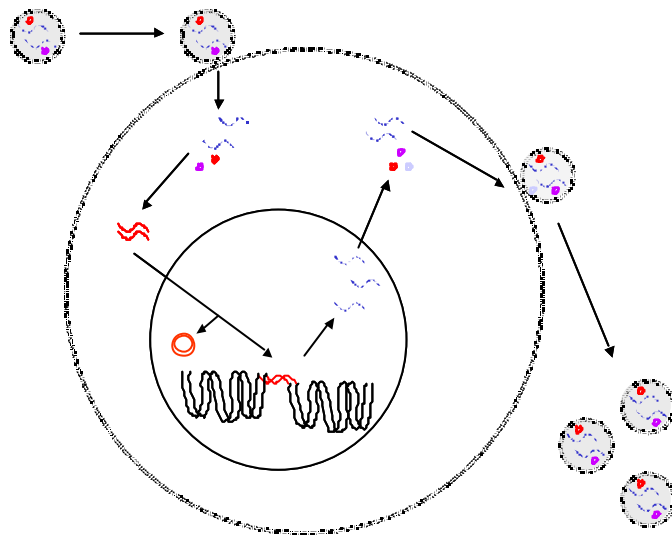


HIV THERAPIES

from health-related quality of
life to DNA levels



Lars E. Eriksson



Stockholm 2003

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Stockholm, Sweden

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to my family,
to all HIV carriers
and to all carers of HIV carriers

ABSTRACT

The treatment of people infected with HIV was revolutionised with the introduction of protease inhibitor based antiretroviral combination therapy. The therapy has greatly reduced the incidence of AIDS and AIDS-related mortality in the industrialised world. However, the necessity of strict adherence to the complicated regimes and the long-term adverse effects have a negative impact on the patient. The aims of this thesis were to study the longitudinal influence of vaccination therapy, antiretroviral therapy and therapy interruption on the health-related quality of life and biomedical variables of HIV-positive patients. Further, to develop methods to monitor HIV DNA during different treatment conditions.

The five-year impact of recombinant envelope glycoprotein 160 (rgp160) vaccination was investigated in 40 HIV-infected patients. During the first six months of therapy, the immunisations were followed by two weeks of double-blind azidothymidine or placebo therapy. Treatment with rgp160 was safe and temporarily improved the levels of CD4⁺ cells but had no apparent long-term effect on disease progression.

In a subsequent study of the rgp160 vaccine, the impact on the health-related quality of life of 72 men was investigated, using the Swedish Health-Related Quality of Life Questionnaire (SWED-QUAL) and the Health Index. Before the initiation of treatment there was a negative effect on quality of life compared with random population groups. The most affected parameters were those related to emotional well-being. During two years of treatment the physical domains deteriorated, unrelated to treatment arm (rgp160 or placebo).

Moreover, 54 patients were measured by the SWED-QUAL before and after two years of protease inhibitor based antiretroviral therapy. We constructed two new comprehensive scores to facilitate evaluation of data obtained by SWED-QUAL. Initiation of therapy improved immune and viral parameters but induced a decrease in the emotional health domain while the physical and social domains remained unchanged. The experience of adverse effects contributed most to the deterioration of emotional health. Further, physical health domains before the start of therapy predicted experiences of adverse effects and adherence. That is why it could be important to consider health-related quality of life when planning strategies and care for antiretroviral therapy.

For the first time, the viral content of CD4⁺ cells was studied during long-term therapy interruption. In order to accomplish this, we developed an unorthodox assay, comparing two viral genomes by real-time PCR. A multiplex PCR was established for measuring the HIV DNA load in CD4⁺ cells by simultaneously amplifying a conserved region of the HIV-1 *pol* gene, an EBV gene and a section of the human albumin gene. The HIV DNA load was monitored in 15 patients undergoing long-term supervised therapy interruption. HIV DNA seemed to decrease unceasingly during efficient treatment. When therapy was interrupted, HIV DNA increased in all patients with an average doubling time of two months. The change in the HIV DNA load (slope) was positively related to the HIV RNA maximum load, the RNA steady state level and the baseline HIV DNA value. The HIV DNA slope was also inversely related to the decrease in CD4⁺ cells both before the start of therapy and during the therapy interruption. The health-related quality of life in the patients undergoing long-term supervised therapy interruption seemed to remain stable or improved slightly after two years of interrupted therapy. The progression rate of the HIV infection before initiation of antiretroviral combination therapy and the health-related quality of life may thus be important considerations when assessing patients for supervised therapy interruption.

ORIGINAL PAPERS

- I** Göran Bratt, Lars E. Eriksson, Eric Sandström, Gustav Gilljam, Jorma Hinkula, Jan Albert, Robert Redfield and Britta Wahren. *Long-term immunotherapy in HIV infection, combined with short-term antiretroviral treatment*. International Journal of STD and AIDS, 1999;10(8):514-521.
- II** Lars E. Eriksson, Gun Nordström, Torsten Berglund and Eric Sandström. *The health-related quality of life in a Swedish sample of HIV-infected persons*. Journal of Advanced Nursing, 2000;32(5):1213-1223.
- III** Lars E. Eriksson, Göran Bratt, Eric Sandström and Gun Nordström. *The longitudinal impact of protease inhibitor based antiretroviral combination therapy (PI-ART) on health-related quality of life*. Manuscript submitted for publication, 2003.
- IV** Lars E. Eriksson, Thomas Leitner, Britta Wahren, Ann-Charlotte Leandersson and Kerstin I. Falk. *A novel way to simultaneously quantify HIV-1 DNA and the human albumin gene in CD4⁺ cells*. Manuscript submitted for publication, 2003.
- V** Lars E. Eriksson, Kerstin I. Falk, Göran A. Bratt, Ann-Charlotte Leandersson, Britta Wahren and Thomas Leitner. *HIV-1 DNA development during long-term supervised therapy interruption*. AIDS Research and Human Retroviruses, 2003;19(4):in press.

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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ART	antiviral therapy
AZT	azidothymidine
CD	clusters of differentiation
ddC	dideoxycytidine
ddI	2'3'-dideoxyinosine
DNA	deoxyribonucleic acid
<i>e.g.</i>	<i>exempli gratia</i> ; for example
<i>et al.</i>	<i>et alii</i> ; och andra
EWB	emotional well-being
HAART	highly active antiretroviral therapy
HI	Health Index
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
<i>i.e.</i>	<i>id est</i> ; that is
LT-STI	long-term supervised therapy interruption
MSM	men who have sex with men
PAUSE	long-term supervised therapy interruption study
PCR	polymerase chain reaction
PI-ART	protease inhibitor based antiretroviral combination therapy
PWB	physical well-being
QoL	quality of life
rgp	recombinant glyco-protein
RNA	ribonucleic acid
RT	reverse transcriptase
SD	standard deviation
STI	supervised therapy interruption/structured therapy interruption
SWED-QUAL	Swedish Health-Related Quality of Life Questionnaire
vs.	versus

GENERAL INTRODUCTION

In the early 1980s, cumulative cases of patients suffering from a syndrome with unusual symptoms related to immunodeficiency began to appear in the medical press. These cases were concentrated to major cities of the USA and consisted mainly of young same-sex oriented men. The syndrome, later named the acquired immunodeficiency syndrome (AIDS), also appeared in intravenous drug abusers, patients with haemophilia and inhabitants of Haiti. In 1983, French researchers isolated a new virus from a patient with lymphadenopathy (Barré-Sinoussi *et al.*, 1983). Shortly thereafter, similar viruses were isolated by researchers in the USA (Popovic *et al.*, 1984). The new virus, later named the human immunodeficiency virus type 1 (HIV-1), has been further divided into several subtypes. Current evidence indicates that HIV entered the human population through multiple zoonotic infections from nonhuman primates infected with simian immunodeficiency virus (Hahn *et al.*, 2000). HIV-1 has been isolated from a stored African plasma sample taken as far back as 1959 (Zhu *et al.*, 1998). However, the introduction of the main group of HIV-1 to the human population must have occurred much earlier, since data suggest that the epidemic may have begun in the 1910s to 1930s (Korber *et al.*, 2000; Salemi *et al.*, 2001). In 1986 an additional type of HIV, HIV-2, was isolated (Clavel *et al.*, 1986). This thesis deals with HIV-1, which is hereafter referred to as HIV.

The estimated number of people living with HIV at the end of 2002 was 42 million according to the World Health Organization (UNAIDS/WHO, 2002). The majority of them are in Sub-Saharan Africa (almost 30 million) but epidemics are emerging in Asia and Eastern Europe. During the past year, 5 million people became newly infected and 3.1 million died in HIV-related illnesses. If there is no radical expansion of global preventive efforts, in the next eight years there will be at least 45 million new HIV cases. In Sweden, 5,640 HIV cases (74% men and 26% women; 40% homosexual, 38% heterosexual, 15% intravenous drug abuse and 7% other/unknown route of infection) have been reported since the beginning of the epidemic and about 3,000 people are estimated to be living with HIV today. A total of 277 new cases were reported in 2001 (57% men and 43% women; 51% heterosexual, 24% homosexual; 13% intravenous drug abuse and 12% other/unknown route of infection; Swedish Institute for Infectious Disease Control, 2002).

HIV is a lentivirus belonging to the family *retroviridae*. The HIV virion contains two RNA strands, each about 9.2 kb in size. The HIV genome is flanked by long terminal repeats and contains the three structural genes *gag*, *pol* and *env* and the six regulatory/accessory genes *tat*, *rev*, *nef*, *vif*, *vpr* and *vpr*. A schematic overview of the virus particle is shown in Figure 1. The nucleic acid strands are, together with associated proteins (*e.g.* reverse transcriptase (RT), protease and integrase), surrounded by a core (p24) and a matrix (p17). The viral surface consists of a lipid membrane with complexes of gp120 and gp41 that form the glycoprotein envelope.

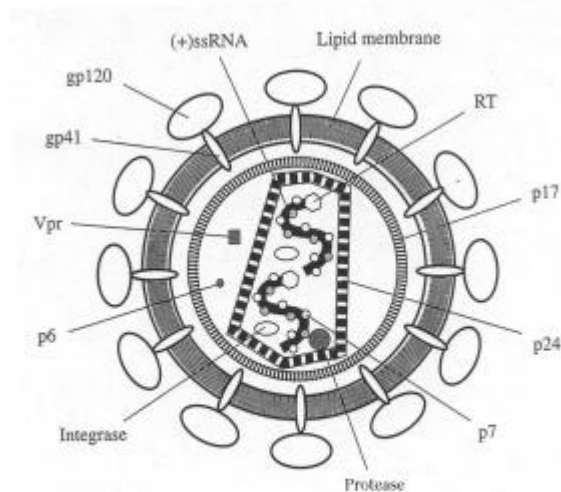


Figure 1. Schematic overview of the HIV particle.
Modified from Leitner (1996).

HIV causes chronic progressive immunological dysfunction. Untreated HIV infection is characterised by a long period with no or minor symptoms and declining levels of T-helper lymphocytes. Low levels of T-helper cells are associated with an increased risk of the acquired immunodeficiency syndrome (AIDS), defined by opportunistic infections and or HIV-related malignancies. HIV infection is also associated with various psychological and neuropsychiatric disorders (Catalan, 1990; Saag, 1994) and decreased health-related quality of life (I; Ragsdale & Morrow, 1990; Wu *et al.*, 1991; Wachtel *et al.*, 1992).

PREFACE TO THE THESIS

This thesis mirrors HIV treatment over a 12-year period from 1991. Paper I deals with the long-term safety and outcome of a therapeutic recombinant glyco-protein 160 (rgp160) vaccine. In paper II we investigated the health-related quality of life in a sample of HIV patients before entering a phase III trial of the rgp160 vaccine. The influence of two years of protease inhibitor based antiretroviral treatment on the health-related quality of life was investigated in paper III. In paper IV we developed a real-time polymerase chain reaction (PCR) method for the quantification of HIV DNA in CD4⁺ cells. This method was used in paper V to monitor HIV DNA levels in patients performing long-term supervised therapy interruptions. In addition, some data, not previously presented, are shown to complete the studies: (a) a 12-year update of the rgp160 vaccinated patients from paper I; (b) the health-related quality of life in a cohort of men who have sex with men; (c) the two-year influence of rgp160 on health-related quality of life in the patients from paper II, and (d) the health-related quality of life outcome of long-term supervised therapy interruption. An overview of the six patient cohorts is given in Appendix A. An overview of the statistical methods used in papers I-III, V and the thesis is given in Appendix B.

