Diploma thesis

HIV-1 SUBTYPES AND TRANSMISSION OF PRIMARY DRUG RESISTANCE IN SOUTHERN AND EASTERN AUSTRIA

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Graz, 18.12.2009 Anna-Maria Sajovitz

Declaration of originality

I hereby declare that this diploma thesis and the work reported herein was composed and originated entirely by myself.
Furthermore, I confirm that no other sources than those acknowledged in the text have been used in the preparation of this thesis and are all mentioned in the list of references.

Graz, 18.12.2009 *Anna-Maria Sajovitz*

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Dedicated to

Mbali, Beniah, Callie, Tholakele, Sibusisiwe, and Siphelsihle

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List of abbreviations

AIDS	Acquired Immunodeficiency Syndrome
CDC	Centers for disease control
cDNA	Complementary Deoxyribonucleic Acid
CRF	Circulating Recombinant Forms
CYP	Cytochrome P 450
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbant Assay
FDA	Food and Drug Administration
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
LAS	Lymphadenopathy Syndrome
MHC	Major Histocompatibility Complex
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PCP	Pneumocystis carinii pneumonia
PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SFV	Simian Foamy Virus
SIV	Simian Immunodeficiency Virus
UNAIDS	Joint United Nations Program on HIV and AIDS
USA	United States of America
WHO	World Health Organization

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1. Abstract

1.1. English

Background: In Austria, the majority of HIV-1 isolates belong to subtype B; however, non-B subtypes may be more prevalent recently due to migration. Additionally, an increased risk for transmission of drug resistant HIV-1 may be observed.

Objectives: To evaluate the prevalence of HIV-1 subtypes in Southern and Eastern Austria. To evaluate the presence of relevant HIV-1 drug resistance in drug-naïve patients. To compare data obtained within a 5-years interval.

Materials and methods: HIV-1 sequences obtained from 54 drug-naïve patients in 2002 were matched with those obtained from another 54 drug-naïve patients in 2007. Automated RNA extraction was done with the AmpliPrep instrument (Roche Molecular Diagnostics). Amplification and sequencing were performed with the TruGene HIV-1 Genotyping Kit (SIEMENS medical) according to the manufacturer's package insert instructions. Sequences were analyzed with the GeneLibrarian module of GeneObjects software (version G12). HIV-1 subtypes were determined by means of the geno2pheno database (www.geno2pheno.org).

Results: Subtype B was found to be the predominant subtype in 2002 (77.8%) and in 2007 (70.4%). In 2002, subtypes CRF01_AE (7.4%), A (5.6%), C, F, G, CRF08_BC, and CRF10_CD (1.9% each) were found additionally. Corresponding data for 2007 were D (9.3%), F (7.4%), CRF02_AG (3.7%), A, G, CRF01_AE, CRF09_cpx, and CRF14_BG (1.9% each). Prevalence of transmitted drug resistance was 20.4% for any drug in 2002 and 22.2% for any drug in 2007. The majority of drug resistance was found for protease inhibitors.

Dual class resistance was observed in 1 patient in 2002 and in 2 patients in 2007. Triple class resistance was not found in any patient.

Conclusions: HIV-1 subtype B is still dominant in Southern and Eastern Austria. The percentage of non-B subtypes and recombination forms is increasing slightly. The transmission rate of drug-resistant HIV-1 has been stable within a 5-years interval.

1.2. German

Hintergrund: In Österreich kann die Mehrheit der analysierten HIV-1 Isolate dem HIV-1 Subtyp B zugeordnet werden. In letzter Zeit könnten Non-B Subtypen migrationsbedingt gehäuft auftreten. Ein erhöhtes Risiko für die Übertragung von therapieresistenten HIV-1 Stämmen wird ebenfalls vermutet.

Studienziele: Die Evaluierung der Prävalenz von HIV-1 Subtypen in Süd- und Ost-Österreich. Die Beurteilung des Auftretens von relevanter HIV-1 Therapieresistenz in unvorbehandelten Patienten. Der Vergleich von Daten innerhalb eines 5-Jahres-Intervalls.

Methoden und Materialien: HIV-1 Sequenzen wurden von 54 unvorbehandelten Patienten 2002 und 54 unvorbehandelten Patienten 2007 bestimmt und miteinander verglichen. Automatische RNA-Extraktion wurde mit dem AmpliPrep instrument (Roche Molecular Diagnostics) durchgeführt. Amplifikation und Sequenzierung wurden unter Befolgung der Gebrauchsanweisung mittels des TruGene HIV-1 Genotyping Kit (SIEMENS medical) durchgeführt. Die Sequenzen wurden mit der GeneLibrarian module of GeneObjects Software (version G12) analysiert. Die HIV-1 Subtypen wurden mittels der geno2pheno database (www.geno2pheno.org) bestimmt.

Resultate: Es stellte sich heraus, dass Subtyp B der vorherrschende Subtyp in 2002 (77.8%) und 2007 (70.4%) war. 2002 wurden zusätzlich die Subtypen CRF01_AE (7.4%), A (5.6%), C, F, G, CRF08_BC, und CRF10_CD (jeweils 1.9%) gefunden. Die Vergleichswerte von 2007 ergaben folgende Subtypen-Prävelenzen: D (9.3%), F (7.4%), CRF02_AG (3.7%), A, G, CRF01_AE, CRF09_cpx, und CRF14_BG (jeweils 1.9%). Die Prävalenz der übertragenen therapieresistenten Stämme betrug 20.4% für jegliches Medikament im Jahr 2002 und 22.2% für jegliches Medikament im Jahr 2007. Der Hauptanteil der

primären Resistenzen wurde für die Klasse der Proteaseinhibitoren gefunden. Zweiklassenresistenz wurde bei einem Patient in 2002 und bei 2 Patienten in 2007 entdeckt. Es wurden keine Dreiklassenresistenzen gefunden.

Konklusion: HIV-1 Subtyp B ist noch immer der vorherrschende Subtyp in Süd- und Ost-Österreich. Der Prozentsatz von Non-B Subtypen und rekombinanten Formen ist leicht angestiegen. Die Übertragung von resistentem HIV-1 blieb innerhalb eines 5-Jahres Intervalls stabil.

2. Introduction

Ever since its detection in 1983, the human immunodeficiency virus (HIV) has become one of the world's most important health issues. The acquired immunodeficiency syndrome (AIDS), the final stage of the HIV-infection, is causing millions of deaths all around the world every year.
A cure or vaccination is not available yet but the improvement of antiretroviral treatment, if available, has made the HIV-infection manageable over decades. In the poorer and most affected regions of the world, especially in Sub-Saharan Africa, HIV/AIDS still remains a deadly disease as treatment is very cost expensive and therefore often not available.
One of the major problems of HIV-infection is that it does not cause symptoms over a long period, so people are not aware of their HIV infection. It is therefore important to indentify infected people and provide them with quick and effective antiretroviral treatment if necessary. Antiretroviral therapy is not only able to transform the affected people's deadly infection into a manageable one but also tremendously reduces the risk of perinatal and sexual transmission of the HIV. Today, "The Universal Access" to antiretroviral treatment by 2010 (set by the World Health Organization, WHO) is far from being achieved.

The introduction of antiretroviral treatment has brought along several challenges despite of its positive effects for the individuals affected. The HIV can mutate very fast when exposed to antiretroviral pressure which usually reduces viral replication significantly. If not almost entirely suppressed, the HIV quickly finds ways to escape from the suppressive effects of the drugs. Insufficient antiretroviral therapy may cause mutations in the HIV genome. Mutated viruses may be transmitted and may lead to *primary* or *transmitted drug resistance* in antiretroviral drug-naïve HIV infected patients. Routine drug resistance testing in the earliest suitable sample is thus highly recommended because infection with drug-resistant HIV may result in an unfavorable response to antiretroviral

standard therapy. Furthermore, the efficiency of post exposure prophylaxis may be limited and vertical transmission may occur more likely in children of mothers infected with a drug-resistant HIV-1 virus.[1]

Drug resistant HIV may be transmitted through different ways including unprotected hetero- and homosexual contact, intravenous drug abuse, and vertical transmission. However, drug resistance may not only be present in antiretroviral drug-naïve HIV patients but more likely be the result of insufficient antiretroviral therapy. In most studies, it is unclear if the drug resistance is really a primary one because those studies lack detailed information about the individuals included.

Determination of the genetic subtype of human immunodeficiency virus type 1 (HIV-1) has shown to be an important tool in analyzing the global pandemic of the virus.[2] Subtype B has been the most common HIV-1 subtype in Europe and Northern America.

This diploma thesis provides data about the present subtypes of HIV-1 and information on the situation concerning primary drug resistance in Southern and Eastern Austria.

[1] Kronawetter M. 2008, p15-16
[2] Kessler H.H et al. Sept. 2001, pp1018-1020

2.1. The History and origin of HIV/AIDS

2.1.1. Introduction

In the early 1980s an accumulation of rare, therapy-refractory opportunistic infections as well as cancers like Kaposi sarcoma were observed in the gay community of California and attracted the attention of doctors and scientists. AIDS did not have its name yet, but it was quite clear, that all of those patients seemed to suffer from the same syndrome.

Soon after, the Human Immunodeficiency Virus (HIV) was discovered and more explored. It became clear that HIV was the cause of the Acquired Immunodeficiency Syndrome (AIDS). Today, most of the scientists agree over this fact and what begun as "gay cancer" in the USA has now grown to one of the most important health issues worldwide, affecting heterosexuals and children as well as the gay community or injecting drug users.

The following pages will give a brief overview over the timeline of HIV/AIDS:

2.1.2. Timeline of HIV/AIDS [3, 4, 5]

1959 – Congolese man

HIV as well as AIDS was discovered in the early eighties, but it seems to be a lot older. The earliest report of a young man dying from a mysterious illness which resembled a lot to what we know as AIDS, dates back to 1956. This case seems to be the first documented AIDS patient in history. He was an African resident living in Leopoldville, Belgian Congo (nowadays Kinshasa, Democratic Republic of Congo), and years after his death, his preserved blood samples were proven to be contaminated with HIV.

[3] http://aids.about.com/od/newlydiagnosed/a/hivtimeline.htm, accessed 24th of November 2009
[4] http://www.avert.org/origin-aids-hiv.htm, accessed 24th of November 2009
[5] Kronawetter M. 2008, pp5-6

1960: Congolese Woman

In 1960 a woman, also living in Leopoldville, Belgian Congo underwent a lymph node biopsy which was later on proved to be contaminated with HIV-1.

1969: Robert R.

Robert R., a 15 year old African American died at the St. Louis City Hospital, USA in 1969, after suffering from extremely aggressive Kaposi Sarcoma. In 1987, it was confirmed that the Kaposi Sarcoma was very likely to be caused by AIDS. Scientists at the Tulane University School of Medicine were able to isolate HIV-1 in his preserved blood samples. Doctors who treated the patient suspected him to be a male prostitute, although the patient himself never confirmed this fact.

1969: Arvid Noe

In 1969 Arvid Noe, a Norwegian sailormen, presented symptoms similar to AIDS. Eight years before, he was working along the West African coast were he got infected with gonorrhea. This shows that he was sexually active during his work-trip to Africa. Finally in 1976, he, his wife and their nine-year old daughter died of what we call nowadays AIDS and samples from Arvid Noe and his wife were tested HIV-1 positive in 1988.

Late 1960s, early 1970s

During this time HIV has definitely arrived on the northern hemisphere. It is not sure how it exactly came to the USA, but the most likely theory is that it spread from Western Africa over Haiti to the northern hemisphere. By the time of the first reported cases in the US, the prevalence of AIDS in the above mentioned regions has already been up to 5%, which supports this propagation path.

Canadian flight attendant theory

Dr. William Darrow at the Centers for Disease Control (CDC) referred Gaetan Dugas, a Canadian airline steward, as “patient 0” in an early AIDS study. It is

known, that 40 of the 248 known AIDS cases in the USA in 1983 have had sexual intercourse either with Dugas himself or with someone who had sex with him. Still this theory is not very likely as HIV had already spread long before Dugas was sexually active, so he could not be the only source for HIV/AIDS in the northern hemisphere. Today a lot of scientists agree that HIV already arrived in Northern America in the late 1960s due to the immigration. These individuals responsible for the spread of HIV are likely to have migrated from Haiti, after working in the Democratic Republic of Congo. Also any other person who had worked in the Congo-region before could have brought HIV to the USA.

1981-1982: AIDS

In 1981 an upcoming of rare diseases was recorded in the US. Kaposi's sarcoma, a rare skin cancer, was diagnosed in at least 8 patients in March of 1981 in New York. All of them belonged to the gay community and the form of their Kaposi Sarcoma seemed to be a very aggressive one.

Also in 1981, an increase of atypical lung infections was monitored in both, New York and California. The patients were also homosexual men and suffered from Pnemocystis carinii pneumonia (PCP), today also known as Pnemocystis jirovecii pneumonia which is caused by the fungus *Pneumocystis jirovecii*. In April the drug technician Sandra Ford noticed an unusual high number of requests for the drug *Pentamine*, which was used to treat PCP:

> *"A doctor was treating a gay man in his 20s who had pneumonia. Two weeks later, he called to ask for a refill of a rare drug that I handled. This was unusual - nobody ever asked for a refill. Patients usually were cured in one 10-day treatment or they died"* (Sandra Ford for Newsweek)[6]

The CDC finally noticed this increase of lung infection and in June a report was published, informing about five men living in Los Angeles who suffered from

[6] Daniel McGinn, 'MSNBC: AIDS at 20: Anatomy of a Plague; an Oral History', Newsweek Web Exclusive

PCP. At this time no identifiable cause was visible for the recent increase of this disease.
A couple of days after publishing the report a task force group, called the KSOI (Kaposi Sarcoma and Opportunistic Infections) was formed to discover what caused these deadly conditions.
It seemed that there was a new disease to be discovered, but at the same time very little was known about the transmission or contagiousness. Also it was thought to be a condition only affecting homosexual men and the New York Times even quoted Dr. Curran who was working on this issue:

> *'The best evidence against contagion', he said, 'is that no cases have been reported to date outside the homosexual community or in women'"* (The New York Times)[7]

So the disease finally found a name: GRID, which stands for "Gay-related immune deficiency".
In December 1981, the first cases of PCP occurring in injecting drug users were reported and it became clear that the disease was not only affecting homosexual communities. The same month, the disease seemed to have reached Europe - the first case was reported in the UK.
By the time not only injecting drug users and homosexuals were affected by the immune deficiency, but also heterosexuals and hemophiliacs and Haitian Immigrants. Therefore in August 1982, the GRID was renamed into AIDS, which stands for "Acquired Immunodeficiency Syndrome" or "Acquired Immune Deficiency Syndrome".

1983-1984: HIV

At the Pasteur Institute in France, a retrovirus was found that researchers believed to be the cause of AIDS. A little later Dr. Robert Gallo also isolated a retrovirus that he named HTLV-III and announced that it is related to AIDS.

[7] Altman L.K. 1981

Those two viruses turned later out to be exactly the same virus and an international committee of scientists renamed the virus HIV.
During those two years another 33 countries announced cases of AIDS.

1985-1986: First tests

Dr. Robert Gallo's laboratory patented the first approved HIV test kit. The Pasteur Institute on the other hand claimed its rights to this virus and wanted half of the royalties from this new test. But still AIDS rested the "gay cancer" in the eye of the society. A comment of Jonathan Man kind of summarizes those early years of HIV/AIDS:

> *"The dominant feature of this first period was silence, for the human immunodeficiency virus (HIV) was unknown and transmission was not accompanied by signs or symptoms salient enough to be noticed. While rare, sporadic case reports of AIDS and sero-archaeological studies have documented human infections with HIV prior to 1970, available data suggest that the current pandemic started in the mid- to late 1970s. By 1980, HIV had spread to at least five continents (North America, South America, Europe, Africa and Australia). During this period of silence, spread was unchecked by awareness or any preventive action and approximately 100,000-300,000 persons may have been infected."*
> (Jonathan Mann)[8]

Only when celebrities like Rock Hudson begun to die of AIDS, the public finally started to draw a little attention to the disease. Also, the school boy Ryan White was expelled from elementary school in Indiana in 1985 because of being HIV positive. He was a hemophilic and received factor VIII transfusions every day, which were the source of his infection. His family sued the school and Ryan White was finally entitled by the court to continue his education. Before his death in 1990 he had become a prominent young activist and spokesperson for people affected with HIV/AIDS.

