dynamic process of initiating cART by allowing individuals to contribute information to multiple trials rather than just considering a single point in time. This provided estimates of the average benefit of initiating cART compared with deferring cART at particular levels of CD4 cell counts and cumulative exposure to HIV-RNA. Our estimates can therefore be used to inform trade-offs between initiating treatment at varying points in disease progression compared with the lifelong challenges of initiating therapy, such as adherence and adverse effects.

The availability of HIV-RNA data from HIV seroconversion allowed us to investigate when to start treatment based on a variety of measures of viremia captured during the life course of HIV infection. Of particular importance, there is potential for lead-time bias[33] when measuring cumulative exposure to viremia in sero-*prevalent* cohorts which is essentially eliminated in this sero-*converter* study as we have serial HIV-RNA measurements taken from the date of seroconversion. This is, therefore, the first study, to our knowledge, that has compared the benefit of cART initiation

by these levels of viremia in combination with CD4 cell count. Nevertheless, despite nearly 10,000 seroconverters being included, we were not able to assess the impact of initiating versus deferring within the CD4 strata where decisions on whether cART should be initiated have previously been most controversial (CD4 >350 cells/mm^3).

In addition to AIDS and death, there are several other non–AIDS defining conditions that can affect morbidity and mortality. Increased exposure to viremia has been shown to be associated with cardiovascular disease,[34] multimorbidity,[35] and AIDS and non-AIDS malignancies,[5,36,37] so had these data been available, our estimates could have shown a stronger benefit of cART initiation. CASCADE does not currently collect data on non–AIDS conditions.

Like all observational studies, our estimates rely on the assumption of no unmeasured confounding. We adjusted for some of the most important factors in deciding when to initiate therapy, but it is possible that other unmeasured factors, such as comorbidities or likelihood of adherence, played a role in the initiation of cART in our population. The HRs above one for cART initiation versus deferred treatment, albeit with wide confidence intervals, in the group with low current HIV-RNA suggest we may lack information on some confounders; this could be a particular concern among those with a CD4 count ≥350 cells per cubic millimeter, a group for which not all treatment guidelines recommended initiation of cART during the study period.

It is unlikely that randomized evidence will ever be available on when to initiate cART by these measures of viremia, so applying robust statistical methods to large observational data sets presented here will likely provide the best evidence that will ever be available. Our data suggest that deferring cART in an individual unwilling or unable to start treatment immediately may not impact the risk of AIDS/death provided a healthy CD4 cell count (≥350, 500 cells/mm^3) and low VCY (<50,000 copy-years/mL) are maintained. However, we found consistently that AIDS and death were delayed among those who initiated treatment with CD4 cell counts ≥350 cells per cubic millimeter and VCY >100,000 copy-years/mL.

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Boosted Lopinavir– Versus Boosted Atazanavir–Containing Regimens and Immunologic, Virologic, and Clinical Outcomes: A Prospective Study of HIV-Infected Individuals in High-Income Countries

The HIV-CAUSAL Collaboration[a]

Background. Current clinical guidelines consider regimens consisting of either ritonavir-boosted atazanavir or ritonavir-boosted lopinavir and a nucleoside reverse transcriptase inhibitor (NRTI) backbone among their recommended and alternative first-line antiretroviral regimens. However, these guidelines are based on limited evidence from randomized clinical trials and clinical experience.

Methods. We compared these regimens with respect to clinical, immunologic, and virologic outcomes using data from prospective studies of human immunodeficiency virus (HIV)-infected individuals in Europe and the United States in the HIV-CAUSAL Collaboration, 2004–2013. Antiretroviral therapy–naive and AIDS-free individuals were followed from the time they started a lopinavir or an atazanavir regimen. We estimated the 'intention-to-treat' effect for atazanavir vs lopinavir regimens on each of the outcomes.

Results. A total of 6668 individuals started a lopinavir regimen (213 deaths, 457 AIDS-defining illnesses or deaths), and 4301 individuals started an atazanavir regimen (83 deaths, 157 AIDS-defining illnesses or deaths). The adjusted intention-to-treat hazard ratios for atazanavir vs lopinavir regimens were 0.70 (95% confidence interval [CI], .53–.91) for death, 0.67 (95% CI, .55–.82) for AIDS-defining illness or death, and 0.91 (95% CI, .84–.99) for virologic failure at 12 months. The mean 12-month increase in CD4 count was 8.15 (95% CI, −.13 to 16.43) cells/μL higher in the atazanavir group. Estimates differed by NRTI backbone.

Conclusions. Our estimates are consistent with a lower mortality, a lower incidence of AIDS-defining illness, a greater 12-month increase in CD4 cell count, and a smaller risk of virologic failure at 12 months for atazanavir compared with lopinavir regimens.

Keywords. lopinavir; atazanavir; HIV; mortality; observational studies.

Most clinical guidelines for treatment of patients with human immunodeficiency virus (HIV) recommend first-line regimens consisting of either a ritonavir-boosted protease inhibitor (bPI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI) in combination with 2 nucleoside reverse transcriptase inhibitors (NRTIs). Two of the most commonly prescribed bPIs are lopinavir and atazanavir. The European AIDS Clinical Society, the US Department of Health and Human Services, the British HIV Association, and the International AIDS Society–USA panel all currently recommend atazanavir over lopinavir [1–4], but have recommended lopinavir over atazanavir in the past. The World Health Organization recommends atazanavir and lopinavir equally as part of second-line therapy [5].

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However, these guidelines are based on limited evidence as these regimens have not been extensively examined in previous studies. In particular, the guidelines are largely based on clinical experience and the results of a single randomized trial, the CASTLE study [6, 7]. This trial compared ritonavir-boosted atazanavir with ritonavir-boosted lopinavir in combination with tenofovir and emtricitabine and found that the proportions with HIV RNA <50 copies/mL and the mean increases in CD4 cell count were similar between arms at the end of follow-up. However, follow-up was limited to 48 and 96 weeks, clinical outcomes such as death and AIDS-defining illness were not assessed, and estimates for AIDS-free individuals (who are an increasing proportion of initiators of antiretroviral therapy) were not able to be estimated.

A more recent trial, the NORTHIV study, compared ritonavir-boosted atazanavir vs ritonavir-boosted lopinavir in combination with 2 NRTIs of the physician's choice. The estimates for immunologic and virologic outcomes were similar to those of the CASTLE study [8]. Again, follow-up was limited (48 and 144 weeks), no clinical outcomes were evaluated, and estimates were not reported for AIDS-free individuals.

Here we aim to complement the randomized trials by providing new evidence on clinical outcomes. We examine deaths and AIDS-defining illnesses among AIDS-free patients who start a first-line regimen consisting of either ritonavir-boosted lopinavir or ritonavir-boosted atazanavir with an NRTI backbone in a large collaboration of prospective cohort studies from the United States and Europe. We also study short-term immunologic and virologic outcomes for comparison with the CASTLE and NORTHIV studies.

METHODS

Study Population

The HIV-CAUSAL Collaboration has been described elsewhere [9]. In brief, the collaboration includes several prospective cohort studies from 6 European countries and the United States: UK CHIC (United Kingdom), ATHENA (the Netherlands), FHDH-ANRS CO4 (France), Aquitaine (France), SHCS (Switzerland), PISCIS (Spain), CoRIS (Spain), VACS-VC (United States veterans), AMACS (Greece), UK Register of HIV Seroconverters (United Kingdom), ANRS PRIMO (France), and GEMES (Spain). All cohorts included in the HIV-CAUSAL Collaboration were assembled prospectively and are based on data collected for clinical purposes within national healthcare systems with universal access to care. Each cohort in the collaboration collects data on all CD4 cell counts, HIV RNA levels, treatment initiations, AIDS-defining illnesses, and deaths.

For each individual, follow-up started at the initiation of an eligible antiretroviral regimen (baseline). Our analysis was restricted to HIV-infected individuals who met the following eligibility criteria at baseline dates between 2004 and 2013; age ≥18 years, previously antiretroviral therapy naive, no history of an AIDS-defining illness [10], no pregnancy (when information was available), and CD4 cell count and HIV RNA measurements within 6 months prior to baseline. For the analysis of clinical outcomes, follow-up ended at the occurrence of the outcome, 12 months after the most recent laboratory measurement (ie, we considered an individual to be lost to follow-up if and when he/she had no new CD4 or RNA measurements for 12 months), pregnancy (if known), or the cohort-specific administrative end of follow-up (ranging from September 2010 to March 2013), whichever occurred first. For the analysis of immunologic and virologic outcomes, follow-up ended on average at 12 months after baseline.

Outcomes

We considered clinical, immunologic, and virologic outcomes. The clinical outcomes of interest were death from any cause and clinical AIDS-defining illness [10] or death. Dates of death were identified using a combination of national and local mortality registries and clinical records as described elsewhere [9], and AIDS-defining illnesses were ascertained by the treating physicians.

The immunologic outcome of interest was the 12-month change in CD4 cell count after baseline. If CD4 cell count was not measured exactly 12 months after baseline, we used the closest measurement within 2 months. Similarly, the virologic outcome of interest was virologic failure defined as HIV RNA >50 copies/mL at 12 ± 2 months.

Antiretroviral Regimens

We considered 2 types of first-line regimens: lopinavir and atazanavir regimens. The analysis was restricted to individuals who started ritonavir, an NRTI backbone, and either lopinavir or atazanavir at baseline. Individuals were excluded if they started an ineligible drug (ie, an NNRTI, an integrase inhibitor, a fusion inhibitor, or a PI other than ritonavir, lopinavir, or atazanavir) or both lopinavir and atazanavir at baseline.

Statistical Methods

We fit pooled logistic models to estimate the hazard ratio of each clinical outcome for atazanavir vs lopinavir regimens. Both models included a regimen indicator (1: atazanavir, 0: lopinavir), cohort, month of follow-up (modeled as a restricted cubic spline with 4 knots at 1, 6, 24, and 60 months), and the following baseline covariates: sex, age (<35, 35–49, ≥50 years), race (white, black, other, or unknown), geographic origin (Western countries, sub-Saharan Africa, other, or unknown), mode of HIV acquisition (heterosexual, homosexual/bisexual, injection drug use, other/unknown), CD4 cell count (<200, 200–299, 300–399, 400–499, ≥500 cells/μL), HIV RNA (<10 000,

10 000–100 000, >100 000 copies/mL), calendar year (2004–2007, ≥2008), and time since HIV diagnosis (<1 year, 1–4 years, ≥5 years, or unknown). For the immunologic outcome, we fit a linear regression model with the same covariates to estimate the 12-month change in CD4 cell count for atazanavir vs lopinavir regimens among those with measurements at 12 ± 2 months. For the virologic outcome, we fit a modified Poisson regression model [11] with the same covariates to estimate the risk ratio of virologic failure at 12 months for atazanavir vs lopinavir regimens among those with measurements at 12 ± 2 months.

Under the assumption that we measured and successfully adjusted for all confounders, the estimated coefficient for the regimen indicator in the adjusted models can be interpreted as the 'intention-to-treat' effect that would have been estimated from an open-label randomized trial with similar adherence and follow-up. Because we defined the clinical regimens of interest in terms of the first-line regimen only, it was unnecessary to adjust for joint determinants of switching and death. The Supplementary Appendix Table shows estimates from unadjusted models.

For the 2 clinical outcomes, we also estimated absolute risks by fitting adjusted models such as the one described above that also included product ("interaction") terms between the regimen indicator and month of follow-up with spline terms. The models' predicted values were then used to estimate the 5-year survival and 5-year AIDS-free survival curves from baseline.

For death, we also estimated the hazard ratio in subsets defined by baseline calendar year, sex, age, mode of HIV acquisition, baseline CD4 cell count, and baseline HIV RNA. For AIDS-defining illness or death, we also estimated the hazard ratio in subsets defined by NRTI backbone. Because of limited numbers of deaths, we were not able to look at subsets defined by NRTI backbone for our death-only outcome.

Sensitivity Analyses

Because the lower limit of detection was unknown in <5% of observations with HIV RNA between 50 and 400 copies/mL, we conducted a sensitivity analysis in which we defined virologic failure as HIV RNA >400 copies/mL.

In another sensitivity analysis, we allowed a 6-month grace period for individuals to complete one of the regimens of interest as opposed to requiring individuals to start all of the drugs in their regimen simultaneously. Individuals were artificially censored if and when they started an ineligible drug before completing a regimen or at 6 months from baseline if their regimen was not yet complete. As previously described, to adjust for potential selection bias due to the artificial censoring, we estimated unstabilized inverse probability weights [12] via pooled logistic models for artificial censoring that included the time-fixed covariates and time-varying CD4 cell count (restricted cubic spline with 5 knots at 10, 200, 350, 500, and 1000 cells/μL), HIV RNA (<10 000, 10 000–100 000, >100 000 copies/mL), AIDS-defining illness (when the outcome was death alone), and time since last laboratory measurement (0, 1–2, 3–4, 5–6, ≥7 months). Note that inverse-probability weighting was not necessary in our main analysis, as treatment was determined at baseline.

Several other sensitivity analyses were also performed. For all 4 outcomes, we used continuous as opposed to categorical baseline covariates, weighted by the inverse probability of remaining uncensored due to infrequent laboratory measurements, and investigated the effect of including chronic hepatitis C infection [13] as a baseline covariate. For the immunologic and virologic outcomes, we also weighted by the inverse probability of remaining alive and having a measurement at 12 ± 2 months after baseline as a form of competing risk analysis.

All 95% confidence intervals (CIs) were estimated via a nonparametric bootstrap with 500 samples. All analyses were conducted with SAS software version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

The dataset included 10 969 individuals, of whom 6668 followed a lopinavir regimen and 4301 followed an atazanavir regimen. Table 1 shows the characteristics of the study population by regimen type at baseline. Women, individuals aged <35 years at baseline, those from non-Western countries, those with the lowest baseline CD4 cell counts, those with the highest baseline HIV RNA levels, and those starting treatment before 2008 were more likely to initiate lopinavir than atazanavir.

In the mortality analysis, the median follow-up time was 40 (interquartile range [IQR], 20–61) months for the lopinavir regimens and 27 (IQR, 14–45) months for the atazanavir regimens. In the AIDS-defining illness or death analysis, the median follow-up time was 37 (IQR, 18–60) months for the lopinavir regimens and 26 (IQR, 13–44) months for the atazanavir regimens. There were 3322 individuals lost to follow-up in the death analysis, of whom 2366 followed a lopinavir regimen and 956 followed an atazanavir regimen. In the AIDS or death analysis, 3228 were lost to follow-up, of whom 2290 followed a lopinavir regimen and 938 followed an atazanavir regimen.

As shown in Table 2, 213 and 83 individuals died and 457 and 157 individuals developed an AIDS-defining illness or died among those initiating a lopinavir and an atazanavir regimen, respectively. Compared with lopinavir, the hazard ratio for atazanavir was 0.70 (95% CI, .53–.91) for death and 0.67 (95% CI, .55–.82) for AIDS or death.

Table 2 also shows the 12-month adjusted mean change in CD4 cell count and the number with virologic failure at 12 ± 2 months. Compared with lopinavir, the estimated mean change in CD4 cell count for atazanavir was 8.15 (95% CI,

Table 1. Characteristics of 10 969 Therapy-Naive HIV-Infected Individuals at Baseline, HIV-CAUSAL Collaboration, 2004–2013

Characteristic	No. of Individuals (%)		
	Lopinavir (n = 6668)	Atazanavir (n = 4301)	Total (n = 10 969)
Sex			
Male	4429 (66.4)	3372 (78.4)	7801 (71.1)
Female	2239 (33.6)	929 (21.6)	3168 (28.9)
Age, years			
<35	2741 (41.1)	1435 (33.4)	4176 (38.1)
35–50	2877 (43.1)	2035 (47.3)	4912 (44.8)
>50	1050 (15.7)	831 (19.3)	1881 (17.1)
Geographic origin			
Western countries	4295 (64.4)	3215 (74.8)	7510 (68.5)
Sub-Saharan Africa	1526 (22.9)	601 (14)	2127 (19.4)
Other	629 (9.4)	348 (8.1)	977 (8.9)
Unknown	218 (3.3)	137 (3.2)	355 (3.2)
Acquisition group			
Heterosexual	3118 (46.8)	1444 (33.6)	4562 (41.6)
Homosexual	2254 (33.8)	1892 (44)	4146 (37.8)
Injection drug use	362 (5.4)	187 (4.3)	549 (5)
Other/unknown[a]	934 (14)	778 (18.1)	1712 (15.6)
CD4 count, cells/μL			
<200	2634 (39.5)	1226 (28.5)	3860 (35.2)
200–299	1808 (27.1)	1245 (28.9)	3053 (27.8)
300–399	1124 (16.9)	1030 (23.9)	2154 (19.6)
400–499	537 (8.1)	427 (9.9)	964 (8.8)
≥500	565 (8.5)	373 (8.7)	938 (8.6)
HIV RNA, copies/mL			
<10 000	1290 (19.3)	827 (19.2)	2117 (19.3)
10 000–100 000	2579 (38.7)	1958 (45.5)	4537 (41.4)
>100 000	2799 (42)	1516 (35.2)	4315 (39.3)
Calendar year			
2004–2007	4220 (63.3)	1396 (32.5)	5616 (51.2)
≥2008	2448 (36.7)	2905 (67.5)	5353 (48.8)
Cohort			
UK CHIC	1055 (15.8)	704 (16.4)	1759 (16)
ATHENA	498 (7.5)	380 (8.8)	878 (8)
FHDH-ANRS CO4	2558 (38.4)	1491 (34.7)	4049 (36.9)
Aquitaine	433 (6.5)	322 (7.5)	755 (6.9)
SHCS	723 (10.8)	421 (9.8)	1144 (10.4)
PISCIS/AMACS	491 (7.4)	182 (4.2)	673 (6.1)
CoRIS	271 (4.1)	152 (3.5)	423 (3.9)
Seroconverters[b]	489 (7.3)	553 (12.9)	1042 (9.5)
VACS-VC	150 (2.2)	96 (2.2)	246 (2.2)
HCV infection			
Definite/probable	119 (1.8)	116 (2.7)	235 (2.1)
Possible	324 (4.9)	139 (3.2)	463 (4.2)
None	6225 (93.4)	4046 (94.1)	10 271 (93.6)

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus.

[a] Other/unknown acquisition group included all VACS-VC participants.

[b] Includes the UK Register of HIV Seroconverters, ANRS PRIMO, and GEMES (Grupo Español Multicéntrico para el Estudio de Seroconvertores-Haemophilia) cohorts.

−.13 to 16.43) cells/μL. The mean CD4 cell count would have increased from 269 to 470 cells/μL over 12 months had all individuals taken a lopinavir regimen, and from 269 to 478 cells/μL had all individuals taken an atazanavir regimen.

Among those initiating lopinavir and atazanavir regimens, 26% and 24%, respectively, had HIV RNA >50 copies/mL at 12 months. Compared with lopinavir, the risk ratio of virologic failure for atazanavir was 0.91 (95% CI, .84–.99).

Figure 1 plots the estimated 5-year survival and 5-year AIDS-free survival. The survival was 96.1% (95% CI, 95.5%–96.7%) for the lopinavir regimens and 97.1% (95% CI, 96.5%–97.8%) for the atazanavir regimens. The 5-year survival difference was 1.0% (95% CI, .1%–1.9%). The AIDS-free survival proportion was 92.3% (95% CI, 91.5%–93.1%) for the lopinavir regimens and 94.4% (95% CI, 93.5%–95.4%) for the atazanavir regimens. The 5-year AIDS-free survival difference was 2.2% (95% CI, .9%–3.4%).

In subset analyses, the mortality hazard ratio was 0.45 (95% CI, .26–.77) when we restricted to baseline calendar years 2008 and beyond; 0.65 (95% CI, .49–.88) when we restricted to men, 0.59 (95% CI, .41–.87) when we restricted to individuals aged <50 years; 0.69 (95% CI, .52–.91) when we restricted to non–injection drug users; 0.62 (95% CI, .46–.84) when we restricted to those with baseline CD4 cell counts <350 cells/μL; 0.57 (95% CI, .37–.88) when we restricted to those with baseline viral loads >100 000 copies/mL; and 0.72 (95% CI, .54–.96) when we restricted to those from Western countries.

Table 3 shows the number and percentage of individuals taking recommended NRTI backbones by regimen type. Backbones consisting of abacavir/lamivudine and tenofovir/emtricitabine were more frequently used with atazanavir, whereas backbones of zidovudine/lamivudine and tenofovir/lamivudine were more frequently used with lopinavir. Table 3 also shows the hazard ratio for AIDS or death by NRTI backbone. Compared with lopinavir, the hazard ratio for atazanavir ranged from 0.50 (95% CI, .38–.65) for tenofovir/emtricitabine to 1.12 (95% CI, .48–2.60) for zidovudine/lamivudine.

None of the sensitivity analyses yielded appreciably different results (data not shown), with the exception of the alternative definition of virologic failure. When we defined virologic failure as HIV RNA >400 copies/mL, 14% and 10% of those initiating lopinavir and atazanavir, respectively, had HIV RNA >400 copies/mL at 12 months. The risk ratio of virologic failure (HIV RNA >400 copies/mL) was 0.79 (95% CI, .69–.90) for atazanavir vs lopinavir (see Supplementary Appendix Table).

DISCUSSION

The clinical effectiveness of ritonavir-boosted atazanavir vs ritonavir-boosted lopinavir has not been directly studied in randomized trials, which have focused on short-term immunologic

Table 2. Clinical, Immunologic, and Virologic Outcomes for Regimens Based on Atazanavir (n = 4301) Versus Lopinavir (n = 6668), HIV-CAUSAL Collaboration, 2004–2013

Outcome	Treatment	No. of Outcomes	HR (95% CI)
Death	Lopinavir	213	1 (Ref.)
	Atazanavir	83	0.70 (.53–.91)
AIDS or death	Lopinavir	457	1 (Ref.)
	Atazanavir	157	0.67 (.55–.82)
		Adjusted Mean Change, Cells/μL[a]	Change From Baseline, Cells/μL (95% CI)
CD4 cell count	Lopinavir	201.11	1 (Ref.)
	Atazanavir	209.26	8.15 (–.13 to 16.43)
		No. Failed[b]	Risk Ratio[c] (95% CI)
Virologic failure (HIV RNA >50 copies/mL)	Lopinavir	1260	1 (Ref.)
	Atazanavir	683	0.91 (.84–.99)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; Ref., reference.

[a] Based on 4881 and 2864 individuals with CD4 cell count measurements at 12 ± 2 months in the lopinavir and atazanavir arms, respectively.

[b] Based on 4812 and 2878 individuals with HIV RNA measurements at 12 ± 2 months in the lopinavir and atazanavir arms, respectively.

[c] Adjusted for the baseline covariates (sex, age, race, geographic origin, mode of acquisition, CD4 cell count, HIV RNA, calendar year, and years since HIV diagnosis).

and virologic outcomes. Our study compared atazanavir vs lopinavir regimens with respect to clinical outcomes among antiretroviral-naive, AIDS-free individuals in Europe and the United States. We estimated a 30% mortality reduction and a 33% reduction in a combined endpoint of death and AIDS-defining illness for atazanavir vs lopinavir. We also found that atazanavir had a beneficial but modest effect on immunologic and virologic outcomes.

Unlike previous observational studies [14, 15], we designed our observational analysis to emulate the intention-to-treat analysis of a randomized clinical trial in which antiretroviral-naive, AIDS-free adults are randomized to receive either ritonavir-boosted lopinavir or ritonavir-boosted atazanavir with an NRTI backbone. Our estimates are based on less restrictive criteria, and therefore are potentially more relevant to the general population of HIV-infected patients than those of the CASTLE

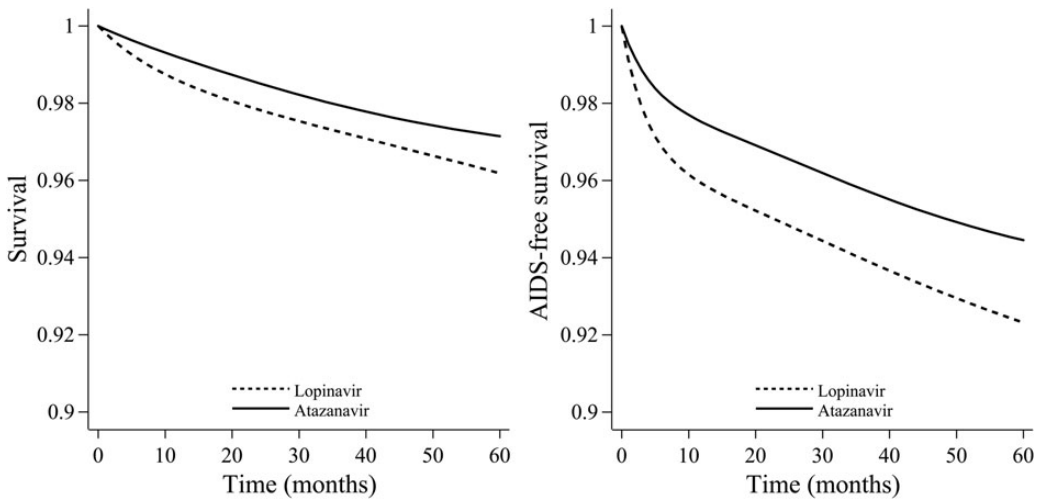

Figure 1. Survival (left) and AIDS-free survival (right) for atazanavir vs lopinavir, HIV-CAUSAL Collaboration, 2004–2013. The curves are standardized by the baseline covariates listed in the Table 2 footnotes.

