Diploma Thesis

Prevalence of drug resistance mutations against dolutegravir, lamivudine, and rilpivirine in ART-naïve residents of South-East Austria

submitted by

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Declaration of Originality

I, hereby, declare that the following diploma thesis has been written only by the undersigned and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself.

Graz, May 6th, 2020 *Anna Benezeder eh.*

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Contents

Abstract

Background: Although a lot of progress has been made regarding antiretroviral therapy, no cure for HIV has been found and lifelong treatment is needed. Long-term drug exposure is accompanied by various side effects and toxicities. With the successful introduction of two-drug regimens in recent years, lowered cumulative drug exposure while maintaining viral suppression has been made possible.

Objectives: To ensure successful treatment, anti-retroviral therapy (ART) should ideally start as soon as possible after HIV-1 diagnosis. In order to guarantee high efficacy of initial combination regimens, data on local prevalence of drug resistance mutations is of great importance. The aim of this project was to identify transmitted HIV-1 drug resistance mutations (DRMs) against drugs currently recommended for two-drug regimes, dolutegravir (DTG), lamivudine (3TC), and rilpivirine (RPV). In addition, the local transmission network of HIV-1 in South-East Austria was reconstructed.

Material and Methods: In this study, 192 HIV patients who had been diagnosed between 2013-2018 living in South-East Austria were included. Conventional Sanger sequencing analysis was performed and sequences were analyzed for DRMs including DTG, 3TC, and RPV. Molecular network analysis was carried out to determine presumed relations and to identify shared DRMs.

Results and Conclusion: Of all ART-naïve patients, 16% showed clinically relevant DRMs against DTG, 3TC, and/or RPV. The prevalence of these DRMs was significantly higher in genetic transmission clusters when compared to non-genetically linked individuals. Within clusters, the majority of DRMs were shared, indicating an elevated risk of transmission of resistant HIV-1 strains. However, of all patients with clinically relevant DRMs, only 2% were not eligible for at least one of the currently suggested two-drug regimens.

Keywords: HIV; dolutegravir; lamivudine; rilpivirine; two-drug regimen; drug resistance mutation; transmission network; molecular epidemiology

Zusammenfassung

Hintergrund:
Obwohl bereits große Fortschritte im Bezug auf antiretrovirale Therapie gemacht wurden, gibt es bisher noch keine Heilung für HIV und eine lebenslange Therapie ist notwendig. Eine Langzeitmedikamenteneinnahme wird von einer Reihe von Nebenwirkungen und Toxizitäten begleitet. Mit der erfolgreichen Einführung von Zweifachtherapien in den letzten Jahren wurde jedoch eine Senkung der kumulativen Medikamentendosis ermöglicht, bei der die Virussuppression dennoch erreicht wird.

Zielsetzung: Um eine erfolgreiche Behandlung zu gewährleisten, sollte die antiretrovirale Therapie idealerweise so früh wie möglich nach einer HIV Diagnose begonnen werden. Dabei ist es wichtig, die lokale Prävalenz der Mutationen, die für Arzneimittelresistenzen verantwortlich sind, zu kennen, um eine hohe Effektivität der anfänglichen Kombinationstherapie zu erreichen. Das Ziel dieser Studie war es, die übertragenen HIV-1 Resistenzmutationen gegen Dolutegravir (DTG), Lamivudine (3TC) und Rilpivirine (RPV) zu erfassen. Zusätzlich wurde das lokale Übertragungsnetz von HIV-1 in Süd-Ost Österreich rekonstruiert.

Material und Methoden: In dieser Studie wurden 192 Patientinnen und Patienten aus Süd-Ost Österreich inkludiert, die eine HIV-1 Diagnose zwischen 2013 und 2018 erhalten haben. Konventionelle Sanger Sequenzierung wurde durchgeführt und auf Resistenzmutationen bezüglich DTG, 3TC und RPV analysiert. Zusätzlich wurden mithilfe einer molekularen Netzwerkanalyse angenommene Relationen festgestellt und geteilte Resistenzmutationen ermittelt.

Ergebnisse und Schlussfolgerung: Von allen untersuchten Patientinnen und Patienten wiesen 16% klinisch relevante Resistenzmutationen gegen DTG, 3TC oder/und RPV auf, bevor die antiretrovirale Therapie begonnen wurde. Im Vergleich war die Prävalenz der Resistenzmutationen in genetischen Übertragungsclustern signifikant höher als in der Gruppe ohne genetische Ähnlichkeiten. In diesen Clustern wurde die Mehrheit der Resistenzmutationen von zwei oder mehr

Personen geteilt, was auf ein erhöhtes Risiko der Übertragung bereits resistenter HIV-1 Stämme hinweist. Obwohl in 16% der Fälle klinisch relevante Resistenzmutationen gefunden wurden, waren nur 2% der Patientinnen und Patienten nicht geeignet für eine Behandlung mit zumindest einer der zwei derzeit empfohlenen Zweifachtherapien.

Abbreviations

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
AZT	azidothymidine
cDNA	complementary deoxyribonucleic acid
CRF	circulating recombinant forms
DNA	deoxyribonucleic acid
DRM	drug-resistance mutations
EACS	European Aids Clinical Society
EBV	Epstein-Barr virus
ELISA	enzyme linked immunosorbent assay
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
HLA	human leukocyte antigen
IDU	injection drug user
IN	integrase
LAS	lymphadenopathy syndrome
mRNA	messenger ribonucleic acid
MSM	men having sex with men
NAT	nucleic acid amplification testing
NHP	nonhuman primates
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors

NtRTI	nucleotide reverse transcriptase inhibitors
PCR	polymerase chain reaction
PLWH	people living with HIV
PrEP	pre-exposure prophylaxis
RNA	ribonucleic acid
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
rtPCR	real-time polymerase chain reaction
ssRNA	single stranded ribonucleic acid
SIV	Simian immunodeficiency virus
STD	sexually transmitted disease
UNAIDS	Joint United Nations Programme on HIV and AIDS
URF	unique recombinant forms
WHO	World Health Organization

1 Introduction

Infection with the human immunodeficiency virus (HIV) causes the acquired immunodeficiency syndrome (AIDS), resulting in progressive damage of the immune system if untreated. AIDS hereby refers to the late, symptomatic stage of the infection with HIV caused by the failure of the immune system to defeat common and opportunistic infections (1). Medical knowledge of the virus and the disease is steadily expanding; however, a cure for AIDS has not yet been found. The currently available antiretroviral therapy (ART) improves the patient's prognosis and extends life expectancy by many years (2).

Access and adherence to ART is preventing viral transmission because the drugs are effectively inhibiting HIV replication. Despite the enormous therapeutical progress, transmission of the virus continues to be high in specific populations. Social issues may prevent people from access to prevention and treatment services, the virus is spreading and the number of new infections per year is still high. Maximum prevalence is found in groups with certain risk factors, as for example men having sex with men (MSM) and injection drug users (IDU). Controlling the ongoing spread of the virus in these groups would be the most effective response to the pandemic (1,3).

The global estimates for adults and children living with HIV in the year 2018 amounted approximately to 38 million people (4). The 37th report of the Austrian HIV Cohort Study calculated an estimation of 8055 to 8560 people living with HIV in Austria in 2018 (5).

Even though the rate of new infections has been declining in the last few years, a lot needs to be done to control the pandemic. Besides the set up of continuous information and prevention programs and the establishment of preventive options, more research needs to be done focusing on the development of new and simple treatment options, with the hope of finding a potential cure for HIV (3).

1.1 The Human Immunodeficiency Virus

In the 1980s, the first clinical observations of what later became known as AIDS were reported; a disease, which would become one of the most dangerous and devastating pandemics in human history (1,3).

The syndrome has been associated with different risk groups over time. Initially, MSM got sick, then injection drug users (IDUs), people who had obtained blood transfusions and eventually, AIDS became a disease affecting the general population. The scientific and medical community was urged to study the disease and elucidate its origin. Within two years of AIDS research, the causative virus was discovered. In 1983, the retrovirus was isolated for the first time. Three decades of research followed, resulting in a broad knowledge about the virus, its interaction with the host cells, and the treatment and prevention of an infection with HIV (1,3).