[8] Mann JM. 1989

1987 – Fist treatment

In the early 1980ies little could be done for the patients suffering from AIDS. Doctors basically had to watch their patients die. After those very frustrating years for both, doctors and patients, suddenly there was hope. *Retrovir (Zidovudine)* was approved for therapy and was given in high doses to HIV-positive people.

Ronald Reagan and the world leaders realized that HIV/AIDS had become a serious problem all over the world and public finally started to draw more attention to the pandemic. At this time about 100 000 - 150 000 cases of HIV and AIDS were confirmed worldwide.

1990: One million affected

People living with HIV/AIDS rose to one million.

1992: Combination Therapy

In 1992 the first drug to be used in combination with *Zidovudine* was approved by the United States' Food and Drug Administration (FDA). The drug *Hivid (Zalcitabine)* marks the beginning of combination therapy.

1993: Concorde Trials

Huge confusion arose in 1993 when a British study, the Concorde Trials, proved that *Zidovudine* used as mono-therapy does not delay the progression of AIDS in asymptomatic patients. Because of the Concorde Trials the so called "re-thinker movement" was born. They did not only doubt the effectiveness of *Zidovudine*, but also the relation between HIV and AIDS.

1996: Protease Inhibitors

The introduction of the protease inhibitors (PIs) as a treatment in the fight against HIV/AIDS was another step forward. Used in combination with the already existing drugs, the PIs were proven to be effective in the therapy of affected

people. So finally from mono-therapy to triple therapy, scientists, clinicians and patients hoped to be able to eliminate HIV in the affected patients.

1997: Elimination impossible

The hopes of eliminating HIV/AIDS were destroyed in 1997, when scientists found that HIV hides in reservoirs in the body which makes total elimination of the virus impossible.

Also that year research studies were able to prove that *Zidovudine* used in pregnant HIV positive women dramatically reduced the vertical transmission of the virus.

1998-1999

First unsuccessful trials to develop a vaccine against HIV started. In effort to get drugs into the hardest affected areas of Africa, European drug companies ignored the US patent laws and started to produce generic versions of antiretroviral medication.

2000: Durban Declaration

South Africa's president Thabo Mbeki doubted the effectiveness of HIV medication and the relation between HIV and AIDS. The "re-thinker movement" got lots of attention but finally the international scientific community responded to this with the *Durban Declaration*, which offers proof that HIV and AIDS are indeed connected.

2001: Generics and entry inhibitors

In 2001 US companies dropped patent lawsuits, after scientist claimed concerns over the toxicity and effectiveness of antiretroviral drugs. This enabled the European drug companies to produce cheap generic drugs to be used in the fight against HIV/AIDS even in poor countries.

Also that year the first fusion inhibitor, *Fuzeon (Enfuvirtid)* was released as a new option of antiretroviral therapy.

In 2001 it was announced that 21 million people have died from AIDS so far, most of them (17 million) in Sub-Saharan Africa. Another 31 million were living worldwide with HIV/AIDS that year.

2004: Combination drugs

Two new combination drugs, *Truvada (Emtricitabine plus Tenofovir)* and *Epzicom (Abacavir plus Lamivudine)* were introduced in 2004, which was a relief to the patients who had to take up to 40 or more medicaments every day.

Also two new PIs were released, *Reyataz (Atazanavir)* and *Lexiva (Fosamprenavir)*. In December 2004 the first generics for antiretroviral medication were approved by the FDA and the hope for lower prices for antiretroviral medication was soon to be reached.

2005: Risk behavior

Statistics were alarmingly high in 2005. 4,9 million were newly infected with HIV and 40,3 million were worldwide living with HIV/AIDS, most of them in Sub-Saharan Africa.

The public seemed to have forgotten about HIV/AIDS and other STDs. Risky behavior as well as infection rates with all STDs including HIV/AIDS were proven to have risen recently, especially in heterosexual contacts.

2006: Origins of HIV/AIDS

In 2006 experts agreed that HIV has its origin in the jungles of Africa among wild chimps. The several existing theories about the origin of HIV/AIDS are discussed below.

2009: HIV genome

In 2009 scientists at the University of North Carolina at Chapel Hill decoded the structure of an entire HIV genome. This might affect not only HIV therapy but also prevention and education in the next years and can bring up a whole lot of new options to solve of the global epidemic of HIV/AIDS.

2.1.3. Origins of HIV/AIDS [9, 10, 11]

Scientists all over the world agree today, that HIV was passed onto humans from non-human primates in the late 19th or early 20th century in Sub-Saharan Africa. The two existing types of HIV in humans, HIV-1 and HIV-2, show similarities with lentiviruses found in primates in Africa. Those viruses are known as the Simian Immunodeficiency Virus (SIV) and are retroviruses. HIV-1, the more virulent and predominant type of HIV in humans, is closely related to *SIVcpz* which was found in chimpanzees who live in equatorial Africa. Due to the molecular phylogenetics, it is very likely that HIV-1 was passed onto humans between 1884 and 1924. The less transmittable HIV-2 has its origins in West Africa and resembles very much to the *SIVsm* found in the Sooty *Mangabey (Cercocebus atys)* a monkey who lives in Guinea-Bissau, Gabon and Cameroon. (Figure 2, page 20)

What we know today about the origin of HIV is closely related to a finding of researchers from the University of Alabama. In February 1999 they announced the detection on *SIVcpz* in a frozen plasma sample of *Pan troglodytes troglodytes (P. t. troglodytes)* a chimpanzee once common in west-central Africa. *SIVcpz* is almost identical to HIV-1. After 10 years of research they finally claimed to prove that HIV-1 has its origin in the *SIVcpz*.
They published their findings two years later in the *Nature Magazine*:[12]

- Wild chimps had been infected simultaneously with two different simian immunodeficiency viruses.
- Those two viruses formed a new virus which was able to be transmitted amongst chimps.
- This hybrid could also be passed on humans causing AIDS.

[9] http://aids.about.com/od/newlydiagnosed/a/hivtimeline.htm, accessed 24th of November 2009
[10] http://www.avert.org/origin-aids-hiv.htm, accessed 24th of November 2009
[11] Kronawetter M. 2008, p6
[12] Gao F, Bailes et al. 1999, pp436-444

The two different SIVs which formed the new SIV could be traced back to the red-capped mangabeys, which were infected with one of the two strains and to the other virus found in spot-nosed monkeys. The researchers believe that the hybridization took place inside the chimps. Those great apes got infected with both strains of SIV due to hunting and killing of spot-nosed monkeys and red-capped mangabeys. Further they claim that each group of HIV-1 (M, N and O) comes from the *SIVcpz*, representing the separate crossover-events from chimps to humans.

Methods of spread

A transfer of a pathogen from animals to humans (zoonosis), as well as the following spread amongst humans requires certain conditions:

- A human population
- A nearby populations of a host animal
- An infectious pathogen in the host animal that can spread from animal to human
- Interaction between the species to transmit enough pathogen to humans to establish a human foothold which could have taken millions of individual exposures
- Ability of the pathogen to spread from human to human (perhaps acquired mutation)
- Some method allowing the pathogen to disperse widely preventing the infection from "burning out" by either killing off its human hosts or provoking immunity in a local population of humans.

Based upon those requirements there are several theories that exist to explain how HIV could have spread to humans. The most important and most likely theory is the so called "hunter theory":[13, 14, 15]

[13] http://aids.about.com/od/newlydiagnosed/a/hivtimeline.htm, accessed 24th of November 2009
[14] http://www.avert.org/origin-aids-hiv.htm, accessed 24th of November 2009
[15] Kronawetter M. 2008, p6

The hunter theory:

The hunter theory is based on the assumption, that the virus was transmitted onto humans due to hunting, killing and eating of chimps, which in fact happened in Africa at the supposed time of transmission. Also the contact with blood even if not eating raw meat is likely if the hunter got wounded during hunting and cutting the chimps meat. If this theory is true, it would also support the fact that there are several different strains of HIV-1 existing, each being genetically slightly different. These differences could have occurred when the virus adapted to being HIV and to work as a virus in humans on different occasions.

In 2004 an article was published in The Lancet[16] in which researchers found that this mode of infection (hunters being infected with primate viruses) still seems to take place in Africa: SFV (Simian Foamy Virus), another illness that was thought to only affect primates, was found in 10 of 1099 tested individuals in Cameroon. Those discoveries led to calls for forbidding bush meat hunting, to prevent simian viruses being passed onto humans.

Most of the other existing theories are extensions to the hunter theory or speculations that do not offer proof at all.

According to the extensions of the hunter theory, there are several conditions that could have facilitated the spread of HIV in humans at those times. Those were: reuse of needles in poor African countries, immune suppression caused by harsh living conditions and urbanization in the affected regions. All of this contributed to the increased infection rates of HIV. Globalization including migration, trade and travel, provided the opportunities for the virus to spread geographically.

[16] Wolfe ND et al. 2004, p932

2.2. HIV: The Human Immunodeficiency Virus

2.2.1. Introduction

The Human Immunodeficiency Virus belongs to the genus of *Lentiviruses* in the family of *Retroviruses*. The integration of the retro-transcript of the viral RNA (provirus) is the basis for further replication. HIV attacks T-lymphocytes, monocytes, macrophages, eosinophil granulocytes, dendritic cells, and microglia cells. This causes, after a long time of incubation, a failure of the immune system, better known as Acquired Immune Deficiency Syndrome (AIDS).

2.2.2. Subtypes[17, 18]

After the isolation of HIV in 1983 by Montagnier in Paris, another HIV was found in Western Africa in 1986. The European isolate that has its origins in Central Africa was called HIV-1 and the new isolate, which genotypically and phenotypically differs from the European HIV, was called HIV-2.
The glycoproteins of the envelope are totally different for HIV-1 and HIV-2, the polymerase and the capsid antigens on the other hand are related, so HIV-1 and HIV-2 belong to the same group of viruses.

HIV-1[19]
HIV-1 is the predominant subtype in the world and accounts for 99% of worldwide HIV infections. It is classified into three groups:

- Group M, for “major”
- Group N, for “non-M, non-O”
- Group O, for “outliners”

[17] Kronawetter M. 2008, pp6-16
[18] Hahn H. et al. 2009, p521
[19] Kronawetter M. 2008, pp6-16

Group M can be divided into 11 Subtypes, A-K:[19]

- Subtype A: mainly in Africa (Rwanda, Zaire, Uganda, Kenya, Djibouti), but also in Europe
- Subtype B: the most common subtype in Western Europe, Northern America and Australia, also common in Southern America, India, Thailand and Japan.
- Subtype C: South Africa, Central Africa, Ethiopia, India, Thailand, Europe
- Subtype D: Zaire, Eastern Africa
- Subtype E: Thailand and Asia, but also India, Japan and Brazil.

Additionally, 13 Circulating Recombinant Forms (CRF) can be found. Those are recombinations from the different Subtypes A-K.
The following figure shows the worldwide prevalence of HIV-1 subtypes.

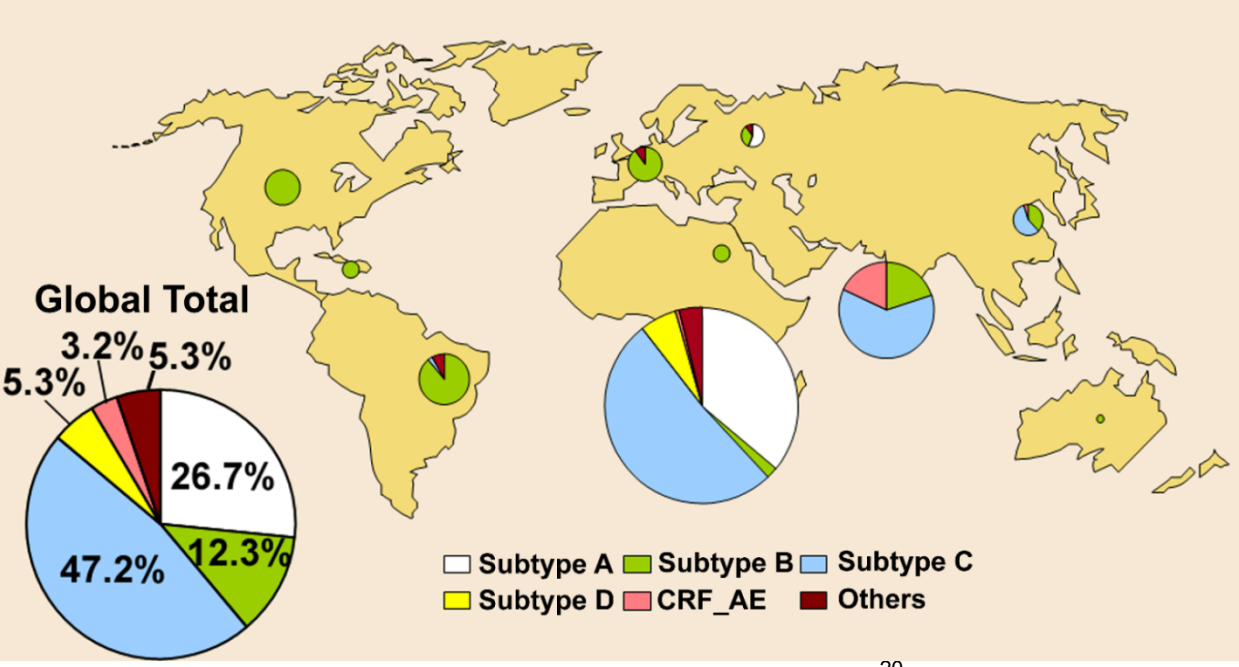

Figure 1: Prevalence of the HIV-1 subtypes[20]

In Austria, up to 30% of non-B-subtypes have been detected in certain regions, which is very likely to be caused by migration and will be discussed later.

[20] http://upload.wikimedia.org/wikipedia/commons/e/e7/HIV-1_subtype_prevalence_in_2002.png, accessed 7th of December 2009

HIV 2[21]

HIV-2 can be divided into 5 Subtypes A-E. Subtype 2A can be found worldwide, Subtype 2B mostly in Western Africa, Europe and India. Subtypes 2C, 2D and 2E have a very low prevalence and only exist in Westerns Africa so far.

Generally HIV-2 is responsible for only 1% of worldwide HIV infections and its pathogenicity is lower compared to HIV-1. The viral spread is for this reason slower, incubation time longer and perinatal transmission is under 4%.

HIV-2 has a natural resistance to *Nevirapine* and can not be detected in RT-PCR.