Table 3. AIDS or Death by Recommended Nucleoside Reverse Transcriptase Inhibitor Backbone, HIV-CAUSAL Collaboration, 2004–2013

Backbone	No. of Individuals (%)		HR for Atazanavir vs Lopinavir[a] (95% CI)
	Lopinavir (n = 6668)	Atazanavir (n = 4301)	
Abacavir/ lamivudine	719 (10.8)	658 (15.3)	0.83 (.48–1.42)
Tenofovir/ emtricitabine	2875 (43.1)	3286 (76.4)	0.50 (.38–.65)
Zidovudine/ lamivudine	2407 (36.1)	88 (2)	1.12 (.48–2.60)
Tenofovir/ lamivudine	237 (3.6)	140 (3.3)	0.67 (.30–1.46)

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Adjusted for the baseline covariates (sex, age, race, geographic origin, mode of acquisition, CD4 cell count, human immunodeficiency virus [HIV] RNA, calendar year, and years since HIV diagnosis).

[6, 7] and NORTHIV studies [8]. When we more closely emulated the design and inclusion criteria of the CASTLE study (ie, baseline HIV RNA ≥5000 copies/mL, and an NRTI backbone of tenofovir and emtricitabine), we estimated a risk ratio of virologic failure of 1.00 (95% CI, .89–1.13), similar to 1.06 (95% CI, .73–1.53) from our meta-analysis of the 2 trials. Our study, however, may still differ from the CASTLE and NORTH IV studies in several ways.

In the CASTLE Study, both arms had a backbone of tenofovir and emtricitabine. In the NORTHIV study, as in our study, the prescribing physician could select the backbone. We conducted analyses restricted to 4 recommended backbones for the outcome AIDS-defining illness or death. Our estimates differed by NRTI backbone. Although we found little difference between lopinavir and atazanavir for those on the NRTI backbones abacavir/lamivudine, zidovudine/lamivudine, and tenofovir/lamivudine, our results may suggest an interaction between lopinavir and tenofovir/emtricitabine that results in lower regimen potency. As this is the most commonly used NRTI backbone, this interaction merits further investigation.

In the CASTLE and NORTHIV studies, individuals received 400 mg/100 mg of lopinavir/ritonavir twice daily. We do not know whether individuals on lopinavir regimens in our study were taking their medication once or twice daily. Although once-daily regimens are generally associated with better adherence, this is unlikely to be a source of bias as both schedules performed similarly in randomized clinical trials [16–18].

As with all observational estimates, ours rely on the untestable assumption that we have successfully measured and adjusted for all confounders. In this analysis, we measured and adjusted for sex, age, race, geographic origin, mode of HIV acquisition, CD4 cell count, HIV RNA, calendar year, and years since HIV diagnosis. If further adjustment is necessary to account for confounding factors responsible for large prognostic differences between patients initiating lopinavir vs atazanavir, the assumption would not hold.

One of these confounding factors might be adherence if lopinavir was more often prescribed to individuals whose future adherence was questionable (eg, because of markers of poor health such as hepatic diseases) even in the absence of a clinical indication for switching or treatment discontinuation. However, we measured and adjusted for several proxies for adherence, including HIV RNA, calendar year, intravenous drug use, years since HIV diagnosis, and time since last laboratory measurement.

Another potential confounding factor is concomitant medication use. For example, ritonavir-boosted atazanavir is not recommended for use with antacids and other drugs that raise gastric pH [2], whereas ritonavir-boosted lopinavir may lead to increased statin use because of unfavorable lipid changes and increased risk of myocardial infarction [19–21]. Although we could not adjust for non–antiretroviral drug use, the magnitude of the reported associations makes it unlikely that our immunologic and virologic estimates can be fully explained by use of antacids, statins, or other drugs.

In summary, our findings extend those of randomized trials from immunologic and virologic outcomes to clinical outcomes. Although we provide new evidence upon which the next set of guidelines can be based, our findings do not support changes to the current guidelines. Future studies need to consider the effects of lopinavir and atazanavir on other clinical outcomes including non-AIDS-defining illnesses, when paired with specific backbones, particularly tenofovir/emtricitabine, and over longer periods.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Temporal trends of transmitted HIV drug resistance in a multinational seroconversion cohort

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Background: The rate of transmitted drug resistance (TDR) may increase with wider use of antiretroviral therapy and can contribute to therapeutic failure. We analysed time trends in TDR among HIV seroconverters.

Methods: Using CASCADE data of individuals with well estimated dates of HIV seroconversion, we examined HIV nucleotide sequences collected prior to antiretroviral therapy use from 1996–2012. All samples were taken within 12 months of testing HIV positive. Using logistic regression, we examined the association between TDR and year of seroconversion, adjusting for confounders.

Results: Of 4717 individuals seroconverting between 1996 and 2012, median (IQR) age at seroconversion was 33 (27, 39) years. The majority (3839; 92%) were male, mainly exposed through MSM (3767; 80%), and infected with subtype B (3464; 73%). Overall, 515 (11%) individuals had at least one drug resistance-related mutation; 280 individuals with nucleoside reverse transcriptase, 185 with nonnucleoside reverse transcriptase, and 144 with protease inhibitor mutations. Estimated TDR prevalence was 19.4% (8.2, 36.0) in 1996, significantly decreasing to 8.5% (5.9, 11.9) in 2012 [odds ratio (OR; 95% confidence interval (CI)) = 0.92 (0.90, 0.95) per year increase]. Individuals exposed through sex between men and women were significantly less likely to have been infected with a drug-resistant strain [OR (95% CI) = 0.59 (0.41, 0.87) compared with MSM], and there was marginal evidence that sampling during acute infection was associated with higher odds of resistance [OR (95% CI) = 1.20 (0.97, 1.7), $P = 0.093$] compared with later sampling.

Conclusion: TDR has decreased over calendar time although a significant proportion of new infections still carry resistance-related mutations.

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Keywords: HIV drug resistance, HIV seroconverters, temporal trends, time trends

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Introduction

Combination antiretroviral therapy (cART) is effective at suppressing plasma HIV RNA to undetectable levels [1] thereby improving patient prognosis [2,3] and reducing the risk of onward transmission of HIV when viral suppression is achieved [4]. However, poor adherence [5–9] can lead to the development of mutations [10] which are associated with HIV drug resistance and subsequent cART failure. Individuals failing treatment have worse health outcomes [11–13], are less likely to benefit from newer drugs, and can pass drug resistant strains of HIV to others [14]. Given this concern, international guidelines recommend that newly diagnosed individuals are tested for evidence of resistance to optimize the selection of first-line cART regimes [15,16].

Recent data from cART-naive seroprevalent cohorts suggest the prevalence of TDR has either stabilized [17,18] or decreased from 2002 to 2009 [19–21]. Given that timing of HIV infection is not known for individuals in seroprevalent cohorts; however, estimated TDR rates may reflect historical trends but not necessarily trends among those recently infected. Furthermore, because of the reversion of a number of mutations to wild-type over time in the absence of cART [22], analysis of TDR rates among seroprevalent cohorts may under-estimate actual TDR prevalence. Trends of TDR among HIV seroconverters are unclear with some studies showing increased TDR between 1987 and 2003 [23] or stability between 1996 and 2007 [24].

Temporal trends of transmitted drug resistance (TDR) among individuals recently infected need to be monitored as new drugs and classes are introduced to inform clinical decision making. We aim to describe the temporal trends of TDR among recently infected individuals using CASCADE data of HIV seroconverters, and to identify predictors of TDR.

Methods

Study population

We used pooled data from the Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) 2014 data release on HIV-1 seroconverters in EuroCoord (www.EuroCoord.net), which has been described in detail elsewhere [25]. Briefly, CASCADE is a cohort collaboration of 31 772 HIV-1 seroconverters from 16 countries across Europe (95%), Australia (1%), Canada (1%), and Sub-Saharan Africa (3%). Date of HIV seroconversion was estimated most commonly (87%) as the midpoint between the last documented negative and the first documented positive HIV antibody test dates with an interval of less than 3 years between the two dates. The remaining individuals had seroconversion dates estimated through laboratory evidence of seroconversion (PCR positivity in the absence of HIV antibodies or antigen positivity with fewer than four bands on western blot – 10%), or as the date of seroconversion illness with both an earlier documented negative and a later positive HIV test not more than 3 years apart (2%).

We restricted our analysis to those with documented seroconversion in the cART era (>1995) with at least one viral genetic sequence within the first year of testing positive for HIV while still being ART naive. Additionally, we restricted the analysis to those seroconverting before 1 January 2013 as to allow at least 1 year of follow-up.

Resistance and subtype analysis

Genotypic resistance data were derived from sequencing of the protease and reverse transcriptase genes performed by laboratories in the country of care using a variety of in-house and commercial resistance assays. The Stanford HIVdb algorithm 7.0 was used centrally to analyse all nucleotide sequences (http://hivdb.stanford.edu); updated on 27 February 2014) [26]. Subtype was analysed and assigned centrally using the REGA algorithm [27].

An individual was categorized as having a transmitted HIV-1 drug resistance-associated mutation if their virus contained one or more mutations from the Surveillance Drug Resistance Mutations list defined by the WHO [28]. We further derived susceptibility to antiretroviral drugs using the Stanford HIV database algorithm. Individuals were considered to have high level of resistance if the Stanford score was higher than 3. Using this algorithm, we further identified mutations associated with drugs of current first-line recommendations according to the European AIDS Clinical Society guidelines (categories A and B) [29].

Statistical methods

Proportions and their associated 95% confidence intervals (CI) were calculated using exact CIs for binomially distributed data. Linear logistic regression was used to assess the time trends of TDR as there was no statistical evidence for departures from linearity using natural cubic splines [30]. Time trend models were adjusted for sex, HIV transmission risk group, seroconversion age, and HIV diagnosis during acute HIV infection, defined as laboratory evidence of HIV seroconversion or having an HIV test interval of less than 30 days. Age at HIV seroconversion was modelled linearly as there was no evidence for departures from linearity using natural cubic splines. Owing to small numbers, we were not able to evaluate the time trends of individual mutations. Instead, we list the most common mutations over the calendar period.

In a sensitivity analysis, we restricted our analysis to include only individuals infected with subtype B as our cohort consists predominantly of subtype B (>70%), and HIV genetic diversity may influence the emergence and type of resistance mutations.

Results

Baseline characteristics

We analysed data from 4717 seroconverters in CASCADE with at least one ART-naive nucleotide sequence available during the first year following HIV seroconversion. Median age at HIV seroconversion was 33 (IQR = 27, 39) years, and the most common HIV transmission risk group was MSM (80%) followed by sex between men and women (MSW, 15%), people who inject drugs (PWID, 3%) and unknown ($n = 101$, 2%). HIV subtype was mainly B ($n = 3464$, 73%), followed by C ($n = 288$, 8%), A ($n = 240$, 6%), and a recombinant form ($n = 176$, 4%), Table 1. Median (IQR) time from HIV seroconversion to sample collection was 124 (44, 256) days, and did not differ between those with and without mutations associated with HIV drug resistance ($P = 0.31$, data not shown). Of the 4717 seroconverters, 1222 (26%) were diagnosed with HIV during acute HIV infection, a proportion which did not differ between those with and without mutations associated with HIV drug resistance ($P = 0.26$, data not shown). The majority of individuals were receiving care in Germany (34%), the UK (21%), or Sweden (12%).

Transmitted drug resistance

Overall, 203 (4.3%; 95% CI = 3.7–4.9) individuals had one mutation and 515 (10.9%; 95% CI = 10.0–11.8) had one or more mutations associated with TDR. Among these 515 individuals, 93 (2.0%; 1.6–2.4), 98 (2.1%; 1.7–2.5), and 67 (1.4%; 1.1–1.8) had one mutation associated with nucleoside reverse transcriptase inhibitors (NRTI), non-NRTIs (NNRTI), or protease inhibitors, respectively, and 280 (5.9%; 5.2–6.6), 185 (3.9%; 3.4–4.5), and 144 (3.1%; 2.6–3.6), had one or more mutations associated with NRTI, NNRTI, or protease inhibitors, respectively.

The most frequent mutations (>5% of individuals with mutations) related to NRTIs were 41L ($n = 91$; 18%), 215S ($n = 61$; 12%), 184V ($n = 34$; 7%), 67N ($n = 30$; 6%), 210W ($n = 28$; 5%) 219Q ($n = 27$; 5%). For NNRTIs, the most common mutation was 103N ($n = 119$; 23%) and, for protease inhibitors these were 90M ($n = 39$; 8%), 46I ($n = 31$; 6%), and 46L ($n = 26$; 5%) (Supplementary Table 1; http://links.lww.com/QAD/B203). In total, 436 (9%) individuals had mutations associated with a single class, 79 (2%) had mutations associated with two or more classes, and 15 (<1%%) had mutations associated with three classes (NRTI, NNRTI, and protease inhibitor).

Table 1. Baseline characteristics for individuals in CASCADE data of HIV seroconverters between 1996 and 2012; a comparison of individuals with at least one ART-naive nucleotide sequence available within 1 year of testing positive for HIV, and the remaining individuals.

Characteristic	Individuals with <1 nucleotide sequence $N = 4717$	Individuals without sequences $n = 17\,574$	*P* value
Seroconversion year	2007 (2004, 2010)	2004 (2000, 2008)	<0.001
Seroconversion age	33 (27, 39)	33 (27, 39)	0.89
Males	4327 (92%)	13,911 (80%)	<0.001
HIV risk group[a]			
MSM	3767 (80%)	10,611 (60%)	<0.001
MSW	715 (15%)	5056 (29%)	
PWID	134 (3%)	1001 (6%)	
OTH/UNK	101 (2%)	906 (5%)	
Acute HIV infection[b]	1222 (26%)	2340 (13%)	<0.001
HIV test interval (days)	179 (26, 381)	278 (108, 541)	<0.001
Country/continent of cohort			
Germany	1,607 (34%)	775 (4%)	<0.001
UK	1,029 (22%)	1,061 (6%)	
Sweden	524 (11%)	299 (2%)	
Spain	400 (8%)	795 (5%)	
Africa	323 (7%)	590 (3%)	
Austria	229 (5%)	150 (1%)	
Netherlands	197 (4%)	241 (1%)	
France	183 (4%)	10,257 (60%)	
Italy	93 (2%)	2,293 (13%)	
Canada	70 (1%)	111 (1%)	
Greece	62 (1%)	192 (1%)	
Subtype			
B	3465 (73%)		
C	288 (6%)		
A	240 (5%)		
CRF01_AE[c]	120 (3%)		
CRF02_AG[c]	112 (2%)		
Other recombinant forms	226 (5%)		
Other/unknown	268 (6%)		

All numbers are *N* (%) or median (interquartile range).
[a]MSM; MSW, sex between men and women; OTH/UNK, other/unknown; PWID, people who inject drugs; UK.
[b]HIV test interval <30 days or laboratory evidence of acute HIV infection.
[c]Circulating recombinant form.

Table 2. Predictors of transmitted HIV drug resistance for individuals with at least one ART-naive nucleotide sequence within 1 year of testing positive for HIV: CASCADE data of HIV seroconverters.

	Any TDR OR (95% CI)	*P*	NRTI TDR OR (95% CI)	*P*	NNRTI TDR OR (95% CI)	*P*	PI TDR OR (95% CI)	*P*
SC year	0.92 (0.90, 0.95)	<0.001	0.89 (0.86, 0.91)	<0.001	0.96 (0.93, 1.001)	0.059	0.93 (0.89, 0.97)	0.001
sex (female vs. male)	1.19 (0.73, 1.93)	0.48	1.47 (0.72, 2.98)	0.29	0.65 (0.30, 1.37)	0.25	2.03 (0.92, 4.47)	0.08
risk group		0.03[b]		0.002[b]		0.95[b]		0.34[b]
MSM	1		1		1		1	
MSW	0.59 (0.41, 0.87)		0.38 (0.21, 0.70)		1.08 (0.65, 1.81)		0.56 (0.28, 1.11)	
PWID	0.62 (0.33, 1.16)		0.46 (0.19, 1.12)		0.79 (0.28, 2.20)		0.63 (0.21, 1.87)	
OTH/UNK	1.01 (0.54, 1.87)		1.23 (0.58, 2.62)		1.03 (0.37, 2.87)		1.04 (0.37, 2.96)	
Age group		0.60[b]		0.95[b]		0.96[b]		0.003[b]
<25	1		1		1		1	
25–34	1.11 (0.84, 1.47)		0.94 (0.66, 1.35)		1.03 (0.67, 1.59)		1.34 (0.78, 2.31)	
35–45	1.02 (0.76, 1.38)		0.92 (0.63, 1.35)		0.95 (0.59, 1.52)		1.12 (0.61, 2.04)	
45 and above	1.22 (0.86, 1.74)		1.03 (0.65, 1.63)		0.94 (0.53, 1.66)		2.61 (1.42, 4.77)	
Acute HIV infection[a]	1.20 (0.97, 1.47)	0.093	1.10 (0.83, 1.46)	0.50	1.15 (0.83, 1.60)	0.40	1.14 (0.78, 1.67)	0.49

CI, confidence interval; MSW, sex between men and women; NRTI, nucleoside reverse transcriptase inhibitors; OR, odds ratio; OTH/UNK, other/unknown; PI, protease inhibitor; PWID, people who inject drugs; SC, seroconversion; TDR, transmitted drug resistance.
[a]HIV test interval <30 days or laboratory evidence of acute HIV infection.
[b]*P* value for heterogeneity.

We observed a significant decline in the prevalence of TDR to any class during 1996–2012, the calendar year of seroconversion; odds ratio (OR) = 0.92 (95% CI; 0.90, 0.95) per year, starting at 19.4% (8.2, 36.0) in 1996 and falling to 8.5% (5.9, 11.9) in 2012. The same decreasing trend over time was observed for transmitted NRTI resistance, OR = 0.89 (0.86, 0.91) per year, NNRTI resistance, OR = 0.96 (0.93, 1.00) per year, and protease inhibitor resistance, OR = 0.93 (0.89, 0.97) per year (Table 2, Fig. 1).

In more recent years (2007–2012), data were available on 2546 individuals, 216 [8.5% (7.4, 9.6)] of whom had a mutation associated with TDR. Among these individuals, 98 (3.8%; 3.1–4.6), 89 (3.5%; 2.8–4.2), and 62 (2.4%; 1.8–3.1), had one or more mutations associated with NRTI, NNRTI, or protease inhibitor, respectively. The most common mutations in this time period include NTRI mutations 41L ($n = 34$; 16%), 215S ($n = 26$; 12%), and 215D ($n = 15$; 7%); NNRTI mutation 103N ($n = 56$; 26%); and protease inhibitor mutations 90M ($n = 19$; 9%) and 46L ($n = 12$; 6%).

In a sensitivity analysis, restricting to those infected with subtype B, we observed the same trends of TDR decreasing over the calendar period (data not shown). Findings were also consistent across all CASCADE participating cohorts.

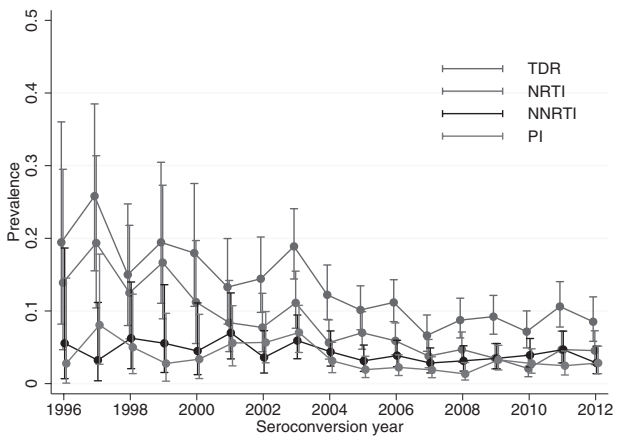

Fig. 1. Temporal trends in transmitted drug resistance over time for individuals with at least one ART naive nucleotide sequence within one year of testing positive for HIV: CASCADE data of HIV seroconverters. NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; TDR, transmitted drug resistance. Statistically significant decline ($P < 0.01$ for TDR, NRTI, and PI) in the prevalence of transmitted drug resistance over time using linear mixed models.

Drug susceptibility

Of 4717 individuals, 296 (6.3%; 5.5–7.0) had a transmitted mutation associated with high-level resistance to a drug according to the Stanford HIV database algorithm, 190 (4.0%; 3.5–4.6) of these were associated with an agent in a recommended first-line treatment regimen with efavirenz having the highest proportion of high-level resistance, Fig. 2. In total, 102 (2.2%; 1.8–2.6), 163 (3.5%; 2.9–4.0), and 83 (1.8%; 1.4–2.2), individuals had at least one transmitted mutation associated with high level of resistance to NRTIs, NNRTIs, and protease inhibitor, respectively. Among the 2546 individuals seroconverting more recently (2007–2012), 154 [6.0% (5.2, 7.0)] had a transmitted mutation associated with high-level resistance; 93 (3.7%; 3.0–4.5), 42 [1.6% (1.2, 2.2)], 91 [3.6% (2.9, 4.4)], 46 [1.8% (1.3, 2.4)] with high-level resistance associated with a first-line regimen, NRTIs, NNRTIs, and protease inhibitors, respectively.

During the calendar period of observation, the rate of transmitted high-level drug resistance declined; OR = 0.97 (95% CI; 0.94, 1.0005) per year, $P = 0.054$.

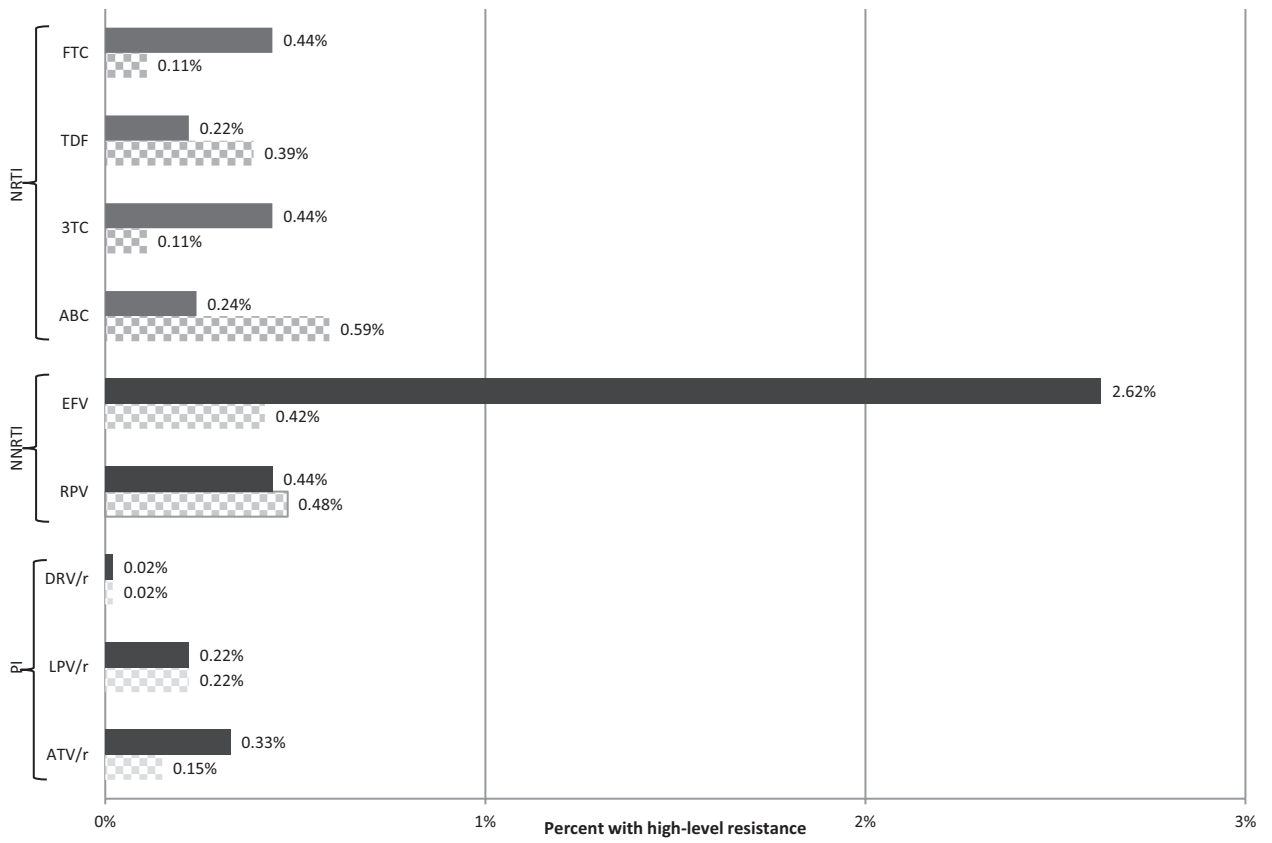

Fig. 2. High level resistance (Stanford scores >3; solid bars indicate a score of 5, checked bars indicate a score of 4) associated with first-line antiretroviral drugs recommended by the European AIDS clinical Society for individuals with at least one ART-naive nucleotide sequence within 1 year of testing positive for HIV: CASCADE data of HIV seroconverters. ABC, abacavir; ATV/r, atazanavir; DRV/r, darunavir; EVF, efavirenz; FTC, emtricitabine; LPV/r, lopinavir; NRTIs, Nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; RPV, rilpivirine; TDF, tenofovir; 3TC, lamivudine. Adapted with permission [29].

