Antiretroviral treatment of HIV-1 in Sweden with focus on virological aspects

Erik Sörstedt

Department of Infectious Diseases
Institute of Biomedicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2021

Cover illustration: Adapted by Erik Sörstedt from original artworks at Vectorstock by illustrators *31moonlight31* and *Anastasia8*: https://www.vectorstock.com/royalty-free-vector/database-storage-server-vector-9412591, https://www.vectorstock.com/royalty-free-vector/flat-icon-on-stylish-background-gay-hiv-ribbon-vector-16476223

Antiretroviral treatment of HIV-1 in Sweden
with focus on virological aspects

erik.sorstedt@vgregion.se

ISBN 978-91-8009-176-3 (PRINT)
ISBN 978-91-8009-177-0 (PDF)
http://hdl.handle.net/2077/67127

Printed by Stema Specialtryck AB
Borås, Sweden 2021

To Tabitha, for making me realize how

desperate we need to fight the HIV epidemic

ABSTRACT

From a clinical standpoint, there are many factors to consider when optimizing the care for people living with HIV (PLWH). With help from clinical guidelines, most obstacles can be addressed. Expanded knowledge is however in constant demand, from local conditions to universal processes. This thesis emerged from a demand for both clinical and virological data about the effect of antiretroviral treatment (ART) in Sweden. All data were derived from the national InfCareHIV database.

The current goal of ART is to achieve lasting suppression to < 50 HIV RNA copies/mL. Transient episodes of viremia up to 500 copies/mL, so-called viral blips, are not uncommon. We sought to investigate the clinical importance and outcome of this phenomenon. Through two large retrospective studies, **Paper I and IV**, we concluded that it is more common with blips in PLWH with higher baseline viral load and ART based on boosted Protease Inhibitors (PI). Blip incidence during Integrase Strand Transfer Inhibitors (INSTI) and Non-Nucleoside Reverse Transcriptase Inhibitor-based ART was lower at a similar level. In PLWH who reached HIV RNA suppression after initiating their first ART, blips were relatively common (10–20% of all participants) but not associated with an increased risk of virological failure.

Before the introduction of the INSTI dolutegravir, PLWH with resistance mutations to Nucleoside Reverse Transcriptase Inhibitors were often restricted to PI-based treatment. PIs are characterized by many drug interactions and often tolerability issues. **In Paper II**, 244 participants with either dolutegravir or traditional PI-based ART were retrospectively studied. Dolutegravir has pharmacological benefits and we concluded that it was an equivalent alternative.

Treatment recommendations are not affected by different levels of baseline viremia. Most clinical studies compare the outcome in participants with higher or lower than 100,000 HIV RNA copies/mL. Considerably higher levels of viremia are sometimes observed. In **Paper III**, we included 2,956 PLWH of whom 394 (13%) had baseline > 500k HIV RNA copies/mL. We found that participants with that high initial viremia needed longer time to reach viral suppression. Initial treatment with INSTIs was associated with faster viral decline. Higher baseline viral load was not associated with an increased risk of virological failure.

Keywords: HIV-1, antiretroviral therapy, transient viremia, viral blip, nucleoside reverse transcriptase inhibitor resistance, dolutegravir, baseline viral load, HIV RNA, virological failure

ISBN 978-91-8009-176-3 (PRINT)
ISBN 978-91-8009-177-0 (PDF)

SAMMANFATTNING PÅ SVENSKA

Det har nu gått fyra decennier sedan den globala hivepidemin upptäcktes och hiv har under dessa år orsakat att miljontals människor har mist livet. Sedan upptäckten har dock stora vetenskapliga framsteg gjorts och forskare har utvecklat effektiva läkemedel. Detta har gjort att den förväntade medellivslängden för personer som lever med hiv idag närmar sig den som hos dem utan sjukdomen. Hiv behöver tillgång till immunceller för att föröka sig och spridas. Genom behandlingen pressas virusnivåerna ner, vilket gör att det inte kan spridas till nya celler och immunförsvaret kan därmed återhämta sig.

Denna avhandling bygger på data från den unika nationella databasen InfCareHIV, där medicinska fakta från alla som lever med hiv i Sverige har samlats under många år. I delstudie I och IV undersökte vi s.k. blippar, ett fenomen där virusnivån plötsligt stiger trots pågående effektiv behandling. Vi upptäckte att dessa finns hos 10–20% av alla som lever med hiv i Sverige. Vi fann även att blipparna var vanligare hos personer som i början av behandlingen hade högre virusnivåer än genomsnittet, samt hos personer som behandlats med en läkemedelsgrupp kallad proteashämmare. Vi klargjorde att om man tar sina läkemedel som planerat så är blippar ofarliga och utgör med stor sannolikhet inte något tecken på att behandlingen kommer att sluta fungera på sikt.

Vanligen består hivbehandling av två s.k. nukleosidanaloger i kombination med ytterligare en medicin. I delstudie II undersökte vi om det nya läkemedlet dolutegravir fungerar hos personer med en variant av hiv där dessa nukleosidanaloger pga resistens hos viruset inte längre fungerar fullt ut. Vi upptäckte att kombinationsbehandling med dolutegravir fungerade lika bra som det tidigare behandlingsalternativet. En bonus med detta alternativ är att det både har färre biverkningar och interaktioner med andra läkemedel.

I delstudie III studerade vi hur behandlingsresultaten skiljde sig åt beroende på hur mycket virus man hade i blodet vid behandlingsstart. Efter att ha studerat data från nästan 3 000 personer som samlats in under 20 års tid fann vi att personer med mer än 500 000 viruskopior per ml blodplasma behöver längre tid för att nå behandlingsmålet jämfört med övriga. Därefter var behandlingsresultaten lika goda och man såg inte någon ökad risk för behandlingssvikt hos denna grupp.

Sammanfattningsvis fann vi att två relativt vanliga fenomen, blippar och höga virusnivåer vid behandlingsstart, är ofarliga. Vidare resulterade våra fynd i att personer med en viss sorts resistenta virus idag kan erbjudas en enklare men lika effektiv behandling.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Sörstedt E, Nilsson S, Blaxhult A, Gisslén M, Flamholc L, Sönnerborg A, Yilmaz A. **Viral blips during suppressive antiretroviral treatment are associated with high baseline HIV-1 RNA levels.** BMC infectious diseases. 2016 Dec 1;16(1):305.

II. Sörstedt E, Carlander C, Flamholc L, Hejdeman B, Svedhem V, Sönnerborg A, Gisslén M, Yilmaz A. **Effect of dolutegravir in combination with nucleoside reverse transcriptase inhibitors (NRTIs) on people living with HIV who have pre-existing NRTI mutations.** International Journal of Antimicrobial Agents. 2018 May 1;51(5):733-8.

III. Sörstedt E, Nilsson S, Nowak P, Treutiger CJ, Månsson F, Änghagen L, Gisslén M, Yilmaz A. **Less than half of patients with chronic HIV-infection and baseline HIV- RNA > 500,000 copies/mL reach treatment goal of < 50 copies/mL within six months.** Submitted manuscript.

IV. Sörstedt E, Nilsson S, Sönnerborg A, Svedhem-Johansson V, Treutiger CJ, Månsson F, Änghagen L, Berggren H, Gisslén M, Yilmaz A. **Viral blips are more common in patients on antiretroviral therapy containing protease inhibitors in comparison to integrase inhibitors and non-nucleoside reverse transcriptase inhibitors – a retrospective nationwide study in Sweden 2007–2020.** In manuscript.

PREFACE

Tabitha was supposed to meet me at the small health clinic she had started a few years earlier in Kibera, a neighborhood in Nairobi's outskirts and the largest urban slum in Africa. Tabitha Atieno Festo, a widow and mother of four, struggled to make ends meet. She was a registered nurse but lacked employment and supported her family by buying and selling vegetables. One day she approached an American student, Rye Barcott, in Kibera for a research project. Tabitha convinced him that by investing $26 in her small business, she could make enough money to succeed with her lifelong dream of opening a small health clinic in Kibera. Rye consented, and after just six months, she had raised enough money to start Rye´s Clinic in a small house attached to her home. Her goal was to provide high-quality maternal care, which was not available in the community at the time. She also wanted to help patients with common infections like malaria, yellow fever and cholera, and started a home-based program for people living with HIV. Two years later, the clinic outgrew its first location and moved to a slightly larger facility nearby.

As a third year medical student, I had the opportunity to spend some time outside of the university in 2004. Through a mutual friend, I was put in contact with Rye, and the non-governmental organization called Carolina for Kibera (CFK) he had co-founded. With his aid, a month-long internship at the clinic and the Kenyatta national hospital was arranged.

Tabitha was not at the clinic when I arrived. Claire, an American CFK-volunteer, told me Tabitha was not feeling well. Instead, I visited her in her home later that day. I arrived at the same time as a physician who came to examine her. Tabitha urged me to stay during the check-up to learn. She told me she was not used to being ill but was now suffering from a chronic wound. The doctor prescribed antibiotics, but her condition kept deteriorating. After a few days, she was admitted to one of the best hospitals in Nairobi. Soon after, Claire told me that Tabitha had been tested positive for HIV. Despite initiating antiretroviral therapy, she kept getting weaker, and after just a few weeks, Tabitha passed away.

The tragedy of her passing, far too early, and leaving her children orphaned had a significant impact on me. I decided to continue her struggle to help patients in need and contribute so that we, someday, will be able to end the terrible HIV epidemic. This thesis is one small step on that journey.

Rye's clinic later changed its name to Tabitha's clinic. It still uses her motto, sacrificing for success, and has grown to serve more than 40,000 patients each year.

Tabitha Atieno Festo, 1962–2014

Image source: Wikimedia Commons

CONTENT

ABBREVIATIONS

ART	Antiretroviral therapy
DNA	Deoxyribonucleic acid
DTG	Dolutegravir
GSS	Genotypic Susceptibility Score
INSTI	Integrase Strand Transfer Inhibitor
IQR	Interquartile range
MSM	Men who have sex with men
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PI	Protease Inhibitor
PLWH	Person/People living with HIV
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-based polymerase chain reaction
VF	Virological failure
VL	Viral load

INTRODUCTION

1. INTRODUCTION

1.1 HIV-1 DEMOGRAPHICS

The human immunodeficiency virus (HIV) epidemic started 40 years ago when previously young and healthy gay men developed rare forms of cancer, pneumonia, and other opportunistic diseases in large cosmopolitan cities such as New York, Los Angeles, and San Francisco. In 1982, the term gay-related immune deficiency (GRID) was suggested for the syndrome. However, the term was changed to acquired immunodeficiency syndrome (AIDS) when the same symptoms also were noticed among heterosexual people with hemophilia (and others in need of blood products), people who inject drugs, immigrants from Haiti, and in children (1). It was not until later that it was understood that the virus had already been present for many years. Due to the long delay between transmission and symptom onset, the disease was already rapidly spreading in key populations (2).

In 1983–84, a novel retrovirus was isolated from lymph nodes in affected patients and suspected to be the causative agent for AIDS (3-5). A few years later, in 1986, it was finally named HIV (6).

Since then, HIV has caused one of the most devastating infectious diseases in humankind's history. At the end of 2019, 75.7 million people had tested positive for HIV, of which 32.7 million have died. Tuberculosis is the most common AIDS-defining disease and accountable for a third of all deaths. It is estimated that more than 38.0 million people are living with HIV, of whom 68% have access to antiretroviral treatment (ART) (85% in pregnant women). Among people living with HIV (PLWH) with access to ART, 88% are estimated to have reached viral suppression < 50 HIV RNA copies/mL. In total, 7.1 million people worldwide are predicted to be HIV positive without knowing it (7).

Although a global disease, HIV is still considerably more common in low-income settings. Two-thirds of all PLWH live in Sub-Saharan (Figure 1) (7). The majority of adult PLWH are women (55%) while children <15 years old represent 5% of all PLWH. In 2019, one-third of all new infections were in young people (15–24 years old) (7).

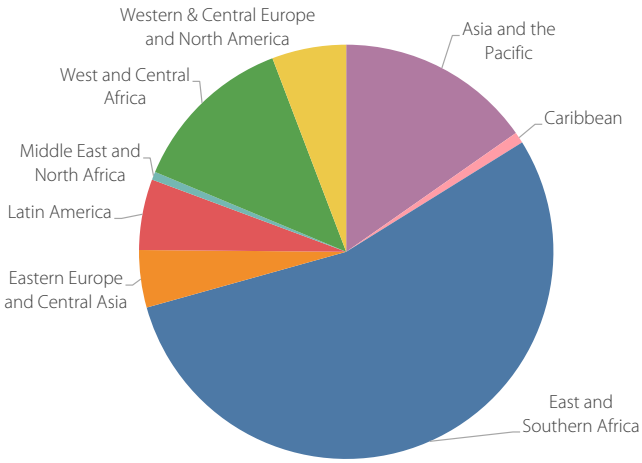


Figure 1. People living with HIV (all ages) by region.
Data source: UNAIDS epidemiological estimates, 2020

HIV is transmitted across mucosal surfaces through sexual contact (worldwide primarily through heterosexual transmission) and vertical transmission from mother to child. It can also spread through direct contact with shared needles among people who inject drugs or via infected blood products. The infectiveness of HIV is highly dependent on access to ART. With modern ART, the risk of transmitting the virus to others is close to zero (8-10). The overall risk of contracting HIV is higher in certain key populations: 30 times higher for sex workers, 29 times for people who inject drugs, 26 times for men who have sex with men (MSM), and 13 times for transgender people (7).

Despite the grim numbers, there is also good news. Access to ART with few side effects is increasing, and through combined interventions the yearly incidence of people diagnosed with HIV has been reduced by 40% compared to 1998, the year when most new cases were diagnosed. AIDS-related deaths have also declined. Compared to the peak in 2004, the number of deaths has been reduced by 60% (7).

1.1.1 HIV IN SWEDEN

The Swedish epidemic is believed to have started in Stockholm among MSM in 1979 (11). The first case reports are from 1983, the same year AIDS was declared a public health hazard according to the Swedish Communicable Diseases Act (12).

Between 1983 and 2019, almost 13,500 people have tested positive for HIV in Sweden (L. Van Leest, The Public Health Agency of Sweden, personal communication, Dec 12, 2020). Out of these, 8,157 PLWH are presently receiving HIV-related health care in Sweden (InfCareHIV December 2020). The remaining individuals are either deceased or have emigrated. The overall Swedish HIV prevalence is thus low (0.08%). The majority of PLWH in Sweden are men (61%) and the median age is 49 years (IQR 40–57). Thirty-five percent of the population originates from Sweden, and 27% of all transmissions have occurred in Sweden. The most common transmission mode is through sexual contact (51% heterosexual, 32% MSM). The remaining infections with known routes of transmission have been acquired from sharing of injection drug preparation equipment (5%), vertical transmission from mother to child (3%), and infected blood products (1%).

In 2016, Sweden became the first country in the world to reach the 90-90-90 goal set by The Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) (13, 14). The numbers represent three objectives; 90% of all PLWH shall be aware of their HIV status, 90% of these shall have access to ART, and 90% of these shall reach HIV RNA < 50 copies/mL (14).

The yearly HIV incidence has during the last decade been stable, with 400–500 new diagnoses per year. Sweden has one of the most diverse HIV subtype compositions outside Africa (15). This diversity is a consequence of migration and the fact that most new cases were diagnosed in another country before the arrival in Sweden. There has unfortunately also been a slow increase in transmission within Sweden in the last ten years (16).

1.2 HIV VIROLOGY

HIV is a virus from the *lentivirus* genus within the *retroviridae* family (17). It closely resembles the simian immunodeficiency virus (SIV), found in primates (18, 19). Phylogenetic studies have shown that it crossed over to humans in central Africa about a hundred years ago, most likely the consequence of monkeys being hunted for their meat (20).

There are two different kinds of HIV capable of causing disease in humans, HIV-1 and HIV-2 (21). HIV-1 is most common, responsible for the global HIV epidemic, the focus of this thesis and onwards referred to as HIV.

HIV-2 is mainly found in West Africa, less contagious, and requires a longer time to deplete patients' immune systems which eventually can also lead to AIDS (22).

HIV is further categorized into four genetically different groups (M, N, O, and P), resulting from separate zoonotic transfers (19, 23). Group M is the most widespread and the origin of the current epidemic (19). It is further categorized into subtypes A–K. Co-infections with more than one HIV subtype have resulted in about 20% of all PLWH having recombinant forms (24). The distribution of subtypes mirrors historical and current human migration. Recombinant forms are increasing, but subtype C is still the most frequent globally. It is most common in Sub-Saharan Africa and India, B is mostly found in Western Europe and America, and A predominates in eastern Europe and central Asia (25).