The following figure schematizes the different HIV subtypes including its origins:

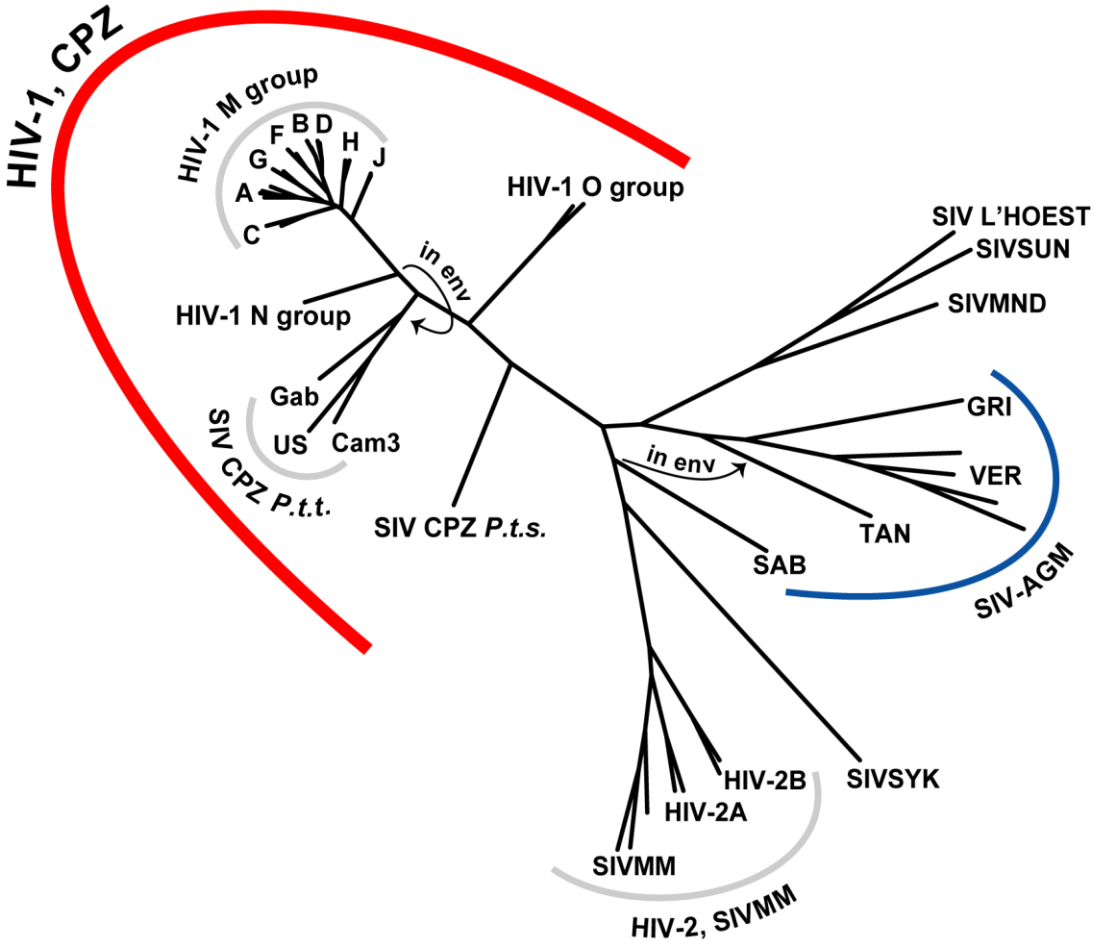

Figure 2: HIV-SIV phylogenetic tree[22]

[21] Kronawetter M, 2008, pp6-16

[22] http://upload.wikimedia.org/wikipedia/commons/2/2f/HIV-SIV-phylogenetic-tree.png, accessed 7th of December 2009

The different subtypes of HIV may have an influence on the severity and progression of the disease. For HIV-1 subtype D or recombinant forms of the HIV-1, a faster progression from infection to the onset of AIDS has been reported.[23] However, under antiretroviral therapy, there are no reports that subtypes have an influence on the clinical outcome.

2.2.3. Structure and Ethiopathology

Structure[24]

HIV is an enveloped, single-stranded RNA virus approximately 100nm in diameter. Inside the viral capsule, which is formed by the protein *p24,* are 2 identical copies of the nucleic acid bound via the molecules of *p6* and *p9*. Beside the viral genome, viral capsule contains important enzymes essential for viral replication, reverse transcriptase, integrase and protease. The inner capsid itself is surrounded by a layer of the protein *p17* that forms the isometric second capsid of the virus. HIV viral envelope contains two crucial glycoproteins, *gp120* and *gp41*, which form a complex important for viral internalization. The origin of these two glycoproteins is the precursor-molecule *gp160*. The interaction between the cellular receptors and HIV is accomplished via *gp120* (SU for surface unit), whilst the *gp41* (TM for trans-membrane) is responsible for the fusion of the viral envelope with the host cell membrane. (Figure 3, page 20)

Ethiopathology[24, 25]

T-lymphocytes, monocytes, macrophages, esosinophile granulozytes, dendritic cells, and microglia cells all expose a CD4-receptor on their surface, which makes them the target cells of HIV. HIV can bind to the Cd4-receptor via the V3 loop of its surface protein gp120. To enter the target cell, an additional co(chemokine)-receptor (either CCR5 or CXCR4) is necessary:

[23] Kiwanuka N et al, 2008; 197(6):707-13
[24] Hahn H. et al. 2009, pp521-523
[25] Troppan K. 2009, p16

- CCR5 is responsible for most of the infections. Due to a deletion (Δ32) mutation, which alternates the expression of CCR5 on the cell surface, a natural resistance against HIV can be found in some individuals.[26]

- CXCR4 is mainly used by the virus in late stages of infection.

After the binding of the HIV to the receptor, fusion takes place and the viral core is released into the target cells' cytoplasm. After the so called "uncoating", the virus' reverse transcriptase converts the viral RNA into cDNA, which itself is integrated into the host cells' DNA via the viral integrase. After insertion of the viral cDNA, the target cell starts to produce the viral proteins which move towards the cell surface. The new viruses are assembled by means of the protease which cuts the proteins into appropriate parts. The new viral particles are released via budding.

[26] Galvani AP et al. 2005

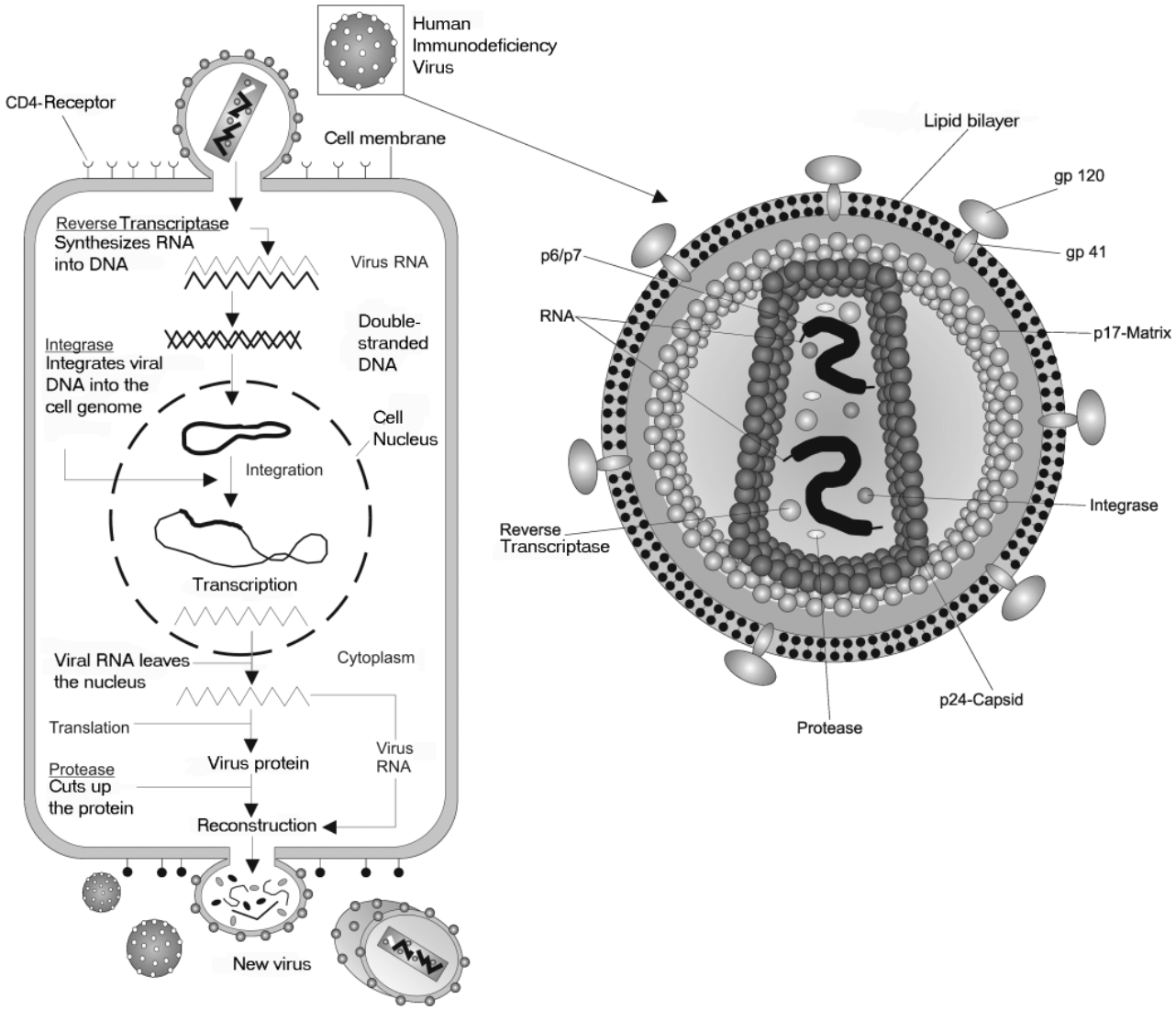

Figure 3: Structure and Replication cycle of the HIV-1[27]

2.2.4. Genome[28]

The viral RNA consists of 3 structural genes (*gag, pol, env*) that encode nucleocapsid, viral enzymes and envelope, as well as 6 regulating accessory genes (*ref, tat, vif, vpr, vpu, nef*), that ensure viral replication.

[27] http://en.wikipedia.org/wiki/File:HIV_gross_cycle_only.png, accessed 12th December 2009
[28] Hahn H. et al. 2009, pp521-523

- *LTR* (5'-ending)*:* It contains the promoters of the genetic expression and enhancer-elements.

- *Gag*(group)-gene*:* The primary gene product is cut into split products, that are used to form the two viral capsids. They are responsible for the group determination.

- *Pol*(ymerase) gene*:* Codes for the reverse transcriptase, for the protease and the integrase.

- *Env*-gene: The glycoproteins *gp120* (responsible for the receptor binding) and *gp41* (responsible for the cell fusion) are the gene products of this region. Their precursor-molecule is *gp160* which is the direct gene product of the *env*-region*.*

- *Tat*-gene: Tat stands for "transactivation transcription" and the gene product is responsible for a positive feedback causing an increase of transcription.

- *Rev*-gene: Regulating function on the expression of the viral proteins.

- *Vif*(virus infection factor)-gene: Increases the infectivity of the virus, by enabling the virus to evade the cellular defense mechanisms.

- *Nef*(negative factor at the 3'ending): Responsible for an increase of viral mRNA-synthesis. It might be toxic to glia-cells and astrocytes. It reduces the expression of MHC- and CD4-factors on the target cell membrane.

- *Vpu* and *vpr:* They increase the replication and virus release and stop the MHC-synthesis. *Vpr* leads to apoptosis

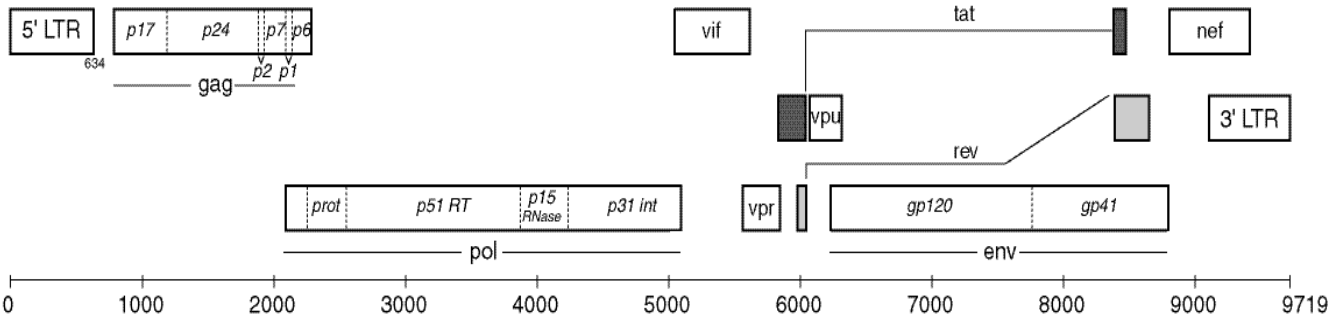

Figure 4: Genomic structure of the HIV-1

2.2.5. Transmission[29]

HIV can not be transmitted through normal social contacts of daily life including:

- Contact with sweat, tears, urine, saliva
- Skin contact
- Insect bites
- Coughing, sneezing

The infectious body fluids are:

- Blood
- Vaginal secretion
- Seminal fluid
- Breast milk
- Liquor

For the different common ways of transmission, the likelihood of an HIV infection is as follows:[30]

[29] Kronwetter M. 2008, pp14-16

- Perinatal transmission with HAART ~20%
- Perinatal transmission under HAART <2%
- Receptive anal intercourse (♀♂, ♂♂) 0,8-3,2%
- Inceptive anal intercourse (♂♀,♂♂) 0,02-0,2%
- Vaginal intercourse (risk for the woman) 0,05-0,15%
- Vaginal intercourse (risk for the man) 0,03-0,09%
- Receptive oral intercourse 0,04%
- Needle sharing 0,67%
- Stitch with canula 0,32%

Within the HIV positive population there are different risk groups. Those groups changed a lot since the beginning of the AIDS-epidemic:[30]

Man having sex with men

At the beginning of the HIV pandemic, the former “gay cancer”, later known as AIDS, used to be a disease that only affected homosexual men. This changed fundamentally over the last 20 years. Nowadays homosexual men are the minority in terms of new infections. The risk behavior on the other hand increases again over the last couple of years. Because of the long incubation time and the prolonged life-expectancy due to HAART, homosexuals will for sure remain a big group amongst the people suffering from AIDS.

Injecting drug users

Needle sharing remains a big problem within this increasing risk group. Not only do injecting drug users risk their own health, but also they can spread HIV amongst mostly heterosexual people via unsafe sexual intercourse. Offering clean needles to injecting drug users (especially in prisons), is the most important form of prevention amongst this group.

[30] Kronawetter M. 2008, pp14-16

Blood transfusions

In Austria, screening for HIV in donated blood started in 1985. With serological testing, the number of blood transfusion related HIV infections dropped significantly. Due to introduction of screening donated blood using molecular assays, the risk of HIV infection through receiving a blood transfusion is almost negligible in Austria nowadays. In Austria, every 1-3 years, a person might be infected with HIV through receiving donated blood because the problem of the diagnostic window still remains even though all blood conserves are tested on HIV RNA.[31]

Hemophiliacs

After 1985 there has not been a reported infection with HIV amongst people suffering from hemophilia. Factor-concentrates are nowadays absolutely secure and the risk of getting HIV is almost eliminated.

Heterosexuals

The unsafe heterosexual intercourse is the biggest factor of HIV-transmission worldwide. In Austria, is has also become the most frequent way of getting infected. Although it is very unlikely to get infected with HIV due to one single intercourse with an HIV positive person, the high number of unsafe heterosexual intercourse is nowadays in Austria responsible for 2/3 of the new infections.

Perinatal transmission

The risk of transmission without antiretroviral therapy is very high (14-40%) and depends on the mothers CD4 cell count and viral load. In Western countries perinatal transmission is very low compared to developing countries. Especially if the mother gets infected shortly before or during pregnancy, the risk for the child is very high, as the viral load in early HIV

[31] Kronawetter M. 2008 p16

infection is very high and the mother often does not know she is infected. HIV can be transmitted to the child:

- In utero: 30% (the risk is very high at the end of the pregnancy)
- Perinatal: 50%
- Post natal: 15-30% (due to breastfeeding)

Highly Active Antiretroviral Treatment (HAART) during pregnancy and additional antiretroviral therapy for the baby during the first 4 weeks after birth can reduce the risk to a minimum (0-2,5%).