AIMS OF THE THESIS

The general aims of this thesis were to study the longitudinal influence of vaccination therapy, protease based antiretroviral combination therapy and long-term supervised therapy interruption on the health-related quality of life and biomedical variables of HIV-positive patients. Further, to develop methods to monitor HIV DNA in lymphoid cells of patients during different treatment conditions. The specific aims were:

To evaluate the long-term safety and outcome of rgp160 immunisation with and without antiviral therapy.

To determine the HIV viral kinetics in long-term treatment and treatment interruption.

To assess the health-related quality of life outcome of HIV immunisations.

To assess the health-related quality of life and the subjective health status of HIV-infected persons before and during antiviral therapy and during therapy interruption.

THE HOPE (I)

Azidothymidine (AZT) belongs to the pharmaceutical group of nucleoside analogue reverse transcriptase inhibitors and was one of the first drugs specifically designed for the treatment of HIV infection that became available for testing in clinical trials. The results from the first sizeable double-blind, placebo controlled study presented in 1987, were very promising and the trial was terminated in advance since an interim analysis showed that 19/137 placebo recipients but only 1/145 azidothymidine recipients had died during the study period of up to 24 weeks (Fischl *et al.*, 1987). However, severe adverse effects were reported to be connected with azidothymidine therapy (Richman *et al.*, 1987). Later, other compounds, as for example 2',3'-dideoxyinosine (ddI; Yarchoan *et al.*, 1989), and dideoxycytidine (ddC; Merigan *et al.*, 1989) were added to the arsenal of potential nucleoside analogue reverse transcriptase inhibitors. However, several reports in the early 1990s indicated that the effect of mono or dual therapy with nucleoside analogue reverse transcriptase inhibitors is only temporary. Further, there was a lack of more promising therapeutic candidates in the pipeline. The initial optimism induced by these drugs now changed into feelings of depression and hopelessness both in the communities of HIV-infected persons and among HIV clinicians. In this atmosphere we started a project with a different approach to HIV treatment – therapeutic HIV vaccinations. This approach, which attracted a great deal of medial publicity, revived the hopes of people living with HIV and those taking care of them. As one patient said, “To be chosen for this study is like winning a seat on a space ship escaping from the end of the world.”

MEANING OF HOPE

Hope is a multidimensional phenomenon of central importance for coping with chronic illness (Kylmä *et al.*, 1996). In a concept analysis of hope, Benzein & Saveman (1998b), found that stressful stimuli and loss are antecedents and that its critical attributes include future-orientation, positive expectation, activity, realism and goal-setting. Further, consequences of hope included an ability to cope, renewal, new strategies, peace, improved quality of life and physical health. Hope was also found to be related to well-being, while physical health was shown to be important for the maintenance of hope (Benzein *et al.*, 2000). Moreover, pain and suffering were viewed as threats to hope (Benzein *et al.*, 2000). Sources of hope can be constituted by family, friends and religious or philosophical beliefs (Raleigh, 1992; Kylmä *et al.*, 1996) but also by information, getting care quickly and professional support (Kylmä *et al.*, 1996).

Benzein *et al.* (2001), studied the meaning of the lived experience of hope in patients with incurable cancer and found that these patients' most significant experience was the hope of being cured. Furthermore, a study of nurses' perception of hope among cancer patients has revealed that a significant dimension of the patients' hope is confidence in treatments (Benzein & Saveman, 1998a). The meaning of hope among chronically ill

persons has been described as life, health, the possibility of getting well, mental balance and trust (Kylmä *et al.*, 1996).

LIFE CYCLE OF HIV

An overview of the life cycle of HIV is shown in Figure 2. HIV usually infects CD4⁺ cells (*e.g.* T-helper cells, monocytes, macrophages, dendritic cells and brain microglial cells (Hirsch & Curran, 1996). gp120 attaches to the CD4 molecule on the target cell. The binding to CD4 results in a conformational change in gp120 that exposes the binding sites for cellular chemokine co-receptors, which can be CCR5 or CXCR4. gp41 will then be available to induce fusion of the virion and host cell membranes (Murakami & Yamamoto, 2000). The fusion results in release of the core compartments into the cytosol of the host cell. During its transport to the nucleus, reverse transcriptase transcribes viral RNA to double-stranded linear DNA. The RT contributes to a large genetic variation of different HIV strains by making 3.4×10^{-5} substitutions per base pair and cycle, which corresponds to about one mis-transcription for every third genome transcribed (Mansky & Temin, 1995). The rate of nucleotide substitution has been estimated to be between 2.7 and 6.7×10^{-3} substitutions per site and year in certain parts of the HIV genome (Leitner & Albert, 1999). Once the linear DNA has been transported to the cell nucleus, the viral protein integrase can integrate the viral genome into the host genome, where it forms a provirus. Viral DNA can, however, also exist in circular non-integrated forms, episomes. If viral DNA is successfully integrated in the host cell genome, every cell offspring will carry the integrated HIV genome. The integrated provirus can be transcribed and give rise to both progeny RNA and translation products necessary to produce new virions. Moreover, at any given moment, a very large proportion of the HIV present in a host is non-functional due to genetic or structural errors.

ANTI HIV THERAPY

There is still no cure for HIV infection. Treatment is mainly directed at inhibiting virus replication and preventing and/or treating opportunistic infections. There are several conceivable classes of potential anti HIV drugs. Three classes currently in clinical use are directed specifically against HIV: (a) nucleoside analogue reverse transcriptase inhibitors and (b) non-nucleoside analogue reverse transcriptase inhibitors, both of which inhibit transcription of viral RNA to proviral DNA; and (c) protease inhibitors, which prevent newly produced viral particles from maturing to infectious virions (Figure 2).

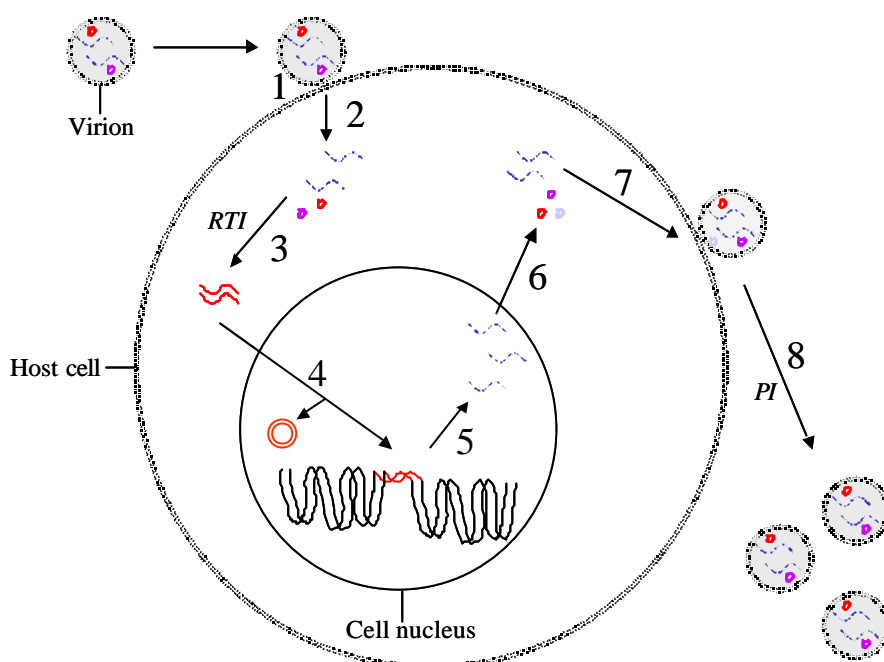


Figure 2. Life cycle of HIV: (1) attachment to the proposed host cell; (2) fusion with the host cell and release of the viral genome (RNA) and enclosed enzymes; (3) reverse transcription of viral RNA to DNA by reverse transcriptase; (4) transport of viral DNA to cell nucleus and integration of the viral DNA into the cellular genome with the help of the viral enzyme integrase. Alternatively, un-integrated viral DNA can form short-lived circular extra-chromosomal DNA; (5) transcription of parts or the whole of the viral genome; (6) viral RNA is transported to the cytoplasm and translated into viral proteins or takes part in the forming of new virions; (7) forming of new virions and budding from the cell surface; (8) release and maturation of the new virions by the viral enzyme protease. Reverse transcriptase inhibitors (RTI) inhibit step 3 and protease inhibitors (PI) inhibit step 7/8 of the viral life cycle.

THERAPEUTIC HIV VACCINES

HIV seroconversion is followed by a strong immune response, including neutralizing antibodies, antibodies inducing antibody dependent cellular cytotoxicity, and cytotoxicity. Antibody reactivities against certain regions of envelope glycoprotein 120 and transmembrane glycoprotein 41 seem especially important. However, specific responses to HIV are lost early in the infection (Wahren *et al.*, 1986; Albert *et al.*, 1990; Shearer & Clerici, 1991). The rationale behind therapeutic HIV vaccinations is that immunisations with various parts of HIV should enhance the natural response to HIV and thus have a positive effect on the natural course of the infection. Immunisation with recombinant glycoprotein 160 (rgp160; *i.e.* uncleaved glycoproteins 120 and 41) has been shown to induce serological and cell-mediated glycoprotein 160 specific responses in HIV negative persons (Orentas *et al.*, 1990; Tacket *et al.*, 1990; Cooney *et al.*, 1991; Dolin *et al.*, 1991; Picard *et al.*, 1992; Salmon-Ceron *et al.*, 1995). Further, new and increased humoral and cellular immune responses have been shown after rgp160 immunisations of HIV positive recipients (Redfield *et al.*, 1991; Redfield & Birx, 1992; Wahren *et al.*, 1994). It has been feared, however, that immunisations also could cause increased HIV replication in infected patients (Fultz *et al.*, 1992).

Therefore, we conducted a long-term phase II study to assess the safety and long-term clinical influence of rgp160 immunisations. Further, we investigated whether additional short-term azidothymidine treatment would be beneficial in therapeutic HIV vaccine therapy.

THE THERAPEUTIC VACCINE TRIAL (I)

An inquiry about participation in an HIV-vaccine trial was made among HIV patients in the Stockholm area who had CD4 counts above 399×10^6 cells/l and also met the remaining inclusion criteria (I). Of the 160 patients who reported an interest, 40 (3 women and 37 men) were randomly selected. These patients received six 160 µg doses of recombinant gp160 (VaxSyn HIV-1, MicroGeneSys, Meriden, Connecticut) intramuscularly over a six-months period (weeks 0, 1, 4, 8, 17 and 26). To test whether azidothymidine would influence the viral outcome of the immunisations, the participants also received either 200 mg azidothymidine or placebo \times 4 double-blindly during two weeks following each immunisation. After this first phase of the study, the patients were followed for nine months before being randomised to get booster injections with 160 µg rgp160 for either every second or every sixth month for two years. In the next phase all patients received injections with either 160 or 320 µg rgp160 every second month for 20 months. Please see Figure 3 for an overview of study I.

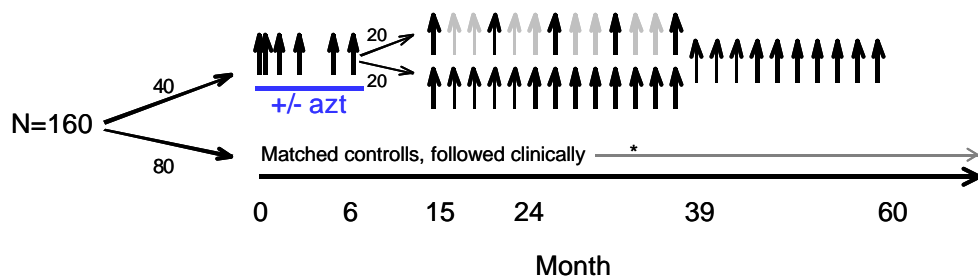


Figure 3. Overview of study I: ↑ indicates rgp160-injection; ↑ indicates placebo injection; half of the vaccinees were treated with azidothymidine (azt) two weeks after the first six immunisations; *43 of the matched controls entered a double-blind study with rgp160 or placebo immunisations (VAC04).

For each of the patients receiving vaccine, two controls matched for sex, CD4 level, mode of transmission, age and approximate duration of HIV seropositivity were chosen from the 120 patients who had reported an interest in participating but were not included in the study. The controls were followed and received treatment according to best clinical practice. Three years after the start of the study, 43 of the 80 controls entered a double-blind study with rgp160 or placebo immunisations.