The HIV genome is located inside a cone-shaped nucleocapsid and stored in two copies of single-stranded HIV RNA with approximately 10,000 base pairs (Figure 2). The genome consists of nine genes: three major genes (*gag, pol, env*) encoding structural proteins and three necessary enzymes protease, integrase, and reverse transcriptase; and six genes (*rev, tat, vif, vpu, nef, vpr*) encoding proteins with regulatory function (26, 27).

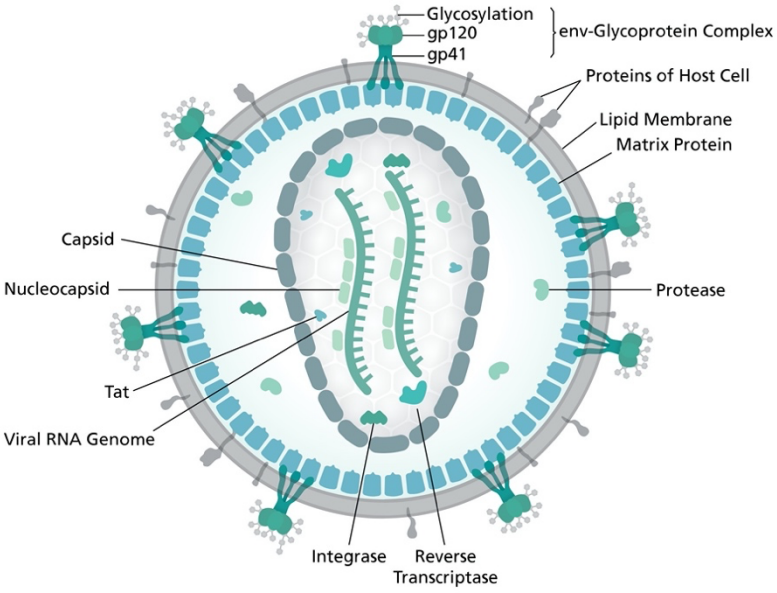


Figure 2. Schematic structure of the HIV-1 virion. Image source: Wikimedia Commons. By: Thomas Splettstoesser (www.scistyle.com)

HIV infects leukocytes that express CD4 receptors and CCR5 or CXCR4 co-receptors on their surface, i.e., $CD4^+$ T lymphocytes, monocytes, microglia, astrocytes, macrophages, and dendritic cells. The HIV virion attaches to these receptors through a surface glycoprotein complex of gp120 and gp41 that brings the virus closer to the cell and eventually fuses with the cell surface membrane (28, 29). The viral enzyme reverse transcriptase (RT) attaches to the released RNA-strands. It first converts the HIV RNA to a complementary single-stranded DNA followed by a second strand, resulting in double-stranded DNA. After this, the viral enzyme integrase binds to the DNA molecule and transfers it to the cell nucleus, integrating the viral genome into the DNA of the cell. At this stage, the chronic HIV infection is established.

The cell can either remain in a latent state or start transcription through the human enzyme DNA transcriptase. The last process continues with the transcription of the integrated HIV provirus into mRNA from the viral DNA. Some of these mRNA relocate to ribosomes and get translated to surface glycoprotein complexes and

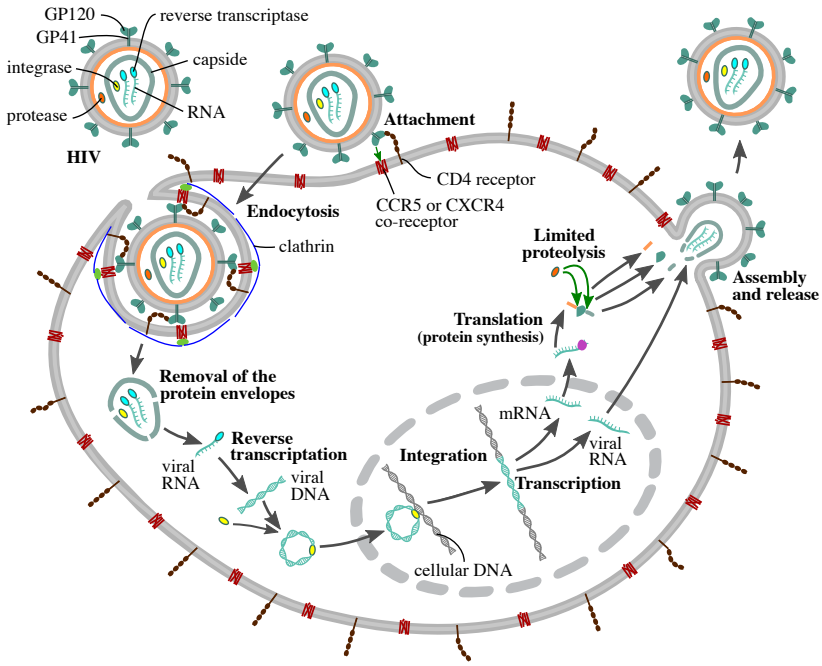


Figure 3. The replication cycle of HIV-1. Image source: Wikimedia Commons. By Jmarchn

viral polyproteins. After migrating to the cell surface, polyproteins and untranslated HIV RNA-strands are assembled and bud off from the host cell. The last step to produce infectious virions involves cleaving of the polyproteins by the enzyme protease to produce functional enzymes like integrase and reverse transcriptase (Figure 3) (27).

Each virion can make up to 10,000,000 copies of itself in one day, and each $CD4^+$ infected cell can produce thousands of new viruses. The cells eventually die, either from bursting by HIV-induced cytolysis or by pyroptosis. The latter inflammatory reaction leads to cell death, possibly triggered when the number of non-integrated DNA-copies becomes too many. The dead cell releases inflammatory cytokines that might attract more T-cells that also get infected, resulting in a vicious circle (27, 30).

1.3 NATURAL COURSE OF HIV

The HIV infection is usually established close to the mucosal surface where the first virion entered the body. During the first couple of weeks, the viral amplification continues and antigen-presenting cells transport the virus to the draining lymph node. The infection is still asymptomatic, and no HIV RNA can be detected in the blood. Within two to four weeks post-transmission, the infection spreads systemically through the body. About 50–65% of infected persons develop flu-like symptoms with fever, sore throat, lymphadenopathy, myalgia, and rash (31, 32). At this stage, referred to as primary HIV or acute HIV syndrome, very high viral loads can be measured in the blood. Simultaneously, the immune system is responding to the infection with antibodies that bind to viral proteins and $CD8^+$ T cells recognizing HIV-antigen presented by MHC class I molecules on infected cell surfaces (33).

After an additional few weeks the symptoms resolve and viral load decreases (34). The infection enters a new phase referred to as chronic HIV or clinical latency phase. Viral amplification and $CD4^+$ T cell depletion continue but are balanced by the immune response. The viral load reaches a constant setpoint that varies greatly between individuals, from < 50 to > 1,000,000 copies/mL (35, 36). Over time, the amount of $CD4^+$ T cells gradually declines, and immune responses consequently deteriorate. The previous asymptomatic person starts developing severe opportunistic viral, bacterial or fungal infections and risks different cancer forms when the CD4 count drops to < 200 $x10^6$/L. This represents the development of AIDS. This is the last phase of the untreated infection and starts on average ten years post-transmission (Figure 4) (36).

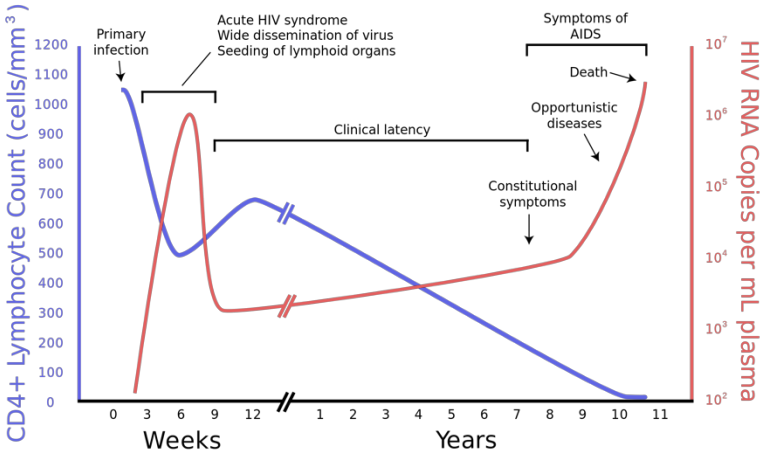


Figure 4. Untreated HIV course. Image source: Wikimedia Commons. By: EternamenteAprendiz

1.4 ANTIRETROVIRAL THERAPY

During the first years of the HIV epidemic, only symptomatic and supportive treatments were available. PLWH suffered from stigma, mental and physical distress, and mortality was very high.

In 1987, zidovudine was introduced as the first antiretroviral drug. It inhibits RNA transcription by incorporating into the complementary single strand DNA, which results in blocking RT from linking additional nucleotides from the cytoplasm. Consequently, natural nucleotides can no longer be added to the growing DNA chain, and the reverse transcription is ended. It was the first drug in a class called Nucleoside Reverse Transcriptase Inhibitors (NRTIs). After initially promising results, it was soon realized that the treatment effect was transient.

In 1996, two new drug classes were introduced: Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) that inhibit RT but in a non-competitive manner, and Protease Inhibitors (PIs) that inhibit the cleavage of viral polyproteins into functioning enzymes. The game-changing concept of highly active antiretroviral therapy (HAART) was introduced the same year. By combing ≥ three drugs from ≥ two classes, a durable effect was finally achieved (37-39). For PLWH with access to these drugs, viral load decreased and the immune system

could slowly start to recover. Clinical trials and mathematical studies confirmed the positive results and for a while there was hope that by using HAART long enough, HIV would finally be eradicated. The combined treatment suppressed the viral load to undetectable levels, but viremia always returned when treatment was discontinued. With the discoveries that HIV also infects long-lived cells like microglia and that it can persist for a very long time in a latent stage, it was evident that life-long treatment was needed (40-42).

Since then, four new drug classes have been introduced. Integrase Strand Transfer Inhibitors (INSTIs) prevent viral DNA from becoming integrated into the human DNA and thus prevent viral amplification. Fusion inhibitors, co-receptor antagonists and post-attachment inhibitors are all designed to block the virion's entry into the host cell. These last three drug classes remain rarely used in clinical practice.

The combination of antiretroviral drug treatment strategy has been unchanged since 1996. However, since then, pharmacodynamic and pharmacokinetic advances and fixed-dose combinations have led to fewer pills per day, less drug interactions and fewer side effects. According to current treatment guidelines, first-line treatment should consist of two NRTIs and one core agent, either an INSTI, PI, or NNRTI (43). As of December 2020, an array of 20 different ARTs from 5 drug classes are available in Sweden: 6 NRTIs, 5 NNRTIs; 4 PIs; 4 INSTIs, and one entry inhibitor (Table 1).

The current treatment goal is durable suppression < 50 HIV RNA copies/mL (43). This threshold used to be the lower limit of detection but has also proved to be a reliable treatment target. The recommendations on when to initiate ART have varied over the years. The initial strategy of "hit hard and early" was replaced by "wait as long as possible" due to troublesome side effects. In 2015, the influential START-study demonstrated that treatment initiation in PLWH with a $CD4^+$ cell count $\geq 500 \times 10^6$/L in comparison to < 350 was significantly associated with lower morbidity and mortality (44). Since then, immediate treatment initiation is once again recommended.

The definition of treatment failure varies between countries. In Sweden, the current recommendation for when to suspect failure is in

PLWH who, six months after treatment initiation, have repeated HIV RNA > 200 copies/mL (43).

Table 1. Antiretroviral drugs currently used in Sweden

Class	Group	Generic name	Abbreviation	Trade name	Year of approval
Reverse transcriptase inhibitors					
	Nucleoside analogues (NRTIs)				
		abacavir	ABC	Ziagen	1998
		emtricitabine	FTC	Emtriva	2003
		lamivudine	3TC	Epivir	1995
		tenofovir alafenamide	TAF	Vemlidy	2015
		tenofovir disoproxil	TDF	Viread	2001
		zidovudine	AZT/ZDV	Retrovir	1987
	Non-nucleoside analogues (NNRTIs)				
		efavirenz	EFV	Stocrin	1998
		nevirapine	NVP	Viramune	1996
		etravirine	ETR	Intelence	2008
		rilpivirine	RPV	Edurant	2011
		doravirine	DOR	Pifeltro	2018
Protease inhibitors (PIs)					
		atazanavir	ATV	Reyataz	2003
		darunavir	DRV	Prezista	2006
		lopinavir	LPV	Kaletra	2000
		ritonavir*	RTV	Norvir	1996
Integrase strand transfer inhibitors (INSTIs)					
		bictegravir	BIC	**	2018
		dolutegravir	DTG	Tivicay	2013
		elvitegravir	EVG	Vitekta	2015
		raltegravir	RAL	Isentress	2007
Entry inhibitor					
		maraviroc	MVC	Celsentri	2007

*only used for boosting other drugs

**Single-drug treatment not available

1.5 HIV DRUG RESISTANCE

Unlike human DNA transcriptase, viral RT lacks proofreading, which is the most important factor to why the mutation rate in HIV is extremely high (1–2 x 10^5 mutations per base and day) (45-47). In combination with the rapid amplification rate, HIV has an enormous capacity for genetic diversity. The heterogeneous HIV pool is one of the reasons why there is still no HIV-vaccination, as surface proteins targeted by the vaccines are in constant evolution. It is also the reason why monotherapy with ART quickly fails. If a mutation successfully modifies the binding site of a drug, the molecule can no longer attach, and viral suppression is immediately stopped.

Acquired drug resistance in PLWH during ART is very uncommon. As the amplification process is blocked, no new mutations can occur. However, amplification can proceed in PLWH with poor adherence, treatment interruptions or drug-interactions that lead to sub-optimal drug levels. At subtherapeutic drug concentrations, resistant clones will enrich until the resistance is fully established.

The risk of developing drug resistance varies as drugs have different genetic barriers, i.e., how many mutations are required for resistance to develop (48). HIV subtypes can also influence the future risk of resistance (49). Drug resistance can affect either a single drug or cause cross resistance for an entire drug class (50). As is often the case with NRTIs, a mutation can also make some drugs less effective while others are potentiated. This discovery can be used to our advantage when designing personalized ART-regimens (50, 51).

Drug resistance can also occur by transmitted resistance. When someone with acquired resistance transfers the mutations to a naïve population it can influence many people's treatment outcomes. As some mutations come at a fitness cost for the virus, if the individual is not exposed to the specific drug there is a chance that the mutation will perish. The persistence varies between different mutations. Resistance has been observed to all drug-classes, but the frequency differs significantly due to the genetic barrier. For example, PI-resistance is still uncommon (52). High genetic barriers have, in recent years, made therapies with only two drugs possible. So far, no treatments based on a single drug have been successful, although it has been suggested as an

option for PLWH switching from an initial treatment after having been successfully suppressed (53).

1.6 HIV RESERVOIRS

Despite the use of effective ART, with the introduction of more sensitive PCR assays, it has been shown that persisting low-level viremia (LLV) (HIV RNA 1–3 copies/mL) is common (54-58). As HIV RNA-strands in plasma have a half-life of six hours, the detectable virus does not reflect pre-ART virus (59). Instead, it is believed that the rate of HIV RNA decay after treatment initiation mirrors the life span of previously infected $CD4^+$ cells. Four stages are identified in the ART-dependent viral decline (Figure 5). The first stage is believed to represent the population of activated T cells. The second stage, with a slightly slower decay, is hypothesized to originate from macrophages. The third phase is considerably slower, with a half-life of 9–15 months. After that, the fourth stage enters where HIV RNA continues to be detectable between 1–3 copies/mL. This phenomenon is often referred to as residual viremia. It is speculated that both the third and fourth phases represent viruses released by latently infected $CD4^+$ T cells (60, 61).