In 1984, a simian lentivirus (SIV) was isolated from rhesus macaques, which showed a remarkably similar disease to human AIDS. The virus SIVmac was suspected to be an origin of HIV due to its analogy of clinical symptoms. Sequencing data showed that HIV possibly originated in the early 20^{th} century in central Africa. There were various cross-species transmissions of SIV from nonhuman primates (NHP) to humans (2). Although the history of SIV evolution is quite complex, infections with SIV of NHP generally occur in a species-specific manner, creating monophyletic lineages in phylogenetic trees. Two subtypes of the human immunodeficiency virus are known: HIV-1 and HIV-2. Close relatives of HIV-1 are SIVcpz from chimpanzees and SIVgor from gorillas; sooty-mangabeys-SIV being the closest relative of HIV-2. This suggests that HIV-1 and HIV-2 most likely originated from NHP species. Subsequent research indicated that the SIVs similar to HIV-1 moved across the barrier of species on four occasions. These subtypes of HIV-1 were named as groups M (main), N (non-M, non-O), O (outliers) and P. Sooty-mangabeys-SIV crossed the species barrier at least nine times, resulting in nine different HIV-2 groups. Nevertheless, only group A and B have expanded to some amount in West Africa, with group A predominating (6).

There are two theories of how HIV-1 could have been transmitted from NHPs to humans. The first hypothesis claims that the infection with the main (M) group of HIV was due to an oral vaccination against polio, which had been obtained from chimpanzee kidneys and was performed mainly in the Congo in the 1950s.

Contrary to this, the second hypothesis indicates that the occurrence of HIV group M took place before the vaccination and therefore is believed to have transferred from chimpanzees to humans in a natural way (7).

Even though there are four subtypes of HIV-1, only group M is blamed for the global pandemic, as the other groups O, N and P are very rare and mainly found in Cameroon. Group M can be further split into nine subtypes (A, B, C, D, F, G, H, J, K) and further more into six sub-subtypes (A1, A2, A3, A4, F1, F2), that are used to label the sister clades. An overview depicting the groups and their subtypes can be seen in Figure 1.

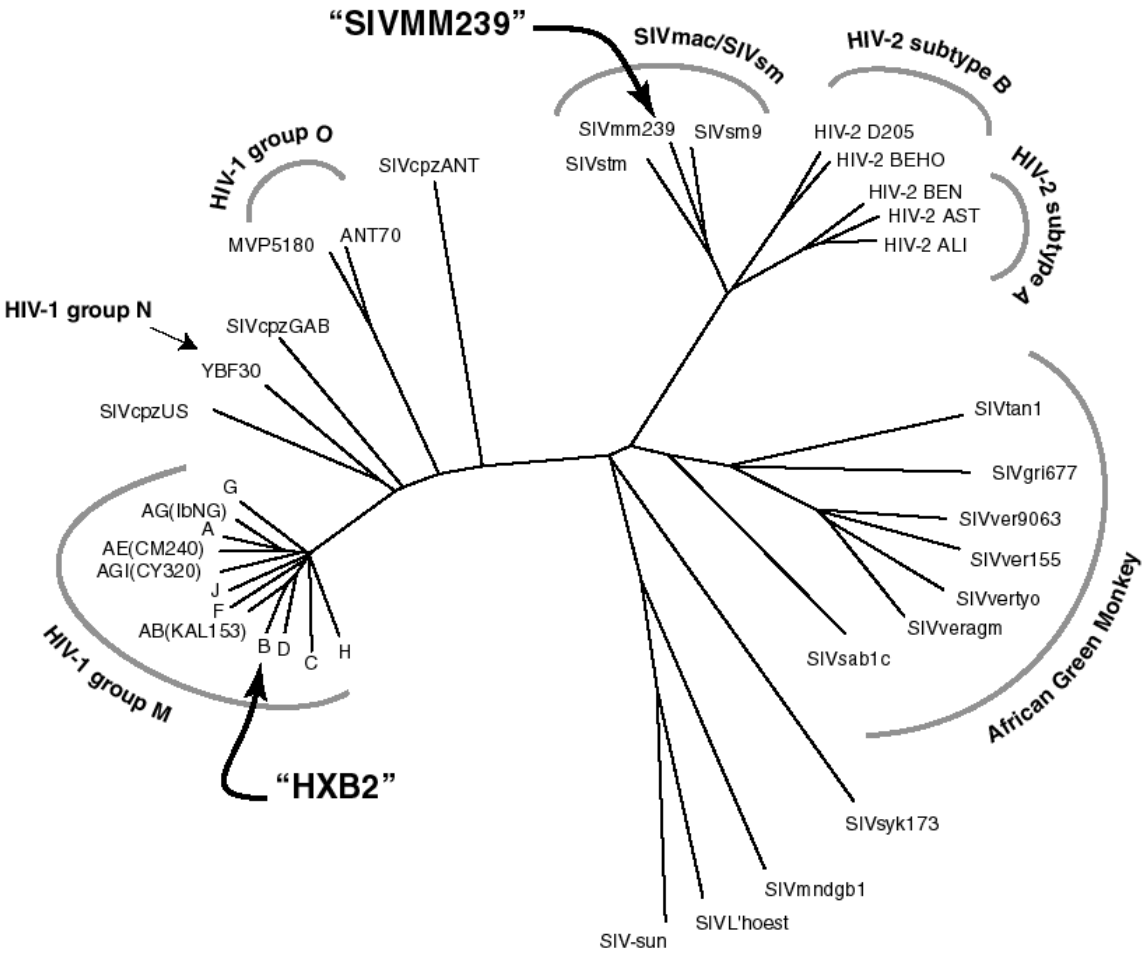

Figure 1: Phylogenetic tree of the primate lentiviruses. From: Calef C, Mokili J et al. "Numbering Positions in SIV Relative to SIVMM239". 2001. Available from: https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/SIV_NUMBERING2001/SivNumbering.html

The occurrence of HIV subtypes is linked to geographical areas. Subtype C dominates and accounts for nearly half of all HIV-1 infections, being found most

commonly in Africa and India. Subtype B is predominately found in Europe, North- and South America and Australia. Subtype A is most common with IDUs in Eastern Europe (2,6).

Various inter-subtype combinations have also been identified, such as unique recombinant forms (URF), which only occur in one individual patient, and circulating recombinant forms (CRF), which are transmitted and therefore arise in at least three patients without any direct epidemiological linkage (6,8,9).

Over time, diversity and complexity of subtypes and numbers of CRFs increases, as recombination of already existing recombinant viruses takes place. Due to the high rates of mutation and recombination, as well as viral replication, genetic variability of HIV is very high (6).

1.1.1 Structure and Morphology

HIV is a retrovirus that belongs to the genus lentivirus and its genetic information is contained as single-stranded ribonucleic acid (ssRNA). A retrovirus is characterized by the ability to rewrite its viral RNA into deoxyribonucleic acid (DNA), allowing the virus to stably integrate itself into the host genome. The lentiviruses are capable of infecting non-dividing cells and are associated with a long incubation period. The target cells of the virus are CD4+ T cells, which it uses to multiply itself. HIV infection leads to the gradual depletion of T cells, which ultimately causes autoimmune deficiency syndrome, if it remains untreated (10).

The HIV virion consists of a lipid membrane as an envelope, matrix protein and a capsid as a core (for a detailed depiction of the structural elements see Figure 2). The lipid membrane forms the outermost layer and is of host-cell origin. While cutting itself off from the host-cell, the virion takes a part of the blood cell membrane with it. The envelope is spiked with two different glycoproteins gp41 and gp120 and human leukocyte antigens (HLA) class I and II. Gp41 is a transmembrane protein and plays a major role in the primary fusion with the host membrane. Gp120 is important for the receptor binding with CD4+ molecules on the surface of leukocytes. The gene encoding for the glycoproteins is called "*Env*".

The matrix protein (MA) p17, the capsid protein (CA) p24 and the nucleocapsid protein (NC) p7 are so-called "gag-derived" proteins. "Gag" is short for group specific

antigen, which is a polypeptide coding for the structural proteins of the matrix and the core. The capsid contains the nucleocapsid proteins, which are tightly bound to RNA and the enzymes reverse transcriptase and integrase. A third important enzyme, the protease, is located outside of the capsid. All three play a major role in the replication of HIV and therefore early stages of infection. The genetic information for these enzymes is stored in the “pol” gene (11).