Clinical and laboratory results – a synthesis

Compared with the baseline CD4 value, the CD4 absolute value was increased at week 17 and the CD4 percentage was increased at weeks 17, 26 and 38 in the whole group (Figure I:1). The CD4 slope in the whole group improved in the first six months after the study start compared with the two preceding years (Table I:2). At month 12, there was also an increase of *in vivo* reactivity to recall antigens as measured by a delayed type hypersensitivity test (Multitest, Mérieux, Lyon, France).

Adherence to the additional zidovudine/placebo treatment was 85% in the zidovudine group and 90% in the placebo group (adherence criterion: >80 of the tablet dosages; pill counting method). The frequency of nausea (Table I:4) was higher in the zidovudine group compared with the placebo group ($p < 0.001$).

Despite the immunological response (see also below), there was no apparent clinical benefit in the rgp160 vaccinated group compared with the matched controls. Furthermore, the viral load did not differ between the rgp160 treated patients and a group of 34 of the matched controls for whom HIV RNA measurements were available. However, since this study was mainly designed to assess the safety of rgp160 vaccine, no definite conclusion can be drawn about clinical efficacy. A later study was designed to measure clinical efficacy (Sandström & Wahren, 1999).

Results from continued investigations on the same patient material have revealed that 80% of the patients were classified as immunological responders on the basis of increased Tcell proliferation and antibody response to rgp160 during the 12 months following the study start (Wahren *et al.*, 1994). Furthermore, the Tcell proliferative responses to rgp160 increased in the whole group, reached a peak six months after the start of the study and declined slowly thereafter (Leandersson *et al.*, 1998). An increase in neutralizing activity and antibody dependent cellular cytotoxicity has also been shown (Broliden *et al.*, 1996).

Viral activation and autoimmunity

There has been concerns that immunisations with recombinant HIV proteins could induce increased viral activity, since studies involving immunisations against other agents, such as influenza, hepatitis B, tetanus and pneumococci, have indicated transient viral activation shortly after the immunisation (Fultz *et al.*, 1992; O'Brien *et al.*, 1995; Staprans *et al.*, 1995; Brichacek *et al.*, 1996; Cheeseman *et al.*, 1996; Stanley *et al.*, 1996). To test for this, we measured the viral RNA levels in the rgp160 group two weeks after the sixth immunisation and compared them with the level just before this immunisation (month six after start). No statistically significant difference was found between the two measurements and there were no indications of an increase in viral load (Table I:3). Another concern was the risk of inducing autoimmunity, since the HIV envelope has many regions with homology to human leukocyte antigen (HLA) class I and II molecules. However, Lundholm *et al.* (1994), investigated material from

the rgp160 group and found no increase in serum IgG to HLA homologous peptides in these patients after the first year of immunisations. Recently, Isaguliantz (2003), has demonstrated the induction of autoantibodies with certain compositions of HIV vaccines where the immunogen is a DNA binding protein. The envelope glyco-protein, however, has no DNA binding properties.

Twelve years later

Twelve and a half years after the start of the study (I), nine of the 40 included patients have died (7 HIV-related and 2 HIV-unrelated; 6 in the vaccine + zidovudine group and 3 in the vaccine + placebo group; Figure 4). There was no statistically significant difference in survival time between those patients who, in addition to the rgp160-vaccine, were treated with intermittent zidovudine therapy and those who were treated with placebo capsules (log rank survival analysis). Twenty-nine of the patients had started HAART (16 in the zidovudine group and 13 in the placebo group). Those in the zidovudine group had a shorter time to start of HAART than the placebo group (median 62 vs. 80 months from study start respectively; $p < 0.01$, log rank survival analysis; Figure 5). This might be due to the fact that the zidovudine group had a steeper CD4 decline before entering the study (Table I:2). The data also indicated that, in a long-term perspective, it was not necessary to include the precautionary azidothymidine in the initial immunisation scheme.

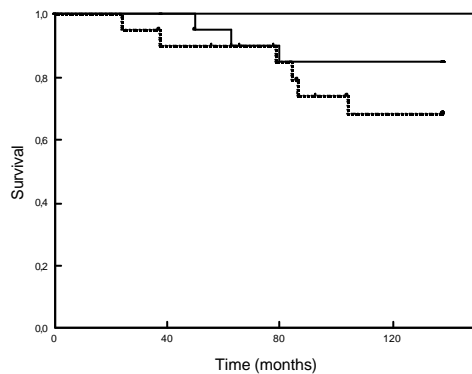


Figure 4. Twelve-year survival of the rgp160 treated patients. The solid line denotes the rgp160 + placebo group, the dotted line denotes the rgp160 + azidothymidine group.

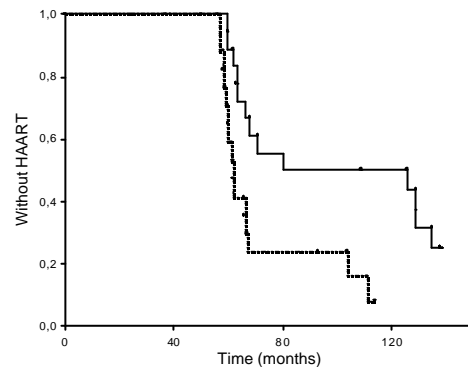


Figure 5. Survival curve of time to initiation of HAART. The solid line denotes the rgp160 + placebo group, the dotted line denotes the rgp160 + azidothymidine group. The rgp160 + azidothymidine group had a shorter time to treatment initiation ($p < 0.01$).

FURTHER STUDIES OF THE RGP160 VACCINE

The clinical effect of therapeutic rgp160 vaccine VaxSyn HIV-1 has later been tested in a number of double-blind, placebo controlled studies with 278–835 participants with CD4 counts ≥ 500 , >400 and $\geq 200 \times 10^6$ cells/l respectively (Tsoukas *et al.*, 1998; Sandström & Wahren, 1999; Birx *et al.*, 2000). Two of the studies showed a better

development of CD4 levels in patients treated with rgp160 compared with placebo (Sandström & Wahren, 1999; Birx *et al.*, 2000). One study showed significantly fewer deaths in patients treated with rgp160 for two but not for three years after initiation of immunisation (Sandström & Wahren, 1999). These studies, however, yielded no long-term clinical benefit of therapeutic immunisations. This treatment modality is certainly worth continued efforts to enhance the immunogenicity and survival effects.

THE HOPE – CONCLUDING REMARKS

To conclude, several studies, together with our own, show that rgp160 vaccine seems to be safe and tolerable, enhances T-cell responses and gives a transient effect on CD4-development but has no apparent effect on disease progression in the absence of efficient antiretroviral therapy. On the other hand, although efficient antiretroviral combination therapy substantially improves immune function, it does not appear to allow major reconstitution of impaired HIV-specific T-cell responses. This might be due to levels of virus specific antigenic stimulation being too low as a result of drug-induced suppression of viral replication. In combination with effective antiretroviral therapy, therapeutic HIV immunisations have been suggested to have the potential to enhance existing antiviral responses, to induce new-HIV specific responses and to reduce viral load and thus promote long-term non-progression (Imami & Gotch, 2002; Kinloch-de Loes & Autran, 2002). It has been suggested that such an approach might even achieve total eradication of virus from all reservoirs of the body (Imami & Gotch, 2002). The ability to enhance virus specific immunity has also been shown with core-based vaccines, (Klein *et al.*, 1997; Åsjö *et al.*, 2002). Therapeutic HIV DNA based vaccinations have likewise proved capable of inducing new cytotoxicity to HIV-infected target cells and might thus be a candidate for such combinatory treatment strategies (Boyer *et al.*, 1999; Calarota *et al.*, 1999). Reports from a Spanish study with the therapeutic HIV vaccine Remune, a gp120-depleted, inactivated HIV vaccine in Freund's incomplete adjuvant, claim that patients treated with the vaccine in combination with antiviral therapy were less likely to experience virological failure than patients treated with placebo vaccine (Burton, 2002).

THE HEALTHY ILL (II)

We had a large group of untreated patients with CD4 levels of $>200 \times 10^6$ cells/l, which meant that they had not reached a state where antiretroviral treatment was requested (this was before the introduction of antiretroviral combination therapy). The group constituted a cross-section of HIV patients and included patients with normal as well as slow or rapid progression of the disease. In our health-care setting these patients were habitually seen as “healthy HIV carriers”. However, work with this patient group led to a growing conviction that these patients were not as healthy as HIV-negative persons. That made it of interest to investigate the health-related quality of life and subjective health status of such patients. We also wanted to investigate the influence of the rgp160 vaccine, used in paper **I**, on health-related quality of life. We therefore followed a group of patients before and during two years of rgp160 vaccine or placebo treatment.

HEALTH AND ILLNESS

Health is a central, multidimensional and relative concept in all health-care professions. The relevance of health as a concept in nursing was pointed out as early as in the mid 19th century by Florence Nightingale (1992). Since then, health has been considered with different degrees of specificity, reductionism and centrality by nursing theorists and scientists and several different models of health with varying levels of abstraction have been presented (Meleis, 1991). Investigating the concept, Willman (1996), concluded that, depending on the perspective chosen, health can be described as a condition (status), a process, a diagnosis, a task, a response, a goal or as investment, *i.e.* proof of prosperity. Further, the meaning of the concept can vary over time, cultures and standards of living. From a medical point of view, health can be seen as freedom from disease and abnormalities. Sociologically, health can be defined as acceptable resources of mental and physical fitness in order to perform one’s social role in society. In addition, optimal autonomy, self-mastery and a positive perception of life are central components of a humanistic view of health (Bowling, 2001). Bowling (1997), states that illness may but does not necessarily result from pathological abnormality. A person can feel ill without medical science being able to detect disease. What matters in the 20th century is how patients feel, rather than how doctors think they ought to feel in the light of clinical measurements. In its constitution, the World Health Organization states that “health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” (WHO, 1948).

HEALTH-RELATED QUALITY OF LIFE

There is neither a single generally accepted definition of quality of life nor a “gold standard” for its measurement (Cella & Tulsky, 1990). However, the World Health Organization has defined quality of life “as an individual’s perception of their position in life in the context of the culture and value systems in which they live and in relation

to their goals, expectations, standards and concerns. It is a broad ranging concept incorporating in a complex way the person's physical health, psychological state, level of independence, social relationships, personal beliefs and their relationship to salient features of the environment" (WHO, 1999). The quality of life concept is multidimensional and since quality of life assessment is essentially subjective, it should include the patient's perspective (Cella & Tulsky, 1990; Aaronson, 1991). Health-related quality of life is one dimension of quality of life and has become increasingly important in recent decades as an outcome measure in health care (Testa & Simonson, 1996), especially when evaluating interventions for patients with chronic diseases (Patrick & Eriksson, 1993). Health-related quality of life usually includes domains of functional status (physical, psychological and social) and health status or health perception. In addition, the broader concept of quality of life may include spiritual, economic and family dimensions (Strickland, 1991). Instruments/questionnaires for measuring health-related quality of life can be generic or disease-specific (Wu & Rubin, 1994) and be based on either patients' self reports or scoring by clinicians (Spitzer, 1987). The information obtained from the instruments/questionnaires can be aggregated into an overall score and/or a number of dimension scores (Wu & Rubin, 1992).

Health-related quality of life and HIV

Several studies (cross-sectional or longitudinal) have focused on the health-related quality of life of HIV-positive individuals in different stages of the HIV infection and under different treatment regimes. The results have varied but in general, HIV infection affects several physical, psychological and social dimensions of health-related quality of life and patients with symptomatic disease and or an AIDS-defining diagnosis are more severely affected than those with other comparable chronic diseases (Ragsdale & Morrow, 1990; Wu *et al.*, 1991; Wachtel *et al.*, 1992). Health-related quality of life has been shown to be related to the CD4 value, viral load and symptoms, so that patients with a more advanced state of HIV infection reported poorer health-related quality of life (Wu & Rubin, 1994; Holzemer & Wilson, 1995; Call *et al.*, 2000; Lorenz *et al.*, 2001). Moreover, symptoms, physical function, role function and sexual function deteriorated over time, while emotional domains were unchanged or improved (Wu & Rubin, 1994; de Boer *et al.*, 1995; Holzemer & Wilson, 1995). Other factors affecting the health-related quality of life in patients with HIV have been reported to be, for example, sense of coherence (Cederfjäll *et al.*, 2001) and coping strategies (Swindells *et al.*, 1999).