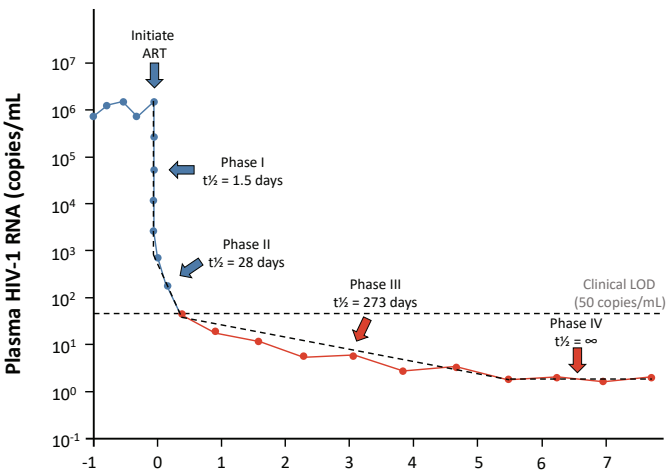


Figure 5. Dynamics of HIV RNA decay after treatment initiation. Blue lines indicate HIV RNA > 50 copies/mL measured with conventional PCR. Red lines indicate low-level viremia detected with ultrasensitive assays and dotted lines a theoretical decay slope. Image source: Curr HIV/AIDS Reports 2012; 9:91-100. Reprinted with permission from Springer Publishing

1.6.1 LATENCY

As described in section 1.3, infected cells can harbor incorporated proviral DNA without replicating. Latent infections also exist in a pre-integrated form, where HIV RNA, transformed to DNA, has entered but is not integrated into inactive lymphocytes (62). However, since unintegrated DNA strands have a half-life of about 1–5 days, this has no significant impact (63-65). Post-integrated viral DNA, on the other hand, can persist much longer. If incorporated in a long-lived resting memory $CD4^+$ memory T cell or possibly in hematopoietic progenitor cells, it can remain unaffected by ART for a very long period of time. Upon activation, HIV replication can resume decades later, representing a significant obstacle for HIV eradication (40-42, 60, 64, 66-68). A possible solution would be if inactive cells could be stimulated into replication, which would facilitate ART. A previous study tried to use intravenous immunoglobulins with promising results (69), but the effect was unfortunately only transient (70).

The current understanding is that the reservoir of long-lived memory $CD4^+$ T cells is established early in the infection (71). The size of the reservoir, as measured by HIV DNA, has been shown to correlate with how prompt ART was initiated and the level of residual viremia (72, 73). A more extensive reservoir also correlates to faster viral rebound after treatment interruptions (74). It is worth noting that the use of HIV DNA as a surrogate marker for the reservoir is debated. As the integrated DNA does not always contain the entire provirus genome, there is a risk of misjudging the results. Several studies have found that only about 10% of integrated proviruses that persists during ART are capable of producing mature and infectious virus (75-77).

1.6.2 SANCTUARY SITES

Much effort has been put into understanding if LLV is a consequence of ongoing replication in specific anatomical compartments not accessible by ART. Gut-associated lymphoid tissue and the central nervous system have been proposed as sanctuary sites where viral amplification might continue (78-82). HIV RNA/DNA during suppressive ART have also been found in lymph nodes, adipose tissue and in the urethra (83-86). Intensified treatment with additional drugs

to target ongoing replication has not convincingly reduced LLV, suggesting that continuous replication is not the reason for this phenomenon (87-89). Also, with some exceptions from small studies in the early ART-era (61, 90), most studies to date have not found convincing evidence of viral evolution, which would have been expected in case of continuous replication (40, 71, 91-93).

1.6.3 VIRAL BLIPS

Case series that described transient episodes of detectable HIV RNA > 50 copies/mL despite effective ART were first published around the turn of the millennium (55, 90, 94, 95). To my knowledge, the retrospective study by Havlir et al in 2001 is the first scientific paper to define these intermittent episodes of viremia as blips (96). Since then, viral blips in plasma have been investigated in several both prospective and retrospective studies. The term viral blip is similarly used for episodes of detectable low-level viremia in cerebral spinal fluid (97, 98). Blips are also described in other infectious diseases caused by other viruses like cytomegalovirus (99), hepatitis B (100) and C (101).

In a prospective study by Nettles et al 2005, 10 participants were followed with blood samples drawn every 2–3 days for 3–4 months. Blips were found in nine of ten participants with a median duration of < 3 days and a median amplitude was 79 HIV RNA copies/mL (102). More recent studies, with less frequent sampling, have found blips to occur in 13–40% of all participants (96, 103-108). However, since PCR assays and blip definitions vary between studies, both incidence and amplitudes are difficult to compare.

The origin of blips is not known. It has been hypothesized to be a result of intermittent activation of latent $CD4^+$ T cells (109, 110), random biological fluctuations (102), assay variations (111), low adherence (102, 112), or emerging drug resistance (113, 114).

AIMS

2. AIMS

The overall aim of this theses was to study the clinical and virological response in PLWH after initiating ART through analysis of the unique Swedish InfCareHIV database.

The specific aims were:

I. To define the incidence and clinical importance of viral blips in PLWH and to identify potential predictive factors for their occurrence

II. To investigate if dolutegravir is an effective treatment option in PLWH with known NRTI-resistance

III. To assess how baseline HIV RNA > 500,000 copies/mL affects treatment outcome with modern ART

IV. To analyze blip incidence in the entire national cohort of PLWH, with particular focus on INSTIs

PATIENTS AND METHODS

3. PATIENTS AND METHODS

3.1 PATIENTS AND STUDY DESIGN

All participants in this thesis are adult PLWH that receive health care and treatment at one of the 30 Swedish HIV clinics.

Due to the low HIV prevalence in Sweden, and the objective to compare large samples of the Swedish cohort of PLWH, some of the different papers' participants are inevitably included in more than one study (Figure 6).

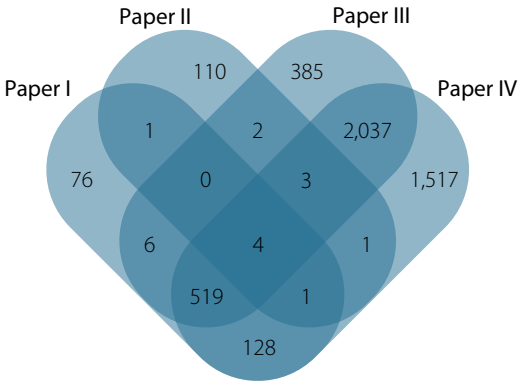


Figure 6. Venn diagram illustrating participant overlap between studies. Each Paper is represented by an oval. The area is not proportional to the number of participants

All results in this thesis are based on retrospective data. No additional blood samples needed to be drawn or analysed. The Regional Ethics Review Board in Gothenburg has approved all four included studies (Dnr 532-11).

PAPER I

In this study, all treatment-naïve participants receiving health care in the five largest HIV clinics in Sweden were eligible for inclusion. This cohort represented about two-thirds of PLWH in Sweden. The study started ≥ six months after ART was commenced when the second HIV RNA sample < 50 copies/mL was registered (Figure 7).

The inclusion period (2007 – 2013) was chosen to ensure that the conditions between the clinics were as similar as possible. All HIV RNA samples had to be collected in ethylenediamine tetraacetic acid test tubes and analyzed with the same HIV RNA quantification method: version 1 or 2 of COBAS TaqMan HIV-1 technique (CAP/CTM1/2; Roche, Molecular Systems, Branchburg, NJ, USA).

The definition of blips was chosen to be in line with what most other studies were using at that time. The definition of virological failure (VF) was also selected in accordance with the majority of previously published studies.

To limit the possibility of blips due to suboptimal treatment, only participants with ART consisting of ≥ 3 components were allowed. ART also had to consist of 2 NRTIs and ≥ 1 core agent, in most cases PI or NNRTI. INSTIs were not commonly used at the time and thus only included in a few cases.

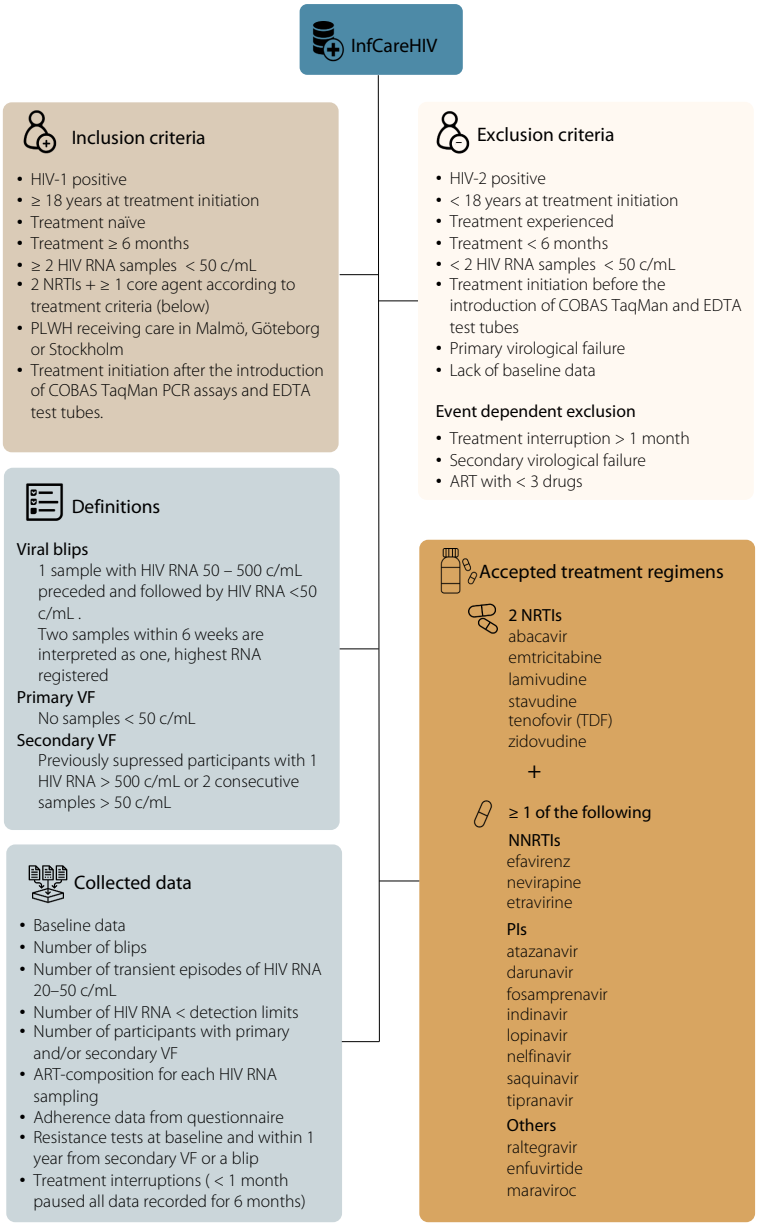


Figure 7. Study design, Paper I

PAPER II

For this study, all PLWH in Sweden with baseline NRTI drug resistance (defined by the Stanford University HIV Drug Resistance Database) and dolutegravir-based ART (50 mg once daily) with 1–2 NRTIs ≥ 1 month were eligible for inclusion. Combinations with dolutegravir and other core agents were not allowed.

NRTI resistance was evaluated using the Genotypic Susceptibility Score (GSS), a theoretical model of expected treatment effect in relation to drug resistance mutations and choice of ART. From an online resistance interpretation guide at the Stanford University HIV Drug Resistance Database (115), a number between 0–1 was calculated for each drug as an indicator of expected drug susceptibility (Table 2).

Table 2. Genotypic Susceptibility Scores

Drug susceptibility	Score
Susceptible	1
Potential low-level resistance	0.75
Low-level resistance	0.50
Intermediate resistance	0.25
High-level resistance	0

As dolutegravir was a novel drug and studies with documented cases of INSTI resistance at the time were very few, dolutegravir was considered to have full susceptibility, i.e., GSS 1. Participants with a combined GSS 1–2.5, and controls matched according to GSS and treatment duration were included. Both treatment-experienced and naïve PLWH were eligible for inclusion.

Participants with HIV RNA > 200 copies/mL in their last available blood sample or that never reached < 50 copies/mL were considered to have VF (Figure 8).

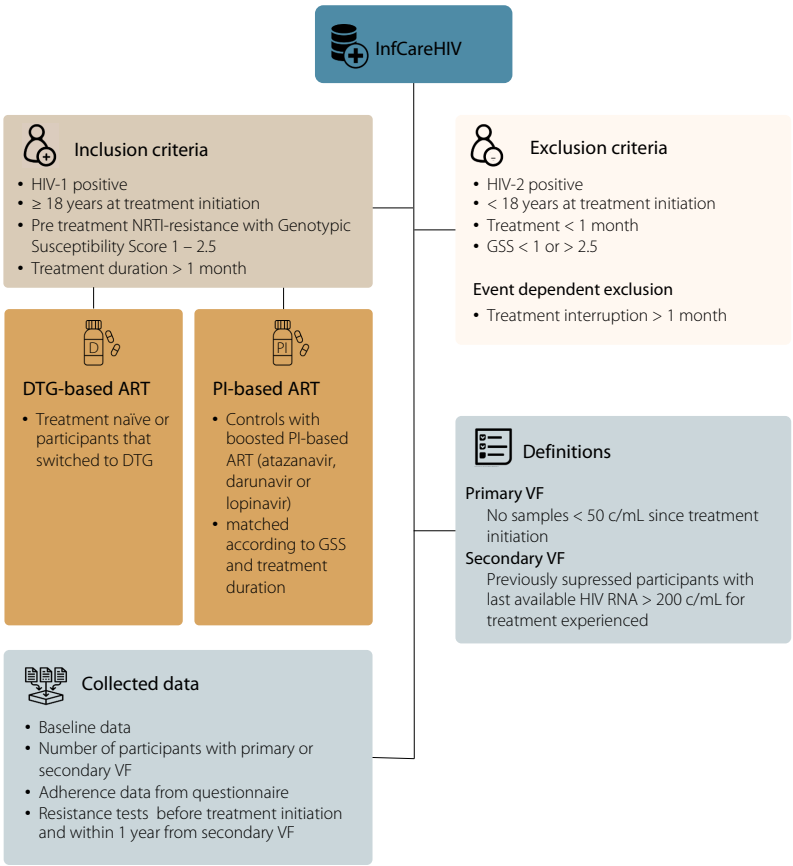


Figure 8. Study design, Paper II

PAPER III

In the third study our aim was to compare the treatment outcome in PLWH with different levels of baseline viremia. Participants were categorized into four groups depending on baseline HIV RNA: < 100k, 100–500k, 500–1,000k, and > 1,000k copies/mL.

The focus of this paper was participants with chronic infection. Participants with primary HIV infections with separate viral dynamics were consequently excluded. To make sure all participants were treatment naïve, those with baseline viral load < 1,000 HIV RNA copies/mL were also excluded.

The study period lasted from 2000 to 2018 and was chosen to secure effective ART with three components (Figure 9). Treatment during the first year was restricted to two NRTIs, with either abacavir or tenofovir as one component. The core agent could be a NNRTI, boosted PI, or an INSTI. After the initial year, any treatment combinations were allowed. Consequently, many individuals with older, potentially less effective NRTIs, and unboosted PIs were excluded.

Primary VF was defined as not reaching viral suppression < 50 HIV RNA copies/mL within one year after ART initiation. Secondary VF was registered in participants with previously registered HIV RNA level < 50 copies/mL treated with ART for ≥ 1 year and who developed ≥ 1 HIV RNA > 1,000 copies/mL or ≥ 2 consecutive samples > 200 copies/mL. To clearly differentiate between primary and secondary VF, participants could not be registered with both of these entities.