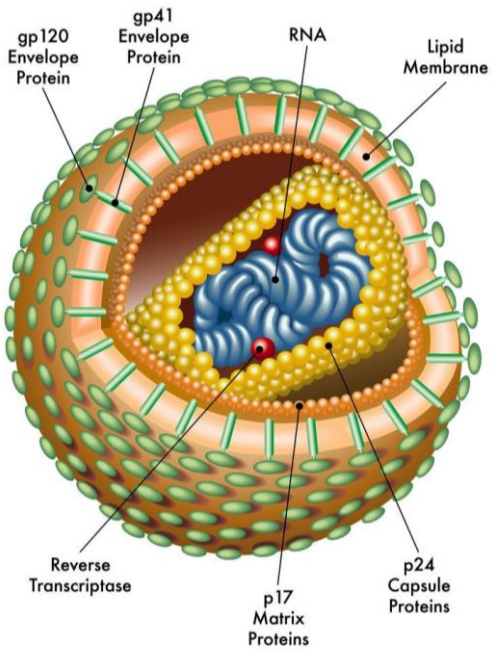

Figure 2: Anatomy of the AIDS Virus. From: CK-12 Foundation.
Available from: https://www.ck12.org/biology/HIV/

1.1.2 Life cycle

The life cycle of HIV can be divided into two phases: an early phase, which describes the process from the binding of the virus to the surface of a host cell to the integration of viral genome in the host cell DNA and a late phase, which includes events from the point of gene expression to the budding off of new, infectious virions (12).

The primary attachment of HIV to the host cell takes place with CD4+ receptors on T-lymphocytes, macrophages and dendritic cells. After the binding of gp120 with the CD4+ receptor, the host cell is activated and thus expresses the chemoreceptors

CCR5 and CXCR4. These are needed for the stronger binding of the virus and the following entry of HIV into the blood cell. Depending on the type of host cell-receptor that the HIV uses, it can be classified as an R5, X4 or R5X4 virus. After the binding with the co-receptors, gp41 is structurally rearranged and leads to the fusion of the virus with the cell membrane and to the release of the viral genome, enzymes and other viral proteins into the host cell (13).

The enzyme reverse transcriptase then transcribes the viral RNA into complementary DNA (cDNA). This ability is one of the major defining features of retroviruses and therefore was eponymous. During the process of transcription, the reverse transcriptase makes errors, which are not corrected because HIV has no proofreading function. This results in a high rate of mutations and immense viral diversity, which plays a major role in the resistance of HIV against ART (14).

After the viral DNA is transported into the nucleus, the enzyme integrase merges it into the host DNA, making the virus impossible to eradicate with therapies existing to this date of time. The integrated DNA then lies dormant to the point of T-cell activation. When the T-lymphocyte is activated, certain transcription factors are expressed, which need to be present to start the transcription of viral DNA. The most important factor is the nuclear factor kappa B (NF-κB) (15). The virus then uses the host's transcription machinery to ensure its own protein production and replication (1). Some of the multiple RNA copies later become the genome of the new viruses; some are used to create new HIV proteins (16). This takes place by the transcription of the viral genome into messengerRNA (mRNA) by RNA polymerase II, which is exported out of the nucleus to the cytoplasm, where it is translated into polypeptide chains.

Early in the course of infection, the regulatory proteins Tat and Rev are produced. These proteins are crucial for the virus production; Tat is responsible for the elongation of transcribed RNA and the promotion of processivity of RNA polymerase II. The purpose of Rev is to export unspliced or partly spliced RNA out of the nucleus (17).

In the cytoplasm, the translation of mRNA takes place, which results in the production of the structural proteins Gag, GagPol, Env and accessory proteins. Gag then initiates the assembly of all viral components at the plasma membrane by binding to the viral RNA. Finally, the viral genome and proteins are packaged and encapsulated and released from the host cell in a process named budding. When

the new virus leaves the host cell, it takes a part of lymphocyte membrane with it, also containing the viral surface proteins gp41 and gp120, which were produced by the endoplasmic reticulum and Golgi apparatus.

Shortly after budding, the virus is still immature. The cleavage of gag polyprotein into smaller fragments by the enzyme protease is called maturation. This results in the formation of the actual nucleocapsid, capsid and matrix proteins and the infectiousness of the virus. The cleavage is accompanied by a morphological transformation, which can be seen in Figure 3 (12).

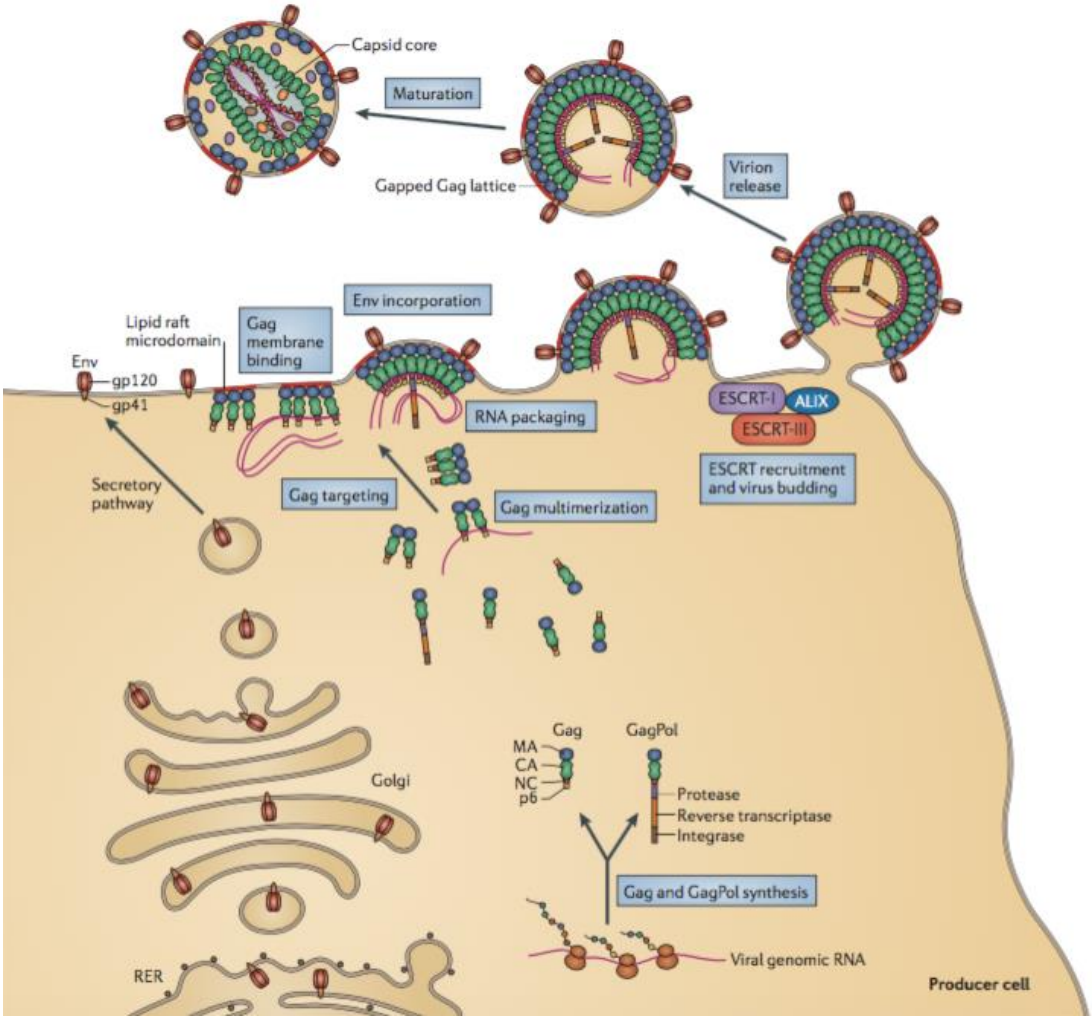

Figure 3: The late stages of the HIV-1 replication cycle. From: Freed EO. "HIV-1 assembly, release and Maturation". Nature Reviews Microbiology. June 2015: 1-13.

1.1.3 Transmission

In a HIV positive person, the virus can be detected in various body fluids, including blood, rectal fluids, vaginal fluids, pre-seminal fluids and semen, and breast milk. When these infectious fluids get into contact with damaged skin or mucous tissue, for example the vagina, penis, rectum or mouth, or have direct contact with the blood stream, there is a certain risk of infection. However, the body fluids of HIV positive persons are not equally contagious. Within six months of daily taking prescribed HIV medication, the amount of virions in the body fluids can be reduced to a level, where it is no longer detectable in the standard lab test. This is called viral suppression and it does not only keep the patients healthy, but also prevents HIV from spreading, as people with an undetectable viral load are not able to effectively transmit HIV to their partners. The higher the viral load of a person infected with HIV or the higher his/her genital secretions, the higher the infectiousness. Other sexually transmitted diseases (STDs) are said to increase the infectiousness too. People who are not under ART have a high viral load and can transmit the virus sexually, parenterally or vertically (1,18,19).