HEALTH-RELATED QUALITY OF LIFE OF HIV PATIENTS (II)

The participants in study **II** were recruited from a group of patients entering a randomised, double-blind placebo controlled study of the HIV rgp160 vaccine VaxSyn HIV-1 at the Gay Men's Health Clinic, Stockholm Söder Hospital. Besides to participating in the pharmaceutical study, the patients were asked to respond to questionnaires regarding their health-related quality of life regularly during a period of

two years (before the start and 3, 6, 12 and 24 months after the start). Out of 87 eligible patients, 73 accepted parallel inclusion in this study of health-related quality of life (for details of inclusion criteria and other aspects of the pharmaceutical- and the present study, please see (Sandström & Wahren, 1999) and paper **II**). A total of 72 men were included in this study; the only recruited woman was excluded from further analyses of the material.

The questionnaires

The Swedish Health-Related Quality of Life Questionnaire (SWED-QUAL)

The SWED-QUAL developed by Brorsson *et al.* (1993), was used to measure health-related quality of life. It consists of 70 items, of which 63 forms two single-item and 11 multi-item dimension scales of Likert type: physical functioning (7 items), mobility (1 item), satisfaction with physical ability (1 item), role limitations due to physical health (3 items), pain (6 items), positive affect (6 items), negative affect (6 items), role limitations due to emotional health (3 items), sleep problems (7 items), satisfaction with family life (relations with parents, siblings, children etc. (4 items)), relation to partner (6 items), sexual functioning (4 items) and general health perception (9 items). Each scale is transformed into a 0-100 index; the higher the score, the better the perceived health-related quality of life. The SWED-QUAL questionnaire was selected because it derives from an instrument that is used internationally and has been tested for reliability and validity in Sweden and because data from a Swedish reference group are available (Brorsson *et al.*, 1993).

The Health Index

The Health Index consists of 10 items concerning energy, temper, fatigue, loneliness, sleep, vertigo, bowel function (the Swedish expression is a blanket term for all aspects of gastro-intestinal function), pain, mobility and general health. For each statement, the participants are asked to rate their health status during the previous week on a four-grade Likert scale, which ranges from 1 to 4 (very poor to very good). The scores are summarised to form a health index ranging from 10 to 40; the higher the score, the better the self-rated health (Nordström *et al.*, 1992). A factor analysis performed by Nordström *et al.* (1992), defined two factors: emotional well-being (EWB), consisting of four items (energy, temper, fatigue and loneliness), and physical well-being (PWB), consisting of five items (mobility, sleep, vertigo, bowel function and pain). The general health item was related to general well-being and was thus excluded from the specific subsets. The Health Index was used because it is short and includes items not found in the SWED-QUAL.

Health-related quality of life of the HIV patients in relation to general population samples

Compared with general population groups (Brorsson *et al.*, 1993; Forsberg & Björvell, 1993), before entering the rgp160 vaccine trial the group of HIV patients reported statistically significant lower scores, indicating worse health-related quality of life and subjective health. Of the 13 SWED-QUAL scales, 10 showed statistically significant lower scores compared with the population group (Table II:2, Figure 6). The most affected parameters were those related to emotional well-being. This amounts to a clear negative effect on the health-related quality of life of the group of HIV patients compared with the general population sample. Further, the HIV patients reported statistically significant lower scores on the Health Index (Table II:3) and thus an impaired health status compared with the general population sample. Similarly, Globe *et al.* (2002), found that haemophilic patients with HIV infection had a worse health status than HIV-negative haemophilic patients.

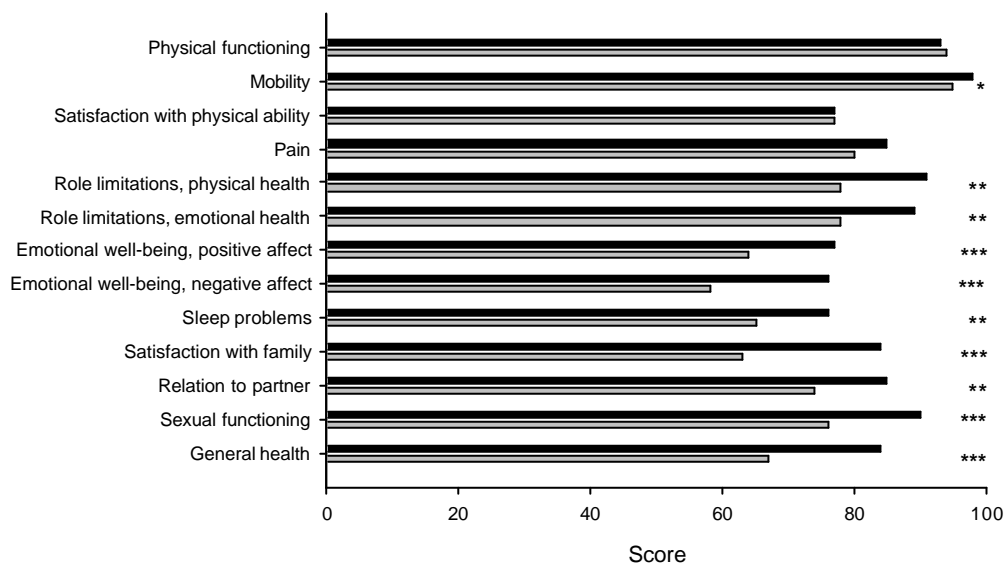


Figure 6. SWED-QUAL. Comparison between the rgp160-group (grey bars) and the Swedish general population sample (black bars). *p <0.05; **p <0.01; ***p <0.001.

Health-related quality of life of different subgroups of the HIV patients

In comparisons according to the medical and demographic variables for different subgroups of the HIV sample, the most prominent differences were found in the physical dimension scores. Symptomatic HIV infection or AIDS, anti-retroviral treatment, sick leave or disability pension, low income and basic education were

associated with lower scores, indicating a poorer health-related quality of life and health status (Tables II:4-6).

Health-related quality of life of the HIV patients in relation to HIV negative men who have sex with men (MSM)

Since a majority of the participants in paper II were infected with HIV by male-to-male sex, we also wanted to compare these patients with a group of non HIV-infected men who have sex with men (MSM). During a three-month period, all patients visiting the Stockholm Söder Hospital information and screening clinic for sexually transmitted diseases among men who have sex with men were asked to participate in a study regarding their health-related quality of life. The SWED-QUAL and Health Index instruments were used. The inclusion criteria were: (a) ability to read and understand the Swedish language, and (b) had not already participated in this study during a previous visit to the clinic. Out of a total of 211 persons visiting the clinic, 12 were not included due to language problems, and six had already answered the questionnaires on a previous visit. Of the remaining 193 patients, 164 (85%) responded to the questionnaires (29 declined participation). Their age ranged between 17 and 74 years and the mean age was 36 (SD 11) years. Further patient characteristics are shown in Appendix A.

The differences in health-related quality of life scores between the MSM group and the rgp160 group were analysed using the Mann-Whitney *U*-test. Compared with the group of HIV patients, the MSM group reported higher scores on the SWED-QUAL scales mobility, pain, role limitations due to physical health, sexual functioning and general health (Figure 7). Further, the MSM also reported higher scores on the total Health Index ($p < 0.05$) and the Health Index physical well-being subscale ($p < 0.05$). No statistically significant differences were found as regards either the SWED-QUAL scales measuring the emotional or family dimensions of health-related quality of life or the Health Index emotional well-being subscale. This means that the HIV-negative MSM group scored their emotional health-related quality of life as being of the same magnitude as reported by the HIV-positive patients (rgp160 group).

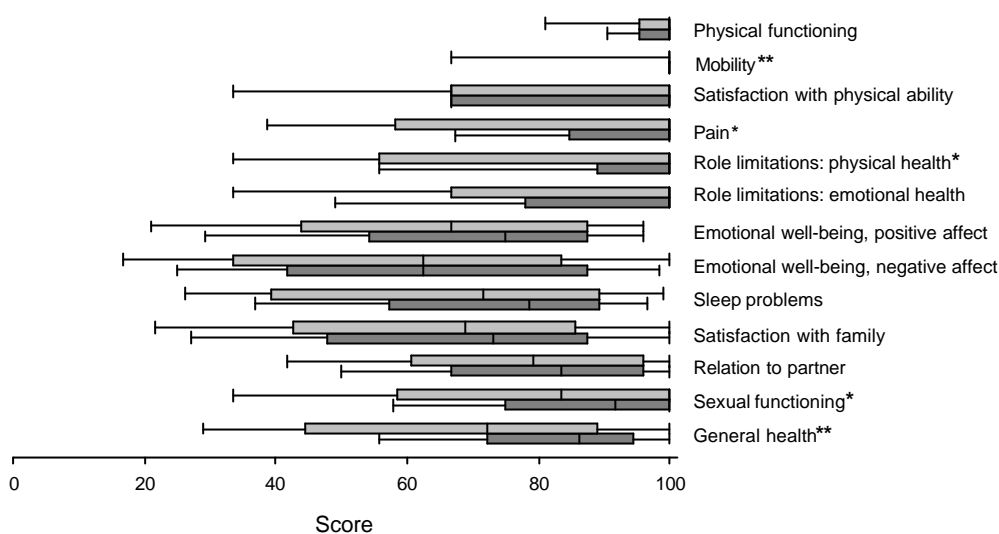


Figure 7. SWED-QUAL, comparisons between the rgp160 group (light gray) and the group of HIV negative men who have sex with men (dark grey). The box boundaries indicate the 25th and the 75th percentiles and the whiskers indicate the 10th and the 90th percentiles. *p <0.05; **p <0.01.

Health-related quality of life in MSM compared with random population

The differences in health-related quality of life scores between the Swedish population sample (Brorsson *et al.*, 1993) and the MSM group were analysed using a one-sample *t*-test. Compared with the Swedish population sample, the MSM group reported higher scores on the SWED-QUAL scales physical functioning, mobility, satisfaction with physical ability and pain (Figure 8). Further, they reported lower scores on the scales emotional well-being positive and negative affect, satisfaction with family life, relation to partner and general health (Figure 8). This indicates that the group of HIV-negative men who have sex with men has a better physical health-related quality of life but a poorer health-related quality of life from an emotional and a family perspective.

Our findings that the HIV-negative MSM group reported lower scores on the emotional well-being and general health domains may have been influenced by the fact that the measurement was performed when the participants were visiting a health care facility for information and screening as regards sexually transmitted diseases. However, a decreased quality of life among same-sex oriented individuals has also been found in other studies. A large Norwegian study (Hegna *et al.*, 1999) investigated the living conditions and life quality among men who have sex with men and women who have sex with women. The participants reported a lower estimation of their health than people in the general population. Further, they had a much higher risk of psychological afflictions. Young age, concealment of sexual orientation and a segregating life style were related to worse psychological health. Moreover, persons with a stable relationship or living with a partner were generally of better psychological health than

those without. A Dutch survey (Sandfort *et al.*, 2003) likewise found a decreased quality of life in men who reported having sex with men compared with men who reported having sex with women. The former group reported worse general health, mental health, emotional role functioning, social functioning and vitality. The decreased quality of life came predominantly from self-esteem and mastery, which were positively correlated to the quality of life scales. Other factors behind the negative effect on the health-related quality of life of the MSM could be the social stigma (Kimmel, 1979), internalised homophobia (Wagner *et al.*, 1996) and increased experience of discrimination, violence, harassment and/or threats (Hegna *et al.*, 1999; Mays & Cochran, 2001) that have been found in investigations of same-sex oriented men.

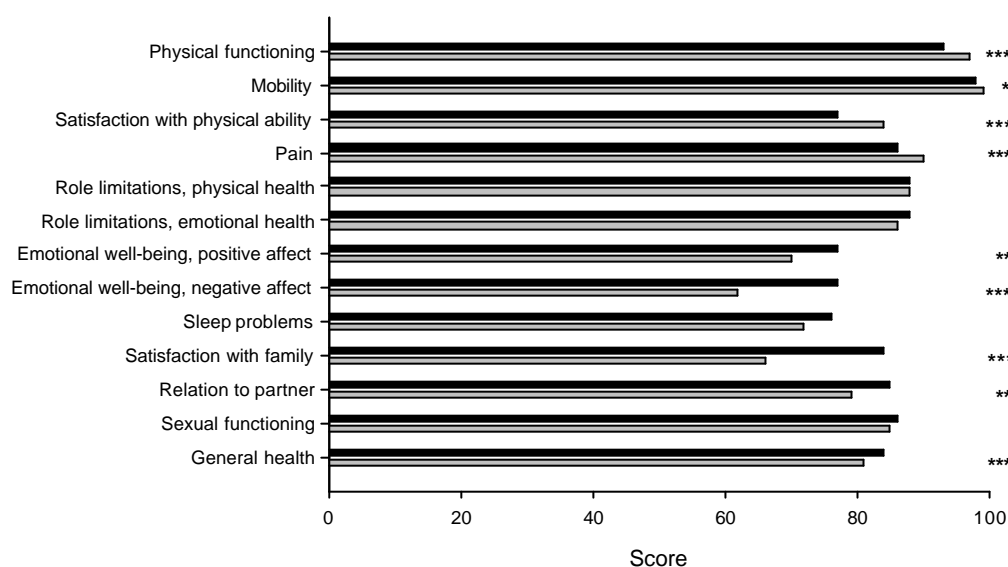


Figure 8. SWED-QUAL. Comparison between the MSM group (grey bars) and the Swedish general population sample (black bars). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The longitudinal impact of rgp160 on health-related quality of life

The health-related quality of life data from before and after two years of double-blind treatment with rgp160 or placebo injections were used for the analysis of the longitudinal response. The questionnaires were completed on these two visits by 38 of the 72 patients; 22 did not complete the two-year follow-up, six did not complete two years in the vaccine trial, four died before the two-year follow-up and two changed clinic during the study period.