A significant decreasing trend over time was observed for high-level resistance to first-line regimens, OR = 0.92 (0.87, 0.97), *P* less than 0.001 per year and high-level NRTI resistance, OR = 0.89 (0.85, 0.93), *P* less than 0.001 per year. The same trend was observed in high-level protease inhibitor resistance, OR = 0.96 (0.91, 1.01), *P* = 0.18 per year. There was no evidence of a decrease in high-level NNRTI resistance over calendar time, although levels have remained relatively low throughout the period of observation of our study at 3.4%.

Predictors of transmitted drug resistance

There was significant heterogeneity between HIV transmission risk group and any TDR and NRTI TDR with those exposed through MSW having a lower probability of being infected with a drug-resistant strain compared to MSM. Older individuals were more likely to have been infected with a protease inhibitor resistant strain (*P* = 0.003) as were females, although the evidence for females was modest [OR = 2.03 (0.92, 4.47, *P* = 0.08)]. Individuals diagnosed during acute HIV infection were slightly more likely to be infected with a resistant strain, OR = 1.20 (0.97, 1.47; Table 2). Of the 1222 individuals in our study diagnosed during acute HIV infection, 144 (11.8%) had at least one mutation associated with TDR compared with 10.9% of individuals with TDR diagnosed later in infection.

When we restricted to those seroconverting in more recent years (2007–2012), older age was the only significant predictor for transmitted HIV drug resistance; OR = 1.58 (1.01, 2.45; *P* = 0.043), 1.50 (0.94, 2.41; *P* = 0.092), and 1.93 (1.13, 3.29; *P* = 0.016) for ages 25–34, 35–44, and 45 and above, respectively, compared with those aged 15–25 years at seroconversion.

Discussion

The prevalence of TDR and high-level resistance among individuals with recent HIV infection decreased between 1996 and 2012. Our estimates provide a realistic estimation of actual TDR in those years as our study was restricted to analysing viral sequences from individuals sampled close to the time of HIV seroconversion.

Our results confirm and expand findings from studies of ART-naive individuals with unknown duration of HIV infection [19,20], and consistent with European reports of TDR with unknown duration of HIV infection [31,32]. Of note, although we show clear evidence for a decline in TDR rates over time, the 8.5% TDR prevalence in the most recent years highlights a moderate but ongoing risk of being infected with drug resistant virus remains.

We detected moderate evidence of an association between TDR and sampling during acute HIV infection. This suggests that TDR may be associated with seroconversion symptoms, possibly leading to presentation to care and HIV diagnosis during acute infection. It may also simply reflect that TDR rates are underestimated if genotypic resistance testing is not performed close to seroconversion because of reversion of mutations to wild type in the absence of drug selective pressure [11]. Of note, we found a similar proportion of TDR, 11.8%, among those diagnosed during acute infection throughout our period of study. There was also a similar association between TDR and acute HIV infection [OR = 1.16 (0.84, 1.58)], although this did not reach statistical significance as fewer individuals contributed to these analyses.

We also found evidence that MSM were more likely to have been infected with resistant strains compared with PWID and MSW. This has been reported by a number of studies in high-income countries [33–36] and may be because of historical access to HIV care, where MSM have been typically more exposed to ART than other risk groups [37], particularly the use of thymidine analogues such as stavudine and zidovudine, the mutations associated with which are known to be persistent [38]. This is supported by the differentially higher rates of NRTI mutation among MSM compared with other risk groups; 6.5, 4.5, and 3.2% among MSM, PWID, and MSW, respectively. The high prevalence of TDR among the MSM especially the last years, could also be because of the high incidence of HIV in this group in Western Europe, where in some cases transmissions may have occurred in transmission clusters of resistant strains in this population.

Our study has a number of limitations. Although we analysed data only from HIV seroconverters to assess actual TDR trends by year of infection, it is known that risk behaviour differs between seroconverters and nonseroconverter HIV-positive individuals [39,40], and that such behaviour may put them at greater risk of becoming infected with drug-resistant HIV. The prevalence of TDR, however, in our cohort was similar to that reported among other (seroprevalent) cohorts in Europe [19,20,24,41] suggesting that our time trends for TDR are generalizable to the HIV-positive population in Europe. However, our numbers outside Europe are small, so although our estimates were consistent across all CASCADE cohorts, our estimates might not be as robust and generalizable in lower income countries. It is also feasible that there were treatment misclassifications and patients with prior ART experience were included in our analysis. Research by the UK HIV Drug Resistance Database suggests that if there is more than a 4% misclassification, time trends could be distorted [42]. Being that integrase inhibitors are a new drug class, we were not able to provide temporal trend estimates for mutations associated with integrase inhibitors, as data on such mutations were limited, where only two individuals had a resistance mutation associated with integrase inhibitor raltegravir. In addition, those with genotypic tests tended to be different than those without genotypic tests, where individuals with genotype tests tended to seroconvert in later years, were more likely to be MSM, present with acute HIV infection, and have shorter HIV test intervals. Also, certain countries tended to test more for genotypic resistance (e.g. Germany and the UK) compared with other countries (e.g. France). We may, therefore, have underestimated the overall prevalence of TDR, given that the risk was higher in earlier years. It is unlikely, however, that the preferential inclusion of MSM among sequenced individuals will have affected our main finding of a decreasing TDR trend given that the proportion of MSM sequined has remained stable at about 60% over the calendar period.

In conclusion, we found a steady decline in TDR among individuals newly infected with HIV between 1996 (19.4% TDR) and 2012 (8.5% TDR). Although the rate of transmitted drug-resistant HIV has decreased, a not insubstantial proportion of newly infected individuals are being diagnosed with drug-resistant strains. Given that resistance testing among such individuals remains cost-effective for baseline resistance above 1% [43], testing for evidence of TDR remains justifiable.

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Conflicts of interest

There are no conflicts of interest.

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OPEN

Virological Blips and Predictors of Post Treatment Viral Control After Stopping ART Started in Primary HIV Infection

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Background: Few individuals commencing antiretroviral therapy (ART) in primary HIV infection (PHI) maintain undetectable viremia after treatment cessation. Associated factors remain unclear given the importance of the phenomenon to cure research.

Methods: Using CASCADE data of seroconverters starting ART in PHI (≤6 months from seroconversion), we estimated proportions experiencing viral blips (>400 copies followed by <400 copies HIV-RNA/mL without alteration of regimen) while on ART. We used Cox models to examine the association between time from ART stop to loss of control (2 consecutive measurements >1000 copies per milliliter) and magnitude and frequency of blips while on ART, time from seroconversion to ART, time on ART, adjusting for mean number of HIV-RNA measurements/year while on ART, and other confounders.

Results: Seven hundred seventy-eight seroconverters started ART in PHI with ≥3 HIV-RNA measurements. Median interquartile range (IQR) ART duration was 16.2 (8.0–35.9) months, within which we observed 13% with ≥1 blip. Of 228 who stopped ART, 119 rebounded; time to loss of control was associated with longer interval between seroconversion and ART initiation [hazard ratio (HR) = 1.16 per month; 1.04, 1.28], and blips while on ART (HR = 1.71 per blip; 95% confidence interval = 0.94 to 3.10). Longer time on ART (HR = 0.84 per additional month; 0.76, 0.92) was associated with lower risk of losing control. Of 228 stopping ART, 22 (10%) maintained post treatment control (PTC), ie, HIV-RNA <50 copies per milliliter ≥24 months after ART cessation.

Conclusion: HIV viral blips on therapy are associated with subsequent viral rebound on stopping ART among individuals treated in PHI. Longer duration on ART is associated with a greater chance of PTC.

Key Words: cure, viral blips, primary HIV infection, post treatment control (PTC)

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INTRODUCTION

Effective combination antiretroviral therapy (ART) controls HIV-1 viral replication to levels below the limit of detection of current laboratory assays,[1–3] confers improved clinical outcome,[4] and prevents onward transmission.[5] However, during suppressive therapy many patients experience transient detectable viremia, or "blips,"[6] defined as detectable plasma viremia >50 copies HIV-RNA/mL which subsequently returns to <50 copies without alteration of ART regimen.[7,8] Among such individuals subsequent viral failure remains infrequent if blip levels remain low[6,9] but, where virological failure ensued, the best predictor was a blip magnitude of >400 copies HIV-1 RNA/mL.[10,11] Furthermore, for most patients achieving HIV-RNA <50 copies per milliliter, approximately 1–3 copies of plasma HIV-RNA can be detected using more sensitive assays.[12]

ART is not a cure for HIV-1 infection—a consequence of an inaccessible reservoir of virally infected cells.[13–15] Novel approaches exploring "HIV-cure" strategies are under development. At present, although not routinely recommended, the only true test of "cure or remission" within the context of these trials is to stop ART, but only where planned and carefully monitored. It remains uncertain which individuals might be best placed to safely interrupt therapy.

For rare individuals initiating ART in primary HIV infection (PHI), plasma viremia remains undetectable after treatment interruption (TI). This phenotype has been termed post treatment control (PTC)[16] and seems to be more common

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among individuals stopping treatment initiated during PHI; a disease stage where the viral reservoir is smaller compared with chronic infection,[17,18] where immune dysfunction is less[19] and ART induced immunological recovery is often better.[20] Assessing PTC necessarily requires a TI. For most individuals, a TI results in viral load rebound,[21–23] which is more rapid among those initiating in chronic infection than in PHI. Furthermore, although this rebound has been shown to confer an increased risk of all-cause mortality for those interrupting ART initiated in chronic infection,[15] viral recrudescence increases the risk of onward transmission after TI, irrespective of disease stage. Therefore, if TI is planned in the context of cure research, it needs to be HIV-RNA guided and closely monitored, as prolonged TI guided by CD4 has been shown to increase morbidity/mortality.[15] Predictive markers that can evaluate individuals at increased likelihood of achieving PTC will be valuable tools in the design of future cure trials.

Although the exact mechanisms underlying PTC remain unknown, important predictors include low levels of viral reservoirs before TI, early initiation of ART, and longer duration of therapy.[16] This is supported by data from the SPARTAC trial[24,25] where pre-TI levels of HIV-1 DNA also predicted viral rebound[26] after ART cessation and data from early treatment studies in primates.[27]

The source and mechanism for viral blips remains uncertain; however,[28] and although blips may reflect transient periods of reduced ART adherence,[29,30] or variations between viral load assays,[31] the frequency and magnitude of blips on ART might also be related to the size of the proviral reservoir[32,33] and intermittent immune activation.[34,35] We, therefore, explored the frequency, magnitude, and predictive value of measured viral blips on the probability of achieving PTC among a cohort of treated HIV-1 seroconverters interrupting ART started initiated in PHI.

METHODS

Data Source

We used pooled data from the CASCADE 2014 data release in EuroCoord (www.EuroCoord.net) of seroconverter cohorts across Europe, Australia, Canada, and Sub-Saharan Africa. The collaboration has been previously described,[36] in brief date of HIV seroconversion in CASCADE is estimated most commonly as the midpoint between the last documented HIV negative and the first HIV-positive antibody test dates with an interval of ≤3 years between the 2 dates (87%). Dates of seroconversion for the remaining individuals (10%) is estimated through laboratory evidence of acute infection (HIV DNA polymerase chain reaction positivity in the absence of HIV antibodies or antigen positivity with <4 bands on Western blot), or as the date of HIV seroconversion illness with both an earlier documented negative and a later positive HIV test not more than 3 years apart (2%). Fiebig staging is not part of the algorithm for estimating date of seroconversion.[37]

All cohorts contributing to CASCADE received ethics approval from their individual ethics review boards.

Inclusion Criteria

Only adults older than 16 years starting ART within 6 months of estimated HIV seroconversion (PHI) with at least 3 HIV-RNA measurements while on ART were eligible for this analysis. Eligibility criteria and numbers, therefore, differ from our previous publication on proportions achieving PTC.[20]

Blips

We characterized the proportion of individuals experiencing blips while on ART initiated in PHI, and the associated exact 95% confidence intervals (CIs) for binomial distributed data. We also identified individuals with multiple blips while on ART. We used a modified definition of blip as a single plasma HIV-RNA measure >400 copies per milliliter in a previously suppressed individual followed by subsequent viral suppression (<400 copies per milliliter) without change in ART regimen.[1] Any magnitude of viremia episode was considered as a blip, as we were interested in the effect of blips regardless of the reasons for them. To be classified as having a blip or not, we included only individuals with HIV-RNA measured with assays detecting ≤400 copies per milliliter. Periods of unsuppressed viremia occurring during ART changes were attributed to the change in regimen and did not contribute to the analysis of blip rates.

In a sensitivity analysis on blip definitions, we defined additional blip thresholds of HIV-RNA >50, >100, and >200 copies per milliliter. The number of individuals included in this sensitivity analysis was smaller than the numbers included in the main analysis as fewer individuals were measured with assays detecting lower values.

Loss of Viremic Control

We used Kaplan–Meier methods to describe time from ART cessation to loss of viremic control and examined associated factors using Cox proportional hazards models. Loss of control was defined as the second of 2 consecutive HIV-1 RNA measurements >1000 copies per milliliter. Factors of interest were time on ART, time between HIV-1 seroconversion to ART initiation, plasma HIV-RNA at seroconversion, ART initiation year, CD4 T-cell count at ART initiation, CD4 T-cell count at ART cessation, ART class, age at HIV-1 seroconversion, sex, HIV-1 transmission risk group, and magnitude and frequency of blips while on ART. As rebound is more likely to be observed in those with more frequent measurements, we also adjusted for the mean number of HIV-RNA measurements/year while on ART. This also served as a proxy for adherence and engagement in care. Linear terms for all continuous variables were used, as there was no evidence for departures from linearity using natural cubic splines.[38]

We preformed several sensitivity analyses for the analysis of loss of viremic control. We defined blips as >50, >100, and >200 copies per milliliter, and we included covariates on the magnitude and frequency of each blip threshold. We also defined loss of control as the second of 2 consecutive HIV-RNA measurements greater than the given blip threshold. In additional, we limited our analysis to individuals who were on ART for at least 1 year before stopping treatment.

TABLE 1. Baseline Characteristics of Individuals Initiating ART Within 6 Months of HIV-1 Seroconversion, Those Subsequently Stopping ART, and Post Treatment Controllers (PTC) in CASCADE

	Started ART	Subsequently Stopped ART	PTC
Total, N	778	228	22
Sex, N (%)			
Male	714 (92)	206 (90)	16 (73)
Female	64 (8)	22 (10)	6 (27)
Risk group			
MSM	581 (75)	167 (73)	10 (45)
MSW	129 (17)	46 (20)	28 (36)
IDU	28 (4)	10 (4)	2 (9)
OTH	40 (5)	5 (2)	2 (9)
ART initiation class, N (%)			
NNRTI	288 (37)	87 (38)	12 (55)
PI	347 (45)	103 (45)	7 (32)
3 N	95 (12)	32 (14)	3 (14)
3 Class	11 (1)	4 (2)	0
Fusion inhibitor	6 (1)	0	0
Integrase inhibitor	30 (4)	2 (1)	0
SC yr, median (IQR)	2004 (2000–2010)	2001 (1999–2005)	2001 (2000–2003)
SC age, yrs, median (IQR)	34 (28–42)	33 (28–41)	35 (28–39)
Time on ART, mo, median (IQR)	16.2 (8.0–35.9)	11.0 (4.2–21.3)	17.4 (6.3–27.6)
Time from SC to ART, mo, median (IQR)	2.3 (0.7–4.1)	2.2 (0.5–3.9)	3.1 (0.6–5.3)
Initial HIV-RNA (log_{10} copies/mL)	5.3 (4.5, 5.9)	5.3 (4.6, 5.9)	4.9 (4.6, 5.6)
# HIV-RNA measurements per year, median (IQR)	3 (1–4)	1 (1–4.2)	3.5 (1–4.0)
HIV-RNA at ART cessation (log_{10} copies/mL), median (IQR)‡	—	0 (0–1.8)	0 (0–1.7)
CD4 at ART initiation	477 (316, 658)	494 (360, 701)	562 (230, 710)
CD4 at ART cessation, median (IQR)	—	709 (519–917)	738 (506–890)
Blips, % (95% CI), % 1 blip, copies/mL			
>50	13 (11 to 16), 78	11 (7 to 18), 87	7 (1 to 44), 0
>100	9 (7 to 12), 85	9 (5 to 16), 85	7 (1 to 42), 100
>200	6 (5 to 9), 79	9 (5 to 15), 77	9 (3 to 43), 100
>400	7 (6 to 9), 84	9 (6 to 14), 89	7 (2 to 36), 100

‡0 indicates undetectable HIV-RNA.

3 class, drugs from 3 or more classes; 3N, 3 nucleoside reverse-transcriptase inhibitors; IDU, injection drug use; MSM, men who have sex with men; MSW, sex between men and women; NNRTI, nonnucleoside reverse-transcriptase inhibitors; OTH, other; SC, seroconversion.

Post Treatment Controllers

PTC was defined as remaining <50 copies per milliliter for at least 24 months after ART stops. Once PTC was achieved, we used a strict definition for loss of PTC status as the first of 2 consecutive HIV-RNA measurements >50 copies per milliliter. Because there were very few PTCs, we did not formally analyze factors related to post treatment control.

RESULTS

Baseline Characteristics

Of 31,772 individuals in CASCADE, 22,688 were defined as PHI in the ART era (≥1995). Of these, 778 started ART within 6 months of seroconversion and had at least 3 HIV-RNA measurements. Of these, 228 (30%) subsequently stopped ART; reasons for stopping ART are unknown.

Among the 778 individuals starting ART in PHI, the majority were male (92%) seroconverting between 1995 and 2013 at median (IQR) age of 34 (28–42) years. Risk factors for HIV-1 infection were sex between men (75%), sex between men and women (17%), injecting drug use (4%), or other/unknown (5%). ART regimens included Nucleoside/Nucleotide Reverse Transcriptase Inhibitors NRTI backbone with protease inhibitor (PI) based (45%) or nonnucleoside reverse-transcriptase inhibitor based (37%) and other triple combinations (18%). Median interquartile range (IQR) time to ART initiation from seroconversion was 2.3 (0.7–4.1) months and median (IQR) time spent on ART initiated in PHI was 16.2 (8.0–35.9) months. Initial HIV-RNA measurement after HIV diagnosis was median 5.3 (4.5–5.9) log_{10} copies per milliliter and median CD4 at ART initiation was 477 (316–658) cells per cubic millimeter, Table 1.

Baseline characteristics for the subset of individuals subsequently stopping ART initiated in PHI (n = 228) were similar to all those starting ART in PHI (n = 778), with the exception of seroconversion year and time spent on ART, as

TABLE 2. Characteristics of Blips Among Individuals Initiating ART Within 6 Months of HIV-1 Seroconversion, Those Subsequently Stopping ART, and Post Treatment Controllers (PTC) in CASCADE

	Started ART	Subsequently Stopped ART	PTC
Total, N	778	228	22
Any blips*, % (95% CI), copies/mL			
>50	13 (11 to 16)	11 (7 to 18)	7 (1 to 44)
>100	9 (7 to 12)	9 (5 to 16)	7 (1 to 42)
>200	6 (5 to 9)	9 (5 to 15)	9 (3 to 43)
>400	7 (6 to 9)	9 (6 to 14)	7 (2 to 36)
Time to first blip†, yrs	0.9 (0.5, 1.9)	0.8 (0.4, 1.2)	1.7 (1.2, 2.3)
Multiple blips‡, %, copies/mL			
>50	22 (14, 33)	13 (3, 45)	—
>100	15 (7, 27)	15 (3, 51)	—
>200	21 (10, 37)	23 (6, 57)	—
>400	16 (9, 29)	11 (2, 39)	—
Time between blips§, yrs	0.7 (0.6, 1.1)	2.6 (1.0, 4.2)	—

*Denominator changes with varying blip thresholds due to different number of individuals with the required lower limits of detection.
†Among those with at least 1 blip >400 copies per milliliter, median (IQR).
‡Percentage of individuals with multiple blips among those with at least 1 blip. One PTC had blips >50,100 copies per milliliter and 2 had blips >200,400 copies per milliliter.
§Among those with multiple blips >400 copies per milliliter, median (IQR).

those subsequently stopping ART seroconverted in slightly earlier years, median (IQR) 2001 (1999–2005), and spent slightly less time on ART, median 11.0 (4.2–21.3) months. Blip rates were similar among individuals starting ART in PHI and individuals subsequently interrupting therapy, Table 1.

Blips While on ART

Of those starting ART in PHI with HIV-1 plasma HIV-RNA measured using assays detecting ≤400 copies per milliliter, we observed 7% (95% CI: 6 to 9) of individuals with 1 blip over 400 copies per milliliter, the majority (84%) of whom we observed only 1 blip. Among those that blipped over 400 copies per milliliter, median (IQR) time to the first blip was 1.0 (0.6–2.5) year and, among those with multiple blips, median (IQR) time between blips was 0.7 (0.6–1.1) years. Median (IQR) time to recover from a blip was 57 (32–111) days. Similarly, we observed at least 1 blip in 13% (11–16), 9% (7, 12), and 6% (5, 9) over 50, 100, and 200 copies per milliliter, respectively, and the majority, again, of whom we observed only 1 blip. Blip rates were similar among those who subsequently stopped ART, Table 2.

Factors Associated With Loss of Control After Stopping ART

Among the 228 individuals stopping ART, 22 (10%) individuals fulfilled the definition of PTC. Viral rebound was observed in 119 (52%) individuals; 23%, 37%, and 45% were observed to have rebounded by 3, 6, and 9 months, respectively. Median (95% CI) time to rebound was 10.3 (7.6 to 16.4) months. Several factors were independently associated with loss of control. Each blip >400 copies per milliliter was associated with a 71% increased risk of loss of control [hazard ratio (HR) = 1.71 (0.94, 3.10)], as was longer interval between seroconversion and ART initiation [HR = 1.16 per additional month (1.04, 1.28)]. More frequent HIV-RNA measurements while on ART were also associated with loss of control [HR = 1.10 per mean additional measurement/year increase (1.02, 1.17)] (Table 3).

Conversely, longer time spent on ART was independently associated with a decreased risk in loss of control [HR (95% CI) = 0.84 per 6 month increase (0.76 to 0.92)], as was later year of ART initiation [HR = 0.91 (0.84, 0.98)] (Table 3). There was no evidence of an association between loss of control and CD4 T-cell count at ART initiation, ART initiation class, seroconversion age, sex, or HIV-1 transmission risk group.

Using different blip thresholds, we observed an increased risk of loss of virologic control per increase in number of blips of similar magnitude to the results presented for blips >400 copies per milliliter in Table 1, although this did not reach statistical significance as fewer individuals contributed to these analyses. For each additional blip we found, HR = 1.96 (0.71, 5.38), 1.66 (0.88, 3.13), and 1.65 (0.90, 3.05) for blips of >50, 100, and 200 copies per milliliter, respectively. Defining loss of control as HIV-RNA >500 copies per milliliter resulted in similar time to rebound (Fig. 1), and factors associated with rebound remained the same as for the main analysis (data not shown). Time from the start of ART to the first blip was not associated with time to virologic rebound (data not shown).