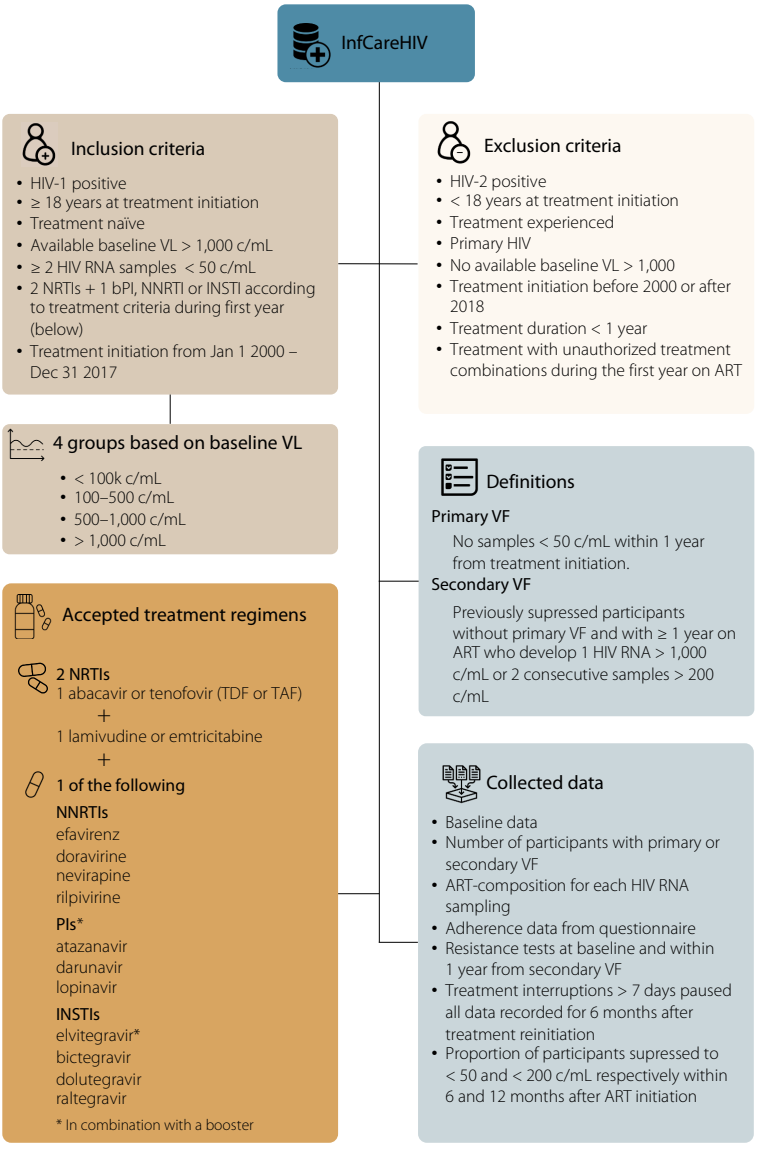


Figure 9. Study design, Paper III

PAPER IV

This study sought to mirror the real-life setting in HIV clinics, where the recommended ART and HIV RNA quantification methods change over time. Due to the very limited use, it was not possible to draw any conclusions from the participants with INTIs in Paper I. We thus wanted to focus on participants with this treatment in this study.

The inclusion period started in 2007, which is the year INSTIs were introduced as a new treatment option. Participants from all Swedish HIV clinics were eligible. As in Paper I, participants were obliged to be treatment-naïve and the observation period started after viral suppression was achieved.

To make sure participants were receiving effective ART, the accepted treatment options were even more limited compared to Paper I. Only one core agent was allowed, i.e., NNRTI, INSTI or boosted PI. The NRTI component had to consist of two drugs, either abacavir or tenofovir, each in combination with either lamivudine or emtricitabine (Figure 10).

Another difference from Paper I was that the definition of VF was changed. Paper III concluded that the initial treatment response takes a longer time than was previously known. Thus, the adapted definition of VFs used in Paper III was also used in Paper IV.

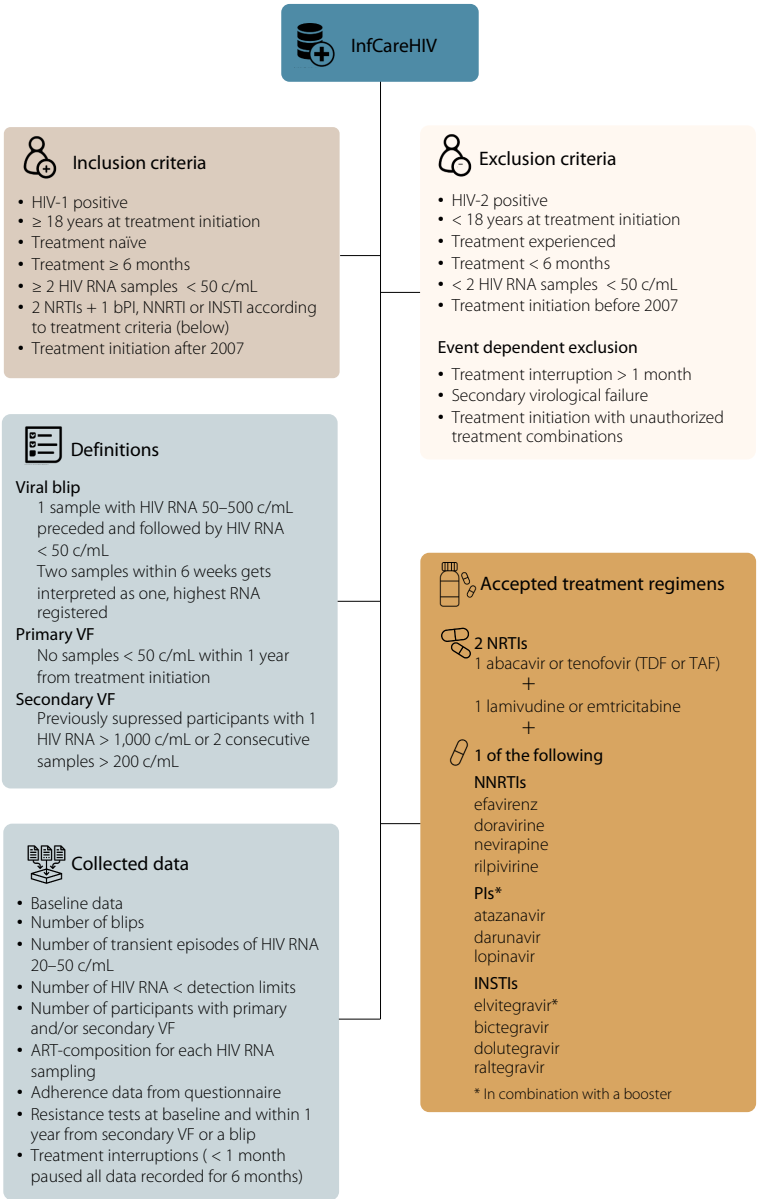


Figure 10. Study design, Paper IV

3.2 INFCAREHIV

Due to a demand for a more effective way to summarize patient's medical history, InfCareHIV was created as a collaboration between Health Solutions AB, Sahlgrenska and Karolinska University hospitals in 2003. InfCareHIV serves as a combined quality assurance, decisions support, clinical and research database, and has since 2008 become a national registry. From 2009 it is considered to have national coverage, and > 99% of all PLWH are registered. It is validated through both a quality index and external processes by IBM Research Haifa, the Mac Planck Institute of Bioinformatics, and from 2011–2015 the Cohere database quality assurance system (HIV Cohorts Data Exchange Protocol).

Data have been added retrospectively from the early 1980s. Thus, InfCareHIV contains almost four decades of relevant demographic, epidemiological, treatment and laboratory data. Patients also regularly fill in a validated questionnaire with questions concerning self-assessed health, treatment satisfaction and adherence (116). The database is a unique and valuable source for researchers who may gain access after permission from the steering committee (117).

3.2.1 DATA EXTRACTION AND SORTING

All data in this thesis are derived from InfCareHIV. Data can be extracted through visual inspection of one patient at a time, through a research section where different parameters can be specified, which renders MS Excel sheets with the desired information. It is also possible to download parts of the database to MS Access.

In Paper I, data were initially collected by visual inspection before switching to computer-aided data handling with the help of a statistician. In Paper II, the smaller group of participants included was manageable in MS Excel. For Paper III and IV, the study populations and related data were considerably larger, and it became apparent that a new method had to be applied. A relational database with anonymized data was established in Filemaker Pro 18 Advanced

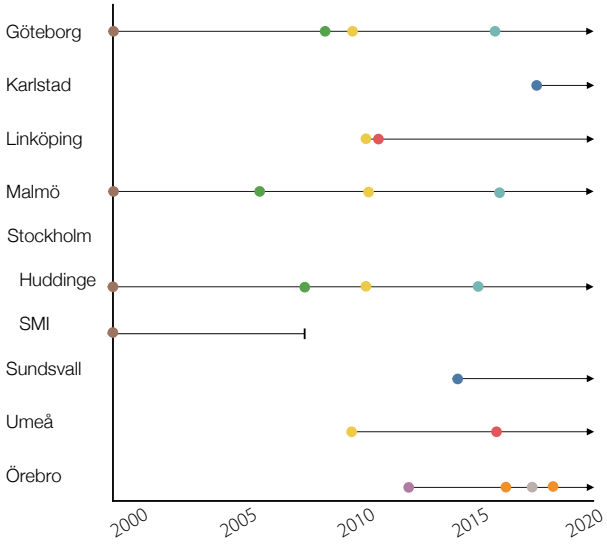
(FileMaker, Inc., Santa Clara, CA, USA). After acquiring knowledge regarding how to write scripts for data sorting, the management of large data sets was much more manageable.

3.3 HIV-1 RNA QUANTIFICATION

HIV-1 RNA quantification based on RT-PCR started in Sweden in 1994 at Sahlgrenska and Karolinska University Hospitals (formerly Huddinge University Hospital). Through the years, different commercially available tests have been in use (Figure 11). The main differences between these tests are the detection limits, e.g., the lowest and highest levels of detectable HIV RNA in plasma, and the amount of manual labour involved in the process.

The quantification process starts with the enzyme RT that converts HIV RNA to complementary single-strand DNA. After this, either manually or by machine, cell-free plasma is combined with a PCR Master mix that contains nucleotides, another enzyme called Taq polymerase enzyme, HIV-1 specific primers, probes that emit fluorescent signals for each duplication, and nuclease-free dilution fluids. Through thermal cycles, the multiplying chain reaction starts with annealing, which causes the DNA primers and probes to adhere to specific parts of the single-strand DNA. In the next step called extension, heat resistant TAQ-polymerase start at the primer and add nucleotides to convert the single-stranded DNA to double-stranded DNA. In the last step called denaturing, the DNA double helix gets separated into two single-stranded DNA copies before the process starts over again (Figure 12). Thus, for every cycle, the amount of DNA doubles, resulting in an exponential amplification.

Lastly, to quantify the viral load, the number of PCR cycles required to reach the lower detection limit of the PCR machine is compared to the slope and intercept of a standard curve, i.e., the number of cycles required for a series of HIV RNA samples with set concentrations that were amplified together with the patient samples.



Test	Lower detection limit*	Upper detection limit*
Abbott RealTime HIV-1	40	10,000,000
Aptima HIV-1 quant dx assay	30	10,000,000
COBAS 4800 HIV-1 test	20	10,000,000
COBAS 6800 HIV-1 test	20	10,000,000
COBAS AmpliPrep/COBAS TaqMan HIV-1 test v 1.0	40	10,000,000
COBAS AmpliPrep/COBAS TaqMan HIV-1 test v 2.0	20	10,000,000
COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test	48	10,000,000
Roche Amplicor HIV-1 Monitor Assay	400	750,000
Veris HIV-1 assay	35	10,000,000

*copies/mL

Figure 11. HIV-1 RNA quantification assays in use in Sweden between 2000 and 2020. The availability of these methods during this period and the number of annual analyses between the laboratories differs significantly. Data received through correspondence with all laboratories.

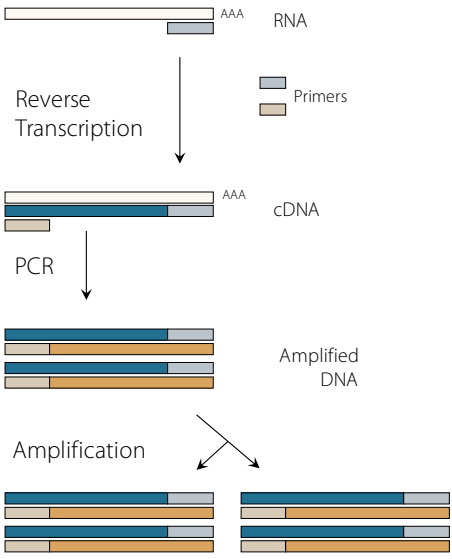


Figure 12. RT-PCR. Image source: Wikimedia Commons. By: Poshul, CC BY-SA 4.0

Today, commercially available PCR assays have a lower quantification threshold of 20 HIV RNA copies/ml (118). Manual assays with ultracentrifugation provide even higher sensitivity and can detect single copies of HIV RNA (54, 119). New assays are rigorously tested for sensitivity and specificity and compared to other assays with concordance analysis (104, 118, 120). In general, a deviation of > 0.5 HIV RNA $\log_{10}$ is considered significant (B. Svennerholm, Department of Clinical Virology, Sahlgrenska University Hospital, personal communication, Dec 9, 2020).

3.4 DRUG RESISTANCE TESTS

To avoid ineffective treatment, most HIV treatment guidelines recommend HIV resistance testing before initiating ART. Testing is equally important if there are signs of treatment failure (121). HIV drug resistance can be determined with phenotypic or genotypic tests. Phenotypic tests involve *in vitro* testing to analyze what effect the mutation will have on the virus on different drugs at different concentrations. This test is however not used much today. Genotypic tests, which are most commonly used, focus on the HIV RNA/DNA sequence and list the corresponding amino acids. An algorithm like Stanford University HIV Drug Resistance Database is then used to compute drug susceptibility.

During the inclusion time for the studies in this thesis, all tests were performed through genotypic resistance tests with population-based Sanger assays of the HIV *pol* gene. In the coming years, this technique is predicted to be replaced by next-generation sequencing, with which the entire HIV genome can be analyzed.

RESULTS

4. RESULTS

4.1 PAPER I & IV

This thesis includes two studies investigating viral blips in PLWH in Sweden (Table 3). In the first study, 735 participants who started ART between 2007 and 2015 met the inclusion criteria. Of these, 76 (10.3%) developed blips. The median follow up time was 3.3 years (range 1.9–4.6). Blip incidence per 100 person years was 3.0 (CI 95% 2.3–3.7).

The second study (Paper IV) included 4,210 participants who started ART between 2007 and 2020; median follow up time was 5.5 years (range 0.5–13.7). With the same blip definition, 853 participants (20.3%) had viral blips. Blip incidence per 100 person years increased to 4.4 (CI 95% 4.0–4.7).

Table 3. Characteristics of participants in Paper I and IV*

	Paper I		Paper IV	
	Participants without blips	Participants with blips	Participants without blips	Participants with blips
№ of participants [%]	659 (89.7)	76 (10.3)	3,357 (79.7)	853 (20.3)
№ of samples [%]	3,729 (83.8)	720 (16.2)	42,500 (70.8)	17,566 (29.2)
№ samples/participant [median (IQR)]	5 (2–8)	9 (7–12)	10 (6–16)	17 (12–24)
Observation time, years [median (IQR)]	3.0 (1.8–4.4)	4.5 (3.7–5.1)	5.0 (2.5–7.8)	7.9 (5.2–10.6)
Baseline HIV RNA log_{10} copies/mL [median (IQR)]	4.6 (3.9–5.0)	4.9 (4.3–5.2)	4.5 (3.5–5.1)	4.9 (4.3–5.4)
Primary virological failure [%]	excluded	excluded	394 (11.7)	73 (8.6)
Secondary virological failure [%]	21 (3.2)	6 (7.9)	321 (9.6)	66 (7.7)

A total of 90 blips were registered in Paper I and 1,180 in Paper IV. Most of the blips registered were < 200 copies/mL: 87% in Paper I and 89% in Paper IV (Figure 13).

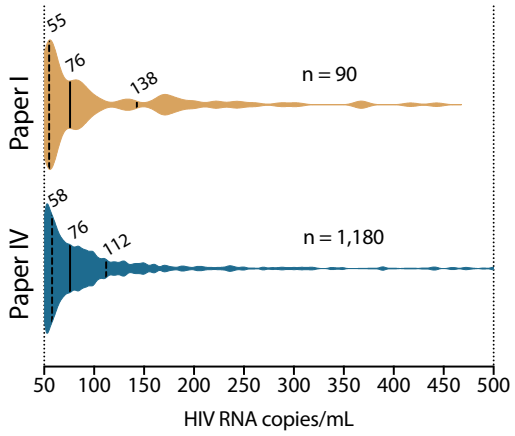


Figure 13. Violin plot depicting all registered blip amplitudes. Wider portions of the diagram translate into the number of samples with corresponding HIV RNA values on the x axis. Vertical lines represent median and IQR.

In both studies, baseline HIV RNA levels were higher in participants with blips than in participants with sustained viral suppression: median 4.9 $\log_{10}$ and 4.6 $\log_{10}$ in Paper I, and 4.9 $\log_{10}$ and 4.5 $\log_{10}$ in Paper IV, respectively (Figure 14).

Blip incidence varied significantly during the inclusion periods. In Paper I, most registered blips occurred during the first two years after the study initiation. However, after comparing years on ART with calendar years, the latter was found to be the most influential factor.