HIV transmission through sexual contacts can occur during unprotected vaginal or anal sex. Anal sex bears the highest risk for the receptive, as well as the insertive part, although the risk for the receptive partner is much higher. A systematic review and meta-analysis published in the International Journal of Epidemiology states that the per-act transmission risk of the receptive partner during anal intercourse may be approximately 18 times higher than during vaginal intercourse (20). Male circumcision has proven to have protective effects against HIV. This might be because of the removal of the foreskin, which has many HIV receptors on its inner surface, and the frenulum, which is a common spot for injuries and other STDs (21). An infection during oral sex is possible, but extremely rare. The risk increases if both partners have mucous lesions, such as sores and bleeding gum (18).

The Joint United Nations Programme on HIV and AIDS (UNAIDS) lists Transgender women, sex workers, clients of sex workers and other sexual partners of key population as potential risk groups (22).

HIV can also be transmitted through infectious blood and blood products. One of the main risk groups for parenteral infection are IDUs, which put themselves at high risk by sharing needles. Even though the virus is not exceedingly viable outside the

body, it can survive several weeks in a syringe. This also bears a risk for health care workers, who might get infected through a needle stick injury.

Various sources list hemophiliacs as a risk group because numerous cases of hemophiliac patients, who were infected with HIV by receiving blood products, were reported since the 1980s. Today, these incidents are extremely rare due to the exclusion of certain risk groups from blood donations and thorough testing of blood donations (18,23).

Vertical transmission from mother to child, most likely happens perinatal, while giving birth. The transmission can also occur during pregnancy or breastfeeding, as breast milk contains infectious virions. Without any form of intervention, the risk for the child to be infected is 15% to 45%. However, the child can be protected through various precautions and treatments, reducing the risk of getting infected with HIV under 5%. These include testing on HIV of pregnant women (which in Austria takes place in the form of mother-child-booklet examinations), ART while pregnancy, caesarian section for untreated mothers, antiretroviral prophylaxis for newborns, as well as avoiding breastfeeding (24,25). Approximately 3 million children were living with HIV in 2017, nearly 90% of them in sub-Saharan Africa (26).

According to the Federal ministry of health and women, there were 510 new infections with HIV in Austria in the year 2017 and 397 in the year 2018 (27).

The 35th Report of the Austrian HIV Cohort Study investigated the modes of transmission correlating to the number of positive HIV tests for the cohort participants. Newly infected patients were sorted into one of the following groups: MSM, IDU, Hetero, Haemophiliac, Transfusion, Perinatal, Other and Unknown. In 2017, MSM covered more than half of the transmissions, followed by heterosexually infected with approximately a quarter. Transmissions of unknown origin were slightly higher than IDUs, who made up about 5%, followed by the other groups mentioned above (28). Worldwide, HIV acquisition happens most frequently during heterosexual intercourse (2).

1.1.4 Diagnosis of infection

Detecting an infection with HIV at an early stage entails benefits for the patient and the society. ART appears to be more effective if started at an early stage. Moreover, it can prevent further transmission of HIV. Therefore, early diagnosis is evidently important to pause disease progression and to further reduce spreading of the virus.

There are three different testing options for the detection of HIV: two indirect methods, the antibody test and the antigen/antibody test, and one direct method, the nucleic acid amplification testing. The indirect methods are immunoassays, the so-called enzyme linked immunosorbent assays (ELISAs), which detect the antibodies against HIV and in case of the antigen/antibody test also the p24 antigen. The combined antibody and p24 antigen test is the fourth generation of immunoassays in HIV diagnosis and is routinely used in screenings. This is the case because it is the fastest method of detection, as its serologic window period, in which time HIV antibodies cannot yet be detected in the plasma, is reduced to only 15 days due to the detection of p24 antigen. The p24 antigen test is combined with the antibody detection because p24 is only temporarily detectable until antibody production starts. HIV antibody testing is performed anonymously and free of charge in Austria at advice centers for AIDS.

Progression of infection can be grouped into six phases, the so-called Fiebig stages, depicted in Figure 4, depending on which test method can be used effectively to detect HIV (29).

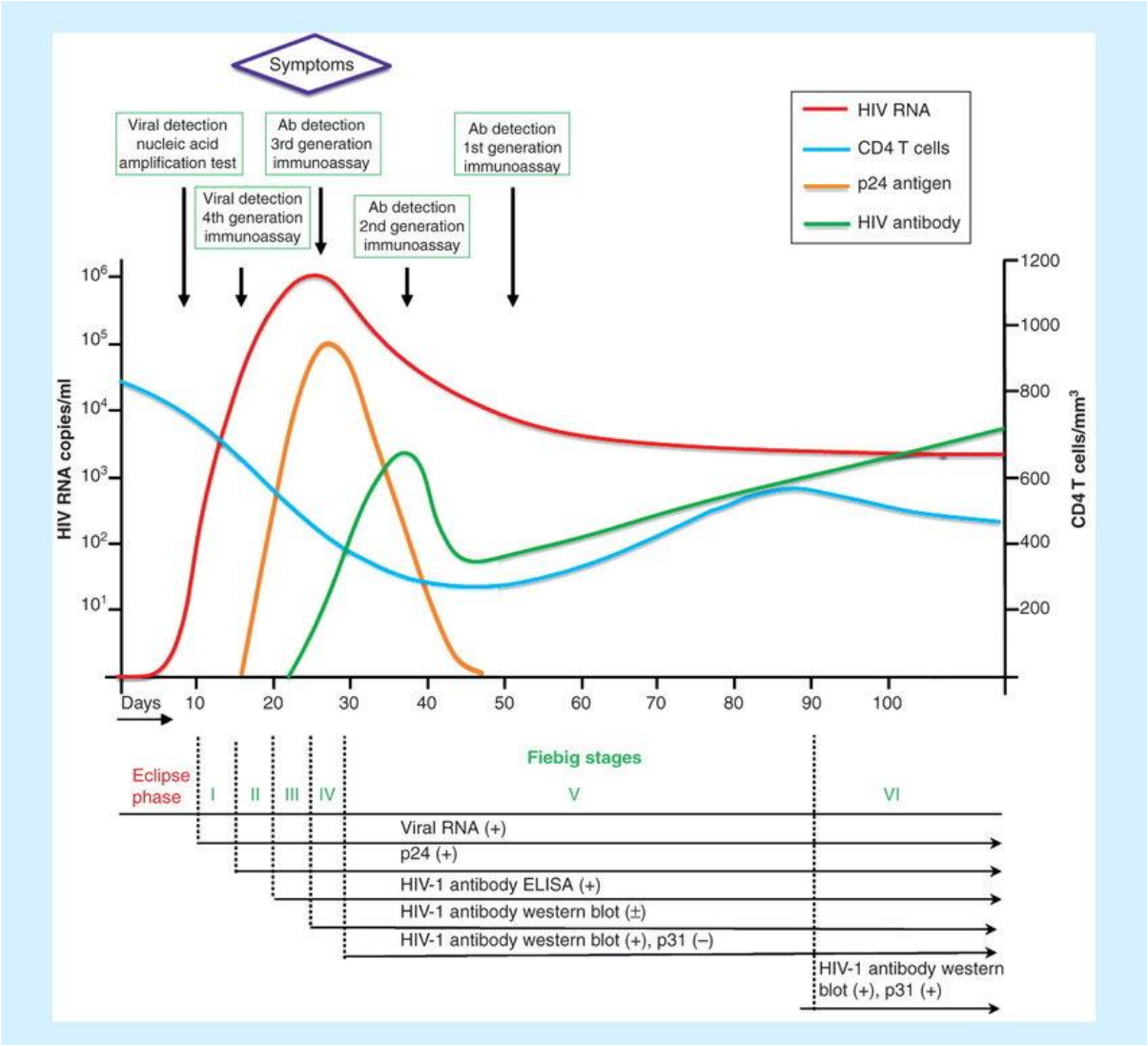

Figure 4: Trajectories of HIV-RNA viremia, CD4 T cells, p24 antigen and HIV antibody over the early phase of HIV infection. From: Routy J, Cao W et al. "Overcoming the challenge of diagnosis of early HIV infection: a stepping stone to optimal patient management". Expert Rev. Anti. Infect. Ther. 2015

Test results are considered reliable and no further testing is required, if the antigen/antibody immunoassay is negative and the window period of two weeks has been taken into consideration. If a test is positive, false positive results have to be ruled out. In Austria two further tests, which are independent of one another, must be done to verify the result. These are mostly a second antibody test (ELISA), which can differentiate between HIV-1 and HIV-2, and a western blot. If the results are unclear, a real-time polymerase chain reaction (rtPCR), which is a NAT, provides certainty. The PCR is not only used as a diagnostic tool for detection of the virus, but also to measure its quantity, the so-called viral load, to monitor progression and

response to treatment (29,30). Furthermore, it is the only reliable diagnostic test for infants under 18 months, as the antibody tests could be false positive due to transfer of antibodies through the placental barrier (25).