The individual SWED-QUAL results from before and after two years of therapy were analysed in a two-way analysis of variance investigating the effects of time and treatment arm (rgp160 or placebo) on the development of health-related quality of life. The analysis revealed a main effect of time on the development of physical functioning ($p < 0.001$; Figure 9) and no interaction between time and treatment arm. This means

that the physical functioning scores decreased during the follow-up period and this decrease was unrelated to treatment arm (rgp160 or placebo). There were no statistically significant differences in the longitudinal development of the remaining health-related quality of life scales.

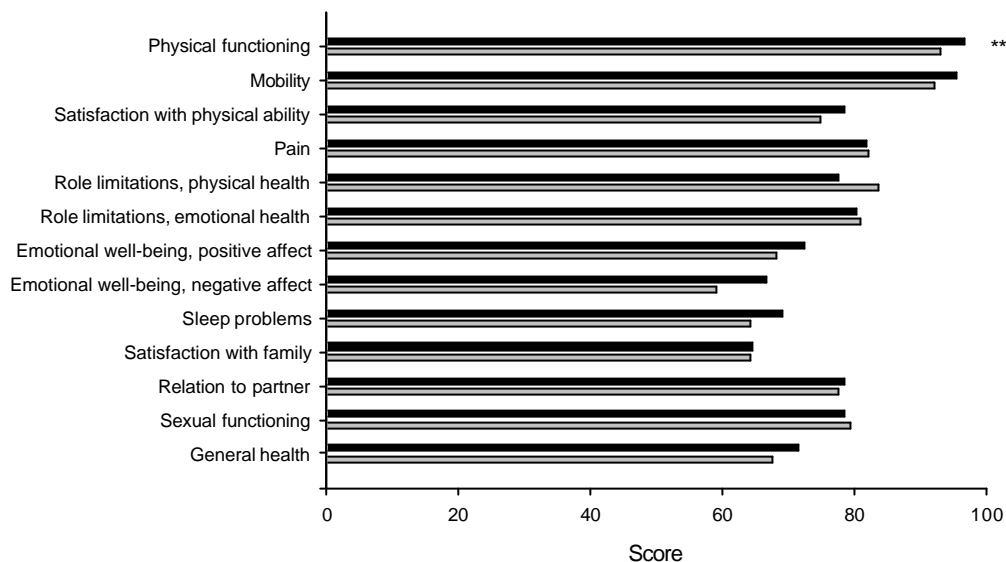


Figure 9. SWED-QUAL, longitudinal development of the patients in the rgp160 vaccine trial (n=38). The results before the patients entered the study are indicated with black bars and the results after two years of therapy are indicated with grey bars. ***p <0.001.

THE HEALTHY ILL – CONCLUDING REMARKS

To conclude, the HIV-positive patients reported substantially lower scores for their health-related quality of life and health status compared with Swedish random population groups. This is especially true for the emotional, social, family and general health domains. However, comparisons between a group of HIV-negative MSM and the HIV-positive patients revealed no differences as regards the emotional and family domains of health-related quality of life but the HIV-positive patients did show decreased scores in the physical and general health domains. This calls for increased attention to psychosocial aspects in the care of these patients. Further, a therapeutic rgp160 vaccine did not seem to have any impact on the development of the health-related quality of life of the HIV-positive patients. Moreover, physical functioning decreased during a two-year follow-up.

BACK TO LIFE (III)

Protease inhibitor based antiretroviral therapy (PI-ART), defined as the combination of at least two nucleoside analogues with at least one protease inhibitor (PI; British HIV Association, 2001), was introduced 1996 and aims to inhibit virus replication and thus protect and restore the immune system (Gulick *et al.*, 1997). Later, combinations without PIs have been added to the broader concept of highly active antiretroviral therapy (HAART; British HIV Association, 2001). The introduction of HAART had a tremendous effect on the prognosis of HIV-infection for patients living in parts of the world where this treatment is available. It led to undetectable HIV RNA levels in the blood of a majority of treated patients and has greatly reduced the incidence of AIDS and AIDS-related mortality (Palella *et al.*, 1998; Mocroft *et al.*, 2000). However, there are dietary restrictions and 100% adherence to the complicated regimens is essential. HAART is also associated with short- and long-term adverse effects, for example gastrointestinal problems, headache, hypersensitivity, metabolic changes, hyperlipidemia and a redistribution of fat tissue from arms, legs and face to the abdomen (Safrin & Grunfeld, 1999; Carr & Cooper, 2000).

CHANGE OF FOCUS

For the patients who started with PI-ART when it first became available, the effects of the treatment led them to reconsider their life expectancies. Prior to the introduction of PI-ART, HIV patients dealt with issues related to death and dying (Tiamson, 2002). Like patients with other life-threatening diseases, they went through different stages of preparing for death. These stages includes: *Initial crisis*: after having been informed about their infection and/or AIDS diagnosis, the individuals are in a stage of shock and intense emotions; the most predominant emotions are denial and intense anxiety. *Transitional state*: a period characterised by distress, confusion and alienation. *Acceptance*: the patients are able to accept their disease, form new identities and reassess their lives and priorities. *Preparation for death*: in the final stage, patients prepare for their forthcoming death. Individual changes, for example the appearance of a new HIV-related symptom, can cause shifts back and forth between the different stages (Nichols, 1985). When effects of PI-ART became apparent, HIV patients who had reached different levels in their preparation for death underwent a change of focus and began to deal with living, an experience that has been termed the Lazarus Syndrome (Scott & Constantine, 1999). The patients could hope again and felt that their lives had been restored but they also experienced a lot of uncertainty about the durability of treatment response, long-term adverse effects, symptoms and their social and professional roles (Brashers *et al.*, 1998; Scott & Constantine, 1999; Trainor & Ezer, 2000).

Effective treatment restores immune function, which improves the body's ability to prevent and fight opportunistic infections. This would be expected to have positive effects on health-related quality of life. On the other hand, health-related quality of life

might be negatively affected by the necessity of 100% adherence. This requirement concerns not only the number of pills but also time schedules and food intake. Further, short- and long-term adverse effects (Safrin & Grunfeld, 1999; Carr & Cooper, 2000) could also have a negative affect on the health-related quality of life. Therefore, we wanted to investigate the impact of PI-ART on the health-related quality of life.

Only a few studies have yet been published on the influence of long-term (>1 year) PI-ART on the quality of life. Nieuwkerk *et al.* (2001), studied how health-related quality of life developed with three different regimes involving protease inhibitor and concluded that in terms of health-related quality of life, patients with higher initial CD4 values experienced less benefit from the treatment.

THE IMPACT OF PI-ART ON HEALTH-RELATED QUALITY OF LIFE (III)

Seventy-two (70 men and 2 women) of the very first patients to receive PI-ART at the Gay Men’s Health Clinic, Stockholm Söder Hospital were investigated before the start of therapy. Fifty-four patients were reinvestigated again after two years of therapy (13 had died and 5 had dropped out). The patients in this group were in a substantially more advanced state of infection than those in the rgp160 vaccine trial (Appendix A). This was also evident when the health-related quality of life of these patients (III) was compared with that of the patients studied in paper II. Compared with the rgp160 vaccine patients, the PI-ART patients reported lower scores on several of the scales measuring the physical and general health domains (Figure 10).

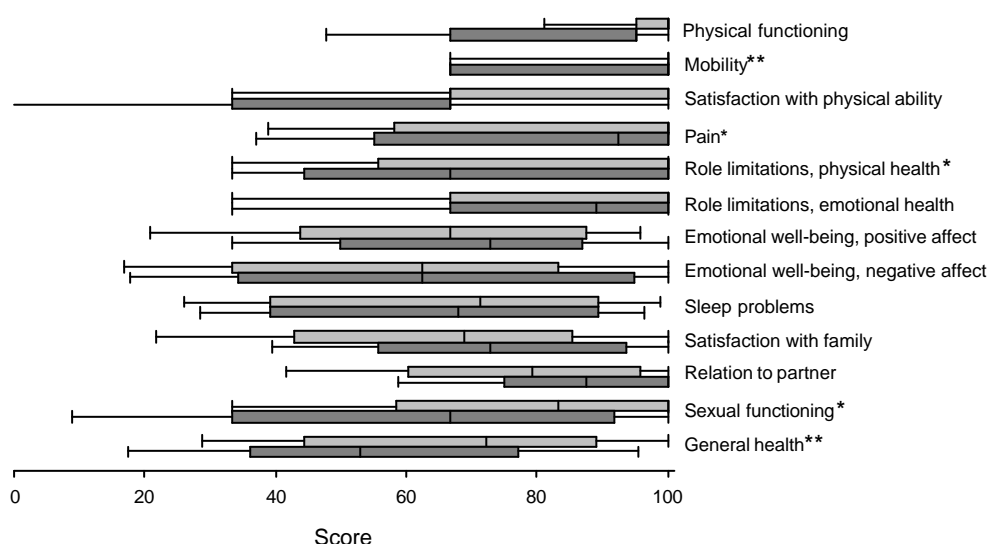


Figure 10. SWED-QUAL. Comparisons between the patients in the rgp160 vaccine study (II; light gray) and the patients in the PI-ART study (III; dark grey). The box boundaries indicate the 25th and the 75th percentiles and the whiskers indicate the 10th and the 90th percentiles. *p < 0.05; **p < 0.01; ***p < 0.001.

Development of health-related quality of life (III)

Our main findings were that the emotional health-related quality of life deteriorated during two years of treatment, while the physical health-related quality of life remained constant (Figure III:1). Multiple linear regression analyses were performed to investigate the relationship between the outcome variables (viral response, adherence and adverse effects) and health-related quality of life. To increase the power to detect relations as a result of the analysis, we created two SWED-QUAL composite scores, PCS (physical health-related quality of life composite score) and ECS (emotional health-related quality of life composite score), through a data reduction procedure (for details see paper III). The results showed that experience of adverse effects contributed most to the deterioration of emotional health-related quality of life. Moreover, physical health-related quality of life scores before the start of therapy predicted both experience of adverse effects and adherence. This agrees with the finding by Osoba (1994), in a review of quality of life research in oncology, that pre-treatment health-related quality of life scores can predict quality of life outcomes during treatment as well as survival. In accordance with other studies in HIV patients (Swindells *et al.*, 1999; Girard *et al.*, 2000), we also found that the patients who died before follow-up had lower scores on several of the SWED-QUAL scales than the patients who survived (Table III:3). These findings illustrate the importance, when planning individual treatment and care, of considering the patient's physical health-related quality of life, before treatment is begun.

The patients' own comments

In the two-year follow-up questionnaire, the PI-ART patients had an opportunity to add a written comment both on their experience of adverse effects of therapy and on their current general well-being compared with their state at the initiation of PI-ART. Some key excerpts are presented below.

In agreement with the results presented in paper III, the comments indicated that adverse effects had a major impact on the patients' daily life. A majority of the comments was related to gastro-intestinal problems. "Diarrhoea!" was a typical comment. One patient stated, "I feel bloody nauseated!" Results from a qualitative study by Brashers *et al.* (1999), support this finding. They investigated revival experiences of HIV patients who had experienced a dramatic treatment response and found that concerns about side-effects of medication were perceived by the patients as a potential threat to normal quality of life.

The comments concerning the longitudinal development of their general well-being after the start of PI-ART reflected the therapy's major impact on the course of the infection but also the problems with adverse effects and restricted liberty. One patient wrote, "I would have been dead without protease inhibitors, but all these bloody adverse effects and restrictions..." Others stated, "Adverse effects [yes], but [I also]

have life” and “[I am] less worried about death”. While many of the patients in the material were in an advanced state of HIV infection when they started with PI-ART, others were in less advanced states and had not yet experienced any symptoms related to the infection. For them, starting a medical treatment that restricts daily life and is liable to produce symptoms from adverse effects could have a negative effect on well-being. Accordingly, one patient stated “I had not reached a state with major problems due to HIV [at the start of therapy] so I suppose the medication has given me a decreased well-being even though I am better off from a medical point of view”.