Restricting to individuals who had been on ART for a year or more before stopping reduced the number of individuals included in analysis to 91. Time spent on ART and number of blips >400 copies per milliliter retained the same magnitude of association, as in the main analysis, although no longer remained statistically significant effects for time spent on ART or number of blips >400 copies per

TABLE 3. Multivariable Analysis of the Factors Associated With Virologic Rebound Among Those Stopping ART Initiated Within 6 Months of HIV Seroconversion Using the CASCADE Dataset

	HR (95% CI)	*P*
Time on ART (per 6-month increase)*	0.84 (0.76 to 0.92)	<0.001
Time from SC to ART (per month increase)†	1.16 (1.04 to 1.28)	0.006
# blips >400 copies/mL (per additional blip)	1.71 (0.94 to 3.10)	0.077
# mean HIV-RNA measurements/year (per additional measurement)	1.10 (1.02 to 1.17)	0.005
HIV-RNA at SC (per $\log_{10}$ increase)‡	1.15 (0.98 to 1.35)	0.086
ART initiation yr (per year increase)	0.91 (0.84 to 0.98)	0.016
Time from ART to viral suppression (per month increase)‡§	0.99 (0.97 to 1.02)	0.93
CD4 at ART initiation (per 100 cells/mm^3 increase)‖	0.99 (0.90 to 1.08)	0.75
CD4 at ART cessation (per 100 cells/mm^3 increase)‖	1.10 (1.01 to 1.20)	0.035
ART class		0.33
NNRTI	1	
PI	0.92 (0.57 to 1.48)	
3 N	1.32 (0.74 to 2.36)	
3 Class	0.24 (0.03 to 1.79)	
Integrase inhibitor	0.77 (0.10 to 6.12)	
SC age	1.00 (0.98 to 1.02)	0.77
Sex		0.49
Male	1	
Female	0.75 (0.33 to 1.69)	
HIV risk group		0.28
MSM	1	
MSW	0.84 (0.44 to 1.58)	
IDU	0.53 (0.14 to 2.03)	
OTH	0.23 (0.03 to 1.73)	

*Per 6 month increase.
†Per month increase.
‡Per $\log_{10}$ increase.
§HIV-RNA <50 copies per milliliter.
‖per 100 cells per mm^3 increase.
3 class, drugs from 3 or more classes; 3N, 3 nucleoside reverse transcriptase inhibitors; IDU, injection drug use; MSM, men who have sex with men; MSW, sex between men and women; NNRTI, nonnucleoside reverse-transcriptase inhibitors; OTH, other; SC, seroconversion.

milliliter [HR = 0.90 (0.78, 1.03) and 2.31 (0.71, 7.48), respectively].

Post Treatment Controllers

Of the 228 individuals interrupting ART, 22 (10.3%) achieved PTC status. ART initiation combinations for these 22 PTCs included nonnucleoside reverse-transcriptase inhibitor-based (n = 12; 55%), PI-based (n = 7; 32%), or triple nucleoside reverse-transcriptase inhibitors (3N) (n = 3; 14%) regimens. The proportion of PTCs for who we observed blips while on ART was slightly lower compared with the 206 individuals interrupting ART not achieving PTC status. We observed only 1 PTC with a blip >50 copies per milliliter, compared with 14 [12% (7, 19)] of all other individuals. Post treatment controllers also spent slightly longer time on ART compared with all other individuals interrupting ART, median (IQR) 17.4 (6.3–27.6) months for PTCs compared with 10.9 (3.6–19.0) months. The first HIV-RNA measurement after HIV diagnosis was slightly lower among PTCs with a median (IQR) 4.9 (4.6–5.6) $\log_{10}$ copies per milliliter compared with 5.3 (4.6–5.9) $\log_{10}$ copies per milliliter and the CD4 at ART initiation was slightly higher among PTCs with a median (562, 230–710) cells per mm^3 compared with 493 (363–690) cells per mm^3 among the remaining 206 individuals interrupting ART. Median number of HIV-RNA measurements per year after ART interruption was similar among PTCs and non-PTCs at 1 measure/year. Heterogeneity in time from HIV-1 seroconversion to ART initiation was small because of the inclusion criteria of starting ART within 6 months of HIV seroconversion and was, therefore, similar between post treatment controllers and all other individuals.

DISCUSSION

Using the large CASCADE dataset of individuals with well-estimated dates of HIV seroconversion, we provide the first evidence that frequency and magnitude of viral blips while on ART initiated in PHI is associated with viral rebound among individuals interrupting ART started in PHI.

The prevalence of PTC (defined by 2 years of undetectable viremia after TI) in our cohort is estimated to be 10.3%. This is not dissimilar to other cohorts reporting PTC[23,39–41] and slightly lower than the VISCONTI study (15.6%).[16] That said, most cohorts report few or none, including among early treated populations.[42–46] In comparison with VISCONTI, the duration of ART was shorter in our cohort, but shorter time from HIV diagnosis to ART initiation was also predictive of PTC in both cohorts.

Although much data exist for the predictive value of blips on subsequent viral failure among individuals on ART in chronic stages of HIV disease,[47–49] it is difficult to extrapolate this to PTC. The source of viral blips on ART is unclear. They may, for example, represent release of virus from transient, random activation of latently infected cells,[34] fluctuations in levels of persistent viral replication on ART,[50] sanctuary sites of suboptimal antiretroviral penetrance,[51] or nonadherence to ART regimens. One explanation for our findings is that initiating ART early in PHI results in fewer viral blips of lower magnitude because of the smaller HIV-1 viral reservoir achieved among these individuals.[52,53] Unfortunately, samples were not available to determine HIV-1 DNA measurements to test this assumption, although this is consistent with data from SPARTAC showing that levels of total HIV-1 DNA measured at TI predict time to loss of control.[24]

The associations observed in our cohort between timing of ART initiation, duration of therapy, and PTC were linear and, accordingly, we were unable to determine an optimal period beyond which ART initiation after seroconversion may be too late to achieve PTC. These are key questions that need to be addressed in prospective studies to inform future cure trial designs and help develop algorithms to predict

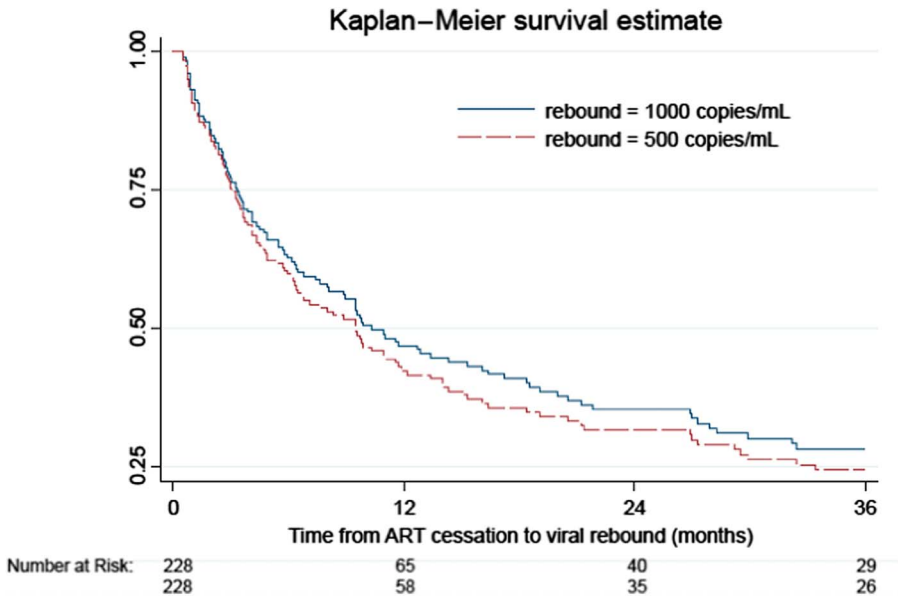

FIGURE 1. Time from ART cessation to virologic rebound, defined as HIV-RNA ≥500, 1000 copies per mL, among those stopping ART initiated within 6 months of HIV seroconversion in CASCADE.

likelihood of PTC. Of note, while HIV-1 HIV-RNA and initial CD4 count measurement at diagnosis of PHI are known to predict disease progression,[54] for those initiating immediate ART in our cohort, these parameters did not appear to influence subsequent PTC status, suggesting that the mechanisms underpinning the 2 processes may be different.

Host genetic factors may also determine PTC status, although we were not able to explore these factors. In the VISCONTI cohort there was no evidence for enrichment of protective HLA Class I alleles and only weak HIV-specific immunity was observed.

The data presented from this large cohort should be interpreted within the limitations of any observational study. First, for those individuals with a measurable viral blip, we assumed that ART was continuous through this period and a viral blip is not the result of temporary poor adherence, absorption, or the assay used; however, irrespective of the cause, the presence of a blip predicted viral rebound and hence must be incorporated into any algorithm for future HIV cure trials.[55] Second, reasons for ART initiation and subsequent cessation for eligible individuals are unknown and those stopping may differ in important characteristics from those not stopping, although short-course ART in PHI was not an uncommon treatment strategy by a number of clinicians during the time.[24,39,40] In any case, baseline HIV-RNA and CD4 measurements at ART initiation were similar for those subsequently stopping and those not stopping ART. It is, therefore, unlikely that reasons for stopping ART initiated in PHI were related to outcome but we acknowledge, as with all observational studies, that unmeasured confounding factors may remain, including in the choice of whether or not to initiate ART in PHI. Third, the absence of data on ART adherence is a limitation of these analyses, and blips may, therefore be, as a result of periods of nonadherence or viral breakthrough. We included the number viral load measurements as a surrogate of adherence in the multivariate analyses. In any case, our findings are of clinical relevance to clinicians as they highlight that patients experiencing blips, regardless of the reason, are more likely to experience viral failure on therapy[56,57] and less likely to achieve PTC if ART is stopped. Finally, frequency of monitoring HIV-RNA and assay variability are likely to affect blip detection, which may account for some of the observed differences in the significance and proportion of intermittent low-level viremia for ART-treated individuals.[8,9] It is also possible that frequency of HIV-RNA monitoring could influence the definition of virologic failure rate in this analysis or clinical practise reflects concerns with ART adherence. The median number of HIV-RNA measurements per year on ART were similar to the frequency off ART [1 (1, 4.2) and 1.6 (1, 2.9), respectively]. We have attempted to correct for measurement frequency by including it as a variable in our Cox models. We were not, however, able to correct for assay variability because of the limited sample size and as it was unknown for >50% of HIV-RNA measurements. In addition, we did not distinguish between boosted and unboosted PIs but, to account for these unmeasured changes in treatment quality over time, we adjusted for ART initiation year. It is possible that newer more potent ART regimens, including integrase inhibitors, not routinely available at the time of this analysis, could additionally impact on size of reservoir and viral blips on therapy.[55]

Stopping ART within the setting of a cure study should be undertaken within close clinical and laboratory monitoring and extrapolation of observational data into a study design in terms of individual health risks and risks of onward viral transmission must be made with caution. Both individual potential risks and the risk of onward viral transmission, should viral rebound ensue, also need to be taken into account.

In conclusion, findings from this large observational cohort of treated seroconverters stopping ART indicate that the absence of viral blips >400 copies HIV-1 RNA/mL in individuals treated with early ART, close to the time of PHI diagnosis predicted a better chance of subsequent after treatment viremic control after ART cessation.

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APPENDIX 1. CASCADE Collaboration in EuroCoord

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CASCADE Clinical Advisory Board: H. C.B., Andrea De Luca, Martin Fisher, R.M.

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ORIGINAL RESEARCH

Abacavir usage patterns and hypersensitivity reactions in the EuroSIDA cohort

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Objectives

Five to eight per cent of HIV-positive individuals initiating abacavir (ABC) experience potentially fatal hypersensitivity reactions (HSRs). We sought to describe the proportion of individuals initiating ABC and to describe the incidence and factors associated with HSR among those prescribed ABC.

Methods

We calculated the proportion of EuroSIDA individuals receiving ABC-based combination antiretroviral therapy (cART) among those receiving cART after 1 January 2009. Poisson regression was used to identify demographic, and current clinical and laboratory factors associated with ABC utilization and discontinuation.

Results

Between 2009 and 2016, of 10 076 individuals receiving cART, 3472 (34%) had ever received ABC-based cART. Temporal trends of ABC utilization were also heterogeneous, with 28% using ABC in 2009, dropping to 26% in 2010 and increasing to 31% in 2016, and varied across regions and over time. Poisson models showed lower ABC utilization in older individuals, and in those with higher CD4 cell counts, higher cART lines, and prior AIDS. Higher ABC utilization was associated with higher HIV RNA and poor renal function, and was more common in Central-East and Eastern Europe and lowest during 2014. During 779 person-years of follow-up (PYFU) in 2139 individuals starting ABC after 1 January 2009, 113 discontinued ABC within 6 weeks of initiation for any reason [incidence rate (IR) 14.5 (95% confidence interval (CI) 12.1, 17.5) per 100 PYFU], 13 because of reported HSR [IR 0.3 (95% CI 0.1, 1.0) per 100 PYFU] and 35 because of reported HSR/any toxicity [IR 4.5 (95% CI 3.2, 6.3) per 100 PYFU]. There were no factors significantly associated with ABC discontinuation because of reported HSR/any toxicity.

Conclusions

ABC remains commonly used across Europe and the incidence of discontinuation because of reported HSR was low in our study population.

Keywords: abacavir, HIV, hypersensitivity reaction

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*The study group is listed in the Appendix.

Introduction

In the absence of genetic screening, hypersensitivity reaction (HSR) presents in approximately 5–8% of persons living with HIV (PLHIV) initiating abacavir [1,2](ABC). HSR can vary in severity and clinical manifestation indicative of multiorgan involvement and includes fever, skin rash, and constitutional, gastrointestinal tract and respiratory symptoms [1–5]. In rare cases, HSR is fatal [6,7]. The risk of ABC HSR is high for patients who test positive for the human leucocyte antigen (HLA)-B*5701 allele [1,8,9]; however, ABC HSRs have been reported at a lower frequency in patients who do not carry this allele and therefore risk for HSR can be reduced by HLA-B*5701 screening [1,3]. ABC should never be initiated in patients with a positive HLA-B*5701 status, and ABC re-challenge among those previously experiencing HSR is contraindicated, as acute onset of potentially fatal symptoms has been reported [2,10–12].

ABC remains a commonly used drug throughout Europe and is recommended as a part of first-line therapy by national and international guidelines [13,14]; it is therefore important to continually examine the safety of ABC over time. A previous 2008 report using data from EuroSIDA, a longitudinal cohort collaboration across 35 countries in Europe plus Israel and Argentina [15], showed an HSR incidence within 3 months of starting ABC of 22.1 [95% confidence interval (CI) 18.7, 25.4] per 100 person-years of follow-up (PYFU) with a decreasing trend for ABC discontinuation because of HSR over time [16]. HSR caused by ABC typically presents within 6 weeks of therapy initiation [4,17], and presentation of HSR later than 8 weeks after ABC initiation is almost always attributable to other causes [18].

The objectives of this study were twofold. First, the aim was to describe the proportion of individuals across Europe on combination antiretroviral therapy (cART) receiving an ABC-based regimen from 1 January 2009 to 1 April 2016 and the factors associated with ABC initiation. Secondly, we sought to describe the cumulative frequency of, incidence of, and factors associated with ABC discontinuation because of reported HSR or because of reported HSR/any toxicity among those starting ABC after 1 January 2009 as part of a cART regimen.

Methods

Study population

EuroSIDA is a longitudinal observational cohort study that was initiated in 1994, and has been previously described [15]. The data collected include start and stop dates for each antiretroviral drug used, reasons for discontinuing an antiretroviral drug and clinical events. Further details on data collected can be found at www.cphiv.dk.

Individuals from the EuroSIDA cohort over the age of 16 years at enrolment receiving cART (at least three drugs from any class, excluding ritonavir) after 1 January 2009 were included in the ABC utilization over time analysis. All persons starting ABC-based cART after 1 January 2009 were eligible for inclusion for analyses of reported HSR-related discontinuation.

Statistical methods

Among those receiving cART and under active follow-up, the proportion of individuals who received ABC at the midpoint of each calendar year (1 July) from 1 January 2009 onwards and by geographical region was summarized using descriptive statistics. Active follow-up was defined as having a first visit date before and last visit date after the midpoint of the year.

Factors associated with ABC utilization were investigated using Poisson regression with generalized estimating equations (GEEs) to control for the inclusion of repeated exposures and events. Baseline was defined as 1 January 2009 or enrolment into EuroSIDA, whichever occurred later. Individuals off ABC contributed follow-up until ABC initiation, the last EuroSIDA visit date or death, whichever occurred first. If an individual stopped ABC they were allowed to re-enter the analysis, and once again were considered eligible for starting ABC. Factors that were significantly associated with ABC initiation ($P < 0.1$) in univariate analyses were included in multivariable models. Factors investigated were gender, age, ethnicity, HIV transmission risk group, region of care, calendar year, CD4 cell count, nadir CD4 cell count, HIV RNA, line of cART regimen, hepatitis B and C status, previous AIDS diagnosis, Framingham 10-year elevated cardiovascular disease (CVD) risk [19], chronic kidney disease (CKD), and Data on Adverse Drugs (D:A:D) CKD risk [20,21]. Line of cART regimen captured the extent of previous antiretroviral treatment and treatment failure and was defined as a change in at least two antiretroviral drugs accompanied by an HIV RNA > 500 HIV-1 RNA copies/mL, or more than 6 months off treatment before starting a new therapy. CKD was defined as two consecutive estimated glomerular filtration rate (eGFR) values < 60 more than 3 months apart using the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) formula [22].

For the second objective, analysing reported HSR-related discontinuation, individuals were included if they initiated ABC as part of cART after 1 January 2009. Individuals with prior ABC exposure were included, and individuals who

started ABC more than once after 1 January 2009 could contribute multiple exposure periods. Baseline was defined as the start of an ABC-containing regimen, 1 January 2009 or recruitment to EuroSIDA, whichever occurred later. This was an on-treatment analysis where individuals contributed follow-up until 6 weeks after ABC initiation, ABC discontinuation for any cause, death or their last visit date, whichever occurred first. The primary outcome was discontinuation because of reported HSR. We also analysed a composite outcome of discontinuation because of reported HSR or any toxicity, as well as investigating all reasons for discontinuation to account for underreporting and potentially undiagnosed HSR cases.

Factors associated with reported HSR or any toxicity-related discontinuation were identified in a multivariable Poisson regression model using GEE to adjust for repeated events; those that were significant ($P < 0.1$) in univariable analyses were included in multivariable models.

Results

Baseline characteristics

Among 10 076 individuals in EuroSIDA receiving cART between 1 January 2009 and 1 April 2016, 3472 (34%;

Table 1 Baseline characteristics of all participants, split by abacavir (ABC) use (total *vs.* no ABC *vs.* ever ABC) in the EuroSIDA cohort from 1 January 2009 to 1 April 2016

	Total		No ABC		ABC		
	n	%	*n*	%	*n*	%	*P*-value
Gender							
Female	7408	74	4926	75	2482	72	0.001
Male	2668	27	1678	25	990	29	
Region of care in Europe							
South	2819	28	1962	30	857	25	< 0.001
West	2478	25	1672	25	806	23	
North	2187	22	1348	20	839	24	
Central-East	1430	14	897	14	533	15	
East	1162	12	725	11	437	13	
Ethnicity							
White	8827	88	5773	87	3054	88	0.291
Black	563	6	361	6	202	6	
Asian	163	2	108	2	55	2	
Other/unknown	523	5	362	6	161	5	
HIV risk group							
MSM	4054	40	2701	41	1353	39	0.171
IDU	2198	22	1438	22	760	22	
Heterosexual	3138	31	2012	31	1126	32	
Other/unknown	686	7	453	7	233	7	
Calendar year*,†	2009	(2009, 2011)	2009	(2009, 2011)	2011	(2009, 2010)	0.001
Baseline age (years)†	45	(37, 52)	45	(37, 51)	51	(38, 52)	0.001
Baseline HIV RNA (copies/mL)†	49	(39, 74)	49	(39, 71)	71	(33, 90)	< 0.001
Baseline CD4 cell count (cells/μL)†	490	(337, 688)	488	(340, 679)	679	(333, 709)	0.385
CKD							
No	7354	84	5597	85	1757	51	< 0.001
Yes	172	2	106	2	66	2	
Missing	1232	14	901	14	1649	47	
AIDS							
No	6848	68	4559	69	2289	66	0.001
Yes	3228	32	2045	31	1183	34	
cART line							
First	5859	58	3815	58	2044	59	< 0.001
Second	2043	20	1307	20	736	21	
Third	964	10	611	9	353	10	
≥ Fourth	1210	12	871	13	339	10	

Baseline is defined as entry to the study which was on 1 January 2009 or enrolment in EuroSIDA, whichever occurred later. Percentages are column percentages. Region of care in Europe includes: South: Argentina, Greece, Israel, Italy, Portugal and Spain; West: Austria, Belgium, France, Germany, Luxembourg and Switzerland; North: Denmark, Finland, Iceland, Ireland, the Netherlands, Norway, Sweden and the UK; Central-East: Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia and Slovenia; East: Belarus, Estonia, Georgia, Latvia, Lithuania, Russian Federation and Ukraine.
CKD, chronic kidney disease; cART, combination antiretroviral therapy; MSM, men who have sex with men; IDU, injecting drug use.
*Calendar year for the first ABC utilization date in the follow-up period.
†Values are median (interquartile range).

95% CI 34–35%) had ever received ABC during follow-up (Table 1). Seventy-four per cent were male and 27% were female, with HIV risk groups of men who have sex with men (MSM) (40%), injecting drug users (IDUs) (22%), heterosexual (31%) and other/unknown (7%). The highest proportion received care in Southern Europe (28%) followed by Western Europe (25%), Northern Europe (22%), Central-East Europe (14%), and Eastern Europe (12%). Median baseline age was 45 years [interquartile range (IQR) 37, 52 years]. In general, demographic characteristics among those exposed to ABC were similar to characteristics in those not exposed, apart from baseline age, with those receiving ABC slightly older than those not receiving ABC. Baseline HIV RNA values among those initiating ABC were slightly higher compared with those not exposed to ABC, and there was also a higher prevalence of AIDS among those initiating ABC.

ABC utilization and factors associated with starting ABC

ABC utilization significantly varied over time, starting at 28% in 2009, dropping to 26% in 2010 and increasing to 31% in 2016 (*P*-heterogeneity from univariate analysis < 0.001) (Fig. 1). There was a significant interaction between region and time, where ABC utilization in Northern Europe decreased, Southern and Eastern Europe increased and Western and Central-East Europe remained relatively consistent with time (*P*-interaction < 0.001).

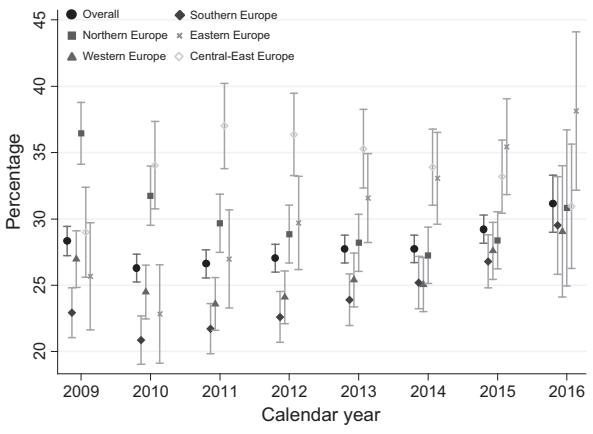

Fig. 1 Percentage of patients prescribed abacavir at the midpoint of each year, overall and in each region by year, in the EuroSIDA cohort from 2009 to 2016. Region of care in Europe includes: South: Argentina, Greece, Israel, Italy, Portugal and Spain; West: Austria, Belgium, France, Germany, Luxembourg and Switzerland; North: Denmark, Finland, Iceland, Ireland, the Netherlands, Norway, Sweden and the UK; Central-East: Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia and Slovenia; East: Belarus, Estonia, Georgia, Latvia, Lithuania, Russian Federation and Ukraine.

Using multivariable Poisson regression, factors associated with lower rates of ABC utilization were older age [incidence rate ratio (IRR) 0.73 (95% CI 0.59, 0.90) for the highest age quintile (> 58 years) compared with the lowest (18–41 years)], higher CD4 cell count [IRR 0.68 (95% CI 0.56, 0.82) for CD4 count > 500 cells/μL compared with < 200 cells/μL), and exposure to more cART treatment regimens [IRR 0.55 (95% CI 0.46, 0.66) for at least fourth-line compared with first-line regimens], and having a previous AIDS diagnosis [IRR 0.9 (95% CI 0.8, 1.0) compared with those without a previous AIDS diagnosis]. Higher ABC utilization rates were associated with higher HIV RNA [IRR 1.92 (95% CI 1.47, 2.51) for HIV RNA > 100 000 copies/mL compared with < 500 copies/mL], CKD [IRR 2.62 (95% CI 2.06, 3.34) compared with those without CKD], and higher D:A:D CKD risk score [IRR 1.18 (95% CI 1.01, 1.38) for those at high risk compared with low risk]. There was heterogeneity in ABC utilization among regions; those in Central-East and Eastern Europe were more likely to initiate ABC compared with those in Southern Europe [IRR 1.58 (95% CI 1.35, 1.84) and 1.71 (95% CI 1.42, 2.05), respectively]. Persons under follow-up in 2014 were less likely to start ABC compared with those under follow-up in 2009 [IRR 0.69 (95% CI 0.57, 0.85)] (Fig. 2).