1.2 Clinical manifestation

In 1993 the Center for Disease Control and Prevention (CDC) published a report named “Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults“ for categorization of HIV infection and following disease, which is still valid today. According to this system, HIV infected adolescents and adults are classified into one of three clinical categories and one of three CD4+ cell count categories (see Table 1).

Table 1: Classification System for HIV Infection among adolescents and adults.

CD4+ cell count	Clinical category		
	A	B	C
> 500/µl	A1	B1	C1
200 – 499/µl	A2	B2	C2
< 200/µl	A3	B3	C3

The beginning of AIDS is defined as contracting AIDS-defining diseases, which includes the categories C1 to C3 by fulfilling the immunologic criteria, as well as CD4+ cell counts with less than 200/µl, which also includes the categories A3 and B3 by fulfilling the clinical criteria (31).

1.2.1 CD4+ cell count category

The CD4+ T-lymphocytes are the targetcells of HIV and therefore cell counts decline with progressing infection. T-cells have crucial functions in cell-mediated immunity and consequently in the general immune response. Studies have shown that the number of CD4+ cells inversely correlates with the occurrence of opportunistic illnesses, characteristic for AIDS. The fewer T-lymphocytes, the more severe the illnesses. For that reason, it is of crucial importance to treat HIV infected patients as soon as possible. The CD4+ cell count is not only used to categorize patients, but also to monitor treatment effectiveness (31).

1.2.2 Clinical category A

Acute retroviral syndrome:

The primary infection is characterized by a peak of HIV RNA in the plasma approximately one to six weeks after the infection. During this period, symptoms differ from patient to patient. Mild symptoms are often misdiagnosed as influenza, since they are uncharacteristic for HIV. About one third of the patients develop manifestations similar to glandular fever, also known as infectious mononucleosis, usually caused by Epstein-Barr virus (EBV). The symptoms can be enlarged lymph nodes, fever, malaise, splenomegaly, sore throat and occasionally muscle pain, a nonspecific rash and diarrhea. Some patients do not develop early symptoms at all (1,32).

Latency period:

Characteristic for this period are virus replication and lacking of symptoms, which on average lasts 10 years without ART. The infection remains undiagnosed in most cases and untreated patients continue to be infectious throughout this time. With effective medication, patients can maintain clinical health without replication of HIV up to several decades. The immune system fights against infection and therefore the viral load decreases after the acute phase to a steady-state level; before eventually slowly increasing again after years. The CD4+ cell count recovers after primary infection, but begins to decrease again slowly after a short period of time (1,32).

About 40% of the patients fall victim to the so-called Lymphadenopathy syndrome (LAS), which is defined by persistent (over three months) and generalized swollen lymph nodes. Approximately 30% of the patients develop seborrheic dermatitis (32).

1.2.3 Clinical category B

The clinical category B is often referred to as the period of AIDS non-defining diseases. Due to further decrease of CD4+ T-lymphocytes, patients become prone to certain illnesses. The developing immunodeficiency is contributing to the spread of the diseases and the risk for complications increases. The lower the CD4+ cell count, the more severe the infections get.

Patients in this category often catch opportunistic infections such as subfebrile temperatures (<38,5°C), weight loss (>10% of total body weight), HIV-associated peripheral neuropathy, pelvic inflammatory disease, chronic diarrhea (> 1 month), idiopathic thrombocytopenic purpura, herpes zoster infection, oropharyngeal and vulvovaginal candidiasis, oral hairy leukoplakia caused by EBV-infection, listeriosis, bacillary angiomatosis and cervical dysplasia/ cervical carcinoma in situ (1,31,32).

1.2.4 Clinical category C

All HIV positive patients, who present with an AIDS defining disease are classified under category C and will remain in this group for classification purposes (31).
In 1993, the CDC published in its report “Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults” following list of conditions as AIDS surveillance case definition (31):

APPENDIX B. Conditions included in the 1993 AIDS surveillance case definition

- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive *
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)
- Lymphoma, primary, of brain
- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary * or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent *
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV
- Added in the 1993 expansion of the AIDS surveillance case definition.

Figure 5: "Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults". From: Kenneth CG, John W et al. CDC. 1993. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

1.3 Antiretroviral therapy

HIV therapy had its origin in 1987, when azidothymidine (AZT), also called zidovudine, was shown to be effective in a clinical trial. AZT, a reverse transcriptase inhibitor (RTI), was the first approved drug against AIDS. Soon viral resistances were found and consequently led to the introduction of combination antiretroviral therapy in 1996. Since then various drugs have been approved, making different therapeutic options available (3).

Currently used drugs can be categorized into 5 classes intervening at different phases of viral replication cycle. Nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and Non-nucleoside reverse transcriptase inhibitors (NNRTIs) interfere during reverse transcription. NRTIs have a similar structure as physiologic nucleosides except for having one OH-group less. They are integrated into the newly transcribed cDNA and lead to a stop in transcription. NtRTIs can be distinguished from NRTIs by having more phosphate groups and therefore are similar to nucleotides. In contrast, NNRTIs interrupt the reverse transcriptase in a non-competitive way. Protease inhibitors suppress the viral enzyme protease, Integrase strand transfer inhibitors (INSTIs) inhibit the integration of cDNA into the host cell genome. Cell entry inhibitors, which include co-receptor inhibitors and fusion inhibitors, interfere with the infiltration of the virus into the target cell. The only available co-receptor inhibitor to date is a CCR5 antagonist and therefore the co-receptor status is required before initiating this therapy. Fusion inhibitors interact with gp41, which plays a central role in the fusion process (2,33).

With the possibility of combining various drugs of classes, which suppress different phases of replication cycle, one can speak of highly active antiretroviral therapy (HAART). The aim of this treatment is to reduce the viral load in blood plasma to levels under detection limit within six months, which is defined as <50 copies/ml. This leads to an increase of CD4+ cell count, and with that a recovery of the immune system, and minimizes the progression of the infection. The combination ART has positive effects such as the reduction of the individual drug dosages, which reduces side effects, the avoidance of resistance development for as long as possible, and a possible synergism between two substances (33). Because of combination ART people living with HIV (PLWH) do not have a shortened life expectancy anymore.

Until a few years ago, the recommendation of initiating ART was depending on the CD4+ cell count. The World Health Organization's (WHO) consolidated guidelines for HIV treatment of 2013 recommended: "initiating ART for all adults with HIV and a CD4 count at or below 500 cells/mm^3, regardless of WHO clinical stage, giving priority to those with severe or advanced HIV disease (WHO clinical stages 3 or 4) or a CD4 cell count at or below 350 cells/mm^3", (34). Since then, studies have laid their focus on how quickly treatment should be started after the confirmed diagnosis of HIV and its benefits on morbidity and mortality.

The WHO guidelines of 2015 changed their recommendation to: "ART should be initiated among all adults with HIV regardless of WHO clinical stage and at any CD4 cell count (strong recommendation, moderate-quality evidence). As a priority, ART should be initiated among all adults with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and adults with CD4 count ≤350 cells/mm3 (strong recommendation, moderate-quality evidence)", (34).

The WHO guidelines of 2017 express the strong recommendation for rapid ART initiation, which is defined as within a week from confirmed diagnosis and even should be offered the same day, to all people living with HIV and stated high quality evidence for adults and adolescents (35).

The European Aids Clinical Society (EACS) recommends as Standard-of-care ART an initial combination regimen of two NRTIs, the so-called backbone, and additionally an INSTI, a NNRTI or a Protease inhibitor as third substance for ART-naïve patients. Until November 2019, only regimens composed of three different drugs were recommended as initial combination ART in the EACS guidelines (36). However, patients under life long drug exposure suffer from side effects and potential long-term toxicities. Thus the interest in two-drug regimens, which would lower the lifetime cumulative drug exposure while maintaining viral suppression, has increased drastically lately. Several studies have been published which imply the successful viral suppression with two-drug regimens including the INSTI dolutegravir (37–40).