More blips were registered between 2008 to 2011 than during the following years. The same trend with declining blip incidences was observed in Paper IV. However, from 2014 the incidence increased again with a new peak in 2017 (Figure 15). The median time until the first blip was 625 days (IQR 452–814) and 946 (IQR 589–1,739) for Paper I and IV, respectively.

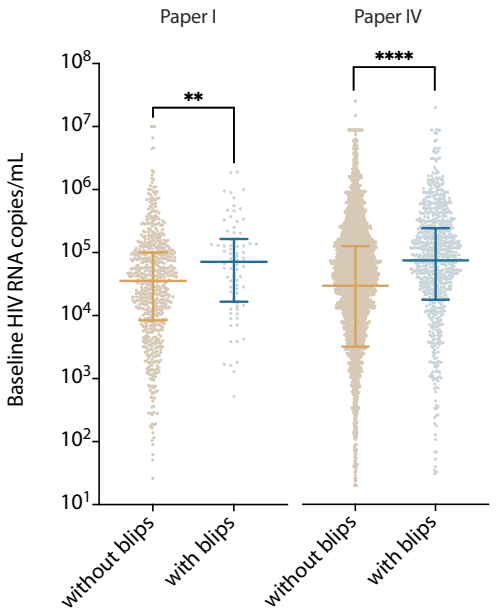


Figure 14. Scatter plots illustrating all included participants without and with viral blips in Paper I and IV. Horizontal bars indicate median and IQR HIV RNA baseline levels. Statistical differences between the groups were calculated using two-tailed Mann-Whitney U-test.

In both Paper I and IV, PI-based treatment at the time of blip occurrence was most common. Blip frequency was comparably lower in INSTI- and NNRTI-based ART (Figure 3, Paper IV). Secondary VF was defined differently in the two studies; in Paper I, VF could be registered six months earlier and at lower HIV RNA levels than in Paper IV. The association between viral blips and VF also differed, with an increased risk of subsequent failure in Paper I and the opposite result in Paper IV.

In both studies, participants with blips were followed for longer periods and had more available HIV RNA samples than participants with continuous viral suppression. In Paper I, HIV RNA samples in participants with blips were drawn with shorter intervals compared to other participants; median 178 days (IQR 144–206) and 217 days (IQR 176–285) respectively ($p < 0.001$). In Paper IV, the opposite occurred: median 150 days (IQR 130–176) between samples in participants with blips and 146 days (IQR 116–174) in participants without blips ($p < 0.05$).

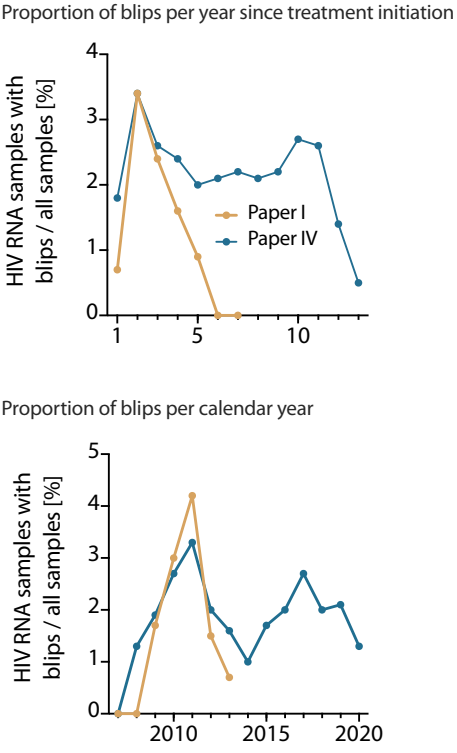


Figure 15. Proportion of HIV RNA samples that met the criteria for viral blips compared to the total number of HIV RNA samples at different years. In the upper figure, the x-axis represents years since treatment initiation. In the lower figure, the x-axis represents calendar year during which the samples were drawn. The number of sampled participants each year differs considerably. Orange lines represent the results from Paper I and the blue lines represent the results from Paper IV. The proportion of blips per year differed significantly between years when analyzed with ANOVA ($p < 0.0001$).

4.2 PAPER II

Through InfCareHIV, two groups with 122 participants each were identified (Table 4). All participants had 1–7 pre-treatment NRTI mutations with median (IQR) GSS 1.5 (1.5–2.0). The first group had DTG-based ART and the second, matched according to GSS and observation time, had boosted PI-based ART. All participants were ART experienced since no treatment-naïve participants with DTG-based ART who matched treatment inclusion were identified. However, all participants in the first group were naïve to DTG. Twenty-six (21.3%) had previous INSTI-experience with raltegravir. The median (IQR) observation time was 1.5 years (0.9–1.9) for participants treated with DTG and 1.4 years (1.0–1.9) for individuals treated with PI.

Table 4. Characteristics of participants in Paper II

	DTG-based ART	PI-based ART
№ of participants	122	122
Women/men [%]	39/61	32/68
Age [median (IQR)]	52 (46–59)	46 (40–51)
Weeks on ART [median (IQR)]	78 (50–98)	75 (50–101)
NRTI composition [%]		
abacavir/lamivudine	59	34
tenofovir/emtricitabine	34	50
other*	7	16
Nadir $CD4^+$ T-cell count [median (IQR)]	260 (171–340)	245 (153–299)
HIV RNA log_{10} copies/mL [median (IQR)]		
Before start of DTG/bPI	<1.3 (<1.3–1.8)	<1.3 (<1.3–2.4)
Last available	<1.3 (<1.3–1.3)	<1.3 (<1.3 – <1.3)

* lamivudine monotherapy, other dual NRTI-combinations or participants who switched NRTI during the study period

In participants with DTG-based treatment, a total of 260 mutations were registered. The majority (53.5%) were thymidine analogs mutations (TAMs), followed by non-TAMs (44.2%) and multidrug mutations (2.3%) (Figure 16).

Abacavir/lamivudine was the most common (59%) NRTI-backbone in participants with DTG. In the controls, tenofovir/emtricitabine was most commonly used (50%).

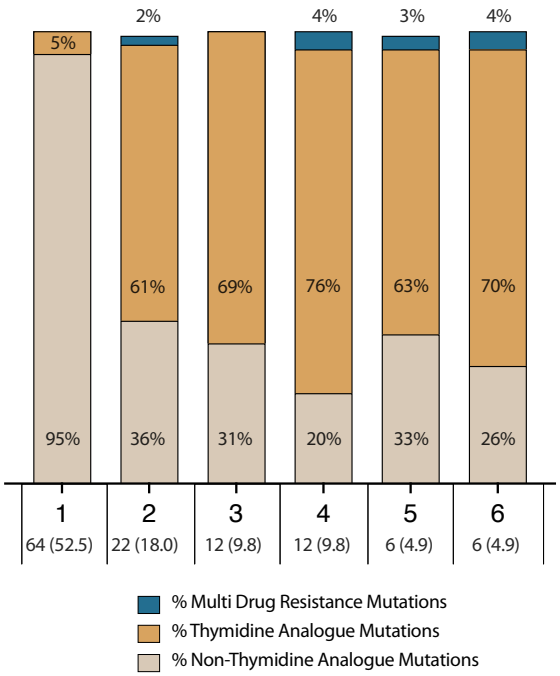


Figure 16. Summary of NRTI-mutations with respect to number and kind in participants with dolutegravir based ART. The x-axis represents the number of mutations and participants with that amount (%).

No participants developed primary VF, but seven cases of secondary VF were observed: four participants with DTG-based ART and three participants from the control group. Three out of four of those on DTG were re-tested without new NRTI- or INSTI resistance mutations. The median GSS for participants with VF was 1.8 for participants with DTG; all three with boosted PI had 1.5 (Figure 17).

In conclusion, participants with pre-treatment NRTI resistance mutations were effectively treated with both DTG and boosted PI-based ART. Post hoc analysis found DTG to be non-inferior to boosted PI with a non-inferiority margin of 6% ($p < 0.05$) (Figure 18.)

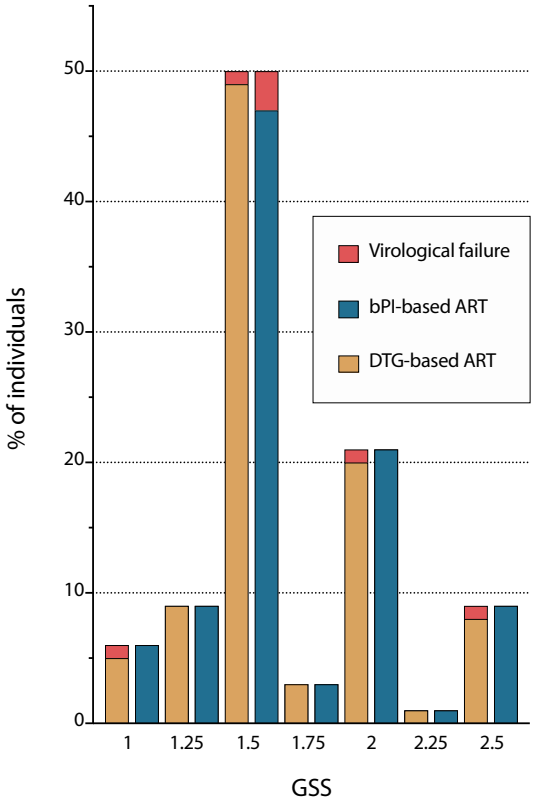


Figure 17. Proportion of virological failure (VF) with respect to Genotypic Susceptibility Score (GSS) for participants with DTG-based ART (orange) and boosted PI-based ART (bPI) (blue). Red areas of the bars indicate participants with secondary VF.

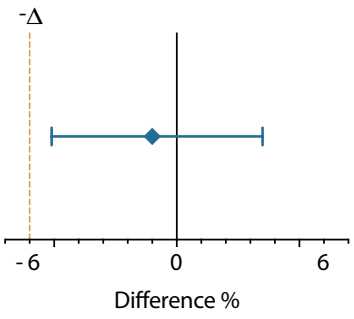


Figure 18. Non inferiority test between dolutegravir and boosted PIs with 90% confidence interval. The dotted line represents the non-inferiority margin (Δ) of 6%.

4.3 PAPER III

During the inclusion period that lasted almost 20 years, 2,956 participants that met the inclusion criteria were identified (Table 1, Paper III). The majority of excluded participants had previous treatment experience or had started ART with disallowed drug combinations.

The most important result was the strong correlation between high pre-treatment viral load and duration until the first HIV RNA < 50 copies/mL. Only 44% of participants with ≥ 500,000 HIV RNA copies/mL had reached the treatment goal within six months, and 20% were still not suppressed after the first year on ART. Although less distinctive, a similar association was seen between baseline viral load and time until first HIV RNA < 200 copies/mL. In total, 76% and 91% of participants with baseline HIV RNA > 500,000 copies/mL reached this threshold within six and twelve months, respectively (Figure 19).

Despite the need for a longer time to reach HIV RNA suppression, participants with baseline VL ≥ 100,000 HIV RNA copies/mL did not have an increased risk of secondary VF. The risk of primary VF failure was however correlated with higher baseline VL.

Treatment regimen differed over the study period, as new drugs were introduced and treatment guidelines updated. Participants with baseline HIV RNA < 100,000 copies/mL were more often started on NNRTI-based ART, while participants with higher baseline viral load were more commonly started with boosted PI-based ART. The time until viral suppression was shorter with INSTI-based ART than other core agents. Abacavir-based NRTI was associated with faster time until suppression, but only in participants with baseline VL<100,000 HIV RNA copies/mL (Figure 3, Paper III).

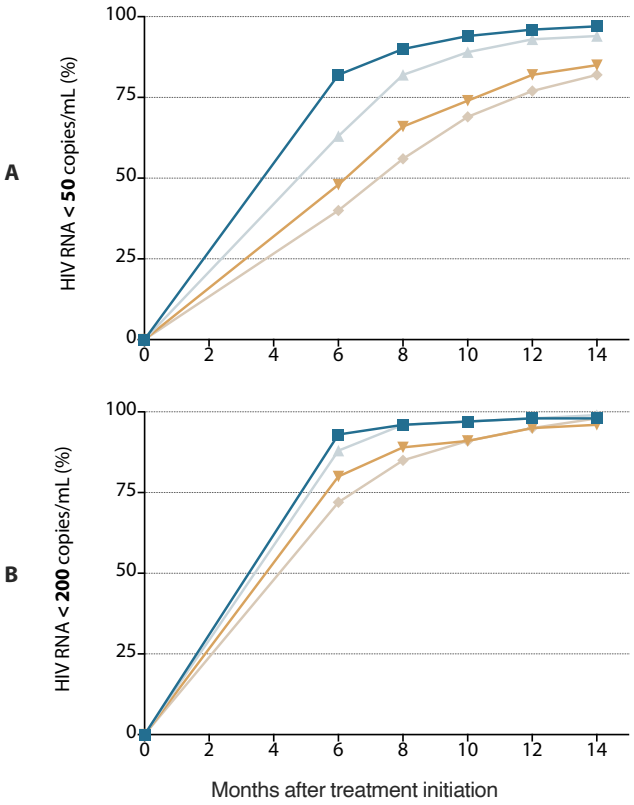


Figure 19. Proportion of participants with different baseline HIV-1 RNA levels that reached HIV RNA < 50 (A) and < 200 copies/mL (B) after ART initiation over time.

DISCUSSION

5. DISCUSSION

This thesis includes two of the largest studies conducted on viral blips in PLWH. Our hope was that these studies would therefore enable us to address the cause of this phenomenon. Unfortunately, in practice it did not turn out to be that simple. The correlation between blips and higher pre-treatment RNA-levels, combined with the finding that blips were less common in PLWH who started treatment early, i.e., participants registered as having primary HIV infection (n = 156 in Paper IV), were what we interpreted as the best clues. Similar associations have been presented in mathematical models and other observational studies (96, 108, 109, 122, 123) and support the hypothesis that blips are a consequence of intermittent activity in long-lived $CD4^+$ leukocytes. In theory, the latent viral reservoir is larger in participants with delayed treatment onset and higher baseline VL (71). In a recent study, Crowell et al. studied the link between reservoir size and blips through observations of blip-incidence in participants that started ART at different well-defined (Fiebig) stages. In line with our results, they found that blips were significantly more frequent if ART was started during chronic infection compared to ART started during earlier phases (108).

Integrated provirus in latent cells is not eradicated with current ART. Due to the lack of viral evolution in the released RNA-strands during blips, they are not believed to originate from replicating cells. However, some concern has been raised about how this was proven (102, 124). With ART, HIV RNA strands detected during blips are not believed to infect and replicate in new cells, and are likely harmless for the infected individual. In all probability, this is not the only blip origin. Subtherapeutic drug concentrations due to tolerability issues or other reasons for low adherence can be a contributing factor to the origin of transient viremia in some PLWH. A previous study, however, showed that even in patients with strict adherence, 25% experienced blips (125). Another important factor to consider is PCR assay variability. Consistencies between assays seem to be particularly difficult in the "blip zone," i.e., in proximity to the lower detection limit (118).

Both Paper I and IV showed that PI-based ART is more common than NNRTIs and INSTIs during blips. Given the inherent limitations of retrospective observational studies, no causality can be proven. Previous studies report inconsistent results concerning drug classes and viral blips. Some found blips to be more frequent in PI-based ART (126-128), others presented high blip-rates in NNRTI-based ART (107, 129, 130), and some studies showed that ART composition had little effect on blip frequency (103, 108, 122). There are multiple parameters to take into account when choosing an ART regimen. Patients expected to have lower adherence are more likely to be recommended ART with a higher genetic barrier for drug resistance (43). To this date, PI-based ART has been associated with the lowest risk of acquired drug resistance (48). In Paper III, we demonstrated that PI-based treatment is also more common in participants with baseline VL > 100,000 copies/mL. As the same group has a higher percentage of blips, this selection bias could explain parts of the association between blips and PIs. As a consequence of diverse study populations, the same bias could possibly explain the inconclusive results between drug classes and blips in previous studies.

Further, the different mechanisms of action between drug classes could be of importance to why blip incidence was higher in PIs. NNRTIs and INSTIs both block replication in the pre-integration phase, while PIs inhibit the protease activity at a later stage. Current PCR assays are not able to detect if the transient viremia that we define as blips originate from functional virus, capable of infecting new cells. Alternatively, particularly during PI-based ART, they could derive from replication-incompetent virus.