2.2.6. Epidemiology

The worldwide situation[32]

The UNAIDS epidemic update for 2009, estimates the worldwide prevalence of HIV at approximately 0,8% of the adult population.[33]

Sub-Saharan Africa remains the region with the biggest number of HIV-positive people (22,4 million people living with HIV). (Figure 5, page 26)

[32] UNAIDS, 2009 epidemic update, Switzerland December 2009
[33] UNAIDS, Report on the global AIDS epidemic 2008, Switzerland, 2008

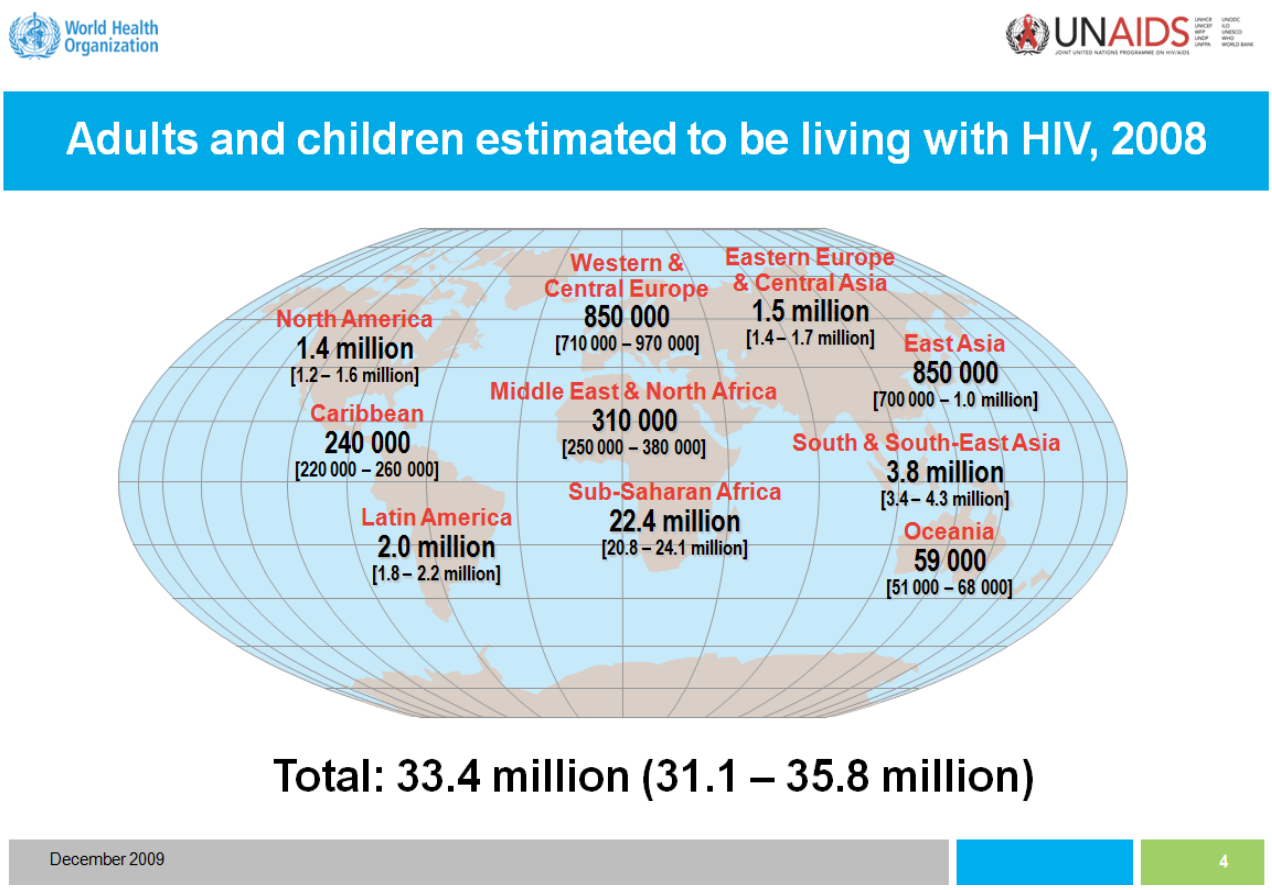

Figure 5: Adults and children estimated to be living with HIV, 2008[34]

People living with HIV in 2008:

- Total 33.4 million (31.1 million–35.8 million)
- Adults 31.3 million (29.2 million–33.7 million)
- Women 15.7 million (14.2 million–17.2 million)
- Children under 15 years 2.1 million (1.2 million–2.9 million)

People newly infected with HIV in 2008

- Total 2.7 million (2.4 million–3.0 million)
- Adults 2.3 million (2.0 million–2.5 million)
- Children under 15 years 430 000 (240 000–610 000)

[34] http://www.unaids.org/en/KnowledgeCentre/HIVData/Epidemiology/2009_epislides.asp, accessed 6th December 2009

AIDS-related deaths in 2008

- Total 2.0 million (1.7 million–2.4 million)
- Adults 1.7 million (1.4 million–2.1 million)
- Children under 15 years 280 000 (150 000–410 000)

The Situation in Austria[33]

UNAIDS suggests that in 2008, there have been 9800 [7600-13 000] HIV positive people living in Austria.[35]

The statistics published every year for Austria by the "AIDS Hilfe Wien", suggest that there are 10 000 to 15 000 people living with HIV in Austria, of which 2/3 are male and 1/3 is female. About half of them are living in Vienna.[36]

Ever since the beginnings of the pandemic in Austria in 1983 there have been 2749 patients diagnosed with AIDS, of which 1502 died. 1247 people have AIDS at the moment. In Styria there are 184 people who suffer from AIDS, a lot more are HIV positive.[36]

Of those AIDS patients the following epidemiologic data could be taken for the whole of Austria:[37]

- 34% Homo/bisexual
- 24,1% Injecting drug users
- 20,6% Heterosexuals
- 0,9% Perinatal transmission

Every day there are 1-2 new infections with HIV in Austria and the infection rate is increasing. In 2009 there is an estimated increase of 5%, compared to 2008. 523 HIV-infections were newly diagnosed in 2009, which is 18 more than in the year before.

[35] UNAIDS, 2009 epidemic update, Switzerland December 2009
[36] http://www.aids.at/index.php?id=15, accessed 07th December 2009
[37] UNAIDS, Report on the global AIDS epidemic 2008, Switzerland, 2008

2.2.7. Testing [38, 39]

Infectivity starts early after HIV infection, but viral isolation is not done on a routine basis at the time. Viral RNA and DNA can be of course detected at that stage of infection, but it is very cost expensive and only done in special situations, for example the testing of blood products.
HIV screening is generally based on antibodies against HIV. Normally antibodies show up in the infected individual already 3-6 weeks after the transmission, but the so called "diagnostic window" can be a lot longer for some individuals. Antibodies are usually developed against envelope antigens (gp120, gp41), capsid antigens and the viral polymerase.

Diagnosis of the HIV infection:[38]

- Initially, screening on HIV infection is done serologically using an antibody ELISA or a combined antibofy/antigen ELISA. These tests show high sensitivity but suffer specificity. They detect HIV- and HIV-2 including all subtypes.
- According to the Austrian AIDS Act, all positive screening results must be confirmed through an immunoblot assay (today usually performed by a recombinant reverse line-probe assay).
- The immediate determination of the viral load is very important as well as the resistance profile. Both are done by molecular assays in specialized laboratories.
- The viral load should be monitored every 3-6 months.
- Testing of newborns of HIV positive mothers is done by molecular assays because they normally have the mothers' antibodies in their blood.

[38] Hahn H. et al. 2009, pp532-533
[39] Kronawetter M. 2008, pp29-30

2.2.8. Prevention [40, 41]

There are four major categories of prevention strategies:

1. Prevention for HIV-1-negative people, especially high risk groups
2. Prevention for people with maximum probability of exposure but before exposure
3. Prevention for people shortly after exposure
4. Prevention for already infected people (=secondary prevention)

The most important prevention strategies are behavioral interventions and education. The use of condoms, fewer sex partners and the avoidance of needle sharing among injecting drug users are other important issues.

Some other forms of prevention that are not done on a regular basis but have been proven in studies are:

- Male circumcision: This can reduce the risk of the HIV-1-transmission by approximately 60%[42]
- Antiretroviral treatment: HAART might become the most important factor to prevent new infections. If the viral load remains very low, transmission is very unlikely to occur.
- Antiretroviral therapy during pregnancy and birth: This strategy is very successful in developed countries, whilst they are not available in most of the developing countries.

Topical microbicides and post exposure prophylaxis may also prevent infections with HIV, but don't show significant outcome yet.

[40] Kronawetter M. 2008, pp14-15
[41] Troppan K. 2009, p16
[42] Quin TC et al. 2007, 20 (1):33-81

2.3. HIV Infection

HIV infection can be divided into four stages:

1. Primary HIV infection
2. Asymptomatic HIV infection/Latency phase
3. Symptomatic HIV infection/AIDS
4. Opportunistic infections/AIDS

2.3.1. Primary HIV infection: [43, 44]

Within days or weeks after infection with the HIV, more than half of the patients start to develop symptoms that resemble to mononucleosis. This illness lasts for about two weeks and shows the following symptoms:[43]

- Fever (90%)
- Lymphadenpathy (50%)
- Pharyngitis (50%)
- Exanthema/Enanthema (50%)
- Mylagia/Arthralgia (50%)
- Diarrhea (32%)
- Headache (27%)
- Nausea, Emesis (27%)
- Further: Weight loss, thrush, neurological symptoms

Antibodies detected by ELISA are usually negative during that time; however the viral load, if measured in molecular assays, is extremely high. Because of the high viral load during that period, the risk of transmission is increased, as most of

[43] Kronwetter M. 2008, pp24-25
[44] Hahn H. et al. 2009, pp528-532

the patients are not aware of their recent HIV infection. Symptoms disappear after a maximum of two weeks with decrease of the CD4 cell count during this period.

2.3.2. Clinical asymptomatic HIV infection

After a few weeks, the patients are again asymptomatic. This is the start of the latency phase. Because of a strong immune defense, viral load is very low during latency phase. The patients remain infectious though, as the immune system is not able to completely eliminate the virus. The immune defense is also responsible for the development of antibodies, which are usually detectable after 1-3 months of acute infection in the blood. The point when antibodies show up in the blood is called seroconversion.

At that stage of infection, HIV is active in the lymphoid organs getting trapped in large amounts in the follicular dendritic cells. About 40% of the patients develop the so called lymphadenopathy syndrome (LAS), with swellings of the lymph nodes that persist more than 3 months, lacking any other symptoms. On the other hand, HIV attacks the CD4 cells, which leads together with the slow destruction of the lymph nodes to a slow decrease of CD4 cell count.

Under good living conditions (no malnutrition etc.), this stage can last up to 10 years or longer.

2.3.3. Symptomatic HIV infection/AIDS

After the latency phase, the patient starts to develop AIDS. The CD4 cell count is steadily decreasing reaching very low levels, and patients start to suffer from constitutional symptoms like moderate and unexplained weight loss, recurring respiratory tract infections, prostatitis, skin rashes or oral ulcerations.

2.3.4. Opportunistic infections/AIDS

When the CD4 cell count finally declines below 200 cells/ µL, the cell-mediated immunity is lost. This leads to opportunistic infections and tumors, causing death.

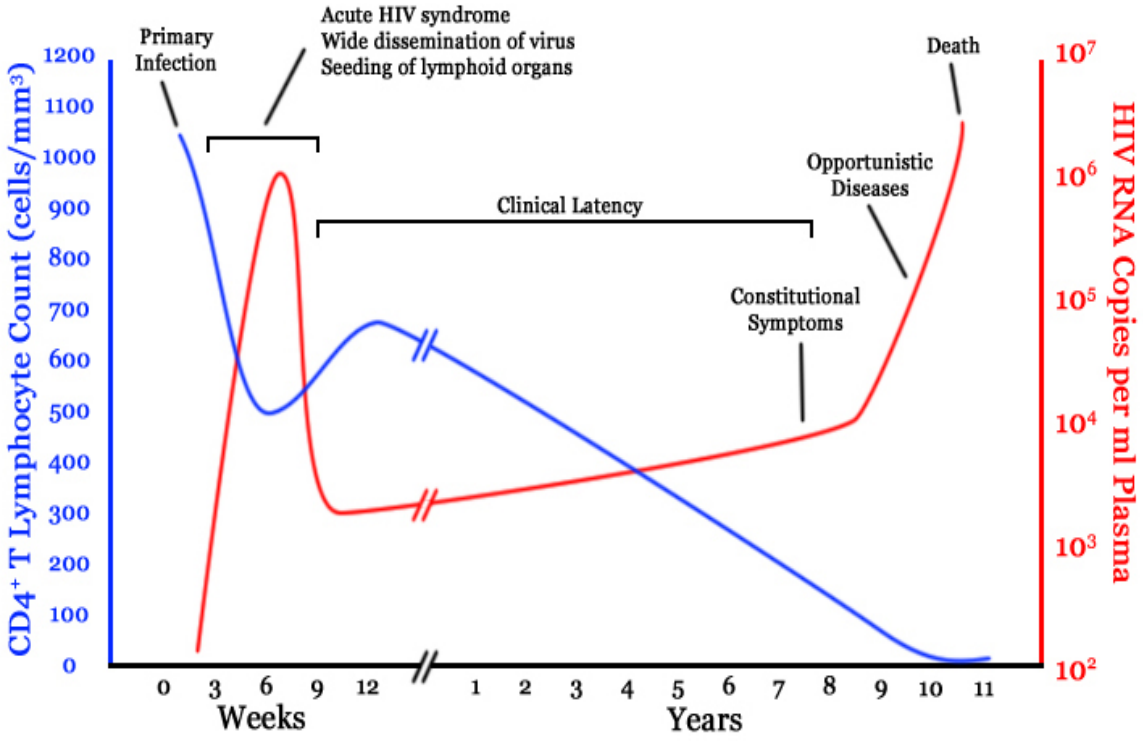

Figure 6: Course of the HIV-infection[45]

[45] http://img.medscape.com/pi/emed/ckb/infectious_diseases/211212-1358427-211316-1377359.jpg)

2.3.5. *WHO Clinical Staging of HIV/AIDS for Adults and Adolescents*[46]

The HIV disease can be divided into 4 clinical stages according to WHO:

HIV-associated symptoms	WHO clinical stage
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe symptoms	4

Table 1: WHO clinical staging of established HIV infection

More specifically the WHO published a staging system for HIV/AIDS based on clinical symptoms, which also useful in countries that are not very well equipped with laboratories. So clinicians can stage the progression of the disease even without the opportunity to count CD4 cells or measure viral loads.

Clinical stage 1
• Asymptomatic • Persistent generalized lymphadenopathy
Clinical stage 2
• Moderate unexplained weight loss (<10% of presumed or measured body weight)I • Recurrent respiratory tract infections sinusitis, tonsillitis, otitis media and pharyngitis) • Herpes zoster • Angular cheilitis • Recurrent oral ulceration • Papular pruritic eruptions • Seborrhoeic dermatitis • Fungal nail infections

[46] World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease In Adults and Children . 2007. Accessed March 30, 2009

Clinical stage 3
• Unexplained severe weight loss (>10% of presumed or measured body weight) • Unexplained chronic diarrhoea for longer than one month • Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month) • Persistent oral candidiasis • Oral hairy leukoplakia • Pulmonary tuberculosis (current) • Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia) • Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis • Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 109 per litre) or chronic thrombocytopaenia (<50 × 109 per litre)
Clinical stage 4
• HIV wasting syndrome • Pneumocystis pneumonia • Recurrent severe bacterial pneumonia • Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site) • Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) • Extrapulmonary tuberculosis • Kaposi's sarcoma • Cytomegalovirus infection (retinitis or infection of other organs) • Central nervous system toxoplasmosis • HIV encephalopathy • Extrapulmonary cryptococcosis including meningitis • Disseminated non-tuberculous mycobacterial infection • Progressive multifocal leukoencephalopathy • Chronic cryptosporidiosis (with diarrhoe) • Chronic isosporiasis • Disseminated mycosis (coccidiomycosis or histoplasmosis) • Recurrent non-typhoidal Salmonella bacteraemia • Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours • Invasive cervical carcinoma • Atypical disseminated leishmaniasis • Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

Table 2: WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection

For children, infected by vertical transmission of HIV-1, these stages are not applicable. Their disease evolution is characterized by prematurity, dystrophia, and severe damages of the central nervous system such as ataxia.[47]

2.4. Antiretroviral Therapy

2.4.1. Introduction

Antiretroviral drugs were designed to specifically blockade antiretroviral replication in the human body. Based on the knowledge about the genomic sequences responsible for the viral replication, there are nowadays five classes of antiretroviral drugs available for the therapy against HIV.

2.4.2. Classes of antiretroviral drugs [48, 49, 50, 51]

1. **Nucleoside (Nucleotide) Reverse Transcriptase-Inhibitors (NRTIs)**
    - NRTIs are the oldest available antiretroviral drugs (Zidovudine is available ever since 1987)
    - NRTIs are nucleoside analogues that lack the 3'OH-group or use a substitute at this position (For example N3 for Zidovudine)
    - They are in concurrence with the natural triphosphates, being used by the Reverse Transcriptase instead of them, in the viral replication.
    - As prodrugs, they undergo two phosphorylations in the host cell and as 5'-triphosphates they can compete with the triphosphates of the host cell for the integration into the viral cDNA.