This treatment simplification has been acknowledged by European and US guidelines and has first been adopted into their guidelines as a two-drug combination of dolutegravir (DTG) and lamivudine (3TC) as alternative regimen among initial combination regimens for ART-naive adult HIV-positive persons

(36,41). DTG and rilpivirine (RPV), another dual regimen, has been added to the switch strategies for virologically suppressed patients in the EACS.

However, in the latest version of the EACS guidelines 10.0, which appeared in November 2019, the dual therapy with DTG + 3TC has been added to recommended regimens as initial combination ART (see Table 2). The dual therapy with DTG + RPV is still listed as switch strategies for virologically suppressed patients (42).

Mutations leading to drug resistance have to be considered, when these drugs are administered as primary combination regimen. Both of the dual therapy regimens are only indicated in absence of known clinically relevant mutations associated with resistance to the included antiretroviral drugs.

Regimen	Main requirements	Additional guidance (footnotes)
Recommended regimens		
2 NRTIs + INSTI (PREFERRED)		
ABC/3TC + DTG ABC/3TC/DTG	HLA-B*57:01 negative HBsAg negative	I (ABC: HLA-B*57:01, cardiovascular risk)
TAF/FTC or TDF/FTC or TDF/3TC + DTG		II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) III Weight increase
TAF/FTC/BIC		
TAF/FTC or TDF/FTC or TDF/3TC + RAL qd or bid		II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) IV (RAL: dosing)
1 NRTI + INSTI		
DTG + 3TC	HBsAg negative HIV-VL < 500,000 copies/mL CD4 count > 200 cells/µL	
2 NRTIs + NNRTI		
TAF/FTC or TDF/FTC or TDF/3TC + DOR TDF/3TC/DOR		II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) V (DOR: HIV-2)
TAF/FTC or TDF/FTC or TDF/3TC + RPV TAF/FTC/RPV TDF/FTC/RPV	CD4 count > 200 cells/µL HIV-VL < 100,000 copies/mL Not on proton pump inhibitor With food	II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) VI (RPV: HIV-2)
2 NRTIs + PI/r or PI/c		
TAF/FTC or TDF/FTC or TDF/3TC + DRV/c or DRV/r TAF/FTC/DRV/c	With food	II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) VII (DRV/r: cardiovascular risk)
Alternative regimens		
2 NRTIs + INSTI		
ABC/3TC + RAL qd or bid	HBsAg negative HLA-B*57:01 negative	I (ABC: HLA-B*57:01, cardiovascular risk) IV (RAL: dosing)
TDF/FTC/EVG/c TAF/FTC/EVG/c	With food	II (TDF: prodrug types. Renal and bone toxicity) VIII (EVG/c: use in renal impairment)
2 NRTIs + NNRTI		
ABC/3TC +EFV	HLA-B*57:01 negative HBsAg negative HIV-VL < 100,000 copies/mL At bed time or 2 hours before dinner	I (ABC: HLA-B*57:01, cardiovascular risk) IX (EFV: suicidality. HIV-2 or HIV-1 group 0)
TAF/FTC or TDF/FTC or TDF/3TC + EFV TDF/FTC/EFV	At bed time or 2 hours before dinner	II TDF: prodrug types. Renal and bone toxicity. TAF dosing) IX (EFV: suicidality. HIV-2 or HIV-1 group 0)

Table 2: Initial Combination Regimen for ART-na ve Adult PLWH: Part 1. From: EACS guidelines 10.0, November 2019.

2 NRTIs + PI/r or PI/c		
ABC/3TC + ATV/c or ATV/r	HLA-B*57:01 negative HBsAg negative HIV-VL < 100,000 copies/mL Not on proton pump inhibitor With food	I (ABC: HLA-B*57:01, cardiovascular risk) X (ATV/b & renal toxicity)
ABC/3TC + DRV/c or DRV/r	HLA-B*57:01 negative HBsAg negative With food	I (ABC: HLA-B*57:01, cardiovascular risk) VII (DRV/r and cardiovascular risk)
TAF/FTC or TDF/FTC or TDF/3TC + ATV/c or ATV/r	Not on proton pump inhibitor With food	II (TDF: prodrug types. Renal and bone toxicity. TAF dosing) X (ATV/b: renal toxicity)
Other combinations		
RAL 400 mg bid + DRV/c or DRV/r	HBsAg negative HIV-VL < 100,000 copies/mL CD4 > 200 cells/µL With food	VII (DRV/r: cardiovascular risk)

Table 3: Initial Combination Regimen for ART-na ve Adult PLWH: Part 2.
From: EACS guidelines 10.0, November 2019.

1.3.1 Mutations of the viral genome

One of the main reasons why HIV became a worldwide pandemic is its genetic variability due to continued mutation, which immensely complicates treatment. Retroviruses have very high mutation rates of about 1 spontaneous mutation per replication cycle, mainly because of their poor fidelity of reverse transcriptase. This error-prone enzyme activity is beneficial for the virus in achieving optimal evolution and spreading, and causes a heterogeneous and ever changing genetic population. This leads to accelerated drug resistance due to favorable mutations, which even can be passed from one patient to another.

Mutations under antiviral therapy occur most when the replication cycle is not fully suppressed but the virus is under selective pressure. Such mutations are linked to every class of antiretroviral drugs. Therefore HIV-1 drug resistance testing is required for therapeutic management of PLWH and is done simultaneously with initiating treatment for ART-naïve patients, and when patients under ART have a detectable viral load in plasma (43,44).

Viral mutations also provide a better understanding of HIV-1 and it's transmission dynamics and epidemiology. Sequence similarities in the viral genome can be compared to either a phylogenetic tree or a sequence alignment. These alignments make transmission events traceable as the viral genome of the newly infected person is partly matching with the viral genome of the person who transmitted the

virus in a nonrandom manner. Phylogenetic analysis combined with clinical and epidemiological data can be used to identify how HIV transmission occurs. More specifically, it is used to analyze how virus lineages are mixed or stay restricted in various subpopulations with for example a different demographical background. DNA sequencing of the viral genome is used to identify transmission clusters, which are highly relevant for public health (45).

2 Objectives

The aim of this study was to identify transmitted HIV-1 DRMs against DTG, 3TC, and RPV. As ART should start as soon as possible after the diagnosis of an infection with HIV-1 and ideally on the same day, the local prevalence of drug resistance mutations is of interest to ensure the efficacy of initial combination regimens, as the individual results of drug resistance testing are not available immediately. Furthermore, the local transmission network of HIV-1 in South-East Austria, which includes an area with more than one million inhabitants, was reconstructed as nationwide assessments of drug-resistance mutations (DRM) are not available.

3 Materials and Methods

This study was conducted in cooperation with the Division of Infectious Diseases, University of California San Diego (UCSD), United States, and according to the principles expressed in the Declaration of Helsinki.

3.1 Study population

In this study, 192 HIV patients living in South-East Austria were included. They had been newly diagnosed with an HIV-1 infection between 2013 and 2018. 90 patients were diagnosed between 2013 and 2015 and 102 patients between 2016 and 2018.

HIV-1 diagnosis included testing on HIV-1 RNA concentration in plasma and resistance testing. HAART was immediately initiated and the plasma HIV-1 RNA concentration was frequently monitored with nucleic acid amplification testing (NAT). The Molecular Diagnostics Laboratory at the Diagnostic and Research Center for Molecular Biomedicine Institute of the Medical University of Graz is the only laboratory conducting HIV NAT in this area, a region with more than one million inhabitants.

All patient-related data was gathered in a de-identified manner, only linked to the 4-digit zip code area of residence, and then associated with the unique HIV sequence of each individual patient. Data included sex, date of birth, date of diagnosis, and residential area for all subjects. There were two sex categories, male and female. Thus, all data presented are not attributable to individual patients.

3.2 Sequence analysis and HIV network analysis

Sequences of circulating RNA from plasma were generated with conventional Sanger sequencing. Analysis was performed using partial HIV-1 pol sequences including the regions encoding reverse transcriptase (RT) and integrase (IN). Sequencing data of the RT and IN genes was uploaded to the Stanford University HIV Drug Resistance Database (46) and therefore is publicly available. Screening

analysis for DRMs including DTG, 3TC, and RPV was done according to the Stanford University Genotypic Resistance Interpretation (47).

The genetic transmission network was identified on the basis of partial pol sequences. The molecular network analysis was carried out to identify presumed linkages between the sequences and performed distance based as previously described (48). Shared DRMs were designated as DRMs present in individuals with genetic linkage.

4 Results

At the time of HIV-1 diagnosis the mean age of patients was 39 (range of 18 to 66 years). The majority of patients (75.5%; 145/192) were male.