BACK TO LIFE – CONCLUDING REMARKS

To conclude, the introduction of PI-ART had a tremendous effect on HIV-related morbidity and mortality. However, the treatment seemed to induce a negative development in the emotional domain of health-related quality of life. A major contribution to the magnitude of this deterioration came from the experience of adverse effects. The patients’ comments also indicated that adverse effects were a major concern. Further, the treatment seemed to interrupt an expected negative development in the physical domain of health-related quality of life. Moreover, lower scores in the physical domain of health-related quality of life preceded poorer adherence and more experience of adverse effects. This underscore the significance of considering the patient’s physical health-related quality of life when planning individual treatment and care.

PLANNING FOR THE FUTURE (IV-V)

The point has now been reached where successful HAART brings HIV RNA lastingly down to non-quantifiable levels (<50 copies/ml) and continuously increases the levels of CD4⁺ cells in a majority of the treated patients. In this context it has been suggested that with chronic treatment, HIV patients may be able to achieve a normal life expectancy. At the same time, a normal life is threatened by treatment risks connected with particular adverse effects and the emergence of viral resistance. As concluded in paper **III**, PI-ART had a negative impact on the patients' emotional health-related quality of life. This emphasises the need for long-term treatment strategies to minimise the risk of negative treatment effects and the development of resistant viral strains. Besides the patients in whom treatment produces favourable results, there are those whose development is a virological failure. Then there are patients who have to discontinue therapy on account of severe drug toxicity. Long-term supervised therapy interruptions may be beneficial for certain groups of patients, for example to limit drug-related side effects in those who have achieved a long-lasting normalisation of CD4⁺ cells. Another motive for supervised therapy interruption could be to reverse signs that the virus has become resistant to treatment. In long-term supervised therapy interruptions, the patients are closely monitored and treatment is re-started if signs of decreased immune function or clinical deterioration appear. We wanted to monitor the viral and quality of life outcome of long-term supervised therapy interruptions. In order to monitor viral outcome, we first established a simple system for measuring the HIV DNA load in CD4⁺ cells.

CHRONIC ILLNESS

Chronic illness has been characterised by Strauss & Corbin (1984), as an experience of multiple problems that may change but do not go away. Symptom control and regimen adherence have been identified as major endeavours for chronically ill persons. A key strategy in living with chronic illness is normalisation. Hymovich & Hagopian (1992), viewed chronic illness as involving multiple and frequently changing stressors that individuals respond to in a variety of ways. They found four categories of such stressors: (a) the disease and its management, (b) external and internal resources of the individual, (c) relationships with family and others, and (d) life-style adjustment. Health professionals view the successful management of chronic illness as reliant on patients' adherence to treatment regimes. Such regimes range from very simple to extremely complex and require patients to change the way in which they manage aspects of their life (Wellard, 1998).

THE EFFECT OF HIV THERAPIES ON THE VIRUS

The viral effect of HIV therapies is commonly measured in terms of HIV RNA levels. A number of commercial quantification assays are available for this. It is well known that the HIV RNA load decreases with successful treatment. However, today's standard

HIV RNA quantification assays cannot be used to follow the viral load during phases of successful therapy since very low HIV RNA levels in plasma are not quantifiable. Here, quantification of the HIV DNA in infected cells could serve as an alternative way of measuring viral load. When HAART is interrupted, the plasma levels of HIV RNA increase rapidly (*i.e.* during some weeks) to much the same levels as before therapy (Garcia *et al.*, 1999; Harrigan *et al.*, 1999; Neumann *et al.*, 1999; Phillips *et al.*, 1999; Ruiz *et al.*, 2000). However, the dynamics of the HIV DNA levels in connection with initiation of HAART or supervised therapy interruption (STI) are poorly understood. Aleman *et al.* (1999), have shown that HIV DNA levels are decreased during up to one year of antiretroviral triple combination therapy. Further, Désiré *et al.* (2001) and Riva *et al.* (2001), report a decreased viral DNA load in patients treated with PI-ART for one year after primary infection

PCR

The polymerase chain reaction (PCR) technique uses the ability of a DNA polymerase to copy DNA by elongation of complementary strands initiated from a pair of oligonucleotide primers (Saiki *et al.*, 1985; Mullis & Faloona, 1987). Repetitive cycles lead to the exponential amplification of a target. A schematic overview of the principle of PCR is shown in Figure 11.

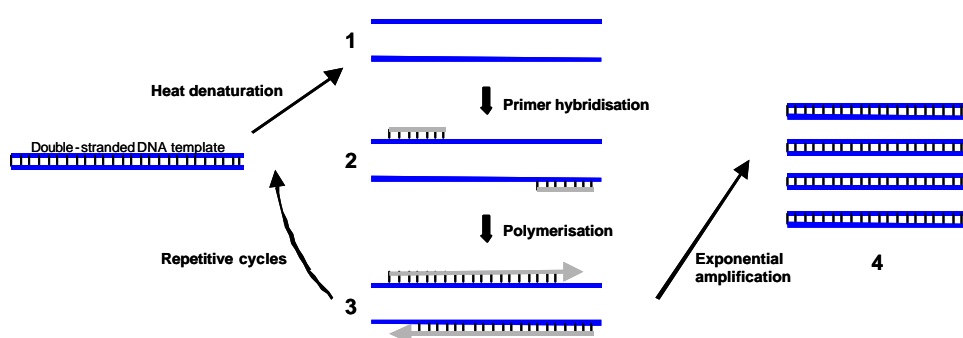


Figure 11. Schematic overview of PCR: (1) the template DNA strains are separated by heat denaturation; (2) the reaction temperature is decreased and the primers anneal to their corresponding template; (3) the polymerase will extend from the primers; (4) the reaction temperature is increased and decreased in repetitive cycles, resulting in exponential amplification of the target.

Real-time PCR

A schematic overview of the real-time PCR system used in this work is shown in Figure 12. The method is based on three oligo-nucleotides (a forward primer, a reverse primer and a probe in between) complementary to a region located in the gene of interest. The probe is marked with a fluorescent dye in the 5'-end and a quencher dye close to or at the 3'-end. When the probe is intact, the short distance between the dyes will result in suppression of the reporter fluorescence signal by Förster-type energy

transfer (Li & Parrish, 1992). The DNA polymerase used is a *T. Aquaticus* polymerase (*Taq* polymerase) with 5'-3' nuclease activity (Holland *et al.*, 1991; Lyamichev *et al.*, 1993). When the primers and probe have annealed to the target, the *Taq* polymerase extends from the primers and degrades the hybridised probe. Thus, the dyes are released (see Figure 12). The distance between the reporter and the quencher then increases, resulting in an increase in the fluorescent signal from the reporter dye (Holland *et al.*, 1991). Since the *Taq* polymerase will not degrade free polynucleotides, an increase in fluorescence is direct evidence of the presence of the specific DNA sequence in the investigated material. The signal increases as the target is amplified. The instrument measures fluorescence continuously (real-time) and no post PCR treatment of the sample is needed to calculate the amount of target. The target gene starting copy number in unknown samples is quantified by using a standard curve from samples with known amounts of target. More than one target can be measured in the same reaction by using probes marked with different reporter dyes.

For other viruses, satisfactory results as regards nucleic acid quantification have been obtained with the real-time PCR technique, *e.g.* for EBV (Enbom *et al.*, 2001; Bossolasco *et al.*, 2002), HHV-8 (Enbom *et al.*, 2001) and HCV (Takeuchi *et al.*, 1999). Further, several groups have recently reported on the possibility of measuring HIV DNA by real-time PCR (Désiré *et al.*, 2001; Riva *et al.*, 2001; Douek *et al.*, 2002). Amplification of the human albumin gene in a separate reaction has been used to normalise for variations in the number of investigated cellular units (Bièche *et al.*, 1998; Désiré *et al.*, 2001; Douek *et al.*, 2002). In paper IV, we developed a real-time PCR method that combines quantification of HIV DNA and the number of cellular genomes in the same reaction (multiplex) for the measurement of HIV DNA in CD4⁺ cells.

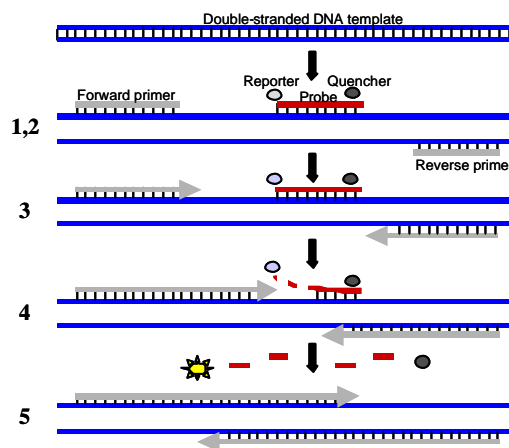


Figure 12. Schematic overview of real-time PCR: (1) the template DNA strands are separated; (2) the primers and probe anneal to their corresponding template; (3) the *Taq* polymerase will extend from the primers; (4) the nuclease activity of the *Taq* polymerase will degrade the probe, resulting in (5) release of the reporter dye and the quencher. The reporter signal will increase as the target is amplified (real-time).

HIV DNA QUANTIFICATION (IV)

Details regarding the set-up of the method, including the sequences of primers and probes, are reported in paper IV. The processing of patient samples is shown in Figure 13. In brief, from stored peripheral blood mononuclear cells of patient samples, CD4⁺ cells were purified by anti-CD4 coated magnetic beads (Dynabeads[®] M-450 CD4; Dynal A S, Oslo, Norway). DNA from the purified CD4⁺ cells was extracted using the QIAamp DNA Blood Mini Kit (Qiagen[®] GmbH, Hilden, Germany). On the resulting material, HIV DNA and the number of cellular genomes were measured with the TaqMan[™] PCR Reagent System and the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, California). By choosing different reporter marker dyes on the HIV probe and human albumin gene probe (*i.e.* 6-carboxyfluorescein (FAM) and VIC[™] (Applied Biosystems, Foster City, California)) we were able to perform the two measurements in one single-well reaction. In addition to the patient samples, material from two different reference strains of HIV, 8E5-LAV (Folks *et al.*, 1986) and U937/HIV-1 MN (Popovic *et al.*, 1984), were used for validation of the method.

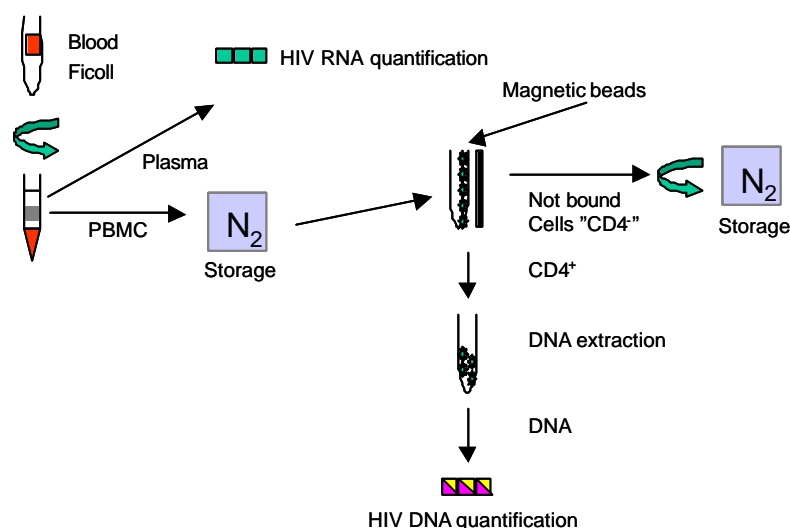


Figure 13. Measurement of HIV. Peripheral blood mononuclear cells (PBMC) were separated from whole blood by Ficoll. HIV RNA levels were quantified from plasma. CD4⁺ cells were separated by anti-CD4 on magnetic beads and DNA was extracted. HIV DNA was quantified by real-time PCR.