[47] Herold G. 2008
[48] Holzer U. 2009
[49] Hoffmann C. Rockstock J Kamps BS. 2008, p18-58
[50] Clotet B et al. 2008
[51] Kronawetter M. 2008, pp40-60

- If the integration of a 5'-Triphosphate is successful, the further synthesis of proviral DNA can not be continued by the reverse transcriptase as it leads to chain determination ("chain terminators").
- Only newly penetrating HIV can be inhibited by NRTIs. If the HIV is already integrated in the host DNA, they loose their effectiveness. For this reason it makes sense to give NRTIs in an early stage of HIV infection.
- The following conditions lead to a decrease or loss of effectiveness:
    1. If the metabolism of the host cell changes, which means no further phosphorylsations
    2. If natural nucleosides are available in a higher number than NRTIs, so that they are not able to compete against the host cells' nucleosides any more
    3. If the drugs are not taken on a regular basis or their dosage is to low
    4. If the virus develops resistance, which usually happens after 6 months.

2. Non Nucleoside Reverse Transcriptase-Inhibitors (NNRTIs)

- They are very small molecules that bind to the catalytic center of the reverse transcriptase.
- This leads to an allosteric inhibition of the reverse transcriptase.
- Furthermore there is a synergism between NNRTIs and NRTIs.

3. Protease-Inhibitors (PIs)

- The one major difference to the human protease is that the viral protease cuts up proteins between phenylalanine and proline.
- They are competitive Inhibitors of the catalytic center of the HIV-Protease.

- Protease Inhibitors hinder the enzyme HIV-protease, to split up the viral gag-pol-preprotein. For this reason the newly produced virus particles are non-infectious.

4. Integrase-Inhibitors

- They are inhibitors of the catalytic activity of the HIV-Integrase, the third important enzyme for the replication of the HIV.
- Viral DNA strands can not be integrated into the host cells DNA any more. (”strand transfer inhibitors”)

5. Entry-Inhibitores

- Entry inhibitors do not inhibit the production of new HIV, but hinder the virus to penetrate new cells.
- HIV normally binds via its surface protein gp120 to the CD4-receptor, which leads to a change of conformation of gp120. This conformational change enables the V3-Loop of the gp120 protein, to bind to the chemokine receptors CCR5 and CXCR4 of the target cell.
- So far, three steps of cellular penetration by the HIV can be inhibited:

    1. Attachment-Inhibitors hinder the binding of HIV via gp120 to the CD4-receptor. Attachment inhibitors rae not available yet.

    2. Co-receptor-Antagonists hinder the definite binding of HIV to the co-receptors CCR5 or CXCR4. At the moment there are only CCR5-antagonists available and they bind to the CCR5-coreceptor, so gp120 can not interact with the receptor, which hinders the entry into the cell. The are not useful in HIV strains that use CXCR4 as a so-receptor.

3. Fusion Inhibitors hinder the Fusion of virus and host cell due to interaction with the intermediate structure of gp41. The only substance used so far is a peptide that exists of 36 amino acids.

2.4.3. Substances accredited in Austria[52]

1. Nucleoside Reverse Transcriptase-Inhibitors (NRTIs)

Nucleoside Analogues

Zidovudine	(thymidine-analogue, first available antiretroviral drug)
Lamivudine	(cytidine-analogue, good side effect profile)
Emtricitabine	(cytidine-analogue, good side effect profile)
Stavudine	(thymidine-analogue, only affective against HIV-1)
Abacavir	(guanosin-analogue)
Didanosin	(analogue to deoxy-adenosine)

- Always used in combination with other drugs as part of a HAART. Partly they are even available as fixed combination drug.
- Absolutely no combination with NRTIs that have the same base as basis. This can cause an antagonism between the two drugs.
- *Zidovudine* is used as post exposure prophylaxis after recent viral exposure and as prophylaxis for vertical transmission in pregnant women.
- *Lamivudine* and *Emtricitabine* are also effective against Hepatitis B.
- Most of the side effects are caused due to inhibition of the mitochondrial DNA-polymerase and are severe. The effect mentioned below can generally occur during therapy with all of the NRTIs:
    - *Zidovudine:* myopathy, bone marrow supression
    - *Didanosine:* pancreatitis in high dosage

[52] Holzer U. 2009

- *Abacavir:* potentially deadly hypersensitivity reactions, lactate acidosis
- *Lamivudine, Emtricitabine:* no therapy limiting toxic side effects, but frequent development of resistances if co-infection with HBV

Nucleotide Analogues

Tenofovir (phosphate related to adenosine, adenosine-monophosphate)

- Renal side effects common, including hypophosphatemia
- Low mitochondrial toxicity
- Increase/decrease of the plasma level of other antiretroviral drugs. This can lead to either loss of effectiveness or increased side effects of the other medicaments.

2. Non Nucleoside Reverse Transcriptase-Inhibitors (NNRTIs)

Efavirenz

Nevirapine

- >90% bind to plasma-proteins
- Only used in combination with other drugs as part of a HAART
- *Nevirapine*
  - is used for the prevention of vertical transmission in pregnant women
  - has a good liquor passage
  - can cause hepatitis
- *Efavirenz:* teratogenic
- Exanthemas including Stevens-Johnson-Syndrome as well as Lyell-Syndrome were observed
- Acceleration of the metabolism of protease-inhibitors and many antibiotics

3. Protease-Inhibitors (PIs)

Lopinavir major substance

Indinavir major substance

Amprenavir

Fosamprenavir

Atazanavir

Darunavir

Nelfinavir

Ritonavir

Saquinavir

Tipranavir

- >90% bind to plasma proteins
- Bad passage into liquor
- Always as part of a HAART
- Effective against HIV-1 and HIV-2
- Cause disturbance of the lipid metabolism: Insulin resistance, hyperlipidemia, lipodystrophia (redistribution of the body fat from the peripheries to the trunk) and coronary heart disease
- *Darunavir, Tipranavir:* favorable in patients with drug resistance
- *Ritonavir:* high hepatic metabolism with CYP3A4; only used in low dosage in combination with other PIs (”boosting”) so the dosage of the other PI can be reduced
- *Indinavir:* nephrolithiasis (sufficient hydration important)

4. Intergrase-Inhibitors

Raltegravir

- High individual variability of the oral bioavailability; better if taken with fatty meals

- Given to pre-treated grown up patients who show viral loads even under antiretroviral therapy
- Raltegravir should only be given in addition to the so called “optimized background therapy”
- Low plasma level if given in combination with *Rifampicin*
- High plasma level if given in combination with Atazanavir and Tenofovir

5. Entry-Inhibitors

a) Attachment-Inhibitors

Still in clinical testing, not available on the Austrian market

b) Co-Receptor-Antagonists

Maraviroc Antagonist to the CCR5-co-receptor

- For combinational therapy in pre-treated grown up patients who are infected with HIV-1 with exclusive affinity to the CCR5-receptor
- Tropism testing necessary before the start of the therapy (Contraindication = Carriers of HIV-1 with affinity to the CXCR4-co-receptor)
- Increase of liver enzymes observed
- Orthostatic hypotonia if given in high dosage

c) Fusion-Inhibitors

Enfuvirtid synthetic peptide consisting of 36 amino acids

- Twice daily subcutaneous application
- Split up into amino acids, no enzyme induction
- Given to AIDS patients who do not respond to classical HAART

- Induces the production of antibodies; this does not seem to impact the viral effectiveness
- Central-nervous system dysfunction observed
- Reports of accumulation of adenopathia and bacterial pneumonia

2.4.4. Highly Active Antiretroviral Therapy – HAART[53, 54, 55, 56]

The goal of every antiretroviral therapy is to prolong the life of each patient in the highest possible health condition with the highest possible quality of life. Two factors are important to achieve this condition:

1. Opportunistic infections and malignant tumors must be avoided

2. The antiretroviral therapy must be chosen individually not only to make this possible, but also to reduce the toxic side effects of the therapy to a minimum.

Indications to start with the HAART have been discussed a lot. At the moment the suggestions are as follows:

- Symptomatic HIV infection
- Start at the latest with the antiretroviral therapy, when the CD4-cell count drops to a minimum of 350/µl blood.[57]
- Patients with a viral load of 10 000 – 20 000 copies/ml independently of the CD4-cell count.

[53] Hoffmann C, Rockstock J, Kamps BS. 2008, pp59-118
[54] www.ifektionsnetz.at, accessed the 07th December 2009
[55] http://akh-consilium.at/indikation/AIDS/Therapie, accessed 07th December 2009
[56] Holzer U. 2009
[57] Lancet 373:1352, 2009, as mentioned in Holzer U. Antiinfektive Therapie Teil 6 – Virustatika, Graz 2009

The most important laboratory parameter for sufficient therapy is the detection of viral RNA in the plasma. The so called viral load should be under 50 copies/ml (this corresponds to the detection limit) to guarantee sufficient therapy success.

HIV is known for its quick development of resistance. For this reason, the HAART always consist of a combination of different drugs.

The typical combination is:

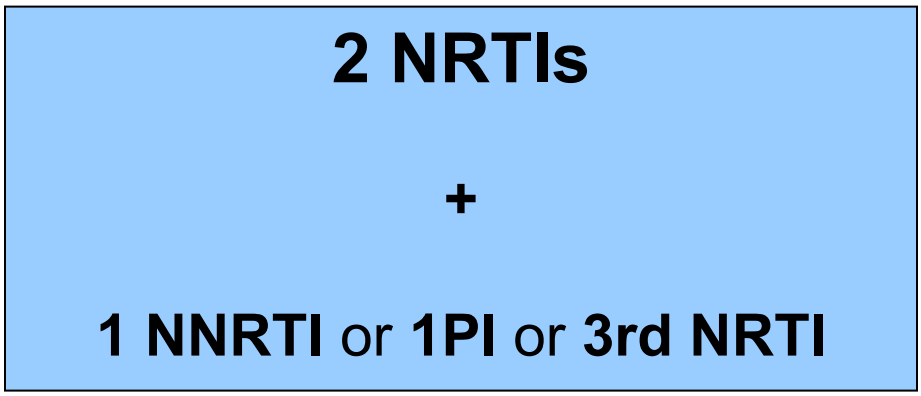

Figure 7: Typical combination of HAART

Some basic rules for HAART:[58, 59]

- Monthly control of the CD4-cell count
- Count of the viral load twice before the start of the therapy, furthermore monthly during the beginning of therapy until the virus is no longer detectable (this should happen within a maximum of 3-6 months[58]), followed by 2-3 monthly controls to guarantee sufficient viral suppression throughout the antiretroviral treatment.
- It is not recommended to start with all 3 common classes of drugs at the same time, to preserve therapy options if resistance should occur.

[58] Holzer U. 2009
[59] Hoffmann C, Rockstock J, Kamps BS. 2008, pp59-118

- A therapy with only one or two drugs on the other hand is insufficient, which was proven in many studies.[58]
- Entry-Inhibitors and Integrase-Inhibitors should be saved for very late stages of antiretroviral therapy.
- Combinations of thymidine-analogues *Zidovudine* + *Stavudine* and the cytidine-analogues *Emtricitabine* + *Lamivudine* must not be given to the patient, as they are in concurrence with the same bases.
- Toxic combinations are must be avoided:
    1. *Stavudine* + *Didanosine* (because of mitochondrial toxicity)
    2. *Nevirapine* + *Efavirenz* (because of additive toxicity)

The patient's compliance remains though the most important factor for sufficient viral suppression (if only 5% of the drugs are not taken, the success of the therapy is already lowered) and the basis of every HAART.

At the start of HAART patients had to take enormous amounts of tablets and capsules every day, which of course lowered their quality of life as well as their compliance. Nowadays the amounts of capsules and tablets to be taken daily are reduced a lot. The first "once daily" drugs were launched during the last couple of years, which can be an advantage for patients with low compliance.

Antiretroviral therapy is not only the most important medical therapy for HIV positive patients, but also the most effective way of prevention of new HIV infections. If an HIV positive person has access to sufficient HAART and its viral load is below the detection level, it is very unlikely that the person can pass HIV to other people.[60] If this goal – the universal access to antiretroviral therapy - set by the World Health Organization can be reached, the problem of HIV/AIDS could be reduced to a minimum.

[60] Hoffmann C, Rockstock J, Kamps BS. 2008, pp190-191

2.5. Drug Resistance of the HIV-1

2.5.1. Introduction

One of the major causes of antiretroviral therapy failure are resistant HIV strains. Because of the high replication rate of HIV, mutations that occur coincidently often become the predominant virus strain in an HIV affected individual. Those strains used to be the reason for severe limitation of the success of antiretroviral therapy. Problems in the therapy of individuals infected with resistant HIV-1 strains occur especially if the strain is resistant to more than one drug.
Recently new substance classes (integrase-inhibitors, entry-inhibitors) were released, which so far show success even in those patients.

2.5.2. Causes of resistance

HIV-1 is well known for the great variability of its strains, both genotypically and phenotypically: Errors occur frequently during the process of transcription of the proviral DNA from the viral RNA. The problem is that the reverse transcriptase of the HIV lacks a proofreading function to correct these errors. During the viral replication, the single stranded RNA-genome is intermediately converted into a double-stranded DNA:RNA hybrid and then finally generated into a DNA copy again. The HIV-1 reverse transcriptase lacks a 3' exonuclease activity which normally enables the proofreading function of a polymerase enzyme to repair replication errors. This causes at least one error per 10 000 bases copied by the reverse transcriptase.[61] The high level of viral replication and turnover, the size of the HIV-1 genome and the frequency of the spontaneous mutations create an opportunity for a large number of genetically distinct strains and quasispecies of HIV to be formed and selected.[62, 63] Usually those resistant quasispecies that

[61] TRUGENE HIV-1 Genotyping Kit, manufacturer's package
[62] Perelson et al. Scienece 1996;271:1582-1586, from Clotet B. et al. 2008, p1
[63] Mansky et al. J Virol 1995, from Clotet B. et al. 2008, p1

occur spontaneously during viral replication finally are selected to be the predominant variant found in an HIV-1 infected individual.[64] The following factors are involved in drug-resistant strain pre-selection:[65]

- Rapid turnover of HIV-1 (half-life free virus <2 hours).
- High error rate of the reverse transcriptase (~1:10^4).
- Large amounts of daily produced viruses (10^{10}) over a long period of time (≥10 years).
- Incomplete suppression of viral replication in subjects under therapy (suboptimal therapies, low adherence, malabsorption, etc…).
- Different genetic barrier among the antiretroviral agents contained in a regimen.
- Different magnitude in the grade of resistance that mutations confer.

Drug resistance can have the following implications on the HIV: [66]

- Changes in viral fitness.
- Interaction in the susceptibility to other drugs.
- Possible changes in viral tropism.

When it comes towards drug resistance of the HIV, 2 terms are used to describe it:[67, 68]

1. <u>Genotypic resistance:</u>

   Genotypic resistance means that compared to the wild type reference, specific change happened in of the nucleotide sequence of a HIV strain. Those changes can go along with a phenotypic resistance and can reduce the sensitivity to one or more drugs.

[64] Drake 1993, from Hoffmann C, Rockstock J, Kamps BS. 2008, p241
[65] Clotet B. et al. 2008, p1
[66] Clotet B. et al. 2008, p2
[67] Perelson et al. Scienece 1996;271:1582-1586, from Clotet B et al. 2008, p1
[68] Kronawetter M. 2008, p70

Such mutations are named by the position they have in a certain gene/codon, preceded by the letter corresponding to the aminoacid seen in the wild type virus, and followed by the mutated amino acid. [69]

First letter	Number	Last letter
Amino acid in wild type target protein	Mutated codon	Amino acid in mutant protein

A	Alanine
C	Cystein
D	Aspartate
E	Glutamate
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysin
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Trytophan
Y	Tyrosine

Examples:

- **D30N** Change from Aspartate to Aspargine at codon 30 of the protease gene.
- **M184V** Change from Methionine to Valine at codon 184 of the reverse transcriptase.
- **K219Q/E** Change from Lysine to Glutamine or Glutamate at codon 219 of the reverse transcriptase.