In the following chapter all mutations detected in the screening analysis will be listed. However, not all DRMs listed are clinically relevant and could lead to a resistance against DTG, 3TC or RPV.

4.1 Prevalence of drug resistance mutations

Of 192 patients, 12 (6.3%) showed a DRM against DTG (Table 4). Eleven of them had a single mutation, while one patient showed 2 DRMs regarding DTG. The E157Q DRM was detected in 7 patients, T97A in 4 patients, T66I and G140S in one patient each.

Table 4: Drug resistance mutations against DTG found in 192 HIV-1 positive patients living in South-East Austria.

Number of patients having DTG DRM	Percentage	DRM
7	3.6	E157Q
4	2.1	T97A
1	0.5	T66I
1	0.5	G140S

Of 192 patients, 8 (4.2%) showed a DRM against 3TC (Table 5). Five of them harbored a single mutation, while 3 patients had 2 DRMs against 3TC. The M184V DRM was detected in all patients, 2 patients additionally showed the K65R DRM, 1 patient additionally the L74V DRM.

Table 5: Drug resistance mutations against 3TC found in 192 HIV-1 positive patients living in South-East Austria.

Number of patients having 3TC DRM	Percentage	DRM
8	4.2	M184V
2	1.0	K65R
1	0.5	L74V

Of 192 patients, 41 (21.4%) patients showed a DRM against RPV (Table 6). Thirty-nine of them obtained a single mutation, while in two patients 2 DRMs against DTG were observed. The E138A/K DRM was detected in 15 patients, the V179D/E DRM in 9 patients, the K101E/P DRM in 8 patients, the V106I DRM in 7 patients, the Y181C DRM in 3 patients and the H221Y DRM in one patient.

Table 6: Drug resistance mutations against RPV found in 192 HIV-1 positive patients living in South-East Austria.

Number of patients having RPV DRM	Percentage	DRM
15	7.3	E138A/K
9	4.7	V179D/E
8	4.2	K101E/P
7	3.6	V106I
3	1.6	Y181C
1	0.5	H221Y

4.2 Drug resistance mutations and susceptibility of DTG, 3TC and RPV

Of 192 patients, 12 (6.3%) showed a DRM against DTG. The E157Q DRM that was most frequently observed (7 times) in this study was found in about 2-5% of viruses from untreated persons in the United States (49). The mutation results in minimal changes in DTG susceptibility and is not relevant for INSTI therapy if occurring alone (50). The T97A DRM, which was found in 4 patients in this study, occurs in about 1-4% of viruses from ART-naïve patients living in the United States (49). The susceptibility of DTG is not relevantly changed if the mutation occurs alone. However, if this mutation appears paired with another INSTI-resistance mutation, effectiveness is markedly reduced (51). In this study, a combined INSTI-resistance mutation T97A and T66I was found in one patient. The G140S DRM occurred in one patient in this study. Alone, it does not reduce the susceptibility of DTG, only if combined with another INSTI-resistance mutation, which was not the case in this patient (52).

Thus, only one patient showed a clinically relevant resistance against DTG (Table 7). Consequently, one out of 192 patients (0.5%) should not be treated with a two-drug regimen including DTG.

Table 7: DRMs against DTG found in this study. The clinically relevant resistance is colored red.

Number of patients	DRM
7	E157Q
3	T97A
1	T97A, T66I
1	G140S

Of 192 patients, 8 (4.2%) showed a DRM against 3TC. In all of them, the M184V DRM, which reduces 3TC susceptibility >100-fold, was observed. This mutation is responsible for treatment failure regarding 3TC in most patients (53). In this study, two DRMs were observed additionally to M184V. The K65R DRM, which was found in two patients, reduces susceptibility 5-10-fold when appearing alone but is highly

clinically relevant when appearing together with M184V (54). The L74V + M184V DRM was found in only one patient. This combination has been reported to occur most commonly in patients who received 3TC (55).

Consequently, all of the observed DRMs against 3TC were clinically relevant resistance mutations in this study (Table 8).

Table 8: DRMs against 3TC found in this study. The clinically relevant resistances are colored red.

Number of patients	DRM
5	M184V
2	M184V + K65R
1	M184V+ L74V

Out of 192 patients, 41 (21.4%) showed a DRM against RPV. The E138A/K DRM occurred most frequently (15 times), followed by the V179D DRM (9 times). According to the current version of the Stanford University HIV Drug Resistance Database, the E138A/K DRM has been classified as a low-level resistance while the V179D DRM has been classified as a potential low-level resistance. The V106I DRM, which was found in 7 patients, is believed to have little effect on NNRTI susceptibility. In combination with the V179D mutation, it may have a clinically relevant effect on RPV but in this study no combination was observed. The K101E and the K101P DRMs were found in 4 patients each. While the K101E DRM alone reduces RPV susceptibility only about 2-fold, K101P reduces it by >50-fold (56,57). One patient showed the K101E and the E138A DRMs in combination, which may decrease susceptibility markedly. The Y181C DRM was found in 3 patients, reducing susceptibility of RPV about 3-fold (58). The H221Y DRM occurred only once in this study and in combination with Y181C, which is a common mutation pattern. In this combination it is of clinical relevance for ART (59).

Consequently, 25 (13.0%) patients harbored a clinically relevant DRM against RPV (Table 9).

Table 9: DRMs against RPV found in this study. The clinically relevant resistances are colored red.

Number of patients	DRM
14	E138A/K
9	V179D
7	V106I
4	K101P
3	K101E
2	Y181C
1	K101E, E138A
1	Y181C, H221Y

Summarizing, out of 192 patients studied, 30 (15.6%) had DRMs of clinical relevance against DTG, 3TC, and/or RPV before initiating ART. Of 30 patients, 26 (86.7%) showed DRMs against one drug class, whereas 4 (13.3%) patients showed DRMs against two drug classes, all of whom showed 3 DRMs (Table 10). DRMs against all three drug classes or more than 3 DRMs per patient were not observed in this study.

Table 10: DRMs against two drug classes. The clinically relevant resistances are colored red.

	INSTI (DTG)	NRTI (3TC)	NNRTI (RPV)
3 DRMs		M184V, L74V	Y181C
		M184IV, K65R	E138A
		M184V	Y181C, H221Y
	T66I, T97A	M184V	

The prevalence of DRMs in patients diagnosed between 2013 and 2015 was compared to that in patients who were diagnosed between 2016 and 2018. 15/90 (16.7%) patients diagnosed with HIV-1 between 2013 and 2015 had DRMs against DTG, 3TC, and/or RPV before initiating ART. Corresponding data for 2016 to 2018 were 15/102 (14.7%). No significant divergence was observed.

4.3 Transmission network analysis

Transmission network analysis revealed 42.7% (82/192) genetically linked individuals, who are forming 26 clusters, varying between 2 to 10 patients per cluster (Figure 6). The frequency of clinically relevant DRMs differed between clustering (22.0%, 18/82) and non-clustering (10.9%, 12/110) individuals, and was significantly higher with p=0.045 (two-tailed Fishers exact test) among clustering individuals. Consequently, 60% (18/30) of individuals showing one or more clinically relevant DRMs against DTG, 3TC and/or RPV were part of 6 different clusters. Of the 18 DRMs found in clustering individuals, 15 (83.3%) were found in genetically linked partners.

Clustering individuals were categorized into two subgroups, which differed in date of diagnosis. Patients who were diagnosed between 2013 and 2015 showed a frequency of clinically relevant DRMs of 23.5% (8/34), while patients who were diagnosed between 2016 and 2018 had 20.8% (10/48). These two subgroups showed no significant change.