Discontinuation of ABC

Among 2139 individuals initiating ABC after 1 January 2009, contributing 778 PYFU, 113 individuals (5.3%) discontinued ABC within 6 weeks of ABC initiation, an incidence rate (IR) of 14.51 (95% CI 12.07, 17.45) per 100 PYFU. The most common single reason for discontinuation within the first 6 weeks was unknown, followed by the patient's wish/decision, then other causes. Thirteen individuals (0.6%) discontinued because of reported HSR [IR 1.67 (95% CI 0.97, 2.87) per 100 PYFU] and 35 (4.6%) discontinued because of reported HSR or any toxicity [IR 4.49 (95% CI 3.23, 6.26) per 100 PYFU] (Table 2).

As only 13 persons discontinued because of reported HSR, we could not formally investigate factors associated with reported HSR-related discontinuation. Expanding the endpoint to discontinuation because of reported HSR or any toxicity did not identify any factors associated with discontinuation. The strongest factor associated with discontinuation for reported HSR/any toxicity was nadir CD4 cell count, where those with a nadir CD4 cell count of 350–500 cells/μL were at the highest risk of discontinuing because of reported HSR/any toxicity [IRR 1.60 (95% CI 0.59, 4.37) compared with those with CD4 count < 200 cells/μL; *P*-heterogeneity = 0.078]. This analysis had limited power as there were few events (n = 35).

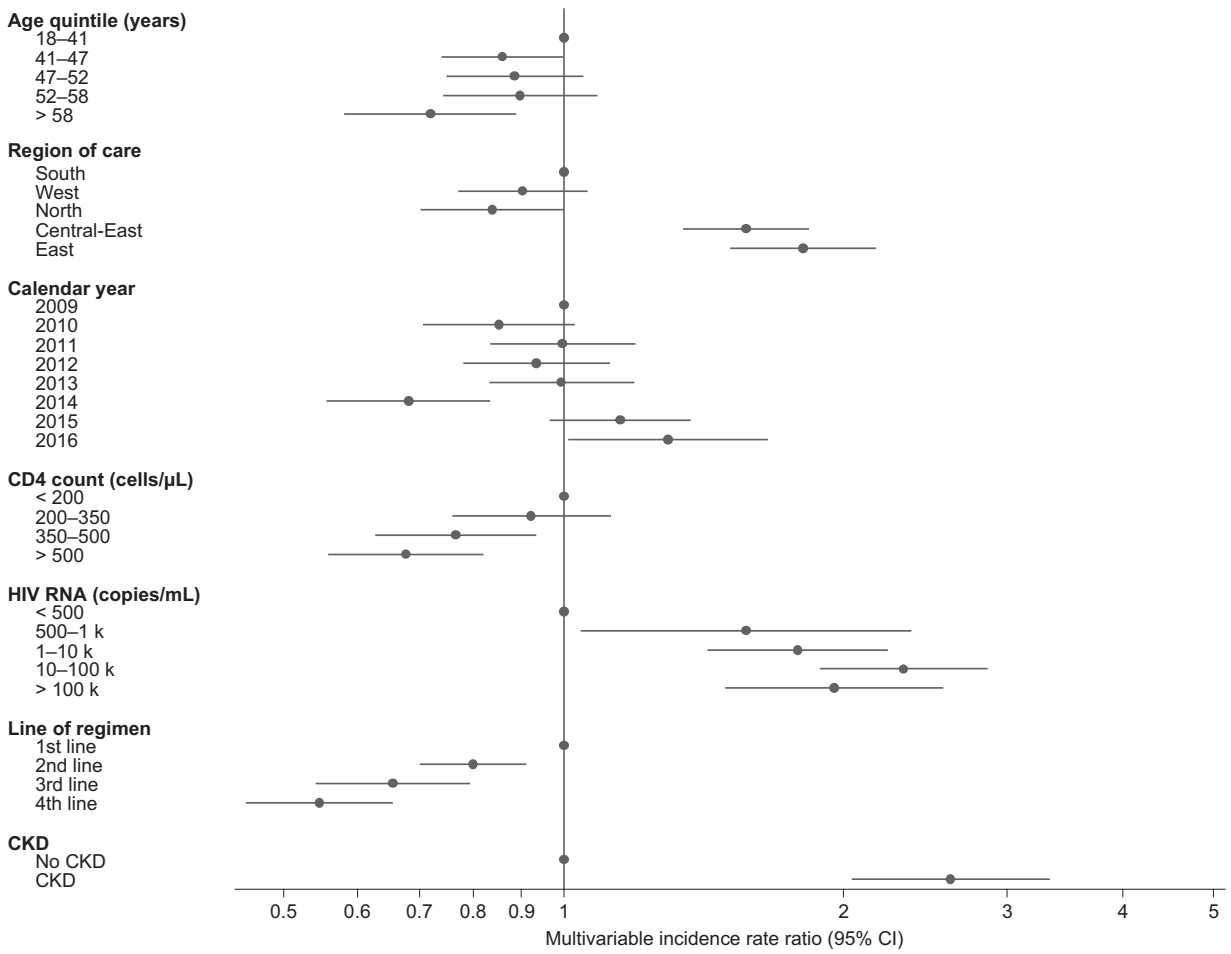

Fig. 2 Multivariable incidence rate ratios for abacavir (ABC) utilization in the EuroSIDA cohort from 1 January 2009 to 1 April 2016. All clinical and laboratory variables are time updated. Chronic kidney disease (CKD) was defined as two consecutive estimated glomerular filtration rate values <60 more than 3 months apart using the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) formula. Other variables in the model include gender, Framingham cardiovascular disease 10-year elevated risk, hepatitis C virus and hepatitis B virus status, Data on Adverse Drugs (D:A:D) CKD risk score and previous AIDS diagnosis. Region of care in Europe includes: South: Argentina, Greece, Israel, Italy, Portugal and Spain; West: Austria, Belgium, France, Germany, Luxembourg and Switzerland; North: Denmark, Finland, Iceland, Ireland, the Netherlands, Norway, Sweden and the UK; Central-East: Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia and Slovenia; East: Belarus, Estonia, Georgia, Latvia, Lithuania, Russian Federation and Ukraine. CI, confidence interval.

Seven individuals died within 6 weeks of initiating ABC, all with advanced HIV disease and other comorbidities. HSR was not reported among these individuals and was unlikely to have been the cause of these deaths.

Discussion

Despite the risk of HSR, ABC remains a commonly used antiretroviral drug across Europe in EuroSIDA. Among individuals initiating ABC-based cART after 2009, there were very few discontinuations within 6 weeks of ABC initiation, and discontinuation rates because of reported hypersensitivity reactions were 0.3 (95% CI 0.1, 1.0) per 100 PYFU. There were seven deaths within 6 weeks of starting ABC, but these were likely to have been from causes unrelated to ABC HSR reactions.

ABC utilization was lower among individuals who had been exposed to more treatment regimens and had previous ABC exposure. Because there is evidence to suggest ABC re-challenge should be avoided [5,12,23], it is possible this result is attributable to lower ABC prescription rates among those already exposed to ABC. Unexpectedly, higher ABC utilization was associated with CKD and higher D:A:D CKD risk scores, although this could be attributed to confounding by indication. Use of tenofovir has been linked to kidney disease [24] and individuals on

Table 2 Reasons and incidence rates for abacavir (ABC) discontinuation by reason for stopping treatment in the EuroSIDA cohort from 1 January 2009 to 4 January 2016. Individuals were censored at 6 weeks after ABC initiation, ABC discontinuation or death, whichever came first

Reason for stopping treatment as reported to EuroSIDA	Failures	Rate	95% CI
Any reason	113	14.51	12.07, 17.45
HSR or any toxicity	35	4.49	3.23, 6.26
Any toxicity	22	2.82	1.86, 4.29
Unknown	21	2.70	1.76, 4.14
Patient's wish/decision	20	2.57	1.66, 3.98
Other causes	17	2.18	1.36, 3.51
Physician's decision	16	2.05	1.26, 3.35
Toxicity, GI tract	16	2.05	1.26, 3.35
HSR	13	1.67	0.97, 2.87
Toxicity, liver	2	0.26	0.06, 1.03
Toxicity, predominantly CNS	2	0.26	0.06, 1.03
Toxicity, predominantly kidneys	2	0.26	0.06, 1.03
Treatment failure	1	0.13	0.02, 0.91
Concern of cardiovascular disease, including dyslipidaemia	1	0.13	0.02, 0.91
Other toxicity	1	0.13	0.02, 0.91
Noncompliance	1	0.13	0.02, 0.91

Total person-years of follow-up (PYFU) = 778; rate is per 100 PYFU.
CI, confidence interval; CNS, central nervous system; GI, gastrointestinal; HSR, hypersensitivity reaction.

tenofovir with decreasing renal function are likely to discontinue tenofovir [25] and be switched to ABC [26], a drug with no reported adverse effect on renal function. Changes over time and within regions in use of abacavir probably reflect marketing and availability of abacavir.

Overall, the rate of discontinuation of ABC in the first 6 weeks after starting ABC was low, being similar to the findings of phase II clinical trials [27,28], but slightly lower than previous EuroSIDA findings [16], although the previous EuroSIDA report had a much larger window to observe reported HSR cases (3 months *vs*. 6 weeks in our study). The rate of stopping because of reported HSR or the composite endpoint of reported HSR or any toxicity was also low, which could indicate the effectiveness of screening for HLA-B*5701, better patient care and a greater understanding of HSR among treating physicians. Screening uptake for the HLA-B*5701 allele has probably avoided many HSR reactions in recent years. EuroSIDA does not collect genetic screening information; thus, it is unknown which individuals were tested for HLA-B*5701, or whether the frequency of testing varied between regions and/or over calendar time.

It is possible that we found low rates of discontinuation as a consequence of using a 6-week window from ABC initiation, but it is well established that this is when HSR is most likely to occur [18]. Even so, compared with early cART, where discontinuation rates at 3 months were reported to be between 10 and 15%[29,30], discontinuation because of ABC was low, indicating the effectiveness of screening for HLA-B*5701, improved patient management, improved antiretroviral regimens, reduction in toxicities, and improved adherence [31]. We found no factors significantly associated with ABC discontinuation because of reported HSR/any toxicity, although this might partly be attributable to low power as few patients discontinued.

Along with lack of data on HLA-B*5701 screening, there are other limitations to our study. Most notably, the symptoms of HSR can be difficult to distinguish from other adverse events in the population, possibly leading to over- or underreporting of discontinuation because of reported HSR. EuroSIDA also only collects one reason for discontinuing a drug, so if HSR and another simultaneous reason for discontinuation occurred, HSR may have not been reported as the reason for discontinuation in our data. We have investigated underreporting by using a composite outcome of reported HSR or any toxicity, with consistent results. Finally, the validity of our models depends on the assumption that we appropriately adjusted for confounding; it is, however, possible that our models have residual confounding by indication. Nonetheless, EuroSIDA is in a unique position to compare and describe treatment patterns as a consequence of the standardized nature of the data collection and the inclusion of countries for which there are no national cohorts or surveillance structures.

This study also has several strengths, including the use of a large data set from a heterogeneous population and the inclusion of data from Eastern Europe. In addition, EuroSIDA covers the period 1994–2016 with consistent records of all antiretroviral use and reasons for stopping, allowing comparisons of temporal trends of ABC utilization and subsequent HSR. There has also been consistency in the way data on antiretrovirals and the subsequent reasons for stopping over our study duration have been collected.

In summary, ABC remains a commonly used drug throughout Europe, and the incidence of reported HSR among those on ABC is low, probably attributable to screening for HLA-B*5701, improved patient care and a greater understanding and awareness of HSR.

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Appendix: the EuroSIDA study group

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Antiretroviral penetration into the CNS and incidence of AIDS-defining neurologic conditions

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ABSTRACT

Objective: The link between CNS penetration of antiretrovirals and AIDS-defining neurologic disorders remains largely unknown.

Methods: HIV-infected, antiretroviral therapy–naive individuals in the HIV-CAUSAL Collaboration who started an antiretroviral regimen were classified according to the CNS Penetration Effectiveness (CPE) score of their initial regimen into low (<8), medium (8–9), or high (>9) CPE score. We estimated "intention-to-treat" hazard ratios of 4 neuroAIDS conditions for baseline regimens with high and medium CPE scores compared with regimens with a low score. We used inverse probability weighting to adjust for potential bias due to infrequent follow-up.

Results: A total of 61,938 individuals were followed for a median (interquartile range) of 37 (18, 70) months. During follow-up, there were 235 cases of HIV dementia, 169 cases of toxoplasmosis, 128 cases of cryptococcal meningitis, and 141 cases of progressive multifocal leukoencephalopathy. The hazard ratio (95% confidence interval) for initiating a combined antiretroviral therapy regimen with a high vs low CPE score was 1.74 (1.15, 2.65) for HIV dementia, 0.90 (0.50, 1.62) for toxoplasmosis, 1.13 (0.61, 2.11) for cryptococcal meningitis, and 1.32 (0.71, 2.47) for progressive multifocal leukoencephalopathy. The respective hazard ratios (95% confidence intervals) for a medium vs low CPE score were 1.01 (0.73, 1.39), 0.80 (0.56, 1.15), 1.08 (0.73, 1.62), and 1.08 (0.73, 1.58).

Conclusions: We estimated that initiation of a combined antiretroviral therapy regimen with a high CPE score increases the risk of HIV dementia, but not of other neuroAIDS conditions. ***Neurology*®** **2014;83:134–141**

GLOSSARY

cART = combined antiretroviral therapy; **CI** = confidence interval; **CPE** = CNS Penetration Effectiveness; ***ICD-9*** = *International Classification of Diseases, ninth revision*; **NNRTI** = nonnucleoside reverse transcriptase inhibitor.

AIDS-defining neurologic disorders, or neuroAIDS, include HIV dementia and the opportunistic infections toxoplasmosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy.[1–3] The incidence of neuroAIDS in developed countries decreased after the introduction of combined antiretroviral therapy (cART) in 1996,[2,4–9] but antiretroviral drug–related neurotoxicity remains a concern.[2,10–12]

The risk of neuroAIDS may depend on the concentration of antiretrovirals in the CNS, which is a function of their ability to penetrate the blood-brain barrier. Greater exposure of antiretrovirals in the CNS may decrease the HIV RNA in the CSF,[13] but may also be neurotoxic.[14,15] One proposed method to assess a drug's penetrative ability into the CNS is via the CNS Penetration Effectiveness (CPE) ranking system. In cohort studies to date, lower CPE ranks are associated with higher CSF HIV RNA after adjusting for a number of clinical variables.[13,16–18]

While the association between CPE rank and CSF HIV RNA is reported, the connection between CPE rank and clinical outcomes remains unclear.[17,19–22] A randomized controlled trial comparing a CNS-targeted therapy to a non-CNS–targeted therapy among 49 individuals with HIV-associated neurocognitive disorders found no difference in improvement of neurocognitive

Editorial, page 109

Supplemental data at Neurology.org

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performance after 16 weeks.[23] An observational study in the United Kingdom found a similar incidence of neuroAIDS in individuals with high and low CPE scores.[24]

Herein, we present estimates of the effect of CPE score on the incidence of 4 neuroAIDS conditions among individuals with HIV-1 infection included in a large multinational collaboration of cohort studies from Europe and the United States.

METHODS

Standard protocol approvals, registrations, and patient consents. Research using the HIV-CAUSAL Collaboration was determined to be nonhuman subjects research by the Institutional Review Board of the Harvard School of Public Health because it involves the study of existing data that are analyzed in such a manner that the subjects cannot be identified, as set forth in US federal regulations. Written informed consent from patients was not required because all data were completely anonymized.

Study population. The HIV-CAUSAL Collaboration includes prospective cohort studies from 6 European countries and the United States. All cohorts included in the HIV-CAUSAL Collaboration were assembled prospectively and are based on data collected for clinical purposes from national health care systems that offer universal access to care. Each cohort in the collaboration collected data prospectively, including all CD4 cell counts, HIV RNA measurements, treatment initiations, deaths, and AIDS-defining illnesses (including the events of interest: HIV dementia, toxoplasmosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy).

The individual cohort studies used in these analyses are UK CHIC (United Kingdom), ATHENA (the Netherlands), FHDH-ANRSCO4 (France), SHCS (Switzerland), PISCIS (Spain), CoRIS/CoRIS-MD (Spain), VACS-VC (US veterans), AMACS (Greece), and AQUITAINE (France). Four cohorts of seroconverters with relatively high CD4 counts did not have any neuroAIDS events and were excluded from the analyses.

We restricted our analyses to individuals with HIV-1 infection who met the following criteria at baseline (starting in January 1998): age 18 years or older, no history of AIDS (defined as the onset of any category C AIDS-defining illness),[25] antiretroviral therapy naive (as defined elsewhere[26]), no pregnancy (when information was available), CD4 cell count and HIV RNA measured within the previous 6 months, and initiating a complete antiretroviral regimen (see below) consisting only of drugs with known CPE ranks.

We conducted separate analyses for the following neuroAIDS events: HIV dementia, toxoplasmosis, cryptococcal meningitis, or progressive multifocal leukoencephalopathy. Non-Hodgkin lymphoma was not included as an event because we could not differentiate primary brain lymphoma from other types of non-Hodgkin lymphoma. We also looked at a combined endpoint of any of the 3 opportunistic infections (toxoplasmosis, cryptococcal meningitis, or progressive multifocal leukoencephalopathy). The date of neuroAIDS was identified by the treating physicians. One of the contributing cohorts (VACS) used *ICD-9* codes to identify incident neuroAIDS cases. The other contributing cohorts used diagnostic procedures that reflect standard clinical practice in Europe rather than standardized research criteria.

For each patient, follow-up started on the date of initiation of a complete antiretroviral regimen—defined as treatment with at least 2 nucleoside reverse transcriptase inhibitors plus either one or more protease inhibitors, one or more nonnucleoside reverse transcriptase inhibitors (NNRTIs), one entry/fusion inhibitor, or one integrase inhibitor—and ended at death, 12 months after the most recent laboratory measurement, pregnancy (if known), the cohort-specific administrative end of follow-up (ranging between December 2003 and February 2013) or the event of interest, whichever occurred earlier.

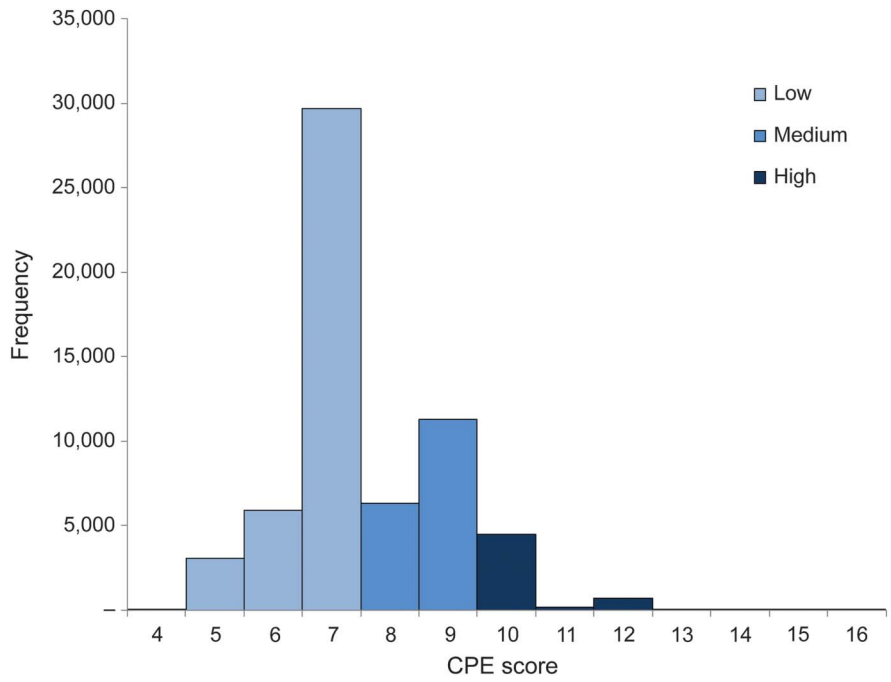
Assessment of antiretroviral CNS exposure. The 2010 CPE ranking system is a proposed method for measuring the penetrative ability of different antiretroviral drugs into the CNS. Each drug is given a rank ranging from 1 to 4 based on pharmacokinetic and pharmacodynamic data, drug characteristics, results of clinical studies, and effectiveness in reducing CSF viral load or improving cognition. A rank of 4 represents the best penetration or effectiveness.[14,16] The CPE score for a given regimen is calculated by summing the ranks of each drug in the regimen. We categorized the CPE score for a regimen as low (<8), medium (8–9), or high (>9) based on the distribution of the data (the cut points were approximately at the median and the 75th percentile) (figure 1). Because our estimates may be sensitive to the chosen cut points, we also treated the CPE score as a continuous variable.

Statistical methods. Using a pooled logistic regression model, we estimated the average "intention-to-treat" neuroAIDS hazard ratio for a high and a medium baseline CPE score compared with a low baseline CPE score. Under the assumption that the monthly probability of an event is small (a condition satisfied in our study), the parameters of our pooled logistic model closely approximate the parameters of a Cox proportional hazards model.[27] We computed these estimates separately for each of the 4 neuroAIDS conditions as well as for the combined endpoint of opportunistic infections. The model included month of follow-up (restricted cubic splines with 4 knots at 1, 6, 24, and 60 months) and the following baseline covariates: CD4 cell count (<200, 200–299, ≥300 cells/μL), HIV RNA level (<10,000, 10,000–100,000, >100,000 copies/mL), sex, acquisition group (heterosexual, homosexual/bisexual, injection drug use, other or unknown), calendar year (1998, 1999–2000, 2001–2003, ≥2004), age (<35, 35–50, >50 years), geographic origin (North America or Western Europe, Sub-Saharan Africa, other, or unknown), race (white, black, other, or unknown), years since HIV diagnosis (<1, 1–4, ≥5 years or unknown), whether or not the regimen was an NNRTI-based regimen, and cohort.

The variables we adjusted for in our models are associated with CPE score and widely known to be associated with the outcomes of interest. For example, a baseline CD4 cell count <200 cells/μL, a baseline HIV RNA >100,000 copies/mL, and a baseline age >50 years were associated with an increased odds of both HIV dementia and the combined endpoint of opportunistic infections compared with a baseline CD4 cell count ≥300 cells/μL, a baseline HIV RNA <10,000 copies/mL, and a baseline age <35 years, respectively (data not shown).

To adjust for potential selection bias due to infrequent follow-up, we computed inverse probability weights. Each patient in the above logistic models received a time-varying weight inversely proportional to the estimated probability of not being censored, for each month that patient was followed.[28,29] We fit a pooled logistic model using the baseline covariates listed above, the baseline CPE score category, and the most recent measurement of the following time-varying covariates: CD4 cell count (restricted cubic spline with 5 knots at 10, 200, 350, 500, and 1,000 cells/μL), HIV RNA level (<5,000, 5,000–10,000, 10,000–100,000, >100,000 copies/mL), time since last laboratory measure (0, 1–2, 3–4, 5–6, ≥7 months), and AIDS (any category C AIDS-defining illness other than the neuroAIDS condition of interest), and estimated each patient's

Figure 1 Number initiating treatment by CPE score



Number of individuals initiating treatment by CNS Penetration Effectiveness (CPE) score (range 4–16), HIV-CAUSAL Collaboration, 1998–2013.

probability of remaining uncensored in each month of follow-up. The models for the weights were fit before the final models. The weights were stabilized as described elsewhere[28] and were then used to fit the final weighted regression model. The estimated weights for each of the 5 outcomes had mean 1.00 (first percentile: 0.96; 99th percentile: 1.20).