But why were PIs, at least until the introduction of DTG, more common in PLWH with high baseline VLs? Previous guidelines have recommended tenofovir-based NRTI backbones rather than abacavir to participants with baseline VL > 100k copies/mL as this was shown to decrease the risk of VF (131, 132). Due to an increased risk of renal impairment, tenofovir is not the drug of choice in combination with boosted PIs (133). Consequently, we would expect to see more participants with NNRTI-based treatment, as this does not affect the tenofovir concentration and the related effect on the kidneys. However, unlike many other countries, this recommendation was never

incorporated in the Swedish treatment guidelines and is therefore of limited importance. Another explanation is the possibility that higher baseline VL acts as a confounder for more advanced disease. In Paper III, higher baseline viremia was associated with lower baseline $CD4^+$ T-cell counts and the probability of AIDS-defining diagnoses in the medical history. Swedish treatment guidelines have, however, never favored boosted PIs even in this group. More likely, the higher genetic barriers of PIs have been translated to an overall perceived robustness that clinicians were inclined to use in patients where they felt extra caution was indicated. The therapeutic margins are inevitably smaller in participants with a more advanced stage of disease, and immediate treatment can be indicated, i.e., before the result from the resistance test has arrived. With the highest genetic barrier, this clearly favors PIs as empiric treatment. If the initial treatment is successful, it is then likely to be continued.

There are two major differences between the results from paper I and IV: the blip frequency and the association between participants with blips and secondary VF. Blip incidence per 100 person years increased from 3.0 in Paper I to 4.4 in Paper IV. The introduction of INSTI-based ART, which has become first-line treatment over time, was associated with a decreased risk of viral blips compared to PI-based ART. Hence, this would have the opposite effect on the blip incidence. During the first couple of years of inclusion in Paper IV, not all clinics had changed their test tubes to ethylene diamine tetraacetic acid test tubes. As a result, some blips registered during this period risk being incorrect (134). However, since less than 3.5% of all blips registered in the study came from this period, this alone cannot explain the increased incidence. Instead, the introduction of new and more sensitive PCR assays is probably the primary factor. Previous studies have found that shifting to more precise PCR assays has been associated with higher RNA levels close to the lower detection limit and consequently increased blip rates (111, 118, 127, 135). Our studies show a significant association between higher blip rates and specific calendar years. The years with most registered blips, 2011 and 2017, were about one year after PCR assays were changed to more sensitive methods.

The increased blip incidence could also be a consequence of the different observation periods. The median time until the first blip was 2.6 years in Paper IV. A larger portion of the included participants were possibly observed long enough to develop blips with a longer observation period. In line with this theory is the observation from both blip studies, that participants with detected blips were both observed for a longer period and consequently had more RNA samples compared to those with continuous viral suppression. It is possible that most studies, like ours, fail to detect many blips due to infrequent sampling. As blips are not related to any physical signs or symptoms, they may go unnoticed. In a study by Nettles et al., ten courageous participants underwent HIV RNA sampling twice a week for 3–4 months. Blips with short duration (< 3 days) were found to be very common and registered in 9 out of 10 participants (102). This implies that, similar to our results, the chance of detecting blips increases with the number of RNA samples available.

Primary VF is evaluated to examine the capacity of ART to achieve initial viral suppression. Secondary VF is instead assessed when the long-term suppressive ability of ART is evaluated. Thus, they serve different purposes but are not always used consistently between studies. Both phenomena are highly affected by drug susceptibility in relation to resistance mutations and drug adherence. In Paper I, participants with primary VF were excluded and secondary VF (in Paper I, simply referred to as VF) was defined as two consecutive HIV RNA samples > 50 copies/mL or a single sample > 500 copies/mL to be in line with existing studies. In Paper IV, the definition of secondary VF was changed to an event with a single sample > 1000 copies/mL or consecutive samples but with a higher cut off at > 200 copies/mL. The rationale was to resemble a clinical setting in which two samples of HIV RNA just above 50 copies/mL seldom raise questions about emergent treatment failure with subsequent treatment modifications. As a consequence, the registered cases of secondary VF are not directly comparable between these studies. Another important factor when comparing our results to others is that in both Paper I and IV participants were censored when they developed secondary VF. Consequently, these participants often had shorter observation periods and thus arguably, a decreased risk of viral blips. Similarly, to our

results, a recent study by Joya et al. also found blips to be protective in relation to VF (107). It is possible that they also censored participants after VF, but this is not clarified.

Viral blips are often defined differently between studies, which further complicates outcome comparisons. We chose a definition of blips that would decrease the risk that HIV RNA fluctuations during the initial treatment response were misinterpreted as blips, and that was in line with what the majority of existing studies were using when this project was started. Since then, there is still no consensus on which definition of blips to use. Some have stratified blips into different categories, often 50–199 and 200–499 copies/mL (106), while others include intermittent viremia from 20–1000 copies/mL (105, 108). Some studies show that the virological outcome is dependent on blip amplitude (127), while others claim the opposite (102, 106). For continuity reasons and to improve the ability to compare our results, we kept the same blip definition in both our studies.

The VL measured during blips in our studies were mostly < 200 HIV RNA copies/mL. In the other study that like us found blips to be protective in terms of VF, blips were per definition restricted to HIV RNA levels < 200 copies/mL (107). The same study also investigated transient episodes of higher viremia (200–1,000 copies/mL). Interestingly, this group had a higher risk of VF. It is possible that the amplitudes of the blips are related to different blip origins, e.g., that lower HIV RNA levels mostly derive from latent $CD4^+$ T cells, while higher levels to a larger extent are the consequence of suboptimal drug concentrations. As the latter theoretically is more likely to cause VF, the lower blip magnitudes found in our studies could be an additional factor to why secondary VF was less common in Paper IV. In line with this speculation, the previously mentioned study with blips during optimized adherence surveillance by Miller et al. defined blips as transient viremia between 40–1,000 copies. Despite this broad definition, the majority of blips registered were in the lower end of this spectrum (median 80 copies/mL, range 40–374) (125). Further studies are needed to determine the need for a stratified approach when assessing the origin and importance of blips. In light of the close association between blips and temporary detectable viremia between

20–50 HIV RNA copies/mL described in Paper IV, future studies are recommended to not refer to these findings as separate entities.

The possible consequences of very high baseline VL were addressed in Paper III. The most important finding was that the treatment outcome during the study period was not affected by the level of initial viremia. Although the time to reach viral suppression took considerably longer with higher initial VL, 99.5% of all participants ultimately reached the treatment goal of HIV RNA < 50 copies/mL. The observed delayed treatment response is in accordance with previous studies (136-140).

For optimal clarity, primary and secondary VF were clearly separated in this study, i.e., participants with primary VF in our study could not also be registered with secondary VF. We showed that participants with higher baseline viremia had an increased risk of primary VF. This was an anticipated consequence of the definition, as HIV RNA had to reach suppressive levels of < 50 copies/mL within a year from treatment initiation. After one year, 96% of participants with baseline HIV RNA <100k copies/mL had reached the treatment goal. In contrast, 23% of participants with baseline VL > 1,000k copies/mL were still not suppressed after the same time. Given our present knowledge, it is not apparent that the slower treatment response should be labeled as failure. This illustrates the importance of finding suitable definitions that make comparisons between studies possible. This is, however, not always easy. This thesis is based on four studies in which VFs are managed in four different ways. In Paper II, a single sample of HIV RNA > 200 copies/mL was defined as VF. For consistency and differentiation between developing blips and VF, it would have been better with consecutive samples > 200 copies/mL. When later reviewing the participants with VF from this study, six out of seven with defined VF had multiple samples > 200 copies/mL. The participant with a single increased HIV RNA has later continued to have similar episodes with a pattern usually seen during suboptimal drug adherence. Thus, these participants likely represent real VF.

In Paper III, we demonstrated that higher baseline HIV RNA levels were not associated with an increased risk of secondary VF. We could not predict the long-term risk, but many of the included participants were observed for more than a decade. However, opposite results were

recently published in a retrospective study by Chen et al. (140). From 758 treatment-naïve participants, 48 with baseline VL ≥ 500k HIV RNA copies/mL were identified. VF was significantly associated with baseline VL ≥ 500k HIV RNA copies/mL. Although their results originated from a substantially smaller study population, shorter observation periods and ART limited to combinations of NRTIs and NNRTI, the major difference from our study was the definition of VF. Aside from viral rebound ≥ 50 copies/mL, they chose to categorize participants that had not reached < 200 HIV RNA copies/mL after 24 weeks as VF. In our study, 99.9% of all participants eventually reached < 200 copies/mL, however, at week 24, 25% of the participants with baseline VL > 500k HIV RNA copies/mL still had HIV RNA ≥ 200 copies/mL. Our study shows that this subgroup did not have treatment failure but rather experienced a much slower viral decay due to the high initial baseline viremia, but due to the difference in study populations and ART composition, the results from these two studies are difficult to compare. Despite this, we believe that it is important that other groups take the substantially longer treatment response described in Paper III into account when interpreting the results of future studies so that VF is not declared prematurely.

Substantially higher baseline VL was not restricted to a particular part of the study period. Time until treatment initiation in participants with baseline VL > 500k HIV RNA copies/mL was also consistent, but substantially shorter in comparison to those with lower levels of viremia. This is most likely a consequence of the correlation between higher baseline HIV RNA and lower $CD4^+$ T cell count, as immediate treatment was recommended for this group even before the implications of the START study (44). It is unknown why some PLWH experience substantially higher viremia, but the finding from Paper III that ART is equally effective regardless of baseline HIV RNA is fortunate. We further learned that INSTIs provided the fastest viral decline. The clinical impact of this besides a shorter period of infectiousness is, however, uncertain. After DTG, NNRTIs provided the second fastest viral suppression, which is in accordance with previous studies (141).

In Paper II, we investigated if DTG was an effective alternative for participants with pre-treatment NRTI resistance. Previously, the first generation INSTI raltegravir had been found to be inferior to boosted PIs in PLWH with previous treatment failure (142). The genetic barrier for drug resistance for DTG was significantly improved compared to raltegravir but had not been evaluated outside of clinical studies (143, 144). Our results showed that VF was uncommon in participants treated with DTG and in the matched controls treated with boosted PI. There were also no new mutations in the few participants with VF, further strengthening the robustness of the treatment. We used GSS as a model to predict drug susceptibility with an algorithm from the Stanford University HIV Drug Resistance Database. Previous studies have demonstrated that available drug resistance data renders a better treatment outcome (145). With multiple resistance mutations that can have both synergistic or antagonistic effects, an algorithm to help guide the clinician is essential (146, 147). There are multiple GSS algorithms available, both unweighted like the one we used and weighted. In the latter, the calculated GSS can for example be multiplied with 1.5–2 for PIs and 0.5 for NRTIs to compensate for different genetic barriers (148). To the best of my knowledge, there is no set factor for weighting different INSTIs. More recent studies have, similar to our results, found DTG-based ART to be robust even with GSS < 1.5 (149). However, in both studies, participants with GSS scores in effect close to DTG monotherapy were few. In clinical practice, many of these patients would likely be treated with dual core agents and would thus not be included in our study.

The use of GSS as a predictor of virologic success is still debated due to conflicting results in existing studies (150-152). In a comprehensive study, Rhee et al. found the combined GSS score for each ART to be the strongest predictor of virologic response (148). An alternative approach to investigate this population's treatment outcome would be to perform a retrospective non-inferiority study, with boosted PIs as an active comparator. In a post hoc analysis reported in the results section of this thesis, DTG would have met the non-inferior criteria with a *p*-value of < 0.05 and a non-inferiority margin of 6%. The effect of DTG would hence be comparable to boosted PIs.

Paper II was analyzed with an intention to treat approach with a binary outcome. Similar results, although without reported degrees of susceptibility, have recently been reproduced (153). To our knowledge, Paper II was the first real-life study with DTG-based ART in PLWH with previous NRTI-resistance. The advantage of this kind of study is that it makes it possible to study the outcome in a more heterogeneous population than those in clinical trials. The downside is the non-randomized design with a risk of selection bias and that the frequency of blood sampling and resistance tests are not consistent. However, for a limited duration of time and in an early INSTI-era, we concluded that DTG constitutes a safe and, in terms of tolerability, promising alternative to boosted PIs for this subpopulation. As DTG has become part of the first line treatment in many treatment guidelines (43, 154-156), the prospect for further studies in this group with longer durations and in treatment-naïve populations is promising.

The studies in this thesis are based on data collected for many years, which increases the scientific reliability of the results. During the study periods, both treatment recommendations and PCR assays have changed, which complicates the interpretation of the results. By the use of the unique InfCareHIV database, a large part of PLWH in Sweden have been included. However, the study populations included inevitably overlap as we wanted to include many participants and the fact that Sweden has a low HIV prevalence (8,157 receiving care in December 2020). For example, it was expected that the majority of participants from Paper I were also included in the follow-up study presented in Paper IV as we used partially overlapping study periods and similar inclusion criteria. However, we believe that a considerably extended study period and focus on INSTI-based ART justifies their re-inclusion. In clinical trials, the use of highly selected subpopulations lower external validity and jeopardizes the scientist's ability to generalize the study results. Overlapping study populations face the same problem, but the studies in this thesis are based on real-life data with generous inclusion criteria and, in comparison to most other studies with similar approaches, based on substantially larger populations which enables robustness.

CONCLUSIONS

6. CONCLUSIONS

- Viral blips are significantly correlated with higher baseline viral loads and are more common in PI-based ART. In relation to other populations, PLWH in Sweden have a comparable low blip incidence.

- Individuals with INSTI-based ART, similar to NNRTIs, have fewer blips as compared to those on PI-based ART. Blips are not associated with an increased risk of secondary virological failure.

- Dolutegravir is equally effective as PIs in PLWH with baseline NRTI-resistance mutations.

- Individuals with baseline HIV RNA > 500,000 copies/mL have similar treatment results as PLWH with lower initial viral loads but require a longer time to reach viral suppression.

ACKNOW-LEDGEMENTS

ACKNOWLEDGEMENTS

Mitt första tack går till alla patienter runt om i Sverige som har gjort denna avhandling möjlig. Tack vara er medverkan har vi kunnat dra nya slutsatser om behandlingsval och vilka faktorer vi behöver vara särskilt observanta på. Jag ser fram emot att sprida kunskapen om detta och att använda den i praktiken på kliniken.

Jag vill även rikta ett varmt tack till följande personer som har varit viktiga för denna avhandling:

Min huvudhandledare **Aylin Yilmaz**. För knappt tio år sedan frågade du om jag var intresserad av blips, något som jag då aldrig ens hade hört talas om. Föga anade jag då hur mycket tid jag skulle lägga på dem. Tack för all stöttning och kunskap som du har delat med dig av under den här tiden. Du har uppmuntrat mig, gett mig frihet att utvecklas och alltid tagit dig tid att svara på alla möjliga och omöjliga frågor. Din förmåga att i en text enkelt uttrycka essensen har särskilt imponerat på mig.

Min bihandledare **Magnus Gisslén**. Din bottenlösa ämneskunskap och din förmåga att hålla många bollar i luften samtidigt som du inte tappar fokus på de viktiga detaljerna är verkligen inspirerande. Jag kommer också att minnas de många gångerna då jag trodde att jag snart var färdig med något när du hittade en lucka i argumentationen eller föreslog en alternativ angreppsvinkel. Trots merarbetet har dock slutresultaten alltid blivit avsevärt mycket bättre.

Staffan Nilsson som med glimten i ögat väglett mig när jag vid upprepade tillfällen gått vilse i den statistiska snårskogen. Ditt klarspråk har varit en källa till munterhet och jag är både förundrad och tacksam över hur länge du har orkat med mina outsinliga blipfunderingar. Det skall bli skönt att ses i sommarstugorna utan att prata jobb framöver.

Övriga medförfattare, **Anders Sönnerborg**, **Leo Flamholc**, **Veronica Svedhem Johansson**, **Anders Blaxhult**, **Christina Carlander**, **Bo Hejdeman**, **Carl-Johan Treutiger**, **Piotr Nowak**, **Fredrik Månsson**, **Lena Änghagen** och **Helena Änghagen** som alla har bidragit med

klokskap och givit värdefull feedback på mina manus. Ett särskilt tack till **Anders**, **Veronica** och **Emmi Andersson** som har hjälpt mig och delat med sig av ett Stockholmsperspektiv till denna avhandlingen.

Anders Ternhag, **Lilian Van Leest** m.fl. på Folkhälsomyndigheten som har lotsat mig rätt, försett mig med bakgrundsfakta och hjälpt mig att reda ut viktiga pusselbitar till avhandlingen ur ett epidemiologiskt perspektiv.