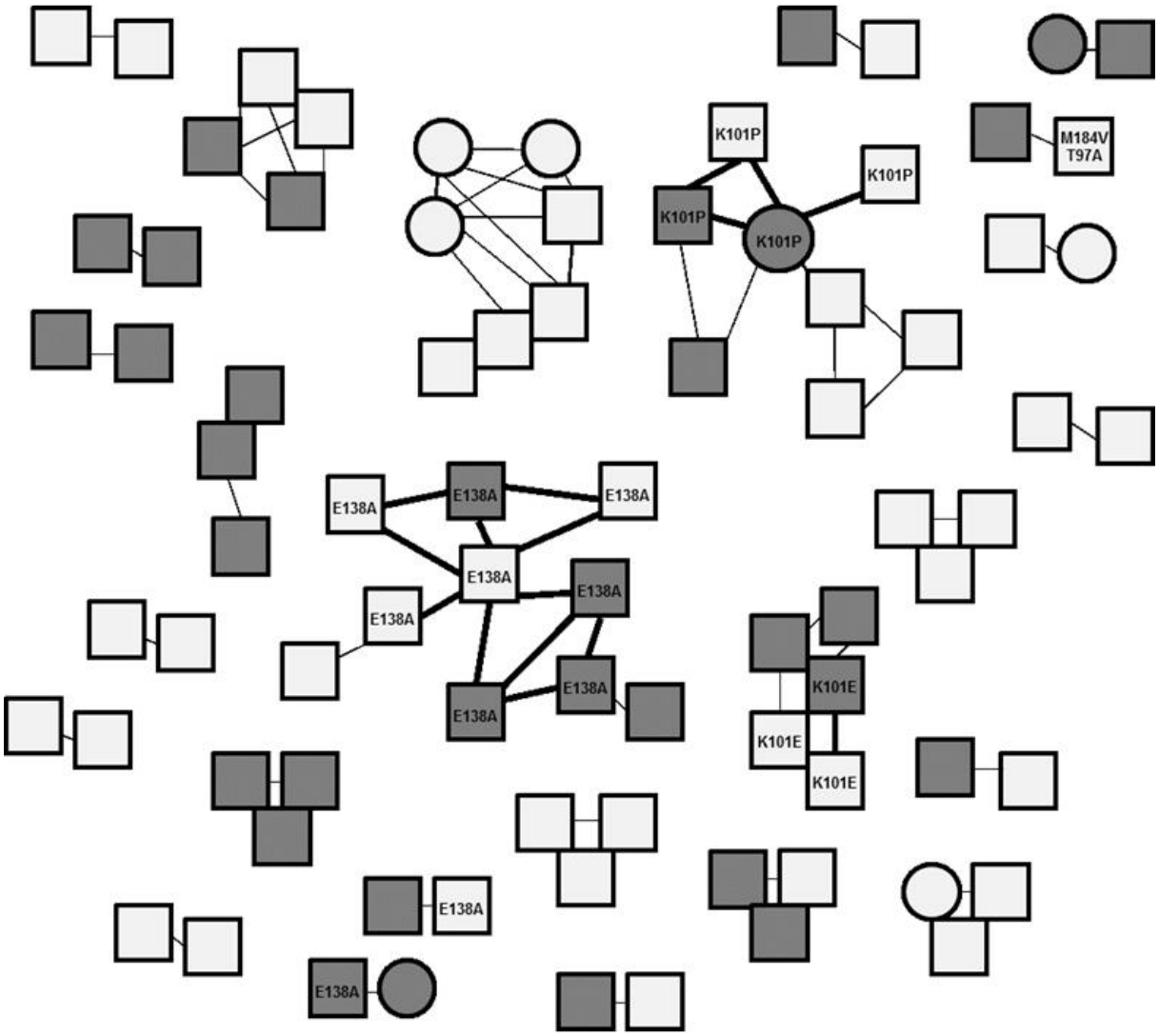

Figure 6: Transmission clusters. Thin lines represent presumed transmission linkages due to sequence similarities, bold lines represent shared DRMs. Only clustering patients are shown. Squares represent men, circles represent women. The transmission network is colored according to time of HIV diagnosis: dark gray for 2013 to 2015, light gray for 2016 to 2018. From: Kessler HH et al. “Antiretroviral Treatment Simplification With 2-Drug Regimens: Impact of Transmitted Drug Resistance Mutations“. Open Forum Infectious Diseases. 2019;7(1).

5 Discussion

Improvement of antiretroviral therapy in the last years has led to a higher quality of life for people living with HIV. Nevertheless, HIV patients need lifelong treatment, as no cure has been found yet. To lower side effects and toxicities, ways of reducing long-term drug exposure have become of increasing interest. Among those, two-drug regimens have been introduced. The aim of this study was to determine transmitted DRMs against the drugs currently used in two-drug regimens by conventional Sanger sequencing. Furthermore, the local transmission network in South-East Austria was reconstructed. Results may contribute to the better understanding of HIV-1 in this region and may be of great importance to ensure an efficient initiation of ART.

In this study, almost 16% of ART-naïve patients had clinically relevant DRMs against DTG, 3TC and/or RPV. However, only one (0.5%) patient had a clinically relevant resistance against DTG and therefore would not be eligible for treatment with a two-drug regimen including DTG. Eight (4.2%) patients had a DRM against 3TC. At this point it should be mentioned that these patients never took pre-exposure prophylaxis (PrEP) as they had been infected with HIV-1 before 2018, when PrEP was first introduced in this region. Twenty-five (13.0%) patients had a clinically relevant DRM against RPV. The high rate of DRMs against RPV may be the consequence of common RPV use in antiretroviral regimens in this region.

The majority of patients showed clinically relevant DRMs against one drug class (26/30), 4 patients showed DRMs against two drug classes. Three of them showed DRMs against 3TC and RPV, one of them against DTG and 3TC. Consequently, only 2% of all patients studied were not eligible for neither of the currently recommended two-drug regimens. This indicates that there may be no contraindication to start with these dual therapy regimens in ART-naïve patients with pending results of drug resistance testing. The two-drug regimen with DTG/3TC should be preferred to the combination of DTG/RPV because of the high rate of DRMs against RPV in South-East Austria.

Transmission network analysis revealed 82 genetically linked individuals, which were part of 26 clusters. Of those 82 individuals, 18 (22.0%) showed clinically relevant resistances, while only 12 (10.9%) of 110 non-clustering patients showed clinically relevant resistances. In comparison, the frequency of DRMs in the group

of clustering patients was found to be significantly higher. Six clusters included patients with clinically relevant DRMs against DTG, 3TC, and/or RPV. Of the 29 individuals forming these 6 clusters, 15 (51.7%) showed shared resistances. Consequently, the mutations leading to drug resistance were very likely transmitted from one person to another within the cluster, rather than occurring spontaneously. Individuals, who are part of a cluster or entering a cluster, are thus at an elevated risk for receiving mutated HIV-1 strains. This could make antiretroviral drugs ineffective sooner than expected.

To investigate the spread of drug resistances, longitudinal studies are suggested including other geographical areas with larger populations. With a transmission network analysis on larger scale, correlations found in this study could be validated, risk factors could be revealed and preventive measure could be set up. As most of the patients included in this study were male (75.5%; 145/192), it may be assumed that the majority of them belongs to the group of men having sex with men, as investigated recently in the same region [59].

Limitations of this study include the relative small number of patients included in a relatively small region with low HIV-1 prevalence and its single center design. Furthermore, in this study only DRMs against three antiretroviral drugs were investigated. The general resistance situation regarding HIV patients might be significantly worse. Thus, different ART prescribing patterns in other geographical areas should be studied to verify the results from this study. Moreover, this study did not investigate risk factors specific for transmitted drug resistance such as age, sexual orientation, or race (60). Finally, it should be investigated if the same risk factors are responsible for transmission of DRMs in Europe.

In this study, the percentage of clinically relevant DRMs against drugs used in two-drug regimens currently recommended was low. With an identical antiviral efficacy as conventional three-drug regimens, patients under these new regimens may benefit due to the reduced drug exposure with less side effects and less long-term toxicities. Furthermore, compliance may be increased as novel two-drug coformulations are available with a single tablet taken once daily. Finally, the costs for lifelong maintenance HIV therapy could be lowered.

In conclusion, this study contributed to the understanding of local drug resistance regarding current antiretroviral two-drug regimens and has shed some light on the role of DRMs against DTG, 3TC, and/or RPV in the transmission network. Of all ART-naïve patients studied, 16% showed any clinically relevant DRM against DTG, 3TC, and/or RPV. The prevalence of these DRMs was significantly higher in clustering patients when compared to non-clustering individuals. Within clusters, the majority of DRMs were shared DRMs, indicating an elevated risk of transmission of resistant HIV-1 strains for patients entering those clusters. Longitudinal studies investigating the spread of DRMs against DTG, 3TC, and/or RPV in this population are suggested. Although 16% of the patients studied showed any clinically relevant DRM against DTG, 3TC, and/or RPV before initiating ART, only 2% were not eligible for at least one of the currently suggested two-drug regimens.

6 Annotation

This study was accepted for publication: Kessler HH, Stelzl E, Blazic A, Mehta SR, Benezeder AS, Genger-Hackl C, Santner BI, Chaillon A and Hoenigl M. Antiretroviral Treatment Simplification With 2-Drug Regimens : Impact of Transmitted Drug Resistance Mutations. Open Forum Infectious Diseases. Published online 2019 Dec 18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6942491/

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