Standards

To transform the measured fluorescence signal from the samples to the number of viral and cellular copies, two different standard curves were used: (a) a standard based on Epstein-Barr virus (EBV), using a FAM labelled probe, for calculating the number of HIV genomes, and (b) a standard based on the human albumin gene, using a VIC[™] (Applied Biosystems, Foster City, California) labelled probe, for calculating the

number of investigated cells. It may seem unusual to employ a virus of another family as the standard but given equal efficacy in the PCR assay of the two different systems, one may be used as the standard for the other and vice versa. We accordingly chose to use the well-established EBV standard (Enbom *et al.*, 2001) to calculate the number of HIV genomes. Cellular DNA from the EBV-positive cell line Namalwa (Cargano *et al.*, 1992) and the EBV-negative cell line BL-28 (Lenoir *et al.*, 1982) were mixed and diluted to form five different dilution points on the dual standard curves.

In paper **IV** we tested and validated our real-time PCR system for quantification of HIV DNA (pol gene) in CD4⁺ cells. The comparison of the two sets of standard curves showed no systematic deviation as regards the human albumin system. Concerning the EBV system, the lowest concentration showed a greater variation between the two estimates than did higher concentrations (Figure **IV:3**). This could have to do with a somewhat too high efficacy in one of the runs ($k=-3.04$), while the expected value for perfect doubling per PCR cycle is $k=-3.32$. Slope values in the range $-3.3 - -3.6$ fulfil one of the criteria for a curve to be used as standard in the calculation of the number of targets. Other such criteria include measures of variation. For instance, the squared correlation coefficient (R^2) should not go below 0.98. This criterion was not always fulfilled for the EBV curves, mostly due to a greater variation in the lowest concentrations. This indicates that the lowest concentration point on the curve lies on the border of the quantification limit and should be set higher in future set-ups.

Kinetic properties

The kinetic properties of the combined HIV DNA and human albumin systems were tested by dilution series of two different HIV subtype B strains and one positive control stock made up of DNA from peripheral blood mononuclear cells. These cells had been infected with viral isolates from five different patients (Figure **IV:2**). Acceptable variation was obtained for the different dilution series with the exception of the 8E5-LAV strain, which showed a greater variation, probably due to the low starting concentration. Slopes within the optimal range were obtained for the dilutions of the two laboratory strains 8E5-LAV and MN. The positive control mixture gave a slightly too steep slope, indicating a somewhat too low efficacy. This could be due to stochastic effects such as an uneven mixture of genetic variants induced by the different isolates in the mixture. The total DNA concentration for the dilution points of the three different dilution series was kept constant (*i.e.* HIV negative cellular DNA was used as the dilution solvent) in this experiment. Earlier tests had indicated that inhibitory effects might influence the efficacy of the HIV PCR if the total cellular DNA content was allowed to vary (k in these experiments varied between $-2.54 - -2.58$ for the three different dilution series). Therefore, to avoid such influences in analyses of clinical samples, the number of cells analysed should be of the same magnitude. Also, results showing very low numbers of target molecules should be interpreted with caution since our results showed a larger variation in the samples with a low concentration of target.

Viral target

We are the first to focus on HIV DNA quantification in the CD4⁺ cells in long-term therapy interruption. We chose to focus on CD4⁺ cells because they are the primary targets of HIV. Certain HIV isolates have, however, also been shown to be able to infect and replicate in, for example, CD8⁺ cells (Saha *et al.*, 2001). Interestingly, when we investigated residual cells from the CD4 purification (*i.e.* cells not selected by the anti CD4 coated beads) in the material from three CD4 purifications, the real-time PCR detected HIV in all three CD4⁻ samples. The magnitude of HIV DNA per investigated cell was about one log₁₀ less than in the corresponding CD4⁺ preparation. However, this could be due to an incompleteness of the anti CD4 coated beads to sort out the complete population of CD4⁺ cells or other non-CD4⁺ cells carrying the HIV genome.

Provirus

Apart from integrated provirus, HIV DNA also exists in non-integrated linear and circular forms (episomes). While our method does not distinguish between integrated and unintegrated viral forms, the results of our patient samples are of the same magnitude as the data from a study on integrated HIV DNA before and after one year of PI-ART (Ibáñez *et al.*, 1999). Our results are also comparable with data on the HIV DNA load in patients under successful treatment and patients with treatment failure (Russell *et al.*, 2001). Other authors claim that more than 90% of the total HIV DNA may not be integrated (Chun *et al.*, 1997; Ibáñez *et al.*, 1999; Vandegraaff *et al.*, 2001). Episomal HIV DNA has, however, been shown to be short-lived (Sharkey *et al.*, 2000). Whether or not episomal HIV DNA is important to consider when following changes in the HIV DNA load in patients over time, our method does seem to provide essential information about the load of HIV in infected cells.

SUPERVISED THERAPY INTERRUPTION

There are various reasons for using supervised therapy interruption: (a) to stimulate the HIV specific immune response after viraemia has been suppressed by treatment (autovaccination); (b) to increase the time off drug; (c) to improve quality of life and limit drug-related side effects; (d) to induce reversion of resistance to wild-type among individuals whose virus has become resistant to treatment, and therefore (e) to improve the chances of success with subsequent salvage therapy (Hirschel, 2001). The following patient categories have been hypothesised to benefit from supervised therapy interruption: (a) patients treated very early in the infection (that is, in the stage of acute infection or close to seroconversion); (b) patients in whom therapy was initiated in the chronic phase of infection and where viraemia is controlled, and (c) patients without controlled viraemia (Gulick, 2002; Lisziewicz & Lori, 2002). Further, supervised therapy interruption can be implemented as (a) shorter repetitive interruptions followed by shorter or longer periods of therapy, or (b) as a single longer period off therapy, long-term supervised therapy interruption (Hirschel, 2001). In this thesis, long-term

supervised therapy interruptions to increase time off drug or due to treatment failure were studied.

The therapy interruption cohort (PAUSE)

A total of 26 patients entered a study of long-term supervised therapy interruption (PAUSE). The patients could enter the study either by meeting successful treatment criteria (*e.g.* HIV RNA <50 copies/ml during at least the last year and $>700 \times 10^6$ CD4⁺ cells/l) or on clinical grounds (*e.g.* severe adverse effects, viral failure or a strong wish to discontinue therapy; for further details regarding the inclusion criteria, see paper V).

Physical examination and blood samples were scheduled for two weeks before treatment interruption, the same day as treatment was interrupted, after two and four weeks, monthly to month three and then at intervals of one to three months. In addition, the patients were asked to complete the SWED-QUAL and Health Index instruments at study entry and after six, 12 and 24 months. Treatment was re-started when two consecutive CD4 counts had decreased to <50 % of baseline or if there were signs of clinical deterioration related to HIV replication.

Viral DNA load (V)

In paper V we developed the real-time PCR to further monitor the HIV DNA load. This was done in samples from a subset of 15 patients from PAUSE study entry and during up to one year of interrupted therapy. Eight patients were included on the basis of successful treatment criteria and seven patients on clinical grounds. Further demographic and medical data are given in paper V and Appendix A. The individual slopes of HIV DNA development were calculated for the first six months of therapy interruption. Slopes of HIV RNA and CD4⁺ lymphocytes were calculated for the following periods (months) after therapy interruption: (a) 0 – 1, (b) 1 – 9 or until the time-point of re-starting treatment if before month nine, and (c) 0 – 9 or until the time-point of re-starting treatment if before month nine. CD4 slopes were also determined for the -periods before the start of and during PI-ART. In addition, in some patients HIV DNA load was measured on stored samples from before the start of therapy and/or during the therapy. The relations between HIV DNA development during therapy interruption and other viral, immunological and treatment-related variables were investigated.

The mean follow-up time was 8.3 months after therapy interruption. Treatment was reinitiated in one patient before month six (*i.e.* five months after study entry), in another patient at month six, in another at month eight and in two at month nine.

The HIV DNA seemed to decrease continuously during PI-ART (Figure V:1) and increased in all patients after therapy interruption (Figures V:1 and 2). Figure 15 shows the individual development of HIV DNA load of the 15 included patients. Three potentially different patterns of HIV DNA increase after therapy interruption could be

discerned: (a) an initial increase and subsequent stabilisation (Figures 15 a-i and V:2A), (b) a continuous increase (Figures 15 j-l and V:2B), and (c) just a slow increase (Figures 15 m-o and V:2C). It is interesting to speculate whether this indicates different processes of HIV DNA regeneration or simply different kinetics. One factor influencing the development of the HIV DNA load in therapy interruption is the phase in which treatment was originally initiated. Patients treated very soon after infection may have a steeper decrease in HIV DNA load during therapy (Andreoni *et al.*, 2000) and better viral control during the therapy interruption (Lisziewicz & Lori, 2002) compared to patients treated in a later phase of infection. Yerly *et al.* (2000), have demonstrated a strong association between HIV DNA load and plasma HIV RNA load after the initiation of combined antiretroviral therapy. Cross-sectional studies have also shown a relation between HIV DNA and plasma viral load or levels of CD4⁺ cells (Burgard *et al.*, 2000; Russell *et al.*, 2001). Other authors claim no such relations (Désiré *et al.*, 2001). Our study clearly showed associations between the treatment interruption HIV DNA slope and both viral and immunological variables. The slope was positively correlated to the HIV RNA maximum, the steady state HIV RNA level and the baseline HIV DNA value and negatively correlated to the total CD4 absolute and percentage slopes (month 0 – 9), the late CD4 percentage slopes (month 1 – 9) and the CD4 absolute slope before start of protease inhibitor based antiretroviral therapy (Table V:1).

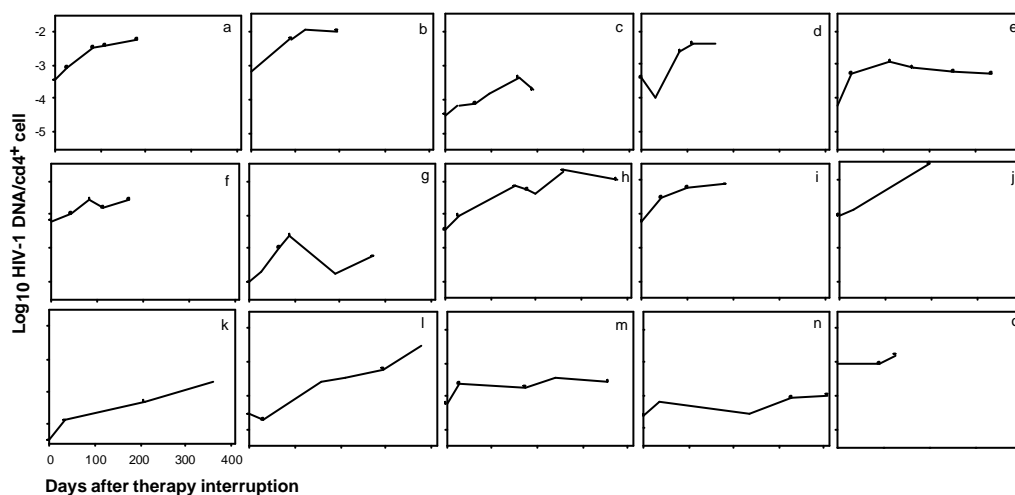


Figure 15. Individual HIV and DNA development in 15 patients initiating long-term supervised therapy interruption. Three tentative patterns of HIV DNA were found. (1) initial increase and later stabilising levels (patient a-i); (2) continuously increasing levels (patient j-l) and (3) only slightly increasing levels (< 0.5 log₁₀; patient m-o).

Interestingly, the HIV DNA increase after therapy interruption had a median slope of 0.156 log₁₀ copies per CD4⁺ cell and month. This result corresponds to a doubling time of 59 days. It has previously been estimated that the HIV DNA in infected patients has

an average half-life of approximately six months (Andreoni *et al.*, 2000; Joos *et al.*, 2000; Yerly *et al.*, 2000; Karlsson *et al.*, 2001). However, the life span of the infected cell population has been shown to vary in different cell subpopulations. The majority of the infected cells die sooner, corresponding to a HIV DNA half-life of one month, while a small fraction may live much longer (Yerly *et al.*, 2000). Finzi *et al.* (1999), have shown the half-life of infected resting CD4⁺ cells to be as long as about 44 months but even half-lives of over 12 years have been reported for certain infected cell populations (Strain *et al.*, 2003). As the previously reported half-lives of HIV DNA were based on the decrease associated with drug treatment, it remains to be seen whether this decrease in HIV DNA and the increase after therapy interruption measure different features of viral replication and latency. For the future, it would be interesting to monitor the evolutionary development of both the HIV RNA and HIV DNA in parallel after therapy interruption to find out the relation between HIV proviral and RNA quasi species.