Table 3: Mutation naming convention[70]

2. Phenotypic resistance:

Phenotypic resistance denotes the ability of the HIV to replicate in vitro under the presence of an antiretroviral drug, in a concentration that would inhibit the replication of the wild type virus. Sensitivity of a phenotype resistant virus to a drug is lower, comparing to the sensitivity of a wild type virus. Those sensitivities can be measured and compared and the ratio is called the Fold change. The inhibitory concentration (IC) determines the susceptibility of a virus to drug(s) in a virus replication assay. IC50 is the concentration of a

[69] Clotet B et al. 2008, p3
[70] Own table, contents from Clotet B et al. 2008, p.3

drug (in µg/ml), necessary to inhibit the viral replication by 50%, IC90 to 90%, IC95 to 95%.

X-Fold rise Or **Fold changes**	=	IC50 from the patient isolate / IC50 from wild type laboratory strain

Table 4: Calculation of the IC50[71]

2.5.3. Types of resistance

Drug resistance can be defined into two types:

1. Primary Resistance or transmitted drug resistance: this means, resistance found in a drug-naïve patient due to spontaneous mutation or transmission of an HIV strain that is already resistant to antiretroviral therapy

2. Secondary resistance or acquired drug resistance: this means, the drug resistance is detected in a patient that receives antiretroviral therapy. Secondary resistance can also occur spontaneously or for example due to insufficient antiretroviral treatment or malabsorption etc.

2.5.4. Causes of mutations

Most of the mutations that cause drug resistance are produced by point mutations but several other ways exist, that can also cause drug resistance:[72]

- Point mutation: Genetic change resulting in a single nucleotide substitution in the DNA/RNA. Changes in amino acids might require more than one nucleotide substitution.

[71] Own table, contents from Clotet B et al. 2008, p3
[72] Clotet B et al. 2008, p4

- Insertion: Incorporation of additional nucleotides or genomic material to the original sequence, derived by duplications, recombinations, etc.
- Deletion: Loss of nucleotides or a gene fragment
- Recombination: Exchange of genetic material fragments between virions

For a better understanding of the mechanisms leading to the mutations mentioned above, it is useful to relate them to the different classes of antiretroviral drugs:

Protease Inhibitors (PIs)[73]

Protease Inhibitors hinder the enzyme HIV-protease to cut up the viral gag-pol-pre-protein. For this reason the newly produced virus particles are not infectious. A PI resistance develops usually slowly, because there must be an accumulation of several mutations. This mechanism is also called "genetical barrier". Two classes of mutation are defined in this context:

1. Major mutations:
   Those mutations are responsible for phenotypic resistance and include:
   - Those mutations that occur first under the selective pressure of a drug
   - Mutations that bind to the active center of the HIV-protease and therefore reduce the PI's ability to bind to the enzyme.
   - Sometimes mutations can lead to a reduced activity of the HIV-protease

2. Minor mutations
   Those mutations can be found outside of the active center and usually only occur in the presence of major mutations. Sometimes they can compensate an eventual loss of viral fitness due to a major mutation.

[73] Hoffmann C, Rockstrock J, Kamps BS. 2008, pp245-247

Nucleoside Reverse Transcriptase Inhibitors (NRTIs)[74]

NRTIs are prodrugs and after two phosphorylations they can compete with the natural dNTPs (desoxynucleotid-triphosphats) for the integration into the viral genome. Once integrated, the further synthesis of proviral DNA can not be continued by the reverse transcriptase which leads to chain determination. Two biochemical resistance mechanisms exist for NRTIs:

1. Steric inhibition:
   Mutations which lead to steric inhibition allow the reverse transcriptase to differ between NRTIs and dNTPs, so the enzyme is able to select the dNTPs for integration in the proviral DNA

2. Phosphorolysis:
   The ATP-mediated Phosphorolysis, leads to the secondary exclusion of already integrated NRTIs from the DNA chain. Depending on the different mutations, this can lead to lower or even higher sensibility to NRTIs.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)[75]

NNRTIs also inhibit the enzyme reverse transcriptase, but chemically differentiate from the NRTIs. They are very small molecules and bind to a place on the reverse transcriptase, that is associated with the catalytic center. For this reason, mutations on the NNRTI binding site of the reverse transcriptase lead to a lower affinity between the NNRTI and the enzyme.

Entry inhibitors:[75]

Entry inhibitors do not inhibit the production of new HIV, but hinder the virus to penetrate into new cells. HIV normally binds via its surface protein gp120 to the CD4-receptor, which leads to a conformational change of gp120. Therefore the V3-Loop of the gp120 protein is able to bind to the chemokine receptors CCR5 and CXCR4 of the target cell.

[74] Hoffmann C, Rockstrock J, Kamps BS. 2008, pp245-247
[75] Hoffmann C, Rockstrock J, Kamps BS. 2008, pp245-247

CCR5-antagonists bind to the CCR5-coreceptor, thus gp120 can not interact with the receptor, which hinders the entry into the cell. Fusion inhibitors hinder the fusion of the viral membrane with the cellular membrane.
Already the exchange of only one single amino acid can highly reduce the effectiveness of those drugs.

Integrase Inhibitors:[75]
Integrase inhibitors hinder the integration of the proviral DNA into the DNA of the host cell. During the process of integration the drugs *Elvitgravir* and *Raltegravir* hinder the strain transfer, as they disturb the function of the integrase molecules. Mutations for both drugs are very specific and always affect the integrase gen. The strain transfer can be affected, as well as the 3'-processing, even though the paths of resistance for the two drugs differ. Cross-resistance between *Elvitgravir* and *Raltegravir* is also possible.

Cross resistance between the different antiretroviral drugs is possible and most likely to develop within one class of antiretroviral drugs.
The mutations in the HIV genome can be classified as follows:[76]

- Neutral: Viral fitness stays the same.
- Deleterious: Mutations lead to viral variants, which have a lower replicative capacity with respect to wild type viruses.
- Resistant: Replicative advantage of the mutated virus over wild type viruses. They usually develop under selective pressure (antiretroviral drugs, immune system etc.).

[76] Clotet B et al. 2008, p5

2.5.5. Types of mutations

Under antiretroviral therapy the virus is forced to develop genetic changes in its genome. Those mutations can give an advantage to the virus and enable it to survive under antiretroviral therapy by allowing it to escape from the drug's inhibitory effect. Per definition, the following two major classes of drug resistance mutations exist:

1. Primary mutations:[77]
    - Selected early in the process of resistance. However, it may be selected or favored after the appearance of secondary mutations.
    - High degree of specificity for one drug class which compromises significantly the susceptibility of the virus for that drug.

2. Secondary mutations:[77]
    - Tend to accumulate in the viral genome already containing one or more primary mutations.
    - May have little or no effect on the level of resistance.
    - May enhance viral replication by increasing viral fitness.

In general, the clinical significance of the concept of "primary versus secondary mutations" remains debatable, as many of the effects of those mutations can not be strictly separated.

2.5.6. Resistance testing

Phenotypic resistance testing

Phenotypic resistance testing is very complex and expensive and will not belong to clinical routine in the near future. As described above (see table 4), the

[77] Clotet B et al. 2008, p5

sensitivity of a virus strain to an antiretroviral drug is directly quantified. The measurement of the viral replication rate under selective pressure from an applied drug (in increasing concentrations) is the basis of phenotypic resistance testing. The result is compared to a wild type virus replication rate under the same conditions (Inhibitory Concentration, IC). The resulting quotient or "Fold Change" is compared to the so called "Cut off". There are three different types of "cut offs":[78]

1. Technical Cut Off:[79]
    - Based on assay reproducibility
    - Not drug specific

2. Biological Cut Off:[79]
    - Based on the upper limit of the susceptibility range observed in the panel of wild type isolates
    - Drug specific

3. Clinical Cut Off:[79]
    - Based on the observation of the virological response (change in viral load) in treated patients.

<u>Genotypic resistance testing:</u>

Genotyping resistance testing means, to analyze genotypically if resistance associated mutations in a virus strain exist. They are determined via amplification and sequencing of the HIV-genome or via specific hybridization with wild type nucleotides. The so called *pol*-region is the target of sequencing, as it encodes the viral enzymes protease, reverse transcriptase and integrase. Another region important for genotyping is the *env*-region. It encodes viral capsid segment (and therefore for the glycoproteins gp41 and gp120) of the HIV gene.[80].

[78] Hoffmann C, Rockstrock J, Kamps BS. 2008
[79] Clotet B et al. 2008, p14
[80] Hoffmann C, Rockstrock J, Kamps BS. 2008

Genotypic resistance testing has become a routinely used method as it enables quick sequencing of the genomic regions that are important for the antiretroviral treatment. At the *Medical University of Graz*, sequencing is performed by one of the approved genotypic test systems, the *"TruGene® HIV-1 Genotyping Ki"t* (for use with the *OpeneGene™ DNA Sequencing System*).

The following table compares the advantages and disadvantages of genotypic and phenotypic assays:

GENOTYPIC ASSAYS	
ADVANTAGES • Relatively simple to perform • Widely available • May detect mutations prior to apparent effect on phenotype • Allow detection of reversal mutations as "signatures" of past drug resistance • Take into account the genetic barrier towards high level resistance • Quick turnaround time and cost effective	DISADVANTAGES • Insensitive for minor variants • Indirect measure for drug susceptibility • Interpretation requires prior knowledge of genetic determinants of resistance • Mutational interactions can not be predicted
PHENOTYPIC ASSAYS	
ADVANTAGES • Provides quantitative resistance information, including assessment of hypersusceptibility and partial susceptibility • Provides information on resistance to new drugs, for which genotypic correlates of resistance are not established • Provides information on resistance in non-type B infection, for which genotypic correlates of resistance are not well established	DISADVANTAGES • Requires proper clinical cut-offs • Less sensitive than genotypes for wild type/mutant mixtures • Insensitive for minor variants • Time-consuming and more costly • The complexity of the assays limits its availability to a smaller number of laboratories

Table 5: Comparison between commercial genotypic and phenotypic assays[81]

[81] Own table, contents from Clotet B et al, 2008, p16

2.5.7. Sensitivity and conditions of resistance testing

The sensitivity of the testing systems can be limited when the plasma samples contain a low number of RNA-copies. Standardized genotyping usually only detects minor populations if they make up a minimum of 20-30% of the whole population. Ultrasensitive detection kits with a detection limit of < 0,1-5% for minor strains are only available in very few and more specialized laboratories. Overall it is not sure whether minor strains are of clinical importance or not.
The plasma sample needs to contain a minimum of 500-1000 copies/ml in order to properly process viral RNA.

2.6. Patient Management and considerations for HIV-1 resistance testing

2.6.1. Introduction

Suboptimal antiretroviral treatment can quickly lead to drug resistant HIV-1 variants. Within days, weeks or in some cases months, resistance will develop if the viral replication is not completely suppressed in the infected individual. Resistance testing has therefore become a very important tool to optimize antiretroviral therapy and to distinguish between virological and non-virological reasons of drug resistance.[82]

2.6.2. Limitation of resistance testing

Availability of genotypic resistance testing can be helpful in the clinical management of the infected individuals, but interpretation of the results remains very complex. Due to lower costs compared to phenotypic resistance testing,

[82] Clotet B et al, 2008, p19

genotypic assays are nowadays broadly introduced in clinical practice, but still neither virologist nor clinicians can predict the success of antiretroviral therapy. Viral strains, with multiple mutations cause difficulties in the clinical course of the treated individual. The calculation of the genotypic background can not always predict the phenotypic impact of particular mutations. Not all resistance patterns have been characterized, which is another limitation of genotypic resistance testing. Also there are no genotypic assays for new antiretroviral agents available. Phenotypic testing might have advantages compared to genotypic testing, but is not performed for every affected patient, as the high cost limits its availability. It is rather used to complement genotyping, and is mostly used for patients with multiple drug resistant strains, since it additionally reflects the actual susceptibility of the viral strain to the drugs.

2.6.3. Clinical management of the patient

Lots of clinical studies have demonstrated the usefulness of genotypic testing, despite of all its limitations.[83] Over the last few years, several tools have been developed, to help the clinician in the interpretation of genotypic assays:

1. Expertise:
   The clinician consults with the expert on a case-by-case basis. However, this is not always possible because many clinicians may not have access to expert advisory. [83]

2. "Rules based" algorithms:
   These algorithms are designed by panels of experts, and they are continuously updated based on newly reported evidence. This kind of interpretation may be generated from a computer as part of genotypic report, such as TruGene™ HIV genotypic assay. The major limitation of these assays lies in the difficulty in agreeing on rules for interpreting novel

[83] Clotet B. et al., 2008, p19

or complex mutation patterns, especially when published evidence is limited or contradictory. Thus substantial discordances in the interpretation of genotypic resistance by several algorithms have been described by some authors.[84, 85, 86]; In particular the interpretation of resistance to selected NRTI, such as *Abacavir*, *Didanosine* or *Stavudine*.[87]

3. The "virtual phenotype":

   This tool was introduced by Virco (vircoTYPE) and interprets automatically genotypic test results. Interpretation is based on the extensive information available in some databases, which include tens of thousands of clinical HIV specimens from which both, genotype and phenotype are known. The patient's phenotype generated is a probabilistic estimation of the real phenotype, which is inferred by an algorithmic approach on these databases. It has been shown, that this kind of interpretation is highly concordant with the real phenotype (90% of the cases), and major discordances were observed in only 3% of the cases.[88]

Proper resistance testing should help clinicians to:[89]

- Avoid unnecessary switching of drugs.
- Rule out adherence problems.
- Perform well-directed switches rather than empirical changes of drugs.
- Use active drugs for longer periods of time. Reduce costs associated with switching drugs.
- Avoid unnecessary toxicities from inactive drugs.

The following table shows the general considerations to take into account for a proper use of resistance tests:

[84] De Luca A, et al. J Infect Dis 2003; 187: 1934-42, from Clotet B. et al, 2008, p20
[85] Stürmer M, et al. Anitvir Ther 2003; 8: 239-244, from Clotet B. et al, 2008, p20
[86] Ravela J, et al JAIDS 2003; 33: 8-14, Clotet B. et al, 2008, p20
[87] Clotet B. et al, 2008, p20
[88] Verbiest W, et al. 40th ICAAC, Toronto, September 2000, Abstract 1257, from Clotet B. et al, 2008, p20
[89] Clotet B et al. 2008, p21

	MAIN CONSIDERATIONS	COMMENTS
1.	Drug resistance testing should be performed on patients with detectable viral load (>500-1000 copies/ml).	
2.	Blood samples should be drawn before the failing regimen is discontinued or within the two weeks following the treatment interruption.	Although TAMs or NNRTIs-related mutations may persist longer, some mutations may disappear rapidly after withdrawal of the drug (e.g. M184V after stopping Lamivudine). However the mutational pattern may persist unaltered during two weeks after the treatment interruption
3.	The test should be performed in a certified laboratory, under a strict quality control and quality assurance standard.	
4.	Predicting phenotypic resistance from genotypic data has considerable limitations.	The phenotype of many combinations of drug resistance mutations is not entirely known. Varying interpretations may be reported regarding the level of resistance conferred by a specific mutational pattern. The many possible complex combinations of multiple mutations may limit the usefulness of rule-based approaches or algorithms interpreting the genotypic results.
5.	Therapeutic failure may be due to non virological reasons.	Resistance testing should always be accompanied by careful questioning of the patient with regard to • Drug history • Adherence • Toxicities • Concomitant medication In addition, whenever possible, determination of plasma levels of PI and NNRTI should be determined 4-12 weeks after staring a new antiretroviral regimen. In case of suboptimal concentrations in plasma, aspects such as adherence, drug interactions or incorrect antiretroviral dosing should be investigated.
6.	The interpretation of genotypic and phenotypic analysis is difficult and requires fluent communication between clinicians and virologists.	The clinical relevance of particular patterns of resistance mutations and different levels of phenotypic resistance is still in debate.
7.	The greater the reduction in phenotypic susceptibility, *in vitro*, the less activity a drug will have against that virus *in vivo*.	However even low-level resistance may predict a lack of response to a particular drug.
8.	Neither genotypic nor phenotypic testing assesses possible cellular mechanisms of drug resistance.	Resistance tests do not assess potential mechanisms of cellular resistance (drug, efflux…).
9.	Phenotypic assays may fail to detect evolving resistance that has not yet led to measurable increases in IC50 values.	E.g.: the K70R mutation conferring zidovudine (ZVD) resistance, emerges within 12 weeks in nearly half of the patients receiving ZVD monotherapy, although its presence may not be associated with measureable increases in ZVD IC50
10.	Susceptibility does not assure clinical efficacy.	In both naïve and experienced patients, when the assay shows resistance to a drug, it is most likely that the drug will not work. However, when the assay shows susceptibility to a drug, there is a reasonable likelihood, but not a guarantee, that the drug will be effective.[90]
11.	Current and previous resistance information should be integrated.	Mutations which were present in previous tests but that have disappeared on the present test, are nevertheless still relevant.