Till all personal på de virologiska laboratorierna runt om i Sverige som outtröttligt förser oss kliniker med livsviktiga data för att kunna hjälpa alla patienter på bästa sätt. Ett särskilt tack till **Bo Svennerholm**, **Ulrika Noborg, Mona Brantefjord** och **Kristina Nyström** på Klinisk mikrobiologi i Göteborg för att ni har tagit er tid att svara på alla mina frågor.

Till **Lars Hagberg** och **Johan Westin**, för att ni upprätthåller och uppmuntrar den höga forskningsaktiviteten som universitetsrepresentanter på infektionskliniken i Göteborg.

Ulrika Snygg-Martin och **Rune Wejstål,** som trodde på min förmåga och anställde mig på infektionskliniken för ca tio år sedan. Och tack till verksamhetschef **Lars-Magnus Andersson** som eftersträvar en hög lägsta-nivå och som har gett mig fortsatt förtroende. Ett varmt tack även till **Marie Studahl**, min mycket uppskattade kliniska handledare under min tid som ST-läkare.

Till alla goa och duktiga arbetskamrater på Sahlgrenskas infektionsklinik. Tack för att ni trots en härjande pandemi gjort det möjligt för mig att komma i mål med detta projektet. Det skall bli väldigt kul att ses snart och få arbeta ihop igen.

Till de fantastiska sköterskorna **Lissie Johansson**, **Mia Mickelsson**, **Marie Börjesson**, **Helena Gisslén** och **Kristina Arrnäs** på Sahlgrenskas HIV-mottagning. Er erfarenhet är guld värd och den har hjälpt mig många gånger.

Till **Teddy Primack** för högt uppskattad korrekturläsning av mina manus. Och ett lika stort tack till **Susanna Leach** för samma insats till

min avhandling. Tack även till **Anna Hansson** som varit bollplank för avhandlingens grafiska uttryck.

Till min första familj, mamma **Kerstin** och pappa **Berthil**. Tack för all kärlek och för att ni planterat en mentalitet om att allt är möjligt om man bara kämpar! Denna gången blev det en doktorsavhandling. Och mina systrar, **Elin** och **Emelie**, tack för all viktig pepp under resans gång! Ett stort tack även till min utökade familj med **Alva**, **Lasse** och **Maj**, **Maja** och **Albert**, **Johan** och **Johanna**, **Klara** och **Ida**.

Till alla fina vänner och släktingar. Tack för all stöttning och för att ni stått ut med blipsnack under snart 10 år.

To **Tabitha Atieno Festo, Rye Barcott**, and **Carolina for Kibera**. Thank you for your dedication, hard- and inspiring work. You all helped me to become a better doctor.

Till **Nils**. Nu är pappas bok äntligen klar så att vi får mer tid till viktigare saker. Tack för all glädje du skänker mitt liv!

Och slutligen till **Anna**. Tack för din kärlek, klokskap och stöttning. Du lyfter mig och jag är så oerhört tacksam för all din hjälp genom denna processen. Du har också varit en måttstock på hur en riktig forskare jobbar och har fått mig att kämpa hårdare när det har behövts.

Arbetet i denna avhandling har genomförts tack vara ekonomiskt stöd från:

ALF-medel från Sahlgrenska Akademin vid Göteborgs Universitet och Västra Götalandsregionen

Göteborgs läkaresällskaps stipendier

Stiftelsen Läkare mot AIDS Forskningsfond

REFERENCES

REFERENCES

1. Marx JL. New disease baffles medical community. Science. 1982;217(4560):618-21.
2. Worobey M, Watts TD, McKay RA, Suchard MA, Granade T, Teuwen DE, et al. 1970s and 'Patient 0' HIV-1 genomes illuminate early HIV/AIDS history in North America. Nature. 2016;539(7627):98-101.
3. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220(4599):868-71.
4. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science. 1984;224(4648):500-3.
5. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science. 1984;224(4648):497-500.
6. Coffin J, Haase A, Levy JA, Montagnier L, Oroszlan S, Teich N, et al. What to call the AIDS virus? Nature. 1986;321(6065):10.
7. UNAIDS. Global HIV & AIDS statisstics – 2020 fact sheet 2020 [Available from: https://www.unaids.org/en/resources/fact-sheet.
8. Bavinton BR, Pinto AN, Phanuphak N, Grinsztejn B, Prestage GP, Zablotska-Manos IB, et al. Viral suppression and HIV transmission in serodiscordant male couples: an international, prospective, observational, cohort study. Lancet HIV. 2018;5(8):e438-e47.
9. Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, van Lunzen J, et al. Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. Jama. 2016;316(2):171-81.
10. Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, Degen O, et al. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. Lancet. 2019;393(10189):2428-38.
11. Von Krogh G, Broström C, Hermanson J, Von Sydow M, Biberfeld G, Sandström E, et al. The introduction of HIV during 1979-80 in a sexually active homosexual population of

Stockholm. Scandinavian journal of infectious diseases. 1987;19(3):285-8.
12. Pehrson P, Lidman K, Bergdahl S, Morfeldt-Månsson L, Blaxhult A, Brostrm C, et al. [AIDS in homosexual men--the 1st cases in Sweden]. Lakartidningen. 1983;80(7):545-8.
13. Gisslen M, Svedhem V, Lindborg L, Flamholc L, Norrgren H, Wendahl S, et al. Sweden, the first country to achieve the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) 90-90-90 continuum of HIV care targets. HIV Med. 2016.
14. WHO. 90–90–90. An ambitious treatment target to help end the AIDS epidemic 2014 [Available from: https://www.unaids.org/sites/default/files/media_asset/90-90-90_en.pdf.
15. Neogi U, Häggblom A, Santacatterina M, Bratt G, Gisslén M, Albert J, et al. Temporal trends in the Swedish HIV-1 epidemic: increase in non-B subtypes and recombinant forms over three decades. PLoS One. 2014;9(6):e99390.
16. Folkhälsomyndigheten. Årsrapport 2019 2019 [
17. Chiu IM, Yaniv A, Dahlberg JE, Gazit A, Skuntz SF, Tronick SR, et al. Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. Nature. 1985;317(6035):366-8.
18. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature. 1999;397(6718):436-41.
19. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1(1):a006841.
20. Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: scientific and public health implications. Science. 2000;287(5453):607-14.
21. Clavel F, Guétard D, Brun-Vézinet F, Chamaret S, Rey MA, Santos-Ferreira MO, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science. 1986;233(4761):343-6.
22. McCutchan FE. Global epidemiology of HIV. J Med Virol. 2006;78 Suppl 1:S7-s12.
23. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med. 2009;15(8):871-2.
24. Tebit DM, Arts EJ. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. Lancet Infect Dis. 2011;11(1):45-56.
25. Hemelaar J, Elangovan R, Yun J, Dickson-Tetteh L, Fleminger I, Kirtley S, et al. Global and regional molecular epidemiology of

HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. Lancet Infect Dis. 2019;19(2):143-55.
26. Strebel K. Virus-host interactions: role of HIV proteins Vif, Tat, and Rev. Aids. 2003;17 Suppl 4:S25-34.
27. Murray PR, Baron EJ. Manual of Clinical Microbiology. Washington, D.C.: ASM Press; 2003.
28. Chan DC, Kim PS. HIV entry and its inhibition. Cell. 1998;93(5):681-4.
29. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science. 1998;280(5371):1884-8.
30. Doitsh G, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature. 2014;505(7484):509-14.
31. Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. Nat Rev Immunol. 2008;8(6):447-57.
32. Lackner AA, Lederman MM, Rodriguez B. HIV pathogenesis: the host. Cold Spring Harb Perspect Med. 2012;2(9):a007005.
33. Alberts B. Molecular biology of the cell. New York: Garland Science; 2002.
34. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of virology. 1994;68(7):4650-5.
35. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373(6510):123-6.
36. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993;362(6418):355-8.
37. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. The New England journal of medicine. 1997;337(11):734-9.
38. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. The New England journal of medicine. 1997;337(11):725-33.

39. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. The New England journal of medicine. 1996;334(16):1011-7.
40. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science. 1997;278(5341):1295-300.
41. Wong JK, Hezareh M, Günthard HF, Havlir DV, Ignacio CC, Spina CA, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science. 1997;278(5341):1291-5.
42. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(24):13193-7.
43. Referensgruppen för Antiviral Terapi (RAV). Antiretroviral treatment for HIV infection: Swedish recommendations 2019: The Swedish Society of Medicine; 2019. Available from: https://www.sls.se/globalassets/rav/rekommendationer/rav_hiv_2019_190216.pdf.
44. Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, Sharma S, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. The New England journal of medicine. 2015;373(9):795-807.
45. Roberts JD, Bebenek K, Kunkel TA. The accuracy of reverse transcriptase from HIV-1. Science. 1988;242(4882):1171-3.
46. Preston BD, Poiesz BJ, Loeb LA. Fidelity of HIV-1 reverse transcriptase. Science. 1988;242(4882):1168-71.
47. Zanini F, Puller V, Brodin J, Albert J, Neher RA. In vivo mutation rates and the landscape of fitness costs of HIV-1. Virus Evol. 2017;3(1):vex003.
48. Gupta R, Hill A, Sawyer AW, Pillay D. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. Clin Infect Dis. 2008;47(5):712-22.
49. Boucher CA, Bobkova MR, Geretti AM, Hung CC, Kaiser R, Marcelin AG, et al. State of the Art in HIV Drug Resistance: Science and Technology Knowledge Gap. AIDS reviews. 2018;20(1):27-42.
50. Tang MW, Shafer RW. HIV-1 antiretroviral resistance: scientific principles and clinical applications. Drugs. 2012;72(9):e1-25.

51. Das K, Arnold E. HIV-1 reverse transcriptase and antiviral drug resistance. Part 2. Curr Opin Virol. 2013;3(2):119-28.
52. Andersson E, Nordquist A, Esbjörnsson J, Flamholc L, Gisslén M, Hejdeman B, et al. Increase in transmitted drug resistance in migrants from sub-Saharan Africa diagnosed with HIV-1 in Sweden. Aids. 2018;32(7):877-84.
53. Katlama C, Soulie C, Caby F, Denis A, Blanc C, Schneider L, et al. Dolutegravir as monotherapy in HIV-1-infected individuals with suppressed HIV viraemia. The Journal of antimicrobial chemotherapy. 2016.
54. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, Polis M, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol. 2003;41(10):4531-6.
55. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L, Jr., Ingerman MJ, et al. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. Jama. 1999;282(17):1627-32.
56. Riddler SA, Zheng L, Durand CM, Ritz J, Koup RA, Ledgerwood J, et al. Randomized Clinical Trial to Assess the Impact of the Broadly Neutralizing HIV-1 Monoclonal Antibody VRC01 on HIV-1 Persistence in Individuals on Effective ART. Open Forum Infect Dis. 2018;5(10):ofy242.
57. Zheng L, Bosch RJ, Chan ES, Read S, Kearney M, Margolis DM, et al. Predictors of residual viraemia in patients on long-term suppressive antiretroviral therapy. Antivir Ther. 2013;18(1):39-43.
58. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, Mican J, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog. 2007;3(4):e46.
59. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science. 1996;271(5255):1582-6.
60. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med. 2003;9(6):727-8.
61. Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanen M, Lewin S, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. The New England journal of medicine. 1999;340(21):1605-13.
62. McCune JM. Viral latency in HIV disease. Cell. 1995;82(2):183-8.
63. Pierson TC, Zhou Y, Kieffer TL, Ruff CT, Buck C, Siliciano RF. Molecular characterization of preintegration latency in human

immunodeficiency virus type 1 infection. Journal of virology. 2002;76(17):8518-31.
64. Siliciano JD, Siliciano RF. A long-term latent reservoir for HIV-1: discovery and clinical implications. The Journal of antimicrobial chemotherapy. 2004;54(1):6-9.
65. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell. 1990;61(2):213-22.
66. Dahl V, Josefsson L, Palmer S. HIV reservoirs, latency, and reactivation: prospects for eradication. Antiviral Res. 2010;85(1):286-94.
67. Chun TW, Justement JS, Lempicki RA, Yang J, Dennis G, Jr., Hallahan CW, et al. Gene expression and viral prodution in latently infected, resting CD4+ T cells in viremic versus aviremic HIV-infected individuals. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(4):1908-13.
68. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med. 1999;5(5):512-7.
69. Lindkvist A, Edén A, Norström MM, Gonzalez VD, Nilsson S, Svennerholm B, et al. Reduction of the HIV-1 reservoir in resting CD4+ T-lymphocytes by high dosage intravenous immunoglobulin treatment: a proof-of-concept study. AIDS research and therapy. 2009;6:15.
70. Mellberg T, Gonzalez VD, Lindkvist A, Edén A, Sönnerborg A, Sandberg JK, et al. Rebound of residual plasma viremia after initial decrease following addition of intravenous immunoglobulin to effective antiretroviral treatment of HIV. AIDS research and therapy. 2011;8:21.
71. Josefsson L, von Stockenstrom S, Faria NR, Sinclair E, Bacchetti P, Killian M, et al. The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(51):E4987-96.
72. Strain MC, Little SJ, Daar ES, Havlir DV, Gunthard HF, Lam RY, et al. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. The Journal of infectious diseases. 2005;191(9):1410-8.
73. Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattoni D, Ferrante P, et al. Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency

virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. Journal of virology. 2003;77(20):11212-9.
74. Piketty C, Weiss L, Assoumou L, Burgard M, Mélard A, Ragnaud JM, et al. A high HIV DNA level in PBMCs at antiretroviral treatment interruption predicts a shorter time to treatment resumption, independently of the CD4 nadir. J Med Virol. 2010;82(11):1819-28.
75. Fourati S, Lambert-Niclot S, Soulie C, Malet I, Valantin MA, Descours B, et al. HIV-1 genome is often defective in PBMCs and rectal tissues after long-term HAART as a result of APOBEC3 editing and correlates with the size of reservoirs. The Journal of antimicrobial chemotherapy. 2012;67(10):2323-6.
76. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DI, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell. 2013;155(3):540-51.
77. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. Nat Med. 2016;22(9):1043-9.
78. Dahl V, Peterson J, Fuchs D, Gisslen M, Palmer S, Price RW. Low levels of HIV-1 RNA detected in the cerebrospinal fluid after up to 10 years of suppressive therapy are associated with local immune activation. Aids. 2014;28(15):2251-8.
79. Spudich S, Robertson KR, Bosch RJ, Gandhi RT, Cyktor JC, Mar H, et al. Persistent HIV-infected cells in cerebrospinal fluid are associated with poorer neurocognitive performance. The Journal of clinical investigation. 2019;129(8):3339-46.
80. Lampinen TM, Critchlow CW, Kuypers JM, Hurt CS, Nelson PJ, Hawes SE, et al. Association of antiretroviral therapy with detection of HIV-1 RNA and DNA in the anorectal mucosa of homosexual men. Aids. 2000;14(5):F69-75.
81. Anton PA, Mitsuyasu RT, Deeks SG, Scadden DT, Wagner B, Huang C, et al. Multiple measures of HIV burden in blood and tissue are correlated with each other but not with clinical parameters in aviremic subjects. Aids. 2003;17(1):53-63.
82. Belmonte L, Olmos M, Fanin A, Parodi C, Baré P, Concetti H, et al. The intestinal mucosa as a reservoir of HIV-1 infection after successful HAART. Aids. 2007;21(15):2106-8.
83. Perreau M, Savoye AL, De Crignis E, Corpataux JM, Cubas R, Haddad EK, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med. 2013;210(1):143-56.