Health-related quality of life

Health-related quality of life was monitored in 14 men and four women of the PAUSE cohort. Thirteen met the successful treatment criteria and five were included on clinical grounds. The patients were asked to complete the SWED-QUAL questionnaire just before treatment interruption as well as at six months and one and two years during interruption. At study entry their mean age was 48 years, the median CD4 value was $735 \times 10^6/l$ and median HIV RNA was <50 copies/ml. Further demographic data are shown in Appendix A. Data from the pre-interruption and follow-up (that is, after two years of interrupted therapy or after one year if therapy was re-started between year one and year two) were used in this analysis. Eleven patients completed the two-year follow-up questionnaire and three more completed the one-year follow-up and then re-started therapy before year two. In total, pre-interruption and follow-up data were available for 14 patients (two re-started therapy before the one-year measure and two did not return the questionnaire).

No statistically significant differences were found for the SWED-QUAL scales (shown as composite scores in Figure 16a). There was, however, a statistically significant difference in the Health Index subscale physical well-being (median 15.5 at pre-interruption and 17.0 at follow-up; $p < 0.05$; Wilcoxon signed-ranks test; Figure 16b). In contrast, when Krentz & Gill (2003), investigated the development of the health-related quality of life for 50 patients with various follow-up times after interruption of antiretroviral therapy, they found a deterioration. It seems that the reason for the interruption is important, since health-related quality of life improved in the cases where the interruption was chosen by the patient.

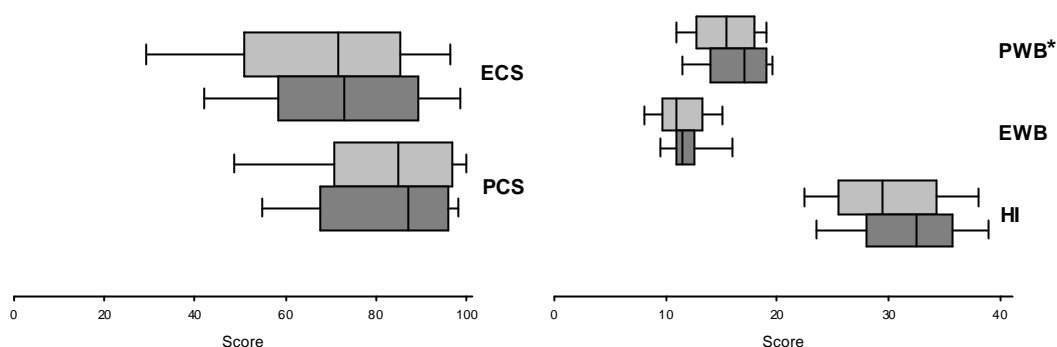


Figure 16a. SWED-QUAL. Results before (pre-interruption) and after up to two years (follow-up) with long-term supervised therapy interruption. Pre-interruption (light gray); Follow-up (dark gray). The box boundaries indicate the 25th and the 75th percentiles and the whiskers indicate the 10th and the 90th percentiles. *PCS* physical well-being composite score; *ECS* emotional well-being composite score.

Figure 16b. Health Index. Results before (pre-interruption) and after up to two years (follow-up) with long-term supervised therapy interruption. Pre-interruption (light gray); Follow-up (dark gray). The box boundaries indicate the 25th and the 75th percentiles and the whiskers indicate the 10th and the 90th percentiles. *HI* total Health Index;

EWB emotional well-being subscale; *PWB* physical well-being subscale; *p <0.05.

The patients generally experienced the therapy interruption positively. Many of them thought that they felt “better than ever”. One patient spontaneously wrote on the form: “PAUSE is the best vacation I have ever had.” A few patients, though, expressed feelings of fear, uncertainty and discomfort when they thought about the virus ravaging freely in their body and one patient even re-started therapy after five months, without conferring with the responsible health-care professionals.

PLANNING FOR THE FUTURE – CONCLUDING REMARKS

The HIV DNA remains in infected CD4⁺ cells despite long-term effective PI-ART and with the therapeutic potential that is currently available, life-long persistence of viral reservoirs seems inevitable (Strain *et al.*, 2003). The HIV DNA levels increased when therapy was interrupted. The magnitude of this increase was related to both viral and immunological parameters during the interruption and also to the pre-treatment development of CD4⁺ lymphocyte levels. The health-related quality of life remained stable after up to two years of therapy interruption. There was, however, a slight increase in one measure of physical well-being. Health-related quality of life could be one factor to take into account in cases where long-term supervised therapy interruption may be indicated. For certain groups of patients, for example those suffering from adverse effects and/or those who are highly motivated to undergo supervised therapy interruption, the effects of the interruption on health-related quality of life may be better than for other groups (Krentz & Gill, 2003). Another factor that may be important to consider is the progression rate before initiation of combination therapy. Patients with a

steeper decrease in CD4⁺ cell levels also had a more rapid increase in HIV DNA levels during therapy interruption (V).

Moreover, the use of therapeutic HIV vaccines and/or immunomodulators, for example hydroxyurea, granulocyte-macrophage colony-stimulating factor and interleukins 2 and 12, may have a potential to enhance viral control and improve T-cell function in supervised therapy interruption (Davey *et al.*, 1999; Hel *et al.*, 2000; Calarota *et al.*, 2001; Imami *et al.*, 2001; Lori *et al.*, 2002; Mitsuyasu, 2002; Garcia *et al.*, 2003).

MAIN CONCLUSIONS AND FUTURE PERSPECTIVES

We monitored the clinical and health-related quality of life in patients with therapeutic HIV vaccinations. The viral content and the quality of life of infected patients undergoing treatment and treatment interruptions were also monitored.

The HIV-positive patients reported substantially lower scores of their health-related quality of life and health status compared with Swedish random population groups. This was especially the case for the emotional, social, family and general health domains. However, compared with a group of HIV-negative patients consisting of men who have sex with men, no differences were found as regards the emotional and family domains of health-related quality of life. Instead, the HIV-negative group showed increased scores in the physical and general health domains. This emphasises the need for increased attention to psychosocial aspects in the care of both these groups.

Several studies, including our own, indicate that therapeutic HIV vaccines are safe and tolerable, enhance T-cell responses and have transient effects on CD4 development. HIV vaccines, however, have little apparent effect on the viral load in the absence of efficient antiretroviral therapy. Further, a therapeutic rgp160 vaccine did not seem to have any impact on the development of the HIV-positive patients' health-related quality of life. Their physical functioning deteriorated during two years of follow-up, most probably related to the natural progression of their disease.

Although efficient antiretroviral combination therapy substantially decreases viral load and may improve general immune functions, it does not seem to lead to any major reconstitution of impaired HIV-specific T-cell responses. This might be because levels of virus-specific antigenic stimulation are too low as a result of drug-induced suppression of viral replication. In combination with effective antiretroviral therapy, therapeutic HIV immunisations have been suggested to have the potential to enhance existing antiviral responses, induce new HIV-specific responses and reduce viral load, thereby promoting long-term non progression (Calarota & Wahren, 2001; Imami & Gotch, 2002; Kinloch-de Loes & Autran, 2002). It has even been suggested that such an approach might totally eradicate virus from all of the body's reservoirs (Imami & Gotch, 2002). Reports from a study with the therapeutic HIV vaccine Remune claim that patients receiving a combination of this vaccine and antiviral therapy were less likely to experience virological failure compared to those treated with placebo vaccine (Burton, 2002). In the light of our data, however, life-long persistence of viral reservoirs seems inevitable.

The introduction of PI-ART had a tremendous effect on HIV-related morbidity and mortality. However, the treatment seemed to have a negative impact on the patients' emotional health. The experience of adverse effects made a large contribution to the magnitude of this deterioration. The treatment seemed to interrupt an expected negative

development of physical functioning. Lower scores in the physical domain of health-related quality of life preceded poorer adherence and adverse effects. It is thus important to consider the patient's physical health-related quality of life when planning individual treatment and care. For the future, more needs to be known about the impact of different treatment regimens on the health-related quality of life. Matters of primary importance are the optimal timing of treatment initiation and the identification of treatment combinations that are optimal in clinical terms as well as for health-related quality of life. Another important issue is which interventions, such as, for example, support groups and educational programs, would facilitate adherence and optimize the quality of life in different patient groups with HIV.

The HIV DNA load decreases with PI-ART but is still present in infected CD4⁺ cells despite a long period of effective treatment. The HIV DNA levels increased when therapy was interrupted. The magnitude of this increase was related to both viral and immunological parameters during the interruption, as well as to the pre-treatment development of CD4⁺ lymphocyte levels. The progression rate before initiation of combination therapy could be important when considering patients for supervised therapy interruptions since the effects may be less beneficial in patients whose initial decrease in CD4⁺ cells was relatively steep. Health-related quality of life seemed to remain stable after up to two years of therapy interruption. There was even a slight increase in one measure of physical well-being. Health-related quality of life could also be a factor to take into account in cases where supervised therapy interruption could be indicated. For certain groups of patients, for example those suffering from adverse effects and those who are highly motivated to undergo supervised therapy interruption, the effects in terms of health-related quality of life may be better than for other groups (Krentz & Gill, 2003). The use of therapeutic HIV vaccines and/or immunomodulators, for example hydroxyurea, granulocyte-macrophage colony-stimulating factor and interleukin 2 and 12, may have a potential to enhance viral control and improve T-cell function in supervised therapy interruption (Davey *et al.*, 1999; Hel *et al.*, 2000; Calarota *et al.*, 2001; Imami *et al.*, 2001; Lori *et al.*, 2002; Mitsuyasu, 2002; Garcia *et al.*, 2003). Since HIV seems to persist and require chronic treatment, several issues remain to be investigated to find individual treatment strategies that have the optimal virological and immunological outcomes with the least discomfort for the patient. Since the pattern of HIV DNA after interrupted therapy differed between different patients, it would be interesting to further explore the kinetics with intense DNA measurements on patients starting therapy, undergoing therapy interruption and re-starting therapy. Another issue is whether DNA kinetics differs between different cell subpopulations. Moreover, the influence of certain patient and viral characteristics on the outcome of therapy interruption, *e.g.* HLA type and viral subtype, requires further exploration, as does viral evolution after interrupted and re-started antiretroviral treatment. All these characteristics may be useful for optimizing immunological treatment.

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APPENDIX A

MEDICAL AND DEMOGRAPHIC DATA REGARDING THE SIX INVESTIGATED COHORTS.

	rgp160 (I) n=40	rgp160 QoL (II) n=72	MSM n=164	PI-ART (III) n=72	PAUSE DNA (V) n=15	PAUSE QoL n=18
CD4 count, cells $\times 10^6/l$: median	534 ^x	390	-	127	755	735
range	126 ^{SD}	110-1080	-	1-660	334-2084	326-1830
Mean age	39	37	36	41	49	47
SD	9	9	11	9	10	9
Ethnic group:						
Caucasian %	-	94	-	-	-	-
Other %	-	6	-	-	-	-
Country of origin:						
Sweden %	-	-	84	82	-	89
Other/not stated %	-	-	16	18	-	11
Mode of transmission:						
Male-male %	75	96	-	96	93	78
Female-male/male-female %	10	4	-	4	7	22
Blood products/IVD%	15	0	-	0	0	0
Working status:						
Full or part-time work/studies %	-	63	83	50	-	56
Sick leave/disability pension %	-	37	7	43	-	39
Other/not stated %	-	0	10	7	-	5
Education:						
Compulsory school %	-	15	7	18	-	17
Upper secondary/high school %	-	40	34	38	-	33
University studies %	-	39	57	38	-	50
Not stated %	-	6	2	7	-	0
Has a partner: Yes/No %	-	56/43	51/48	42/57	-	39/61
Not stated %	-	1	1	1	-	0

^{PI-ART} protease inhibitor based antiretroviral therapy; ^x mean; ^{SD} standard deviation; ^{IVD} intravenous drug abuse
^{MSM} men who have sex with men; ^{PAUSE} long-term supervised therapy interruption study; ^{rgp160} recombinant glyco-protein 160

APPENDIX B

OVERVIEW OF THE DIFFERENT STATISTICAL METHODS USED IN PAPERS I-III, V AND THE THESIS

	I	II	III	V	Thesis
One-sample <i>t</i> -test		X			X
Independent samples <i>t</i> -test	X		X		
Two-way analysis of variance (ANOVA) with repeated measures	X				X
Hierarchical multiple linear regression, single step	X		X	X	
Hierarchical multiple linear regression, stepwise			X		
Factor analysis (rotated component matrix)			X		
Chronbach's α		X	X		
Mann-Whitney <i>U</i> -test	X	X	X	X	X
Kruskal-Wallis test	X	X			
Wilcoxon signed-ranks test	X		X	X	X
Friedman test	X				
Pearson χ^2 -test			X		
Fisher exact test			X		
Log rank survival analysis					X
Spearman rank correlation				X	