Table 6: Considerations for the practical use of resistance tests[91]

[90] Hirsch MS, et al. Clin Infect Dis 2003, from Clotet B. et al., p19
[91] Own table, contents from: Clotet B et al. 2008, pp22-23

2.6.4. Resistance testing in drug naïve individuals

Two things are sure, when it comes to drug resistance. First, the transmission of protease- or reverse transcriptase inhibitors-resistant HIV strains, as well as the transmission of virus strains resistant to 2 or 3 classes of antiretroviral drugs, is occurring. Second the individuals infected with those mutants show impaired treatment response when the regimen is based on drugs to which the virus is already resistant. Another important fact is, that avoiding drugs to which resistance already exists, may prevent the quick development of new resistance to other drugs included in the antiretroviral regimen of a patient. This would preserve future rescue regimens for the affected individual.
A lot of studies that worked with a large number of drug-naïve individuals who recently got infected with HIV, showed that prevalence of primary drug resistance ranges, between 2% and 33%, with observed increasing trend.[92, 93, 94, 95, 96, 97]

As prevalence of drug resistant virus transmission tends to be different in various geographical regions, the question that comes up is, which level of prevalence should be taken as reason for testing drug-naive patients. Data, taken from the GART-study suggest that cost-effectiveness starts with prevalence higher than 4%, while a consensus guidelines panel suggests 5% to 10% prevalence of primary drug resistance, justifies initial testing.[98]

The following table summarizes the suggestions for resistance testing in drug-naive patients.

[92] Boden D et al. JAMA 1999; 282: 1135-1141, from Clotet B et al. 2008, p24
[93] Little SJ, et al. JAMA 1999; 282: 1142-1149, from Clotet B et al. 2008, p24
[94] Puig T, et al. AIDS 2000; 14: 727-732, from Clotet B et al. 2008, p24
[95] Gallego O, et al. AIDS 2001; 15: 1894-1896, from Clotet B et al. 2008, p24
[96] Bennett D, et al. 12th CROI, Boston, February 2005, Abstract 674, from Clotet B et al. 2008, p24
[97] Little S, et al. N Engl J Med 2002, from Clotet B et al. 2008, p24
[98] Clotet B et al. 2008, p24

	CONSIDERATIONS
Primary HIV infection and recent serocenvertors (less than 6 months since HIV seroconversion)	If considered, treatment should be initiated as soon as possible in attempt to minimize immune system damage, particulary during the acute infection (<4 weeks). In such situation, the initiation of treatment should not wait for resistance results. If transmitted resistance is later documented, then the therapeutic approach can be modified accordingly. Information about the index case, when available, may be of great help to design more suitable regimen until the availability of the resistance results.
Naïve patients with established chronic HIV infection (more than 6 months since seroconversion)	Resistance test should be performed a soon as possible after HIV diagnosis, regardless of the need for therapy. Because mutant viruses tend to be more fit than wild type variants, primary infection with resistant viruses may be unrecognized because of outgrow of wild type revertants. Nevertheless, minor resistant species could emerge rapidly when antiretroviral therapy is initiated. Species constituting 20% or less of the amplified product may not be detected with current sequencing of phenotypic assays. The initiation of antiretroviral therapy in chronic infection is almost never an emergency, permitting us to wait for the resistance results before starting therapy.
Post-exposure prophylaxis (PEP)	Resistance tests on the source subject could be useful for healthcare workers accidentally exposed to contaminated fluids and for individuals who had a sexual contact or shared needle with an infected person. To properly design the best prophylactic regimen, the source person should be tested and their antiretroviral history determined. As with primary HIV infection, treatment should be started without waiting for the resistance results, and should be modified accordingly. However, currently recommended length of PEP is hence 4 weeks, if results are not available in less than a week, the effect of antiretroviral therapy will be negligible.
Pregnant women	Because currently the diagnosis of HIV infection in pregnant women is usually antenatal, screening for resistance could help selecting the optimal antiretroviral therapy. Effective antiretroviral treatment during pregnancy, mainly before delivery, significantly reduces the risk of vertical HIV transmission.

Table 7: Role of resistance testing in drug-naïve individuals[99]

[99] Own table, contents from Clotet B et al. 2008, pp26-27

2.6.5. Resistance testing in pretreated individuals[100]

Therapeutic failure is definitely an indication for resistance testing in pretreated individuals, as they are more likely to benefit from exclusion of drugs to which resistances already exist. This does not only prevent the accumulation of additional cross-resistance mutations, but also saves costs and unnecessary exposure to drug side effects and toxicity.

The availability of new treatment options for patients with multiple drug resistant strains is on the other hand limited. The development of new agents will hopefully be an option for those patients, to achieve virological suppression regardless of their already existing resistance. However, also in these cases it is important to treat the patients with a combination of antiretroviral drugs, to maximize the success of their therapy and to minimize the development of new resistance.
Drug resistance is on the other hand not the only reason for therapeutic failure, which means a drug sensitive virus must not necessarily mean a good virological suppression. Various other reasons can lead to insufficient virological suppression and the must also taken into consideration, for example malabsorption.

The following table summarizes the most important considerations for resistance testing in pretreated individuals:

[100] Clotet B et al. 2008, p28

CONSIDERATIONS	EXPLINATIONS
Patients who fail to achieve less than 50 HIV-RNA copies/ml after 3-6 months on therapy	This is straightforward if viral load is >1000 copies/ml, however, the currently available methods will not give result in about 50%-70% of patients who have a measurable viral load below 500-1000 copies/ml. In such cases the test should be repeated few weeks later /8-12 weeks) to see if a further increase in plasma HIV-RNA has occurred and can be amplified. In the case that no genotype is obtained after 2 repeated attempts, there are three possible options for the clinician: 1. Maintaining the same regimen in order to wait for an increase in HIV-RNA large enough to permit resistance testing. 2. Intensification of the current regimen with potent drugs not previously used and with a low risk of cross resistance (E.g.: adding didanosine, tenofovir or abacavir); or boosting the PI through low doses of ritonavir. It is also possible to replace the most probable failing drug (which will be that one with the lowest genetic barrier). 3. Substitution of all agents included into the antiretroviral regimen. Unusual subtypes of HIV, as well as false positive results of viral load determinations, could be other possible reasons of failure on HIV-RNA amplification. Plasma levels of PI and NNRTI and adherence issues should be also evaluated.
Pregnant women receiving antiretroviral treatment and with plasma HIV-RNA >50 copies/ml.	In this setting, the genotype could help to switch to a more potent combination and to minimize the risk of vertical transmission of drug resistant viruses to the child.
First, second or subsequent treatment failure	Genotype may be of great help but, in subjects presenting with three or more treatment failures, the phenotype (real or virtual) should be considered in addition to the genotype.
Structured treatment interruptions in patients with therapeutic failure	In patients who have received different therapeutic regimens and with persistent detectable viral load, the interruption of treatment could theoretically favor the outgrowth of wild type viruses and, hence, a better response to the reintroduction of treatment.[101] If such theory is true, to determine the genotype (and possibly also the phenotype) before interrupting the treatment, could be of great value. Likely resistance tests should be repeated before (reintroducing) the antiretroviral therapy, which should be done according to the susceptibility re-established during the interruption. It should be taken into consideration, that the interruption of treatment in highly experienced patients could be dangerous and very complex and should not be recommended because the risk may overcome the benefits of this method. Also very little data exist for structured treatment interruptions and it is necessary to wait for more information to be published onto this topic.

Table 8: Specific situations for resistance testing in pretreated individuals[102]

[101] Devereux HL, et al. AIDS 1999
[102] Own table, contents from Clotet B et al. 2008, pp33-34

2.6.6. Salvage Therapy[103]

There is no exact definition for salvage therapy but generally it means a final treatment for people who are non responsive to, or can not tolerate standard antiretroviral therapy. The viral load can not decrease under the detection limit with the standard combinations of drugs. If this occurs for all three standard classes of antiretroviral drugs (NRTIs, NNRTIs, PIs), then it is considered as "triple class failure". Most commonly doctors talk about salvage therapy or dual- or triple class resistance, if there is genotypic resistance against at least two or three classes of antiretroviral therapeutics.
Before 2007 it was difficult to reduce the viral load of those patient's as the only salvage therapy available at those times consisted of three drugs: *Lopinavir, T-20* and *Tipranavir.* This changed in 2007, when 4 new drugs were released on the US-market: *Darunavir, Maraviroc, Raltegravir* and *Etravirin.* Those new drugs were very successful in patients with multiple drug resistance and the development of new drugs and classes of antiretroviral medication is continuing. Further US-studies showed that Dual- or Triple class resistance is decreasing.

Salvage therapy is modified very individually and there are no standard rules. The patient must be monitored closer than patients with no or one resistance and the goal remains the same: to decrease the viral load under the detection limit.

[103] Hoffmann C. Rockstock J Kamps BS, 2008, pp152-164

3. Materials and Methods

3.1. Specimens used in this study

In this retrospective study, HIV-1 sequences obtained from a total of 108 patients were analyzed. Fifty-four HIV-1 sequences from drug-naïve patients obtained in 2002 were compared with those from another 54 drug-naïve patients obtained in 2007. All patients had been diagnosed with HIV infection prior to first RNA sequencing at the *Institute for Hygiene, Microbiology and Environmental Medicine* of the Medical University of Graz.
For determination of the HIV-1 subtype, the TruGene® HIV-1 Genotyping Kit (SIEMENS medical) was used according to the manufacturer's package insert instructions.

3.2. Study population

The samples used in this study were taken from drug-naïve patients living in Eastern (Vienna, Lower Austria) and Southern (Styria, Carinthia) Austria.

The study population was analyzed by the following criteria:

- Sex
- Age
- HIV-1 subtype
- Resistance profile

Resistances were classified as:

- Resistance against Nucleoside Reverse Transcriptase Inhibitors (NRTIs)
- Resistance against Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
- Resistance against Protease Inhibitors (PIs)

3.3. Genotypic resistance analysis

Automated RNA extraction was done with the AmpliPrep instrument (Roche Molecular Diagnostics). Amplification and sequencing were performed with the TruGene® HIV-1 Genotyping Kit (SIEMENS medical) according to the manufacturer's package insert instructions. All sequences were analyzed with the GeneLibrarian module of GeneObjects software (version G12). HIV-1 subtypes were determined by means of the geno2pheno database (www.geno2pheno.org).

With the TruGene® HIV-1 Genotyping Kit, detection of mutations which refer to drug resistance of the HIV-1 virus is done by sequencing the genomic regions of the 2 main targets of the antiretroviral drug treatment. The targets are parts of the reverse transcriptase and the protease region. The RT-PCR product is compared to the corresponding genomic regions of the wild type reference standards. DNA Sequencing can be performed at a minimum viral load of 1000 RNA copies/ml.

The TruGene® HIV-1 Genotyping Assay consists of several processes:[104]

- Reverse transcription of target RNA to generate cDNA using RT-PCR amplification of target cDNA using HIV-1 specific primers.
- CLIP sequencing of the PCR amplicons using HIV-1 specific primers
- Separation of the CLIP sequencing reactions by electrophoresis on a polyacrylamide gel, and detection by laser-induced fluorescence
- Analysis of the forward and reverse CLIP sequences using the OpenGene DNA System Software.

Reviewing and editing the CLIP sequence by the user is of major importance as base insertions or deletions will not automatically identified by the OpenGene system software.

In this study, the presence of ≥1 resistance-related mutation was defined as genotypic resistance, matched with the GeneLibrarian module of Gene Objects.

[104] TruGene® HIV-1 Genotyping Kit, manufacturers package, p8

4. Goals of this study

- To evaluate the prevalence of HIV-1 subtypes in Southern and Eastern Austria and to compare data obtained in 2002 with those obtained in 2007

- To estimate the situation of primary drug resistance in Southern and Eastern Austria and to compare data obtained in 2002 with those obtained in 2007

- To estimate the rate of transmitted drug resistance in patients infected with HIV-1 subtype B compared to those infected with HIV-1 subtypes non-B

5. Results

5.1. Prevalence of HIV-1 subtypes in Southern and Eastern Austria

The samples used in this study were derived from patients (12 females, 96 males; mean age 34.8 years in the 2002 population, 35.6 years in the 2007 population; age range, 0-70 years).

In 2002, 42 (77.8%) of 54 patients included in this study were found to be infected with HIV-1 subtype B. In 2007, 38 (70.4%) of 54 patients included were found to be infected with HIV-1 subtype B. The majority of all patients were from Austrian origin while 18 (16.4%) of 110 patients lived in Austria but with migration background.

In 2002, subtypes CRF01_AE (7.4%), A (5.6%), C, F, G, CRF08_BC, and CRF10_CD (1.9% each) were found additionally. Corresponding data for 2007 were D (9.3%), F (7.4%), CRF02_AG (3.7%), A, G, CRF01_AE, CRF09_cpx, and CRF14_BG (1.9% each).

Figure 8 shows the prevalence of HIV-1 subtypes found in this study.

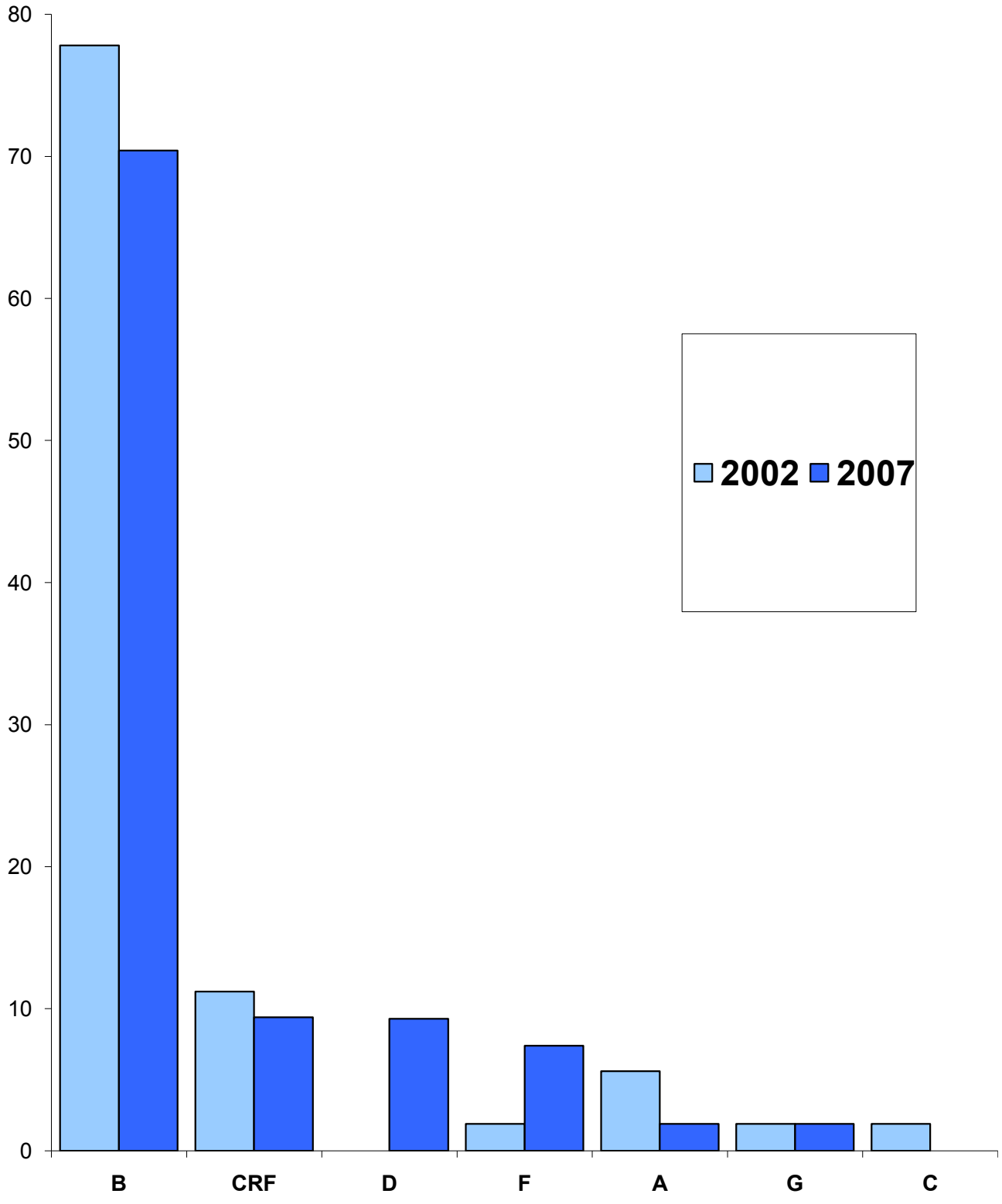

Figure 8: Distribution of HIV-1 subtypes in 2002 and 2007.