We used robust variance estimators that take into account the procedure of weight estimation to compute 95% confidence intervals (CIs) for each of our estimates.[30] Under the assumption that the measured covariates are sufficient to adjust for confounding and selection bias, our approach emulates a nonblinded randomized trial in which patients were assigned to regimens with 1 of 3 CPE score categories.[28]

Several sensitivity analyses were performed for the outcome HIV dementia: we (1) varied the CPE score of regimens that were boosted with ritonavir because the CPE score category for boosted ritonavir is somewhat ambiguous in 2 regimens; (2) restricted the analysis to VACS in order to additionally adjust for the VACS Index[31]; (3) excluded VACS from the analysis; (4) estimated stabilized inverse probability weights to adjust for potential selection bias due to death, a competing risk[32]; (5) excluded cases of HIV dementia occurring in the first year; and (6) restricted the analysis to individuals who initiated therapy before the introduction of the first CPE scoring system in 2008. Exclusion of 0.15% of individuals with unusual treatment combinations (e.g., boosted nelfinavir and unboosted darunavir) did not materially affect the estimates. We also considered an alternative categorization of the CPE score by dividing the lowest category into 2 smaller categories (<6 and 6 to <8), and estimated the average log hazard ratios using a model that included a flexible functional form for the continuous CPE score (restricted cubic spline with 4 knots at 5, 7, 9, and 12). We fit a pooled logistic model containing this form of the CPE score as well as the previously listed baseline covariates, and adjusted for potential selection bias due to infrequent follow-up as previously described.

All analyses were conducted with SAS 9.3 (SAS Institute, Cary, NC). The LOGISTIC procedure was used to fit the weighted regression models, and a nonparametric bootstrap with 500 samples was used to compute 95% CIs.

RESULTS A total of 61,938 individuals met the eligibility criteria for our study; 38,786 (62%) initiated a regimen with a low CPE score, 17,687 (29%) with a medium CPE score, and 5,465 (9%) with a high CPE score. The mean score for individuals on an NNRTI-based regimen was 8.2 and the mean for individuals on a non-NNRTI–based regimen was 7.3. Table 1 shows the baseline characteristics of the study population by CPE score category. Individuals with a high CPE score were more likely to be female, heterosexual, initiating therapy before 2004, and of Sub-Saharan African origin. The median (interquartile range) follow-up time was 37 (18, 70) months.

During follow-up, there were 235 cases of HIV dementia, 169 cases of toxoplasmosis, 128 cases of cryptococcal meningitis, and 141 cases of progressive multifocal leukoencephalopathy. Forty individuals developed 2 of the 4 neuroAIDS conditions, and one individual developed 3. The incidence rate (per 10,000 person-years) was 9.0 for HIV dementia, 6.5 for toxoplasmosis, 4.9 for cryptococcal meningitis, and 5.4 for progressive multifocal leukoencephalopathy. The median (interquartile range) time from cART initiation to the event of interest was 14 (2, 39) months for HIV dementia, 4 (1, 16) months for toxoplasmosis, 10 (1, 25) months for cryptococcal

Table 1 Baseline characteristics by CPE score category, HIV-CAUSAL Collaboration, 1998–2013

Baseline characteristics	Persons, % (n)		
	Low CPE score (n = 38,786)	Medium CPE score (n = 17,687)	High CPE score (n = 5,465)
CD4 cell count, cells/μL			
<200	37.8 (14,674)	44.9 (7,939)	43.5 (2,377)
200 to <300	25.5 (9,890)	25.6 (4,521)	24.2 (1,320)
≥300	36.7 (14,222)	29.5 (5,227)	32.3 (1,768)
HIV RNA, copies/mL			
<10,000	18.6 (7,215)	17.9 (3,172)	20.5 (1,121)
10,000–100,000	41.6 (16,136)	40.6 (7,176)	41.9 (2,292)
>100,000	39.8 (15,435)	41.5 (7,339)	37.6 (2,052)
Sex			
Male	80.0 (31,016)	74.4 (13,157)	66.2 (3,618)
Female	20.0 (7,770)	25.6 (4,530)	33.8 (1,847)
Race			
White	23.9 (9,285)	23.3 (4,125)	17.8 (970)
Black	13.9 (5,373)	20.0 (3,541)	21.9 (1,198)
Other	62.2 (24,128)	56.7 (10,021)	60.3 (3,297)
Age, y			
<35	34.6 (13,418)	35.5 (6,280)	41.9 (2,289)
35–50	47.4 (18,382)	47.1 (8,327)	43.6 (2,384)
>50	18.0 (6,986)	17.4 (3,080)	14.5 (792)
Origin			
North America or Western Europe	60.3 (23,373)	54.7 (9,671)	53.1 (2,903)
Sub-Saharan Africa	13.7 (5,321)	19.9 (3,516)	23.4 (1,277)
Other	9.3 (3,600)	7.5 (1,326)	6.6 (359)
Unknown	16.7 (6,492)	17.9 (3,147)	16.9 (926)
Acquisition group			
Heterosexual	33.1 (12,853)	40.3 (7,129)	45.5 (2,488)
Homosexual/bisexual	41.8 (16,214)	32.2 (5,706)	28.4 (1,554)
Injection drug user	5.9 (2,264)	6.9 (1,213)	7.7 (418)
Other/unknown	19.2 (7,455)	20.6 (3,639)	18.4 (1,005)
Calendar year			
1998	7.4 (2,865)	7.2 (1,283)	6.0 (326)
1999–2000	8.5 (3,291)	14.6 (2,578)	29.1 (1,593)
2001–2003	8.7 (3,362)	24.8 (4,381)	41.3 (2,257)
≥2004	75.4 (29,268)	53.4 (9,445)	23.6 (1,289)
Regimen			
NNRTI-based	47.2 (18,295)	59.4 (10,501)	74.6 (4,079)
non-NNRTI-based	52.8 (20,491)	40.6 (7,186)	25.4 (1,386)

Abbreviations: CPE = CNS Penetration Effectiveness; NNRTI = nonnucleoside reverse transcriptase inhibitor.

meningitis, and 3 (1, 14) months for progressive multifocal leukoencephalopathy.

Table 2 shows the 3 most frequently used cART regimens with high, medium, and low CPE scores. Compared with initiating a cART regimen with a low CPE score, the hazard ratio (95% CI) for initiating a cART regimen with a high CPE score was 1.74 (1.15, 2.65) for HIV dementia, 0.90 (0.50, 1.62) for toxoplasmosis, 1.13 (0.61, 2.11) for cryptococcal meningitis, and 1.32 (0.71, 2.47) for progressive multifocal

Table 2 Most frequently used cART regimens with a low, medium, and high CPE score, HIV-CAUSAL Collaboration, 1998–2013

Regimen	No. of initiators	CPE score (category)
Efavirenz, tenofovir, emtricitabine	14,839	7 (low)
Nelfinavir, zidovudine, lamivudine	3,368	7 (low)
Lopinavir, ritonavir, tenofovir, emtricitabine	3,342	7 (low)
Efavirenz, zidovudine, lamivudine	5,346	9 (medium)
Lopinavir, ritonavir, zidovudine, lamivudine	3,823	9 (medium)
Efavirenz, lamivudine, abacavir	1,837	8 (medium)
Nevirapine, zidovudine, lamivudine	3,373	10 (high)
Indinavir, ritonavir, zidovudine, lamivudine	757	10 (high)
Efavirenz, zidovudine, lamivudine, abacavir	409	12 (high)

Abbreviations: cART = combined antiretroviral therapy; CPE = CNS Penetration Effectiveness.

leukoencephalopathy (table 3). Compared with a low CPE score, the respective hazard ratios (95% CIs) for a medium CPE score were 1.01 (0.73, 1.39), 0.80 (0.56, 1.15), 1.08 (0.73, 1.62), and 1.08 (0.73, 1.58). Figure 2 shows the log hazard ratio against the continuous CPE score for the combined endpoint of opportunistic infections and for HIV dementia (see figure e-1 on the *Neurology*® Web site at Neurology.org for the individual opportunistic infections).

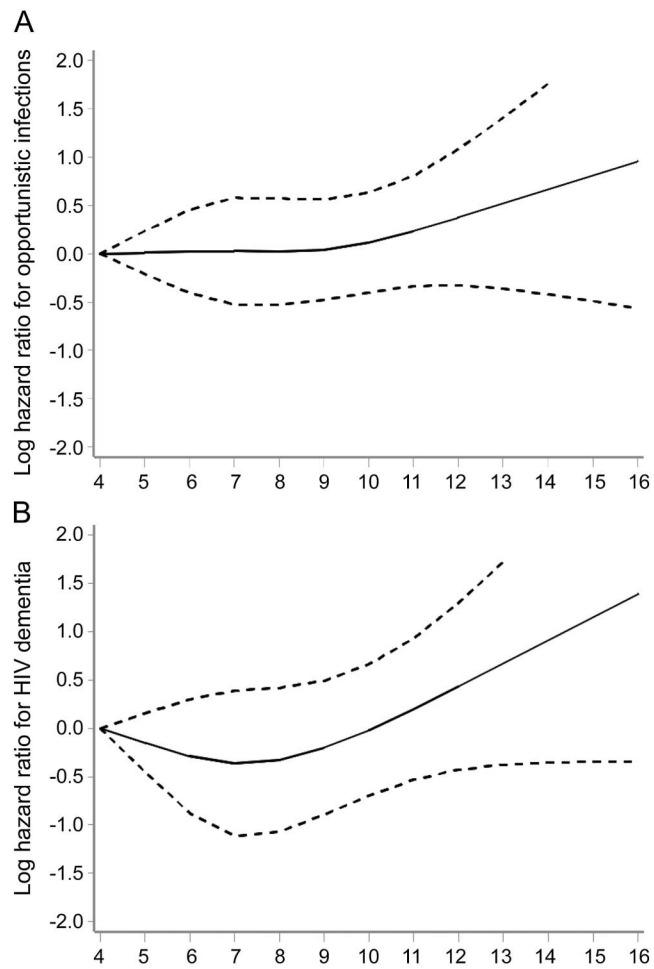
The hazard ratio of HIV dementia for a high CPE score vs a low CPE score did not vary substantially by CD4 cell count, age, sex, and type of cART regimen (NNRTI-based vs non-NNRTI–based), but the 95% CIs were wide (data not shown). The hazard ratios and 95% CIs did not change with different specifications of the functional form for month of follow-up (data not shown). None of the sensitivity analyses described in the previous section yielded appreciably different results (table e-1). Varying the cut points of the CPE score did not materially change the point estimates but resulted in wider CIs. Excluding cases of HIV dementia occurring in the first year of follow-up (table e-2) did not materially change the estimates of the effect of CPE score on HIV dementia.

Table 3 Hazard ratios for CPE score, HIV-CAUSAL Collaboration, 1998–2013

CPE score	Person-years	No. of events	Unadjusted hazard ratio	95% CI	Adjusted hazard ratio[a]	95% CI
HIV dementia						
Low	140,962	127	1.00	Reference	1.00	Reference
Medium	86,799	72	0.97	0.72, 1.30	1.01	0.73, 1.39
High	32,097	36	1.55	1.06, 2.26	1.74	1.15, 2.65
Opportunistic infections[b]						
Low	140,553	245	1.00	Reference	1.00	Reference
Medium	86,455	134	1.09	0.88, 1.34	0.99	0.80, 1.22
High	31,985	49	1.18	0.87, 1.62	1.08	0.77, 1.52
Toxoplasmosis						
Low	140,983	106	1.00	Reference	1.00	Reference
Medium	86,807	45	0.86	0.60, 1.22	0.80	0.56, 1.15
High	32,099	18	0.94	0.57, 1.57	0.90	0.50, 1.62
Cryptococcal meningitis						
Low	141,098	64	1.00	Reference	1.00	Reference
Medium	86,818	48	1.35	0.92, 1.98	1.08	0.73, 1.62
High	32,121	16	1.43	0.83, 2.48	1.13	0.61, 2.11
Progressive multifocal leukoencephalopathy						
Low	141,109	81	1.00	Reference	1.00	Reference
Medium	86,849	43	1.12	0.77, 1.64	1.08	0.73, 1.58
High	32,116	17	1.36	0.80, 2.33	1.32	0.71, 2.47

Abbreviations: CI = confidence interval; CPE = CNS Penetration Effectiveness.
[a] Adjusted for cohort, month of follow-up, baseline CD4 cell count, baseline HIV RNA level, sex, acquisition group, calendar year, age, geographic origin, race, years since HIV infection, and type of drug regimen, as well as time-varying CD4 cell count, RNA level, time since last measurement, and AIDS. Stabilized inverse probability weights were used to account for censoring due to infrequent follow-up.
[b] Includes toxoplasmosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy.

Figure 2 Estimated log hazard ratios and 95% confidence intervals



Estimated log hazard ratios and 95% confidence intervals for opportunistic infections (A) (toxoplasmosis, cryptococcal meningitis, or progressive multifocal leukoencephalopathy) and HIV dementia (B) comparing each CNS Penetration Effectiveness (CPE) score with a CPE score of 4 (lowest), HIV-CAUSAL Collaboration, 1998–2013.

DISCUSSION We estimated that the incidence of HIV dementia increases by more than 70% after initiating an antiretroviral regimen with a high CPE score compared with a low score. However, we found little change in the incidence of toxoplasmosis, cryptococcal meningitis, and progressive multifocal leukoencephalopathy. These results are unexpected, and the interpretation of our effect estimates needs to be tempered by the limitations of our study.

In the only published study that has considered CPE scores in relation to neuroAIDS among HIV-positive individuals, the incidence of neuroAIDS was similar for a baseline CPE score of 10 or greater and for a baseline CPE score of 4 or less, but the small number of events resulted in a wide 95% CI (hazard ratio: 0.95; 95% CI: 0.53, 1.72). Furthermore, the study was not restricted to antiretroviral therapy–naive individuals and effect estimates for individual neuroAIDS conditions such as HIV dementia were not provided.[24] Other studies of the association between CPE scores and risk of cognitive impairment (but not neuroAIDS) have had conflicting results.[17,19–21,33]

Our findings that CPE score does not affect the incidence of opportunistic infections may not be particularly surprising, because the development of these neuroAIDS conditions may be very closely connected to the degree of impaired cell-mediated immunity and not associated with antiretroviral penetration.[4,5] In contrast, antiretroviral penetration into the brain may lead to deposition of β-amyloid plaques, which has been proposed as a possible explanation for a harmful effect of high CPE score on HIV dementia.[34] One study observed a higher percentage of extracellular β-amyloid in cART-treated patients than in untreated HIV-positive individuals,[35] and HIV-positive individuals with HIV-associated dementia have higher levels of intraneuronal β-amyloid immunoreactivity compared with HIV-positive individuals without HIV-associated dementia.[34–36] However, the underlying mechanism through which antiretroviral penetration could cause HIV dementia remains unknown. The hypothesis that antiretroviral penetration increases the incidence of HIV dementia via deposition of β-amyloid plaques requires further research to determine whether these associations are in fact causal. Alternative pathways including antiretroviral-related direct neuronal damage and mitochondrial toxicity should also be evaluated.[15]

Another explanation for the higher dementia risk for regimens with a high CPE score is that these regimens are less effective to treat HIV disease, for example, because of incomplete adherence: 68% of individuals in the study deviated from their initial regimen at some point. However, both the high average proportion of follow-up spent on the initial regimen (58%) and the lack of a strong association between CPE score and opportunistic infections do not support this explanation.

Similar to any other observational study, the validity of our estimates relies on the untestable assumption that the measured covariates were sufficient to adjust for confounding and selection bias. It is possible that consideration of CPE scores is a factor for decisions concerning antiretroviral regimens in patients with neurocognitive symptoms. If individuals with neurocognitive symptoms are more likely to initiate antiretroviral regimens with higher CPE scores, the estimated effect on dementia might be explained by this confounding by indication. If this were the case, we would expect the estimated effect on dementia to disappear or to attenuate after a certain amount of time. However, excluding cases of HIV dementia occurring in the first year of follow-up does not materially change the results. Furthermore, restricting the

analysis to individuals who initiated therapy before the introduction of the first CPE scoring system in 2008 does not affect the results.

Some limitations of our study should be noted. First, with relatively few events, the 95% CIs around our effect estimates are wide. However, our study is the largest one to date on this topic. Second, the cohorts included in this analysis are from developed countries; our results may not be generalizable to resource-limited settings or to other health care systems. Third, with the exception of VACS, the contributing cohorts used diagnostic procedures that reflect standard clinical practice in Europe. Excluding VACS from the analysis, however, did not significantly alter the results. Fourth, while our effect estimates are adjusted for cohort, we were not able to adjust for the individual centers within each cohort. Thus, some residual confounding due to centers within each cohort is theoretically possible. Finally, the average duration of follow-up in our study was approximately 3 years. Future studies will be needed to investigate the effect of antiretroviral penetration on the long-term incidence of neuroAIDS, as well as the effect of newer antiretrovirals that are not well represented in current studies, including ours.

We estimated that initiation of a cART regimen with a high CPE score increases the risk of HIV dementia, but not of other neuroAIDS conditions. These findings should be interpreted cautiously, and additional studies are needed to examine the effect of CPE score on the incidence of HIV dementia more closely. Together with additional data on the safety and effectiveness of different cART regimens, these results may be useful to plan the management of individuals with HIV infection.

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AUTHOR CONTRIBUTIONS

E.C. Caniglia, L.E. Cain, and M.A. Hernán conceived the study and wrote the first draft of the manuscript. E.C. Caniglia completed the literature search and created the figures. All authors contributed to the final draft. A. Justice, J. Tate, R. Logan, C. Sabin, A. Winston, A. van Sighem, J. Miro, D Podzamczer, A. Olson, J. Arribas, S. Moreno, L. Meyer, J. del Romero, F. Dabis, H. Bucher, G. Wandeler, G. Vourli, A. Skoutelis, E. Lanoy, J. Gasnault, and D. Costagliola contributed to data collection. E.C. Caniglia and L.E. Cain completed the analyses and data interpretation with assistance and advice from M.A. Hernán and R. Logan. Members of the Writing Committee include Ellen C. Caniglia, Lauren E. Cain, PhD (Coordinating Center), Amy Justice, PhD, Janet Tate, PhD (VACS), Roger Logan, PhD (Coordinating Center), Caroline Sabin, PhD, Alan Winston, MD (UK CHIC), Ard van Sighem, PhD (ATHENA), Jose M. Miro, PhD, Daniel Podzamczer, PhD (PISCIS), Ashley Olson, PhD (UKREG), José Ramón Arribas, MD, Santiago Moreno, PhD (CoRIS/CoRIS-MD), Laurence Meyer, PhD (PRIMO/SEROCO), Jorge del Romero, MD (GEMES), François Dabis, PhD (Aquitaine), Heiner C. Bucher, MD, Gilles Wandeler, MD (SHCS), Georgia Vourli, MSc, Athanasios Skoutelis, MD (AMACS), Emilie Lanoy, PhD, Jacques Gasnault, MD, Dominique Costagliola, PhD (FHDH-ANRS C04), and Miguel A. Hernán, DrPH (Coordinating Center).

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DISCLOSURE

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Opportunistic infections and AIDS malignancies early after initiating combination antiretroviral therapy in high-income countries

The HIV-CAUSAL Collaboration

Background: There is little information on the incidence of AIDS-defining events which have been reported in the literature to be associated with immune reconstitution inflammatory syndrome (IRIS) after combined antiretroviral therapy (cART) initiation. These events include tuberculosis, mycobacterium avium complex (MAC), cytomegalovirus (CMV) retinitis, progressive multifocal leukoencephalopathy (PML), herpes simplex virus (HSV), Kaposi sarcoma, non-Hodgkin lymphoma (NHL), cryptococcosis and candidiasis.

Methods: We identified individuals in the HIV-CAUSAL Collaboration, which includes data from six European countries and the US, who were HIV-positive between 1996 and 2013, antiretroviral therapy naive, aged at least 18 years, had $CD4^+$ cell count and HIV-RNA measurements and had been AIDS-free for at least 1 month between those measurements and the start of follow-up. For each AIDS-defining event, we estimated the hazard ratio for no cART versus less than 3 and at least 3 months since cART initiation, adjusting for time-varying $CD4^+$ cell count and HIV-RNA via inverse probability weighting.

Results: Out of 96 562 eligible individuals (78% men) with median (interquantile range) follow-up of 31 [13,65] months, 55 144 initiated cART. The number of cases varied between 898 for tuberculosis and 113 for PML. Compared with non-cART initiation, the hazard ratio (95% confidence intervals) up to 3 months after cART initiation were 1.21 (0.90–1.63) for tuberculosis, 2.61 (1.05–6.49) for MAC, 1.17 (0.34–4.08) for CMV retinitis, 1.18 (0.62–2.26) for PML, 1.21 (0.83–1.75) for HSV, 1.18 (0.87–1.58) for Kaposi sarcoma , 1.56 (0.82–2.95) for NHL, 1.11 (0.56–2.18) for cryptococcosis and 0.77 (0.40–1.49) for candidiasis.

Conclusion: With the potential exception of mycobacterial infections, unmasking IRIS does not appear to be a common complication of cART initiation in high-income countries.

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Keywords: HIV, immune reconstitution inflammatory syndrome, incidence, inverse probability weighting, unmasking

Introduction

Combined antiretroviral therapy (cART) has dramatically reduced morbidity and mortality associated with HIV infection [1–4]. cART restores the immune response against opportunistic infections, but some patients experience an inflammatory reaction within weeks or months after cART initiation [5,6]. This immune reconstitution inflammatory syndrome (IRIS), whose pathogenesis is not fully elucidated, can result in clinical worsening of existing opportunistic infections after commencing cART (paradoxical IRIS) or in the appearance soon after cART initiation of a new and previously unrecognised opportunistic infections (unmasking IRIS) [7]. IRIS, may be associated with significant morbidity, is a diagnostic challenge and complicates clinical management [7].

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IRIS has been described in patients with opportunistic infections and AIDS malignancies caused by infections [8–17] as well as in patients with non-infectious conditions, such as rheumatoid arthritis and sarcoidosis, although with a different immunopathogenesis [18,19]. Whereas there is a solid body of literature documenting reactions associated with immune restoration for mycobacterial infections both in the HIV-negative [20] and the HIV-positive population [21–24], our knowledge of IRIS for other conditions is mainly based upon case studies of patients on cART. Most of these describe cases of paradoxical phenomena and only a few studies have reported on unmasking IRIS. Further, because case definitions have not been implemented in large observational databases, it has been problematic to estimate its magnitude.

The HIV-CAUSAL Collaboration has recently reported an increase in tuberculosis incidence shortly after cART initiation which was particularly marked in patients with $CD4^+$ cell counts below 50 cells/μl, a pattern strongly suggestive of unmasking IRIS [25]. This pattern was not seen for *Pneumocystis jirovecii* pneumonia. Here we update and extend our study to the effect of cART on AIDS-defining events suggested to be associated with IRIS in the literature: tuberculosis, *mycobacterium avium* complex (MAC), cytomegalovirus (CMV) retinitis, progressive multifocal leukoencephalopathy (PML), herpes simplex virus (HSV), Kaposi sarcoma, non-Hodgkin lymphoma (NHL), cryptococcosis and candidiasis. For each of these AIDS-defining events, we explore whether changes in incidence after cART initiation are compatible with unmasking IRIS.

Methods

Study population

We used data from the HIV-CAUSAL collaboration, which include HIV-positive individuals from prospective cohorts in six European countries and the United States [25]. All cohorts are based on routinely collected data in clinical practice within settings with universal access to care. Initiation of cART was defined as the date on which an individual initiated treatment with at least two nucleoside reverse transcriptase inhibitors and either one or more protease inhibitors, one non-nucleoside reverse transcriptase inhibitors, one entry/fusion inhibitor or one integrase inhibitor.

Analyses included individuals who were HIV-positive between 1996 and 2013 aged 18 years or more, and had a $CD4^+$ cell count and an HIV-RNA measurement within 6 months of each other while antiretroviral therapy (ART) naive. Individuals' follow-up started at baseline, defined as the date when all the inclusion criteria were met, and ended at outcome diagnosis, death, 12 months after the most recent laboratory measurement or cohort-specific administrative censoring, whichever occurred earlier. To prevent the misclassification of undiagnosed prevalent opportunistic infections and AIDS malignancies as incident cases, and thus, minimise the inclusion of cases of paradoxical IRIS, our analyses excluded HIV-positive individuals who were not AIDS-free during the baseline month.

Outcomes

We considered as primary outcomes all AIDS-events previously suggested to be associated with IRIS. We included tuberculosis, CMV retinitis, cryptococcosis, PML and Kaposi sarcoma because these were the most common AIDS-defining events in a systematic review of IRIS in observational studies [11]. We included MAC because its association with IRIS was observed soon after antiretroviral therapy was introduced [21]. We included NHL because rare manifestations of IRIS have been reported [10,26]. We included candidiasis and HSV (often not considered in association with IRIS) because a large cohort study of cART initiators in the United States [12] reported them as the most common IRIS-related events.

The diagnostic criteria for the AIDS-defining events [27] were those routinely used in clinical practice in each of the participating countries. Information on use of prophylaxis drugs for these conditions is not collected by the HIV-CAUSAL Collaboration because prophylaxis for these conditions is not widely implemented in most of the participating cohorts.

For each outcome, our working definition for unmasking IRIS was a newly diagnosed and non-previously detected AIDS-defining event in the first 3 months after starting cART.