84. Couturier J, Suliburk JW, Brown JM, Luke DJ, Agarwal N, Yu X, et al. Human adipose tissue as a reservoir for memory CD4+ T cells and HIV. Aids. 2015;29(6):667-74.
85. Damouche A, Lazure T, Avettand-Fènoël V, Huot N, Dejucq-Rainsford N, Satie AP, et al. Adipose Tissue Is a Neglected Viral Reservoir and an Inflammatory Site during Chronic HIV and SIV Infection. PLoS Pathog. 2015;11(9):e1005153.
86. Ganor Y, Real F, Sennepin A, Dutertre CA, Prevedel L, Xu L, et al. HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. Nat Microbiol. 2019;4(4):633-44.
87. Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(23):9403-8.
88. Gandhi RT, Bosch RJ, Aga E, Albrecht M, Demeter LM, Dykes C, et al. No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. The Journal of infectious diseases. 2010;201(2):293-6.
89. Archin NM, Cheema M, Parker D, Wiegand A, Bosch RJ, Coffin JM, et al. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS One. 2010;5(2):e9390.
90. Günthard HF, Frost SD, Leigh-Brown AJ, Ignacio CC, Kee K, Perelson AS, et al. Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. Journal of virology. 1999;73(11):9404-12.
91. Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. Jama. 2005;293(7):817-29.
92. Bailey JR, Sedaghat AR, Kieffer T, Brennan T, Lee PK, Wind-Rotolo M, et al. Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T cells. Journal of virology. 2006;80(13):6441-57.
93. Persaud D, Siberry GK, Ahonkhai A, Kajdas J, Monie D, Hutton N, et al. Continued production of drug-sensitive human immunodeficiency virus type 1 in children on combination antiretroviral therapy who have undetectable viral loads. Journal of virology. 2004;78(2):968-79.
94. Günthard HF, Wong JK, Ignacio CC, Guatelli JC, Riggs NL, Havlir DV, et al. Human immunodeficiency virus replication

and genotypic resistance in blood and lymph nodes after a year of potent antiretroviral therapy. Journal of virology. 1998;72(3):2422-8.
95. Ramratnam B, Mittler JE, Zhang L, Boden D, Hurley A, Fang F, et al. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. Nat Med. 2000;6(1):82-5.
96. Havlir DV, Bassett R, Levitan D, Gilbert P, Tebas P, Collier AC, et al. Prevalence and predictive value of intermittent viremia with combination hiv therapy. Jama. 2001;286(2):171-9.
97. Canestri A, Lescure FX, Jaureguiberry S, Moulignier A, Amiel C, Marcelin AG, et al. Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. Clin Infect Dis. 2010;50(5):773-8.
98. Edén A, Nilsson S, Hagberg L, Fuchs D, Zetterberg H, Svennerholm B, et al. Asymptomatic Cerebrospinal Fluid HIV-1 Viral Blips and Viral Escape During Antiretroviral Therapy: A Longitudinal Study. The Journal of infectious diseases. 2016;214(12):1822-5.
99. Lodding IP, Mocroft A, da Cunha Bang C, Gustafsson F, Iversen M, Kirkby N, et al. Impact of CMV PCR Blips in Recipients of Solid Organ and Hematopoietic Stem Cell Transplantation. Transplant Direct. 2018;4(6):e355.
100. Stornaiuolo G, Stanzione M, Brancaccio G, Cuomo G, Precone V, Di Biase S, et al. Viral blips during long-term treatment with standard or double dose lamivudine in HBe antigen negative chronic hepatitis B. World J Gastroenterol. 2007;13(42):5642-7.
101. Guedj J, Dahari H, Perelson AS. Understanding the nature of early HCV RNA blips and the use of mathematical modeling of viral kinetics during IFN-based therapy. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(29):E302; author reply E3.
102. Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. Jama. 2005;293(7):817-29.
103. Sungkanuparph S, Overton ET, Seyfried W, Groger RK, Fraser VJ, Powderly WG. Intermittent episodes of detectable HIV viremia in patients receiving nonnucleoside reverse-transcriptase inhibitor-based or protease inhibitor-based highly active antiretroviral therapy regimens are equivalent in incidence and prognosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(9):1326-32.

104. Wojewoda CM, Spahlinger T, Harmon ML, Schnellinger B, Li Q, Dejelo C, et al. Comparison of Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 (CAP/CTM v2.0) with other real-time PCR assays in HIV-1 monitoring and follow-up of low-level viral loads. Journal of virological methods. 2013;187(1):1-5.
105. Farmer A, Wang X, Ganesan A, Deiss RG, Agan BK, O'Bryan TA, et al. Factors associated with HIV viral load "blips" and the relationship between self-reported adherence and efavirenz blood levels on blip occurrence: a case-control study. AIDS research and therapy. 2016;13:16.
106. Fleming J, Mathews WC, Rutstein RM, Aberg J, Somboonwit C, Cheever LW, et al. Low-level viremia and virologic failure in persons with HIV infection treated with antiretroviral therapy. Aids. 2019;33(13):2005-12.
107. Joya C, Won SH, Schofield C, Lalani T, Maves RC, Kronmann K, et al. Persistent Low-level Viremia While on Antiretroviral Therapy Is an Independent Risk Factor for Virologic Failure. Clin Infect Dis. 2019;69(12):2145-52.
108. Crowell TA, Pinyakorn S, Sacdalan C, Kroon E, Colby DJ, Puttamaswin S, et al. Viral Blips After Treatment Initiation During Acute Human Immunodeficiency Virus Infection. Clin Infect Dis. 2020;70(12):2706-9.
109. Wang S, Rong L. Stochastic population switch may explain the latent reservoir stability and intermittent viral blips in HIV patients on suppressive therapy. Journal of theoretical biology. 2014;360:137-48.
110. Rong L, Perelson AS. Modeling latently infected cell activation: viral and latent reservoir persistence, and viral blips in HIV-infected patients on potent therapy. PLoS Comput Biol. 2009;5(10):e1000533.
111. Taylor N, Grabmeier-Pfistershammer K, Egle A, Greil R, Rieger A, Ledergerber B, et al. Cobas ampliprep/cobas TaqMan HIV-1 v2.0 assay: consequences at the cohort level. PLoS One. 2013;8(8):e74024.
112. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ. Decreased adherence to antiretroviral therapy observed prior to transient human immunodeficiency virus type 1 viremia. The Journal of infectious diseases. 2007;196(12):1773-8.
113. Cohen Stuart JW, Wensing AM, Kovacs C, Righart M, de Jong D, Kaye S, et al. Transient relapses ("blips") of plasma HIV RNA levels during HAART are associated with drug resistance. J Acquir Immune Defic Syndr. 2001;28(2):105-13.
114. Macias J, Palomares JC, Mira JA, Torres MJ, García-García JA, Rodríquez JM, et al. Transient rebounds of HIV plasma viremia

are associated with the emergence of drug resistance mutations in patients on highly active antiretroviral therapy. J Infect. 2005;51(3):195-200.
115. Stanford HIVdb. HIVdb Program. Genotypic Resistance Interpretation Algorithm Version 7.0 2014 [cited 2016. Available from: http://sierra2.stanford.edu/sierra/servlet/JSierra.
116. Marrone G, Mellgren Å, Eriksson LE, Svedhem V. High Concordance between Self-Reported Adherence, Treatment Outcome and Satisfaction with Care Using a Nine-Item Health Questionnaire in InfCareHIV. PLOS ONE. 2016;11(6):e0156916.
117. Häggblom A, Lindbäck S, Gisslén M, Flamholc L, Hejdeman B, Palmborg A, et al. HIV drug therapy duration; a Swedish real world nationwide cohort study on InfCareHIV 2009-2014. PLoS One. 2017;12(2):e0171227.
118. Wiesmann F, Ehret R, Naeth G, Däumer M, Fuhrmann J, Kaiser R, et al. Multicenter Evaluation of Two Next-Generation HIV-1 Quantitation Assays, Aptima Quant Dx and Cobas 6800, in Comparison to the RealTime HIV-1 Reference Assay. J Clin Microbiol. 2018;56(10).
119. Cillo AR, Vagratian D, Bedison MA, Anderson EM, Kearney MF, Fyne E, et al. Improved single-copy assays for quantification of persistent HIV-1 viremia in patients on suppressive antiretroviral therapy. J Clin Microbiol. 2014;52(11):3944-51.
120. Loetscher P, Templer S, Seiverth B, Marins E, Simon C. Performance evaluation of Cobas® HIV-1, a quantitative nucleic acid test for use on the Cobas® 6800/8800 systems. Journal of HIV and AIDS. 2017;3(1).
121. Günthard HF, Calvez V, Paredes R, Pillay D, Shafer RW, Wensing AM, et al. Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society-USA Panel. Clin Infect Dis. 2019;68(2):177-87.
122. Di Mascio M, Markowitz M, Louie M, Hurley A, Hogan C, Simon V, et al. Dynamics of intermittent viremia during highly active antiretroviral therapy in patients who initiate therapy during chronic versus acute and early human immunodeficiency virus type 1 infection. Journal of virology. 2004;78(19):10566-73.
123. Sánchez-Taltavull D, Alarcón T. Stochastic modelling of viral blips in HIV-1-infected patients: effects of inhomogeneous density fluctuations. Journal of theoretical biology. 2015;371:79-89.
124. Jacobs JL, Halvas EK, Tosiano MA, Mellors JW. Persistent HIV-1 Viremia on Antiretroviral Therapy: Measurement and Mechanisms. Front Microbiol. 2019;10:2383.

125. Miller LG, Golin CE, Liu H, Hays RD, Hua J, Wenger NS, et al. No evidence of an association between transient HIV viremia ("Blips") and lower adherence to the antiretroviral medication regimen. The Journal of infectious diseases. 2004;189(8):1487-96.
126. Martin-Blondel G, Saune K, Vu Hai V, Marchou B, Delobel P, Izopet J, et al. Factors associated with a strictly undetectable viral load in HIV-1-infected patients. HIV medicine. 2012;13(9):568-73.
127. Grennan JT, Loutfy MR, Su D, Harrigan PR, Cooper C, Klein M, et al. Magnitude of virologic blips is associated with a higher risk for virologic rebound in HIV-infected individuals: a recurrent events analysis. The Journal of infectious diseases. 2012;205(8):1230-8.
128. Geretti AM, Smith C, Haberl A, Garcia-Diaz A, Nebbia G, Johnson M, et al. Determinants of virological failure after successful viral load suppression in first-line highly active antiretroviral therapy. Antiviral therapy. 2008;13(7):927-36.
129. Zhang T, Ding H, An M, Wang X, Tian W, Zhao B, et al. Factors associated with high-risk low-level viremia leading to virologic failure: 16-year retrospective study of a Chinese antiretroviral therapy cohort. BMC Infect Dis. 2020;20(1):147.
130. Martinez V, Marcelin AG, Morini JP, Deleuze J, Krivine A, Gorin I, et al. HIV-1 intermittent viraemia in patients treated by non-nucleoside reverse transcriptase inhibitor-based regimen. Aids. 2005;19(10):1065-9.
131. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, Peeples L, et al. Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. The New England journal of medicine. 2009;361(23):2230-40.
132. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, Fisher M, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr. 2010;55(1):49-57.
133. Cao Y, Han Y, Xie J, Cui Q, Zhang L, Li Y, et al. Impact of a tenofovir disoproxil fumarate plus ritonavir-boosted protease inhibitor-based regimen on renal function in HIV-infected individuals: a prospective, multicenter study. BMC Infect Dis. 2013;13:301.
134. Stosor V, Palella FJ, Jr., Berzins B, Till M, Leake A, Chmiel JS, et al. Transient viremia in HIV-infected patients and use of plasma preparation tubes. Clinical infectious diseases : an official

publication of the Infectious Diseases Society of America. 2005;41(11):1671-4.
135. Garrett NJ, Apea V, Nori A, Ushiro-Lumb I, Oliver AR, Baily G, et al. Comparison of the rate and size of HIV-1 viral load blips with Roche COBAS TaqMan HIV-1 versions 1.0 and 2.0 and implications for patient management. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2012;53(4):354-5.
136. Lee FJ, Amin J, Carr A. Efficacy of initial antiretroviral therapy for HIV-1 infection in adults: A systematic review and meta-analysis of 114 studies with up to 144 weeks' follow-up. PLoS ONE. 2014;9(5).
137. Stephan C, Hill A, Sawyer W, van Delft Y, Moecklinghoff C. Impact of baseline HIV-1 RNA levels on initial highly active antiretroviral therapy outcome: a meta-analysis of 12,370 patients in 21 clinical trials*. HIV Med. 2013;14(5):284-92.
138. Santoro MM, Armenia D, Alteri C, Flandre P, Calcagno A, Santoro M, et al. Impact of pre-therapy viral load on virological response to modern first-line HAART. Antivir Ther. 2013;18(7):867-76.
139. Di Biagio A, Rusconi S, Marzocchetti A, Signori A, Schiavetti I, Bruzzone B, et al. The Role of Baseline HIV-1 RNA, Drug Resistance, and Regimen Type as Determinants of Response to First-Line Antiretroviral Therapy. Journal of Medical Virology. 2014;86(10):1648-55.
140. Chen S, Han Y, Song XJ, Li YL, Zhu T, Lu HZ, et al. Very high baseline HIV viremia impairs efficacy of non-nucleoside reverse transcriptase inhibitor-based ART: a long-term observation in treatment-naïve patients. Infect Dis Poverty. 2020;9(1):75.
141. Edén A, Andersson LM, Andersson O, Flamholc L, Josephson F, Nilsson S, et al. Differential effects of efavirenz, lopinavir/r, and atazanavir/r on the initial viral decay rate in treatment naïve HIV-1-infected patients. AIDS Res Hum Retroviruses. 2010;26(5):533-40.
142. Eron JJ, Young B, Cooper DA, Youle M, Dejesus E, Andrade-Villanueva J, et al. Switch to a raltegravir-based regimen versus continuation of a lopinavir-ritonavir-based regimen in stable HIV-infected patients with suppressed viraemia (SWITCHMRK 1 and 2): two multicentre, double-blind, randomised controlled trials. Lancet. 2010;375(9712):396-407.
143. Cahn P, Pozniak AL, Mingrone H, Shuldyakov A, Brites C, Andrade-Villanueva JF, et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. Lancet. 2013;382(9893):700–8.

144. Castagna A, Maggiolo F, Penco G, Wright D, Mills A, Grossberg R, et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. The Journal of infectious diseases. 2014;210(3):354-62.
145. DeGruttola V, Dix L, D'Aquila R, Holder D, Phillips A, Ait-Khaled M, et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. Antivir Ther. 2000;5(1):41-8.
146. Wensing AM, Calvez V, Ceccherini-Silberstein F, Charpentier C, Günthard HF, Paredes R, et al. 2019 update of the drug resistance mutations in HIV-1. Top Antivir Med. 2019;27(3):111-21.
147. Larder BA. Interactions between drug resistance mutations in human immunodeficiency virus type 1 reverse transcriptase. J Gen Virol. 1994;75 (Pt 5):951-7.
148. Rhee SY, Fessel WJ, Liu TF, Marlowe NM, Rowland CM, Rode RA, et al. Predictive value of HIV-1 genotypic resistance test interpretation algorithms. The Journal of infectious diseases. 2009;200(3):453-63.
149. Charpentier C, Peytavin G, Lê MP, Joly V, Cabras O, Perrier M, et al. High virological suppression regardless of the genotypic susceptibility score after switching to a dolutegravir-based regimen: week 48 results in an observational cohort. The Journal of antimicrobial chemotherapy. 2018;73(6):1665-71.
150. Cooper DA, Steigbigel RT, Gatell JM, Rockstroh JK, Katlama C, Yeni P, et al. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. The New England journal of medicine. 2008;359(4):355–65.
151. Anderson JA, Jiang H, Ding X, Petch L, Journigan T, Fiscus SA, et al. Genotypic susceptibility scores and HIV type 1 RNA responses in treatment-experienced subjects with HIV type 1 infection. AIDS Res Hum Retroviruses. 2008;24(5):685–94.
152. Marcelin AG, Flandre P, Descamps D, Morand-Joubert L, Charpentier C, Izopet J, et al. Factors associated with virological response to etravirine in nonnucleoside reverse transcriptase inhibitor-experienced HIV-1-infected patients. Antimicrobial agents and chemotherapy. 2010;54(1):72–7.
153. Sangaré MN, Baril JG, de Pokomandy A, Ferreira Guerra S, Carabali M, Laprise C, et al. Treatment Switch to Dolutegravir With 2 Nucleoside Reverse-Transcriptase Inhibitors (NRTI) in Comparison to Continuation With Protease Inhibitor/Ritonavir Among Patients With Human Immunodeficiency Virus at Risk

for Prior NRTI Resistance: A Cohort Analysis of Real-World Data. Open Forum Infect Dis. 2020;7(11):ofaa404.

154. European AIDS Clinical Society (EACS). EACS Guidelines. Version 10.0 - November 2019 2019 [Available from: https://www.eacsociety.org/files/2019_guidelines-10.0_final.pdf.

155. British HIV Association guidelines for the treatment of HIV-1-positive adults (BHIVA). Guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update) 2016 [Available from: https://www.bhiva.org/file/RVYKzFwyxpgiI/treatment-guidelines-2016-interim-update.pdf.

156. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV: Department of Health and Human Services; 2019 [cited 2020. Available from: https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/AdultandAdolescentGL.pdf.