5.2. Situation of primary drug resistance in Southern and Eastern Austria

Prevalence of transmitted drug resistance was 20.4% for any drug in 2002 and 22.2% for any drug in 2007 (Figure 9).

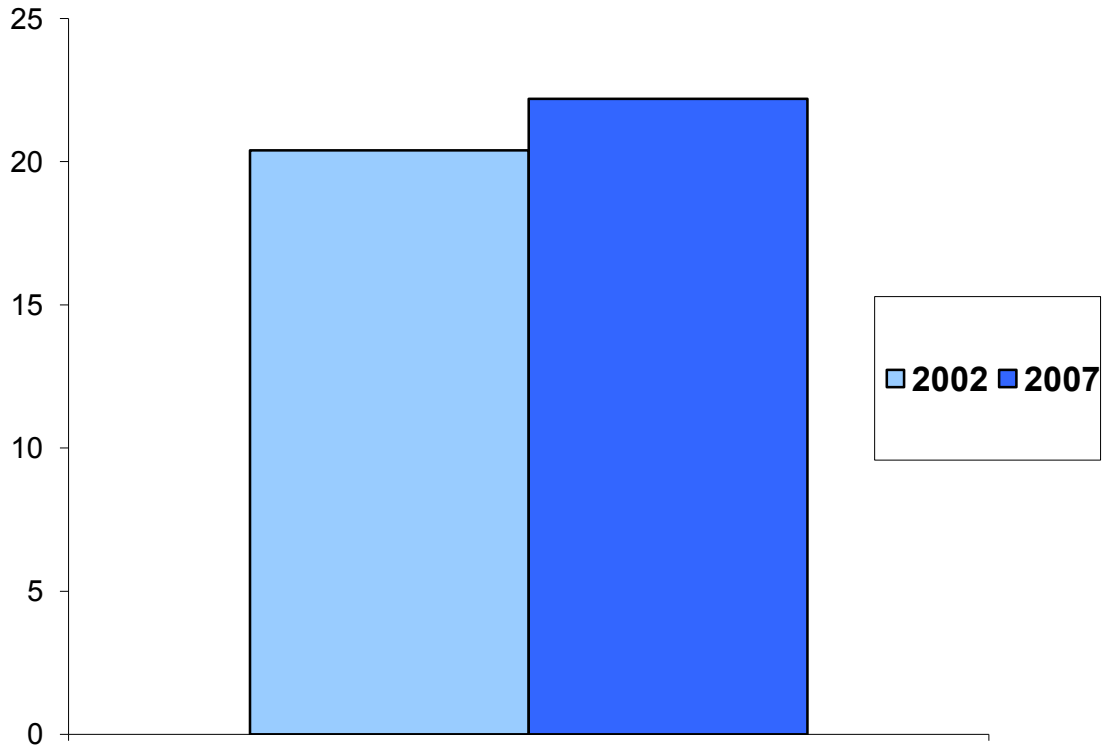

Figure 9: Prevalence of transmitted drug resistance for any drug in 2002 and 2007.

The majority of drug resistance was found for protease inhibitors. Dual class resistance was observed in one patient in 2002 and in two patients in 2007. Triple class resistance was not found in any patient.

Table 9 provides an overview about the single drug mutations detected in 2002.

Mutations in the drug resistance related genomic region	No. of drug resistance mutations detected
HIV-1 reverse transcriptase	
A98G	1
K103	1
K219Q	1
M41L	1
T215S	1
T215Y	1
V108I	1
HIV-1 protease	
L63P	32
I93L	18
M36I	18
I13V	17
I62V	13
V771	13
H69K	11
L89M	9
I15V	8
L10I	4
A71V	3
N36I	3
A71T	2
D60E	2
L10V	2
L33V	2
L63T	2
G73A	1
G73S	1
I02L	1
I54T	1
K20M	1
K20R	1
L33F	1
L89I	1
M36I/V	1
M36L	1

Table 9: Drug mutations found in samples collected in 2002.

Mutations were associated with resistance to the following drugs:

NRTIs:
Zidovudine (1) and Stavudine (1).

NNRTIs:
Efavirenz (1) and Nevirapine (1).

PIs:
Tipranavir/r, possible resistance (9); Atazanavir, possible resistance (2); Indinavir (1); Saquinavir (1).

The following resistance profiles with resistance to more than one drug but within one class only were found:

Patient 1:
PIs: Possible resistance to Saquinavir/Ritonavir, Atazanavir, and Tipranavir/Ritonavir.

Patient 2:
PIs: Possible resistance to Atazanavir and Tipranavir/Ritonavir.

Furthermore, one resistance profile was detected showing dual class resistance:

NRTIs: Resistance to Zidovudine and Stavudine.
NNRTIs: Resistance to Nevirapine and Efavirenz.

Table 10 provides an overview about the single drug mutations detected in 2007.

Mutations in the resitance related genomic regions 2007 (frequency of occurrence)	
HIV-1 Protease and Reverse Transcriptase	
T215S	3
T69N	2
K101Q	1
K103N	1
M184I	1
P225H	1
T69D/N	1
Y188H	1
Protease Inhibitors	
I93L	28
I62V	26
L63P	21
V77I	21
M36I	18
I15V	17
I13V	14
L10I	11
L89M	11
H69K	9
A71T	7
G16E	7
L63T	7
D60E	6
L10V	4
K20I	4
A71V	3
K20I/M	2
L33V	2
A71I/T/V	1
K20M	1
L36P	1
M36L	1
M46L	1
Q58E	1
V11I	1

Table 10: Drug mutations found in samples collected in 2007.

Mutations were associated with resistance to the following drugs:

NRTIs:
Lamivudine/Emtricitabine (1) and Didanosine (1).

NNRTIs:
Efavirenz (1); Efavirenz, possible resistance (1); Nevirapine (1); Nevirapine, possible resistance (1)

PIs:
Tripanavir (1); Tripanavir, possible resistance (5); Atazanavir (1); Atazanavir, possible resistance (4); Saquinavir/r (3); Fosamprenavir (1); Indinavir (1); Indinavir/Ritonavir (1); Amprenavir, possible mutation or Fosaprenavir/Ritonavir (1)

The following resistance profiles with resistance to more than one drug but within one class only were found:

Patient 1:
PIs: Possible resistance to Saquinqvir/Ritonavir; resistance to Atazanavir and Tipranavir/Ritonavir

Patient 2:
PIs: Possible resistance to Saquinqvir/Ritonavir, Atazanavir and Tipranavir/Ritonavir

Patient 3:
Protease Inhibitors: Possible resistance to (P)ATV Atazanavir and (P)TPV/r Tipranavir/r

Patient 4:

Protease Inhibitors: Resistance to Indinavir, possible resistance to Indinavir/Ritonavir, resistance to Fosamprenavir, possible resistance to Amprenavir/Ritonavir OR Fosaprenavir/Ritonavir

Furthermore 2 resistance profiles were detected, showing dual class resistance:

Patient 1:

NRTIs: Resistance to Lamivudine/Emtricitabine

NNRTIs: Resistance to Nevirapine and Efavirenz

Patient 2:

Protease Inhibitors: Possible resistance to Atazanavir

NNRTI: Possible resistance to Nevirapine and Efavirenz

5.3. Rate of transmitted drug resistance in patients infected with HIV-1 subtype B compared to those infected with HIV-1 subtypes non-B

Figure 10 shows the comparison of resistance mutations found in patients infected with HIV-1 subtype B and those infected with HIV-1 subtypes non-B. A total of 11 drug resistance related HIV-1 mutations were observed in 2002 with 8 in patients infected with HIV-1 non-B subtypes, while a total of 12 drug resistance related HIV-1 mutations were observed with 4 in patients infected with HIV-1 non-B subtypes.

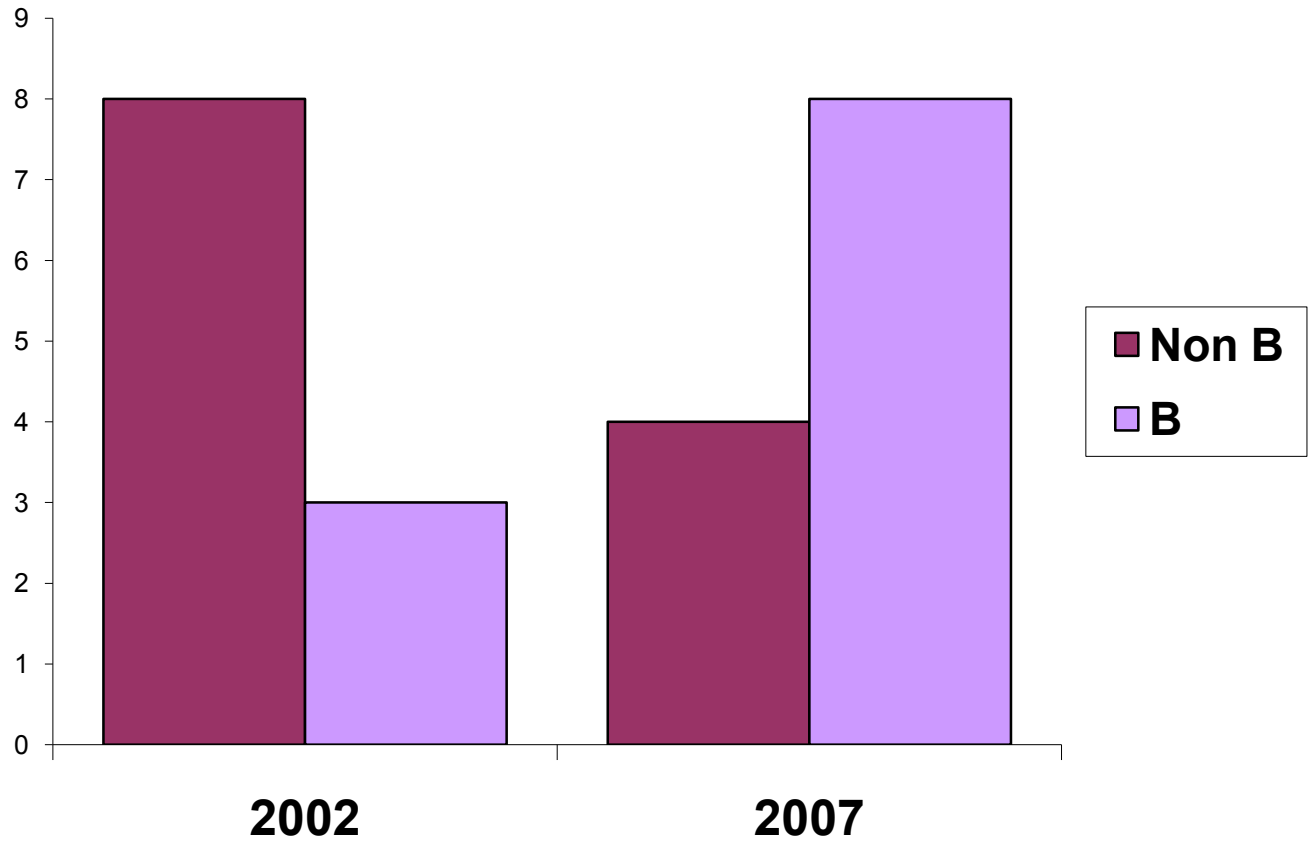

Figure 10: Prevalence of resistance mutations in 2002 and 2007.

6. Discussion

Determination of HIV-1 subtypes may contribute to track viral spread. Furthermore, the HIV-1 subtype may have an influence on the transmissibility and pathogenicity of the infection.
In this study, an increase of HIV-1 non-B subtype infections was found between 2002 and 2007. This may be due to an increased immigration from African countries. An additional reason may be increased sex-tourism to non-European countries.

This study revealed a slight increase of primary HIV-1 drug resistances between 2002 and 2007 which corresponds with data found in most of the other European countries.[105, 106] The most frequently found resistances were those to a single PI. This does not correspond with data reported from other countries with the prevalence for NRTI resistance being the highest.[107] Multiple drug resistances were a rare event in this study.

Antiretroviral treatment was designed for HIV-1 subtype B. This may have an impact on the response to treatment and development of drug resistance in individuals infected with HIV-1 non-B subtypes. In a recent study, it was suggested that a correlation between HIV-1 subtypes and resistance associated mutations can be found.[108] In another study, it was hypothesized that drug resistance mutations in the polymerase gene may have a great impact on the treatment of patients with HIV-1 non-B subtypes, resulting in accelerated resistance.[109] Recombination between drug resistant strains may result in new HIV-1 variants, presenting transmissible dual or triple class resistance.[110] In this

[105] Wensing AM et al. 2005, J Infect Dis; 192: 958-966
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[107] Yerly S. et al. AIDS 2007, 21:2223-2229
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[109] Kantor R. et al. 2005, PLoS Med 2: p. 112
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study, no impact of the HIV-1 subtype on the primary drug resistance was found. However, this might be due to the low number of patients included.

In conclusion, HIV-1 subtype B remains dominant in Southern and Eastern Austria; however, the percentage of HIV-1 non-B subtypes and recombination forms appears to increase slightly. Furthermore, this study shows that primary drug resistance within a defined population in a country with large access to antiretroviral therapy has remained rather stable between 2002 and 2007. Additionally, no significant increase of dual or triple class resistance was observed in HIV infected individuals in Southern and Eastern Austria. This suggests that optimized antiretroviral treatment may contribute to the low spread of primary transmitted drug resistance.

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Curriculum Vitae

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Humanitarian Congress	• 10/2008, Berlin/Germany
Seventh International Symposium on Molecular Diagnostics (ISMD)	• Graz, 05/2008

Memberships and NGO work	
Member of the AMSA, a member of the IFMSA	• NATIONAL OFFICER on REPRODUCTIVE HEALTH including HIV/AIDS (2008/09) • LOCAL OFFICER on REPRODUCTAIVE HEALTH including HIV/AIDS (2005-2009) • PROJECT COORDINATOR for the orphanage project in South Africa (10/2005 – present) • SUPERVISING TUTOR for international incoming students on several occasions • Organization and Coordination of "World AIDS day" and "World women's day" every year ever since 2005 (up to 20 000 people reached each year)
Member of the Austrian Red Cross	Paramedic
School education	
	High school "Untere Bachgasse", Mödling/Lower Austria
1995-1999	High school "Europagymnasium", Klagenfurt/Carinthia-Austria
1999-2002	High school "Bundesrealgymnasium Klagenfurt-Viktring", Klagenfurt/Carinthia-Austria (high school with special focus on music)
2002	High school qualifications: "Matura" (final exam) with honours Graduation 2002
Language skills	
German	Fluently (spoken, written)
English	Very good knowledge (spoken, written)
French	Very good knowledge (spoken, written)
Hobbies	
	Travelling the world European and alternative movies Music (concerts, making music) Sports (Hiking, Skiing, Cycling)