Statistical methods

All analyses were conducted separately for each outcome. We computed incidence rates as number of cases per 1000 person-years and estimated the hazard ratio of each outcome for (i) cART versus no cART and (ii) no cART versus less than 3 and at least 3 months since cART initiation. We then estimated the cumulative incidence up to 3 months after cART initiation [28].

To estimate the hazard ratios, we used a pooled logistic model for risk of the outcome at month $m+1$ that included a time-varying indicator for ever use of cART through month m, month of follow-up m (restricted cubic splines with five knots) and the following baseline covariates: $CD4^+$ cell count (<50, 50–99 100–199 200–349 350–499, or ≥500 cells/μl) HIV-RNA level (<4, 4–5 or >5 $\log_{10}$ copies/ml), sex, transmission group (heterosexual, MSM, injecting drug users, or other/unknown), calendar year (1996–1998, 1999–2000, 2001–2003 or 2004–2013), age (<35, 35–50, or >50 years), geographical

origin (Western countries, sub-Saharan Africa, other, or unknown), time since HIV infection diagnosis (<3 versus ≥3 months) and cohort.

As cART is more likely to be initiated in individuals with a low $CD4^+$ cell count and a high HIV-RNA level, estimates from the previous models have to be adjusted for these time-dependent confounders. Because $CD4^+$ cell count and HIV-RNA are affected by prior treatment, adding them as time-dependent covariates in the logistic regression model may introduce bias [29]. Therefore, we used inverse probability weighting to adjust for time-varying $CD4^+$ cell count and HIV-RNA. Formally, under the assumption that all time-varying predictors of both cART and AIDS were included in the analyses, the weighted model estimates the parameters of a marginal structural Cox model [30].

Each patient in the analysis received a time-dependent weight inversely proportional to the probability of having its own observed history of cART initiation, as described elsewhere [30]. To estimate each patient's probability of cART initiation in each month, we fit a pooled logistic model that included the covariates listed above for the outcome model and the most recent measurement of the following time-dependent covariates: $CD4^+$ cell count (restricted cubic splines with five knot), HIV-RNA level (<4, 4–5 or >5 $\log_{10}$ copies/ml), AIDS (yes or no) and time since last laboratory measurement. Inverse probability weights were also estimated to adjust for potential selection bias because of censoring by infrequent measurement. Both the cART initiation and censoring weights were stabilized and their product used to fit the weighted pooled logistic model. To avoid the influence of outliers on the variance of the estimates, we truncated the weights at a maximum of 10 which affected less than 1% of the individuals. The estimated weights used in the analyses had a mean of 1.01. Truncation did not materially change the hazard ratio estimates. We computed conservative 95% confidence intervals for the log hazard ratio by using a variance estimator that accounts for the estimation of the weights.

We performed several sensitivity analyses: (i) we estimated the hazard ratio of no cART versus time since cART initiation categories less than 4 and at least 4 months, (ii) in addition to censoring follow-up at 12 months without a laboratory measurement, we censored at 18 and 24 months after the last measurement, (iii) the start of follow-up was delayed by 3 months to exclude prevalent cases, iv) we lagged $CD4^+$ cell count and HIV-RNA level 14 or 21 days to ensure that cART initiation was predicted using prior measurements, (v) we estimated inverse probability weights for censoring by death (so that estimates can be interpreted as if all deaths could be prevented) and (vi) we included patients who started cART during the first month after baseline.

All analyses were conducted with SAS, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Our analysis included 96 562 eligible individuals who contributed 377 324 person-years during a median [interquantile range (IQR)] follow-up of 31 [13,65] months. Table 1 shows their baseline characteristics: 78% were men and 70% started follow-up after 2000. The median [IQR] $CD4^+$ cell count, HIV-RNA and age at baseline were 405 [263 570] cells/μl, 4.4 [3.8,5.0] $\log_{10}$ copies/ml and 36 [30,43] years, respectively. Fifty-seven percent of the included patients initiated cART during follow-up; the median [IQR] $CD4^+$ cell count, HIV-RNA and age at cART initiation were 279 [187 380] cells/μl, 4.7 [4.0,5.2] $\log_{10}$ copies/ml and 38 (32,46) years, respectively.

The incidence rate (per 1000 person-years) ranged between 2.3 for tuberculosis and 0.3 for CMV retinitis and PML. For all outcomes, incidence rates were lower for higher $CD4^+$ cell count, younger age and lower HIV-RNA level at baseline (Fig. 1). Appendix 1, http://links.lww.com/QAD/A579 shows the number of cases and incidence rates for each outcome by baseline characteristics. The hazard ratios (95% confidence intervals) for cART versus no cART were less than 1 for all outcomes, and ranged between 0.13 (0.05–0.38) for cryptococcosis and 0.76 (0.58–1.00) for HSV. Appendix 2, http://links.lww.com/QAD/A579 shows the weighted and unweighted hazard ratio estimates.

The median [IQR] $CD4^+$ cell count at event diagnosis was 291 [161 440] cells/μl for tuberculosis, 34 [10 189] cells/μl for MAC, 38 [10 189] cells/μl for CMV retinitis, 185 [72 310] cells/μl for PML, 360 [199 535] cells/μl for HSV, 322 [186 457] cells/μl for Kaposi sarcoma, 318 [192 466] cells/μl for NHL, 55 [19 149] cells/μl for cryptococcosis and 241 [100 399] cells/μl for candidiasis.

Table 2 presents the hazard ratios of each outcome by time since initiation of cART. Compared with non-cART initiation, the hazard ratios up to 3 months after cART initiation were 1.21 (0.90–1.63) for tuberculosis, 2.61 (1.05–6.49) for MAC, 1.17 (0.34–4.08) for CMV retinitis, 1.18 (0.62–2.26) for PML, 1.21 (0.83–1.75) for HSV, 1.18 (0.87–1.58) for Kaposi sarcoma, 1.56 (0.82–2.95) for NHL, 1.11 (0.56–2.18) for cryptococcosis and 0.77 (0.40–1.49) for candidiasis. The hazard ratios ≥3 months since cART initiation compared with non-cART initiation ranged between 0.06 (0.02–0.19) for cryptococcosis and 0.69 (0.51–0.92) for HSV. The hazard ratio up to 3 months after cART initiation for any of the explored AIDS event compared with non-cART initiation was 1.25 (1.05,1.48).

Table 1. Baseline characteristics of study participants, HIV-CAUSAL Collaboration 1996–2013.

	Individuals	Median [IQR] follow-up, months	Person-years	cART initiators (%)
$CD4^+$ cell count (cells/μl)				
<50	3522	27 [11, 64]	13088.33	2784 (79%)
50–100	3061	33 [12, 71]	12360.83	2468 (81%)
100–200	8641	34 [14, 73]	35951.17	7018 (81%)
200–350	21807	33 [14, 70]	88392.08	15648 (72%)
350–500	24553	31 [14, 67]	96822	13854 (56%)
>500	34978	30 [13, 62]	130709.67	13372 (38%)
HIV-RNA, log_{10} copies/ml				
<4	30596	29 [13, 61]	114836.42	12650 (41%)
4–5	43419	32 [14, 67]	171358	25928 (60%)
>5	22547	34 [14, 71]	91129.67	16566 (73%)
Sex				
Male	75049	32 [14, 67]	295646.25	43038 (57%)
Female	21513	29 [13, 65]	81677.83	12106 (56%)
Age (years)				
<35	44698	30 [13, 63]	169760.17	23362 (52%)
35–50	40154	33 [14, 70]	162430.83	23980 (60%)
≥50	11710	32 [12, 67]	45133.08	7802 (67%)
Transmission group				
Heterosexual	30060	31 [14, 67]	117387.58	17814 (59%)
Homo/bi-sexual	39971	35 [16, 71]	166591.83	23014 (58%)
Injection drug-use	8201	24 [11, 56]	29432.17	3817 (47%)
Other/unknown	18330	26 [11, 58]	63912.5	10499 (57%)
Geographical origin				
Western countries	57041	31 [13, 67]	224174.75	32765 (57%)
Sub-Saharan Africa	12497	29 [13, 62]	44686.75	7453 (60%)
Rest of world	7577	27 [13, 55]	25211.42	4054 (54%)
Unknown country	19447	34 [15,74]	83251.17	10872 (56%)
Calendar period				
1996–1998	20317	38 [14, 118]	112796.42	11251 (55%)
1999–2000	8940	40 [14, 110]	44845.33	5276 (59%)
2001–2003	16639	43 [16, 98]	77702	9742 (59%)
2004–2013	50666	27 [12,50]	141980.33	28875 (57%)
Overall	96562	31 [13,65]	377324.08	55144 (57%)

cART, combined antiretroviral therapy; IQR, interquantile range.

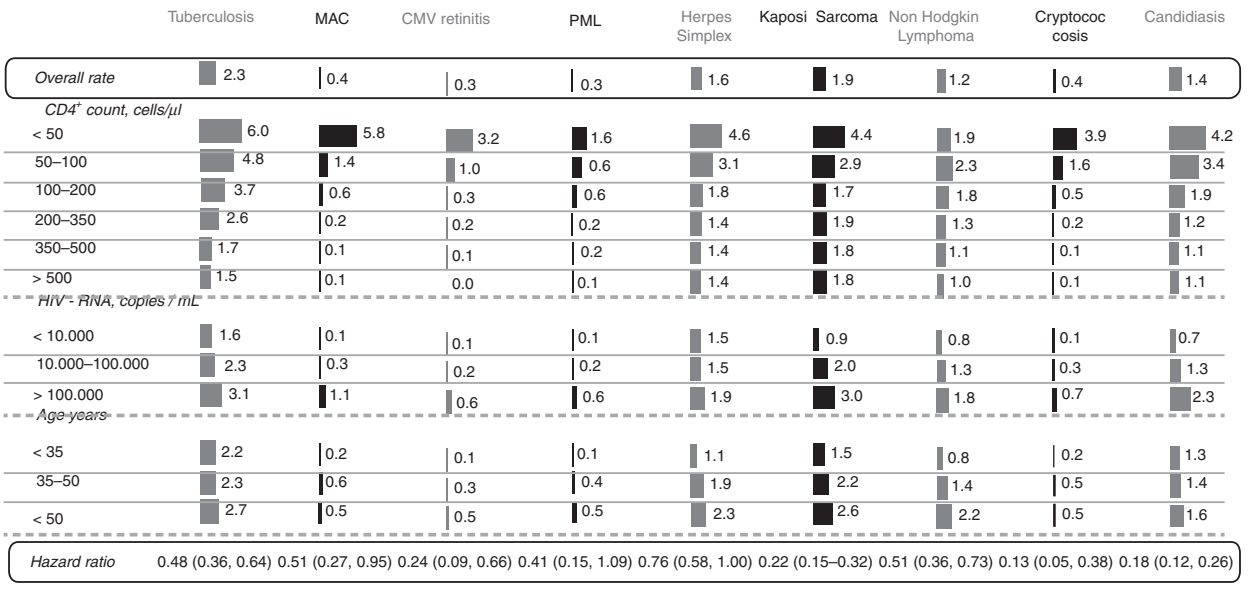

Fig. 1. Incidence rates of AIDS-defining events per 1000 person-year of follow-up, HIV-CAUSAL Collaboration 1996–2013. cART, combined antiretroviral therapy; CMV, cytomegalovirus; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy.

Table 2. Hazard ratios of AIDS-defining events by time since initiation of combined antiretroviral therapy, HIV-CAUSAL Collaboration 1996–2013.

	Time since cART initiation	*N* cases	Person-years	Incidence rates of events per 1000 person-year	Hazard ratio (95% confidence intervals)
Tuberculosis	No cART	422	143523.33	2.9	1
	<3 months	97	9259.00	10.5	1.21 (0.90–1.63)
	≥3 months	379	236095.92	1.6	0.36 (0.26–0.49)
MAC	No cART	46	143936.50	0.3	1
	<3 months	37	9306.83	4.0	2.61 (1.05–6.49)
	≥3 months	80	238799.67	0.3	0.31 (0.16–0.59)
CMV retinitis	No cART	35	143938.83	0.2	1
	<3 months	12	9308.42	1.3	1.17 (0.34,4.08)
	≥3 months	58	238917.67	0.2	0.13 (0.04–0.39)
PML	No cART	38	143944.00	0.3	1
	<3 months	19	9307.42	2.0	1.18 (0.62–2.26)
	≥3 months	56	238960.75	0.2	0.21 (0.06–0.71)
HSV	No cART	254	143476.42	1.8	1
	<3 months	42	9282.00	4.5	1.21 (0.83–1.75)
	≥3 months	324	236713.50	1.4	0.69 (0.51–0.92)
Kaposi sarcoma	No cART	404	143755.17	2.8	1
	<3 months	95	9250.67	10.3	1.18 (0.87–1.58)
	≥3 months	249	236065.50	1.1	0.14 (0.10–0.21)
NHL	No cART	198	143875.92	1.4	1
	<3 months	38	9288.17	4.1	1.56 (0.82–2.95)
	≥3 months	252	237871.50	1.1	0.40 (0.27–0.58)
Cryptococcosis	No cART	60	143924.67	0.4	1
	<3 months	21	9305.67	2.3	1.11 (0.56,2.18)
	≥3 months	58	238860.92	0.2	0.06 (0.02–0.19)
Candidiasis	No cART	275	143745.33	1.9	1
	<3 months	36	9275.67	3.9	0.77 (0.40–1.49)
	≥3 months	224	237213.75	0.9	0.13 (0.09–0.20)

cART, combined antiretroviral therapy; CMV, cytomegalovirus; HSV, herpes simplex virus ; NHL, non-Hodgkin lymphoma; MAC, *Mycobacterium avium* complex; PML, progressive multifocal leukoencephalopathy.

The hazard ratio estimates by time since cART initiation stratified by $CD4^+$ cell count, HIV-RNA level, age and sex for events with more than 500 cases (tuberculosis, Kaposi sarcoma, NHL, candidiasis and HSV) are presented in Appendices 3–7, http://links.lww.com/QAD/A579. The risk of tuberculosis up to 3 months after cART initiation was 1.77 (0.78,4.00) in patients with baseline $CD4^+$ cell count <50 cells/μl, 2.10 (1.07,4.11) in patients with age less than 50 years and 1.21 (0.84,1.74) in males.

The hazard ratio estimates did not materially change in sensitivity analyses (Appendix 7, http://links.lww.com/QAD/A579). The cumulative incidence (95% confidence intervals) at 3 months following cART initiation ranged between 0.17% (0.14–0.20%) for tuberculosis and 0.02% (0.01–0.04%) for CMV retinitis. The cumulative incidence for any of the outcomes at 2 months following cART initiation was 0.67% (0.60–0.74%).

Discussion

Our study suggests that cART initiation reduces the overall incidence of tuberculosis, MAC, CMV retinitis, PML, HSV, Kaposi sarcoma, NHL, cryptococcosis and candidiasis. In spite of this net overall reduction, there was evidence of an increased risk of MAC up to 3 months after cART initiation. The 3-month risk was also slightly elevated for tuberculosis, CMV retinitis, HSV, Kaposi sarcoma and NHL, but the 95% confidence intervals were wide. The epidemiological patterns observed for MAC and tuberculosis are consistent with a relevant proportion of unmasking IRIS among the diagnosis; for the other conditions the evidence is less compelling. For candidiasis, the evidence did not support unmasking IRIS.

Our results build on previous findings reported by the HIV-CAUSAL Collaboration with follow-up through 2007. We now report a lower incidence of tuberculosis (2.3 versus 3.2 cases per 1000 person-years) and a lower increase in tuberculosis incidence soon after cART initiation (21 versus 36%). Since median $CD4^+$ cell count at tuberculosis diagnosis has not increased over time (results not shown), these changes might be explained by a combination of random variability, temporal trends in tuberculosis incidence, and with enhanced pre-cART screening due to increased awareness of tuberculosis-related IRIS.

Although IRIS has been most often reported for opportunistic infections, IRIS associated with malignancies has also been described [9,10]. Like previous studies [31,32], we found small increases in risk of NHL and Kaposi sarcoma up to 3 months of cART initiation, but the 95% confidence intervals were wide. Given that

the development of these cancers should be preceded by exposure to causative agents, the increased incidence for malignancies up to 3 months after cART initiation is consistent with unmasking IRIS leading to increased clinical symptoms, and thus, diagnostic steps in the case of prevalent subclinical cancers.

We also found that the risk at 3 months of cART initiation for any of the events was less than 0.7%. This risk is much lower than that reported in a meta-analysis (between 38% for CMV retinitis and 6% for Kaposi sarcoma) [11] and in the HIV Outpatient Study cohort (between 23% for candidiasis and 0.5% for PML) [12]. As these previous studies were not restricted to AIDS-free patients, their risk estimates encompass both paradoxical IRIS and unmasking IRIS. In fact, the risk of unmasking IRIS may be even lower because our study cannot distinguish cases of unmasking IRIS from new cases unrelated to IRIS. Further ascertainment bias may account for some of the cases recorded early after cART initiation, as these might have been previously undiagnosed cases due to more intensive clinical screening in newly treated patients. However, the fact that we found no significant initial increase in risk despite this potential bias strengthens our conclusions that unmasking IRIS for the explored events is not common after cART initiation in patients starting cART in recent times in the European and North American setting.

Our study had several limitations. First, like all observational studies, the validity of our estimates relies on the assumption of no unmeasured confounding. Although we adjusted our models for $CD4^+$ cell count and HIV-RNA levels, the most important factors used by clinicians to decide whether to start cART, we cannot exclude the possibility that other unmeasured variables related to cART initiation could have also played a role. Second, we assumed that patients remained on therapy once it was initiated. If the diagnoses of the examined AIDS-defining events were largely occurring in individuals who had stopped cART or had poor adherence, then we might have underestimated the effect of cART initiation on the risk of the explored events. On the contrary, this bias is unlikely to have affected our conclusions on the trends in incidence up to 3 months after cART initiation. Finally, given the small number of events occurring during the first months of cART for some of the outcome events, we could not yet explore whether the effect of cART differed by patients characteristics that may be associated with development of IRIS. This is particularly important for baseline $CD4^+$ cell count [11,25] because unmasking IRIS is mainly observed in patients with very low $CD4^+$ cell counts, who were a minority in our study population.

In summary, this study suggests that, with the exception of mycobacterial infections, unmasking IRIS is not common after cART initiation in AIDS-free patients in Europe and the United States. In order to make an early diagnosis and provide adequate treatment, clinicians should rule out MAC and tuberculosis meticulously in patients at risk before starting cART and monitor closely for these opportunistic infections during the early phases of treatment.

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Conflicts of interest

There are no conflicts of interest.

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Appendix 2 – Cohort descriptions

CASCADE cohort collaboration

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Appendix 3 – HICDEP data tables

tblART - Antiretroviral treatment

holds type of antiretroviral drug, start and stop dates and reason for stopping

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	identifies patient
ART_ID	character. see coding table for valid codings.	represents the antiretroviral treatment
ART_SD	`yyyy-mm-dd`	date of initiation of treatment
ART_SD_A	character: see coding of date precision	precision of date "Initiation of Treatment"
ART_ED	`yyyy-mm-dd`	date of stopping treatment
ART_ED_A	character: see coding of date precision	precision of date "Stopping of Treatment"
ART_RS	character. see coding table for valid codings.	reason for stopping treatment

Additional fields

Depending on the aim of the study it might be needed to gather both the dosage and the frequency of the dosage taken. However many cohorts do not collect this date and thus these fields are optional.

Field name	Format	Description
ART_RS2	character. see coding table for valid codings.	Second reason for stopping treatment
ART_RS3	character. see coding table for valid codings.	Third reason for stopping treatment
ART_RS4	character. see coding table for valid codings.	Fourth reason for stopping treatment
ART_DO	numeric	Dosage (mg or mL) per intake unless ART_FR=-1
ART_FR	numeric: -1 = Frequency not known. ART_DO contains dosage per day 0.33 = 1 dose every third day 0.5 = 1 dose every second day 1 = 1 daily dose/qd 2 = 2 daily doses/bid 3 = 3 daily doses/tid 4... = code gives number of daily doses	Frequency
GENERIC	numeric: 1 = Branded 2 = Generic 9 = Unknown	Was this a branded or generic drug?
ART_FORM	numeric: 1 = Tablet/capsule 2 = Syrup/Suspension 3 = Combination of 1 and 2 4 = Powder 5 = Subcutaneous 6 = Intravenous 7 = Intramuscular 9 = Unknown	What formulations of the drug was given?
ART_COMB	numeric: 0 = Individual drug 1 = Part of a fixed-dose combination 9 = Unknown	Was the drug given as part of a fixed-dose combination?
ART_START_RS	numeric: see coding table for valid codings	Reason for starting/receiving ART

It may also be necessary to record the start and end time:

Field name	Format	Description
ART_ST	`hh:mm`	Start hour and minute of the day
ART_ET	`hh:mm`	Stop hour and minute of the day

tblBAS - Basic clinical, background and demographic information

holds basic information such as demographics, basic clinical information, date of AIDS diagnosis, death and drop-out information

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
BIRTH_D	`yyyy-mm-dd`	Birth date
BIRTH_D_A	character: see coding of date precision	optional precision annotation for birth date
FRSVIS_D	`yyyy-mm-dd`	First seen at clinic
ENROL_D	`yyyy-mm-dd`	Date of enrolment into the cohort
ENROL_D_A	character: see coding of date precision	optional precision annotation for date of enrolment into the cohort
GENDER	numeric: `1` = Male `2` = Female `9` = Unknown	Gender/sex
HEIGH	numeric (metric): 999 = Unknown	Height of patient at visit/most current
MODE	numeric. see coding table for valid codings.	Mode of infection
ORIGIN	character (1-3 letter/numeric codes). see coding table for valid codings.	Country or region of birth
ETHNIC	numeric. see coding table for valid codings.	Ethnicity of patient. Please take the additional notes into consideration when using this field.
EDU_LVL	numeric. see coding table for valid codings.	Last completed education Level. ISCED97 refers to the ?1997 International Standard Classification of Education
HIV_POS_D	`yyyy-mm-dd`	Date of first positive HIV test
SEROCO_D	`yyyy-mm-dd`	Date of seroconversion
RECART_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Has the patient ever received antiretroviral treatment? This includes all antiretroviral therapy given as Treatment even if given by another Center or program but excludes antiretroviral drugs given only for PMTCT or other prophylaxis.
RECART_D	`yyyy-mm-dd`	Date of first antiretroviral Treatment Initiation. Leave blank if ART not yet initiated. This should be the first date at which antiretroviral therapy, regardless of Regimen, was given as Treatment irrespective of whether it was given at this center/program or not. It excludes antiretroviral regimens given only for PMTCT or other prophylaxis.
RECART_D_A	character: see coding of date precision	optional date precision annotation for date `RECART_D`.
LTART_D	`yyyy-mm-dd`	Date last assessed for ART. If started ART, last date known to be on ART, or if not on ART, last date ART free.
AIDS_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Has the patient ever been given an AIDS diagnosis? (i.e. WHO stage 3 or 4 or CDC category C diagnosis)
AIDS_D	`yyyy-mm-dd`	If yes, date of AIDS diagnosis
AIDS_D_A	character: see coding of date precision	optional precision annotation for date `AIDS_D`

Additional fields

For mode of infection and origin a set of other fields are often used to capture what cannot be coded. These fields are represented

here as optional fields as it is the intention that the suggested codes applied to the MODE and ORIGIN should be able to cover all possible values.

Field name	Format	Description
MODE_OTH	character	Mode of infection OTHER
ORI_OTH	character	Origin of patient OTHER
CENS_D	yyyy-mm-dd	The last date the database has been updated for this patient
SEROHOW	numeric: 1 = Midpoint between last neg/first pos test 2 = Lab evidence of seroconversion 3 = Seroconversion illness 4 = Other 9 = Unknown	For Seroconverters only: How was the seroconversion date determined?
NAIVE_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Is the patient ART-naïve upon enrollment?
PROPH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Prior to enrollment, has the Patient been exposed to antiretroviral therapy for prophylaxis such as PMTCT, PREP, or PEP?

tblCENTER

holds information about the Center (e.g. geographical localisation, type of clinic) where the patient is receiving HIV care

Core Fields

Note: Fields marked bold form the unique identifier for a record of the table.

Field name	Format	Description
CENTER	character	Code for Clinic/Center/Hospital where patient is seen. Needs to be unique within each Region.
PROGRAM	character	Program or region with which the center is associated. Links to tblPROGRAM.
NAME	character	Proper name to identify center
COUNTRY	character	3-letter ISO code
PROVINCE	character	(Optional) Proper name to identify province
DISTRICT	character	(Optional) Proper name to identify district
CITY	character	(Optional) Proper name to identify city
GEOCODE_LAT	numeric	Latitude
GEOCODE_LON	numeric	Longitude
RURAL	numeric: 1 = Urban 2 = Mostly urban 3 = Mostly rural 4 = Rural 9 = Unknown	Code for the site situation (facility location)
LEVEL	numeric: 1 = Health centre 2 = District hospital 3 = Regional, provincial or university hospital 9 = Unknown	Code for level of care
ADULTPED	character: "PED","ADULT", or "BOTH"	Population the center serves
OPEN_D	`yyyy-mm-dd`	(Optional) Date of opening of dataset: earliest date for which data were included from this site
CLOSE_D	`yyyy-mm-dd`	(Optional) Date of closing of dataset
ADD_CENTER	`yyyy-mm-dd`	Inclusion date: date that the site was added to the cohort
DROP_CENTER	`yyyy-mm-dd`	(Optional) Exclusion date: date that the site was dropped from the cohort
SURVEY_INTERNET	numeric: 1 = sufficient Access to complete online surveys 2 = degraded Access making online Survey completion difficult 3 = no internet access 9 = Unknown	Quality of internet access for completing online Surveys.
SURVEY_PAPER	numeric: 1 = site has resources to print and transfer Surveys 2 = site has resources to print, but not Transfer surveys 3 = site does not have resources to print, but can transfer surveys 4 = site Needs assistance in both printing and transferring surveys 8 = not applicable 9 = Unknown	Resources for printing and transferring paper surveys to a central location for data entry.
LAST_REVIEWED_D	yyyy-mm-dd	Date when Center data in this table was last reviewed and/or updated.
LAST_REVIEWED_D_A	character: see coding of date precision	Optional precision annotation for last review date LAST_REVIEW_D

tblCEP - Clinical Events and Procedures

holds type and date of adverse events including serious non-AIDS conditions

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
EVENT_ID	numeric	Unique Event Identifier (foreign key to the different event tables)
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
CEP_D	`yyyy-mm-dd`	date of event
CEP_ID	character. see coding table for valid codings.	identifies type of event
CEP_SPEC	character. see coding table for valid codings.	further specification
CEP_V	numeric. See coding table for interpretation.	Depending on CEP_ID and CEP_SPEC: value of given event
SRCDOC_Y	numeric: 1 = Yes 0 = No	whether the source documentation is available
SRCDOC_D	`yyyy-mm-dd`	date for source documentation verification
VERIFY_Y	numeric: 1 = Yes 0 = No	Has the monitor verified the source documentation?
VERIFY_D	`yyyy-mm-dd`	date for monitor verification
APPROV_Y	numeric: 1 = Yes 0 = No	final verification/approval
APPROV_D	`yyyy-mm-dd`	final verification date
APPROV_S	character	signature for final verification

Additional fields

Field name	Format	Description
CEP_Y	numeric: 1 = Yes 0 = No 9 = Unknown	has the patient had an event?
CEP_NAME	character	full name of the event
CEP_DESCRIP	character	full description of the event
CEP_R_Y	numeric: 0 = not related 1 = definitive 2 = remote/unlikely 3 = possible 4 = probable	relation to treatment

tblDIS - CDC-C and WHO Stage Diseases

holds type and date of CDC-C diseases and malignancies.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
DIS_ID	character. see coding table for valid codings.	Code to identify event
DIS_D	`yyyy-mm-dd`	Start date of event (Date of disease diagnosis)
DIS_WD	numeric. see coding table for valid codings.	Means/Certainty of diagnosis
DIS_OTH[1]	character	Other location, only to be filled out if `DIS_ID` code alone is not sufficient

[1] DIS_OTH might be part of the record's unique identification

Additional fields

Please see tblCEP for specification on optional fields.

Field name	Format	Description
DIS_ED	`yyyy-mm-dd`	End date of Event (If end date is available, disease outcome should be specified)
DIS_ED_A	character: see coding of date precision	optional precision annotation for end of infection date `DIS_ED`
DIS_SITE	numeric. see coding table for valid codings.	Event site
DIS_OUTCOME	numeric. see coding table for valid codings.	Disease outcome

tblLAB - Laboratory values

holds type, date, value and unit of laboratory tests.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
LAB_ID	character. see coding table for valid codings.	Code representing the measurement
LAB_D	`yyyy-mm-dd`	Date of measurement/sample
LAB_D_A	character: see coding of date precision	optional precision annotation for date of measurement/sample
LAB_R	numeric: 1 = Positive (including trace, 1+, 2+, etc.) 0 = Negative 9 = Unknown/borderline	Measurement result
LAB_V	numeric: -1 = undetectable or detection limit as negative value	Value of measurement
LAB_U	numeric. see coding table for valid codings.	Unit of measurement

Additional fields

Other detailed information regarding the patient and the measurement would be relevant, like the proposed fasting information shown below.

Field name	Format	Description
LAB_FA	numeric: 0 = No 1 = Yes 9 = Unknown	Was the blood sample taken while fasting?
LAB_ST	character. see coding table for valid codings.	Code representing the specimen type

tblLAB_BP - Laboratory values - Blood pressure

holds date, diastolic and systolic values and unit of blood pressure measurements.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
BP_D	`yyyy-mm-dd`	Date of Measurement/Sample
BP_D_A	character: see coding of date precision	Precision annotation variable for measurement date
BP_SYS	numeric	Systolic Blood Pressure
BP_DIA	numeric	Diastolic Blood Pressure
BP_U	numeric. see coding table for valid codings.	Unit of measurement

tblLAB_CD4 - Laboratory values

holds date and value of CD4 measurements.

Note: If needed, a CD8 table (tblLAB_CD8) could be formed from the same structure.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
CD4_D	`yyyy-mm-dd`	Date of measurement
CD4_V	numeric (per microliter): -1 = undetectable or detection limit as negative value	Value of CD4 measurement

Additional fields

CD4_V is assumed to contain absolute CD4 cell counts per mL as standard. In case CD4 % (with respect to CD45+ lymphocytes as denominator) should be collected as well, please append the following field to the table:

Field name	Format	Description
CD4_U	numeric with codes (or full string): `1` = cells/µl `2` = %	Unit of measurement
CD4_D_A	character: see coding of date precision	precision of measurement date

tblLAB_RES - Resistance testing

holds background information on the resistance test, laboratory, library, kit, software and type of test

Note: This table is tightly linked to tblLAB_RES_LVL_1, tblLAB_RES_LVL_2 and tblLAB_RES_LVL_3.

Resistance should be reported at lowest level of interpretation possible ? so if the nucleotide sequence is available this should be reported rather than the list of mutations or resistance scores. However, the resistance test results should be captured if they have been part of the physician?s treatment decisions for the patient.

Non-amplifiable resistance tests should not be reported.

These four tables are designed to capture several possible formats the clinics and cohorts might have recorded resistance test data in. Once this data is gathered it should like all other tables be quality assessed.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
TEST_ID	character (or numeric if possible)	An arbitrary value identifying a resistance test result
SAMPLE_D	`yyyy-mm-dd`	Date of the actual sample taken (NOT the test date)
SAMPLE_D_A	character: see coding of date precision	optional precision annotation for date of sample
SEQ_DT	`yyyy-mm-dd hh:mm`	Date and time when the sequencing was performed
SEQ_DT_A	character: see coding of date precision	optional precision annotation for date of sequencing
LAB	character	Name of laboratory where the test was performed
LIBRARY	character	Library/algorithm used to identify resistance mutations
REFSEQ	character	Name/identifier of reference strain used to find mutations
KIT	character	Vendor and version/name of the kit used for the test
SOFTWARE	character	Software and version used to determine resistance
TESTTYPE	numeric: `1` = Genotype (e.g., GeneXpert, NAAT/LPA `2` = Phenotype (e.g., culture) `9` = Other	Type of test
PATHOGENTYPE	character: MeSH terminology ?https://meshb.nlm.nih.gov/#/fieldSearch	Type of pathogen
VIRUSTYPE	numeric: `1` = HIV `2` = HCV	Type of Virus
SUBTYPE	character	Subtype of HIV- or HCV-RNA

Additional fields

As shown with the core fields, the *TEST_ID* is the link between the 3 levels of data and the test background information table. Some

prior assessment of the assigned test identifiers has to be done in order to avoid duplicates.

In a running database the duplicate issues are easily resolved by adding a unique auto-generated key as the identifier between 3 levels of data and the test background information table.

Along with the *TEST_ID* it might be necessary to store the ID assigned to the sample at both the testing laboratory but also the centres laboratory in order to track the sample.

Field name	Format	Description
SAMP_LAB	character (or numeric if possible)	The assigned sample ID at the lab where the resistance test is preformed.

SAMP_INT	character (or numeric if possible)	The assigned sample ID from the centre.

tblLAB_RES_LVL_1 - Nucleotide sequences (PRO, RT, GP41, GP120)

holds nucleoside sequence for the PRO and RT sequences. No entry is made if the test was a phenotype test.

Note: This table is tightly linked to tblLAB_RES.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
TEST_ID	character (or numeric if possible)	Identifier linking this record to tblLAB_RES
SEQTYPE	character: `PRO` = PRO sequence `RT` = RT sequence `GP41` = GP41 sequence `GP120` = GP120 sequence	Type of nucleotide sequence if available
SEQ_START	numeric	Start position for the sequence
SEQ_STOP	numeric	Stop position for the sequence
SEQ_NUC	character	Nucleotide sequence if available

Additional fields

In cases where the amino acid sequence is collected rather than the nucleotide sequence, the field *SEQ_NUC* might be replaced with *SEQ_AA*, which is the nucleotide sequence, expressed in an amino acid sequence:

Field name	Format	Description
SEQ_AA	character	Amino acid sequence if available (empty if test was phenotype)

However using the amino acid sequence does not give the same detail of data as the nucleoside sequence: wobbles in the nucleoside sequence can either complicate the reading and alignment of the amino acid sequence or the wobbles can be lost and silent mutations are lost.

tblLAB_RES_LVL_2 - Mutations

holds mutations and positions of PRO and RT sequences.

Note: This table is tightly linked to tblLAB_RES.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
TEST_ID	character (or numeric if possible)	Identifier linking this record to tblLAB_RES
GENE	character: `PRO` = PRO sequence `RT` = RT sequence `GP41` = GP41 sequence `GP120` = GP120 sequence	Type of sequence/gene (PRO, RT, GP41, GP120)
AA_POS	numeric	Position of the mutation in the sequence
AA_POS_SUB	character: `a` = first `b` = second etc.	Subposition used to code insertions
AA_FOUND_1	character. empty = Amino acid has been deleted.	Mutation (Amino acid) found in the sequence
AA_FOUND_2	character. empty = Amino acid has been deleted.	Mutation (Amino acid) found in the sequence (if more than 1)
AA_FOUND_3	character. empty = Amino acid has been deleted.	Mutation (Amino acid) found in the sequence (if more than 2)
AA_FOUND_4	character. empty = Amino acid has been deleted.	Mutation (Amino acid) found in the sequence (if more than 3)

AA_FOUND_# could be extended if mixtures with more than 4 amino acids are found.

tblLAB_RES_LVL_3 - Resistance test result

holds resistance result in relation to antiretroviral drug.

Note: This table is tightly linked to tblLAB_RES.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
TEST_ID	character (or numeric if possible)	Identifier linking this record to tblLAB_RES
ATC_CODE	character	?ATC code of the medication
RES_SCOR	character	Score of resistance or recommendation given from the test.

Additional fields

For phenotype test results it will be necessary to extend the table with a field to store the cut-off value:

Field name	Format	Description
RES_CUT	character	Cut-off value for phenotype test result
RES_SCOR_ID	character: S = sensitive L = low level I = intermediate H = high level	Coded score of the resistance or recommendation given from the test

However using the amino acid sequence does not give the same detail of data as the nucleoside sequence: wobbles in the nucleoside sequence can either complicate the reading and alignment of the amino acid sequence or the wobbles can be lost and silent mutations are lost.

tblLAB_RNA - Laboratory values

holds date, value, detection limit and type of viral assay.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
RNA_D	`yyyy-mm-dd`	Date of Measurement/Sample
RNA_D_A	character: see coding of date precision	Precision annotation variable for date of measurement
RNA_V	numeric: -1 = undetectable or detection limit as negative value	HIV-RNA measurement value
RNA_L	numeric	Lower Limit of HIV-RNA Assay
RNA_T	numeric. see coding table for valid codings.	IF AVAILABLE, What type of VIRAL ASSAY was used for this measurement?

Additional fields

Field name	Format	Description
RNA_UL	numeric	IF AVAILABLE, Upper Limit of assay

tblLAB_VIRO - Laboratory values - viro-/serology

holds test results for viro-/serological tests (hepatitis etc.)

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
VS_ID	character. see coding table	Viral test
VS_D	`yyyy-mm-dd`	Measurement date
VS_D_A	character: see coding of date precision	optional precision annotation for date of measurement
VS_R	numeric: 1 = Positive 0 = Negative 9 = Unknown/borderline	Measurement result
VS_V	numeric	Measurement value (HCV-RNA & HBV-DNA only) (copies/ml)
VS_U	character. see coding table for valid codings.	Measurement unit

Additional fields

Field name	Format	Description
VS_LL	numeric	IF AVAILABLE, Lower limit of assay
VS_UL	numeric	IF AVAILABLE, Upper limit of assay
VS_T	character. see coding table for valid codings.	IF AVAILABLE, type of ASSAY used for this measurement
VS_ST	character	Specimen type

tblLTFU - Death and drop-out

holds data in death and drop-out

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
DROP_Y	numeric: 0 = No 1 = Yes	Has the patient dropped out?
DROP_D	yyyy-mm-dd	If patient has dropped out, date of last visit
DROP_D_A	character: see coding of date precision	optional precision annotation for date of last visit
DROP_RS	numeric. see coding table for valid codings.	Reason for drop
DEATH_Y	numeric: 0 = No 1 = Yes	Has the patient died?
DEATH_D	yyyy-mm-dd	Date of Death
DEATH_D_A	character: see coding of date precision	optional precision annotation for date of death
SUD_DEATH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Sudden Death?
EXP_DEATH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Expected Death?
AUTOP_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Was an autopsy Performed?
DEATH_R1	character. see coding table for valid codings.	Cause of death
DEATH_RC1	character with codes: I = Immediate cause U = Underlying cause/condition C = Contributing cause N = Not available	Coding of causal relation of the code given in DEATH_R1 to the death
DEATH_R2	character. see coding table for valid codings.	Cause of death
DEATH_RC2	character with codes: I = Immediate cause U = Underlying cause/condition C = Contributing cause N = Not available	Coding of causal relation of the code given in DEATH_R2 to the death
DEATH_R3	character. see coding table for valid codings.	Cause of death
DEATH_RC3	character with codes: I = Immediate cause U = Underlying cause/condition C = Contributing cause N = Not available	Coding of causal relation of the code given in DEATH_R3 to the death
DEATH_SOURCE	character	Source of information for coding of death (e.g. CoDe within own cohort, CoDe from D:A:D, CoDe from ART-CC, etc.)

MOTHERDEATH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Hast the patient's biological mother died?
MOTHERDEATH_D	yyyy-mm-dd	Date of death of the patient's biological mother
MOTHERDEATH_D_A	character: see coding of date precision	optional precision annotation for date of death of patient's mother
FATHERDEATH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Hast the patient's biological father died?
FATHERDEATH_D	yyyy-mm-dd	Date of death of the patient's biological father
FATHERDEATH_D_A	character: see coding of date precision	optional precision annotation for date of death of patient's father

List of *DEATH_R#* and *DEATH_RC#* should be continued for as many reasons that are recorded.

The *DEATH_RC#* fields should enable cohorts to transfer data in accordance with the ?Coding of Death project (CoDe). You are welcome to contact the CoDe group for electronic sample forms for detailed collection of data used for the CoDe review process.

CoDe defines 1 immediate, 2 contributing and 1 underlying cause of death.

Additional fields

Field name	Format	Description
ICD10_1	character	Cause of death as ICD-10 if available
ICD10_2	character	Cause of death as ICD-10 if available
ICD10_3[1]	character	Cause of death as ICD-10 if available
ICD9_1?	character	Cause of death as ICD-9 if available
ICD9_2	character	Cause of death as ICD-9 if available
ICD9_3[1]	character	Cause of death as ICD-9 if available
DEATH_OT	character	Reason for death ? other - description
L_ALIVE_D	yyyy-mm-dd	Last date known to be alive
L_ALIVE_D_A	character: see coding of date precision	optional precision annotation for last date of Information / lknown to be alive

[1]: List of *ICD10_#* and *ICD9_#*inplace of or together with *DEATH_R#* and together *DEATH_RC#* and should be continued for as many reasons that are recorded.

CoDe defines 1 immediate, 2 contributing and 1 underlying cause of death.

tblMED - Other medication

holds type, start and stop dates for other medication/treatments.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
MED_ID	character. see coding table for valid codings.	Code representing the treatment
MED_SD	`yyyy-mm-dd`	Date of Initiation of Treatment
MED_SD_A	character: see coding of date precision	Precision annotation variable for date of initiation of drug
MED_ED	`yyyy-mm-dd`	Date of stopping treatment
MED_ED_A	character: see coding of date precision	Precision annotation variable for date of stopping drug
MED_RS	character. see coding table for valid codings *(identical to stopping reasons for ART)*	reason for stopping treatment

Additional fields

Field name	Format	Description
MED_RS2	character. see coding table for valid codings *(identical to stopping reasons for ART)*	Additional reason for stopping treatment
MED_RS3	character. see coding table for valid codings *(identical to stopping reasons for ART)*	Additional reason for stopping treatment
MED_RS4	character. see coding table for valid codings *(identical to stopping reasons for ART)*	Additional reason for stopping treatment
MED_DO	numeric	Dosage (mg or mL) per intake unless MED_FR=-1 (optional)
MED_FR	numeric: -1 = Frequency not known. MED_DO contains dosage per day 0.33 = 1 dose every third day 0.5 = 1 dose every second day 1 = 1 daily dose/qd 2 = 2 daily doses/bid 3 = 3 daily doses/tid 4... = code gives number of daily doses	Frequency
DOT_Y	numeric: 0 = No 1 = Yes 9 = Unknown / Not performed	Directly observed Treatment (optional)
MEDSTART_RS	numeric: 1 = Treatment (incl. for presumptive dx) 2 = Prophylaxis (Primary or secondary) 9 = Unknown	Reason for starting medication (optional)

tblSAMPLES - Blood Samples

This table contains information on the storage of blood, urine and other samples stored in a laboratory.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	patient cohort identifier
SAMP_LAB_D	`yyyy-mm-dd`	date when the sample was taken
SAMP_TYPE	character: `BS` = blood serum `BP` = blood plasma `C` = viable cells `D` = cell pellet (DNA) `S` = semen `OTH:x` = other sample type `x` (none of the above)	type of the sample
SAMP_ID	character	identification symbol allowing the localization of the sample in the laboratory
SAMP_LAB	character	laboratory where the samples are stored
SAMP_FREEZE_D	`yyyy-mm-dd`	date when the sample was frozen
SAMP_FREEZE_T	`hh:mm`	time when the sample was frozen
SAMP_ALIQ_NO	numeric	number of aliquots available
SAMP_ALIQ_SIZE	numeric	size of the aliquot: in `ml` for serum, plasma and cell pellet aliquots in `millions of cells` for viable cell aliquots
SAMP_ALIQ_U	character: 0 = millions of cells 1 = `ml`	unit of measurement for the `SAMP_ALIQ_SIZE` value

Additional fields

Field name	Format	Description
SAMP_LAB_T	`hh:mm`	time when the sample was taken
SAMP_TEMP	numeric	temperature of the storage unit containing the samples (**in °C**)
SAMP_DEFROST	numeric: 1 = Yes 0 = No 9 = Unknown	have the samples already been defrosted?

tblVIS - Basic follow-up/visit related data

holds visit related information such as weight, wasting, smoking, occupational status etc.

Core fields

Note: Fields marked **bold** form the unique identifier for a record of the table.

Field name	Format	Description
PATIENT	character (or numeric if possible)	Code to identify patient (Cohort Patient ID)
VIS_D	`yyyy-mm-dd`	Date of patient visit
VIS_D_A	character: see coding of date precision	Precision annotation variable for date of visit
CENTER	character	Center the patient visits. Links to tblCENTER.
WEIGH	numeric (metric: kg): `999` = Unknown	Weight of patient at visit
GAIN_Y	numeric: `0` = No `1` = Yes `9` = Unknown	Is the patient gaining fat in the abdomen, neck, breast or other defined locations?
LOSS_Y	numeric: `0` = No `1` = Yes `9` = Unknown	Is the patient experiencing loss of fat from extremities, buttocks or face?

Depending on the collaboration this data might be collected in intervals of a year, e.g. from July last to July this year. In that case all visit dates or a fixed number of visit dates for that period should be gathered, if the patient did not have a visit in the defined period, a record with the PATIENT id and empty fields for VIS_D etc. should be included.

Additional fields

Field name	Format	Description
CDC_STAGE	character. see coding table for valid codings.	Clinical CDC stage at time of visit?
WHO_STAGE	numeric. `1` = WHO Stage I `2` = WHO Stage II `3` = WHO Stage III `4` = WHO Stage IV `9` = Unknown	Clinical WHO stage at time of visit?
FAM_Y	numeric. `0` = No `1` = Yes `9` = Unknown	Family history of CVD: Have any first degree relatives experienced myocardial infarction or stroke before the age of 50 years?

The following optional fields are meant to be used to **document the transition process from adolescent to adult**.

Field name	Format	Description
CLINIC_TYPE	numeric `1` = paediatric `2` = adolescent within paediatric care `3` = adolescent within adult care `4` = adolescent stand alone `5` = adult `9` = missing	Type of clinic/service the patient is currently attending

SPEC_TYPE	numeric 1 = Physician providing paediatric care 2 = Physician providing adolescent care 3 = Physician providing adult care 4 = Physician providing paediatric and adult care 5 = other healthcare provider (e.g. nurse) 9 = missing	Type of specialist providing care. Combinations if multiple specialists are involved (e.g. 23, 45).
TRANS_STAGE	numeric 0 = transition not started 1 = transition in progress 2 = transition completed 9 = not applicable/missing	Stage of transition from pediatric to adult care at current visit. Transition has not yet started when the patient only sees paediatricians. Transition is complete when the patient only sees adult physicians.

The following fields are meant to be used **for adolescents and adults**.

Field name	Format	Description
EMPLOY	numeric. see coding table for valid codings.	What is the patient's current situation regarding labour?
CONTRACT	numeric. see coding table for valid codings.	If the patient is an employee, what is the type of the patient's employment contract?
SMOKING_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Is the patient currently a smoker?
PREG_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Is the patient currently pregnant? If possible, provide additional details in tblPREG
GENDER_ID	numeric: 1 = Male 2 = Female 3 = Transgender male 4 = Transgender female 5 = Other 9 = Unknown	Current gender identification
SCHOOL	numeric: 0 = No 1 = Yes 9 = Unknown	Is the patient currently attending school or on break for customary school holidays?
SCHOOL_LVL	numeric: see coding table	Current level of education (ISCED97 refers to the 1997 International Standard Classification of Education)

The following fields are meant to be used **for HIV-infected children and adolescents only**.

Field name	Format	Description
STATUS_KNOWN	numeric: 0 = No 1 = Yes 2 = Disclosure ongoing 9 = Unknown	Does the patient know his/her HIV status?

The following fields are meant to be used **for children and infants**.

Field name	Format	Description
HEIGH	numeric (metric in m). 999 = Unknown	Height/length of patient at visit in meters (m)
LIVEWITH	numeric. see coding table for valid codings.	Child lives with/in

HEALTHY_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Is child healthy?
FEEDOTH_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Is the Patient currently receiving Foods or liquids other than breast milk?
CAREGIVER	numeric: 1 = Mother 2 = Father 3 = Sibling 4 = Grandparent 5 = Aunt or Uncle 6 = Self 7 = Other family member 8 = Other non-family member 9 = Unknown 10 = Other non-coded	Who is the patient's primary caregiver?
BROUGHT_PATIENT	numeric: 1 = Mother 2 = Father 3 = Sibling 4 = Grandparent 5 = Aunt or Uncle 6 = Self 7 = Other family member 8 = Other non-family member 9 = Unknown 10 = Other non-coded	Who brought the Patient to this clinic visit?
HIV_STATUS	numeric: 1 = HIV exposed, status indeterminate 2 = HIV infected 3 = HIV uninfected	Current HIV status

The following fields are meant to be used **for infants**:

Field name	Format	Description
HEIGH_P	numeric	Height/length of patient at visit in percentiles
WEIGH_P	numeric	Weight of patient at visit in percentiles
HEADC	numeric	Head circumference measured in millimeters (mm)
HEADC_P	numeric	Head circumference in percentiles
BREASTF_Y	numeric: 0 = No 1 = Yes 9 = Unknown	Currently Breastfeeding?

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