

The effectiveness of highly active antiretroviral therapy in HIV-infected patients

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This review is based on the following papers:

- I. Jensen-Fangel S, Kirk O, Larsen L, Blaxhult A, Gerstoft J, Pedersen C, Black FT, Lundgren JD, Obel N. Saquinavir hard gel suppresses viral load insufficiently in HIV-infected patients naïve to antiretroviral therapy: A retrospective cohort study. *Scand J Infect Dis* 1999;31: 489-93.
- II. Jensen-Fangel S, Kirk O, Blaxhult A, Gerstoft J, Pedersen C, Black FT, Lundgren JD, Obel N. The insufficient suppression of viral load by saquinavir hard gel is reversible: A retrospective cohort study. *HIV Clin Trials* Jan 2001;2:122-7.
- III. Jensen-Fangel S, Pedersen C, Nielsen H, Tauris P, Møller A, Sørensen HT, Obel N. Use of the protease inhibitor saquinavir hard gel in human immunodeficiency virus-infected patients in the early period of highly active antiretroviral therapy: does it affect long-term treatment outcome? *Scand J Infect Dis* 2003: Accepted for publication.
- IV. Jensen-Fangel S, Pedersen C, Larsen CS, Tauris P, Møller A, Obel N. Trends in the use of highly active antiretroviral therapy in western Denmark. *Scand J Infect Dis* 2002;34:460-5.
- V. Jensen-Fangel S, Thomsen HF, Larsen L, Black FT, Obel N. The effect of nevirapine in combination with nelfinavir in heavily pretreated HIV-1 infected patients: A prospective, open-label, controlled, randomized study. *J Acquir Immune Defic Syndr* 2001;27:124-9.
- VI. Jensen-Fangel S, Justesen US, Black FT, Pedersen C, Obel N. The use of calcium carbonate in nelfinavir-associated diarrhoea in HIV 1-infected patients. *HIV Med* 2003;4:48-52.
- VII. Jensen-Fangel S, Pedersen C, Larsen CS, Tauris P, Møller A, Obel N. Changing demographics in an HIV-infected population: Results from an observational cohort study in Western Denmark. *Scand J Infect Dis* 2001;33:765-70.
- VIII. Jensen-Fangel S, Pedersen L, Pedersen C, Larsen CS, Tauris P, Møller A, Sørensen HT, Obel N. The effect of race/ethnicity on the outcome of highly active antiretroviral therapy for human immunodeficiency virus type 1-infected patients. *Clin Infect Dis* 2002;35:1541-8.
- IX. Jensen-Fangel S, Pedersen L, Pedersen C, Larsen CS, Tauris P, Møller A, Sørensen HT, Obel N. Low mortality in HIV-infected patients starting HAART: A comparison with the general population. *AIDS* 2004;18:89-97.

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The papers are referred to in the text by their Roman numeral in parenthesis.

The studies which form the basis of this thesis were carried out in the period 1998-2003 during my employment at the Department of Infectious Diseases, Aarhus University Hospital. In the review I have put an effort into working out an overview of the current knowledge in the fields covered by the thesis, in preference to repeating the details from our own studies. These appear from the published or accepted papers, and can on request be required from the author at: fangel@dadlnet.dk. I am indebted to a number of persons who have contributed to the completion of the project. First of all, I particularly want to thank my supervisor and mentor in the best senses, *Niels Obel*, whose steady enthusiasm, efforts, and support have been an invaluable foundation for the entire project. I enjoyed the guidance and critical comments from *Court Pedersen* all along the completion of the studies.

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ABBREVIATIONS

ART:	antiretroviral therapy
HAART:	highly active antiretroviral therapy
LLOD:	lower limit of detection
NNRTI:	non-nucleoside reverse transcriptase inhibitor
NRTI:	nucleoside reverse transcriptase inhibitor
PI:	protease inhibitor
SQVhgc:	saquinavir hard-gel capsule
SQVsgc:	saquinavir soft-gel capsule

1. INTRODUCTION

1.1. HIV/AIDS IN DENMARK

Along with several other countries in Western Europe and the United States, Denmark noted the first AIDS cases in the early 1980's. Soon after, in 1983, AIDS became a mandatory reportable disease to the national surveillance unit at the Statens Serum Institute. Up til January 1, 2003, a total of 2435 AIDS cases have been reported (1). With the change in the course of HIV infection brought by the introduction of potent antiretroviral treatment, the relevance of the AIDS case surveillance system has diminished. To outline the epidemic in Denmark, the HIV case surveillance system is currently of far more value. Confirmed HIV infection has been mandatory only since August 1990, with 3678 HIV cases reported as of January 1, 2003 (1). Despite considerable shifts in reported mode of transmission and in the demographic features of the HIV-infected population during the years, the annual number of reported new HIV diagnoses remains quite stable with a mean of 274 (range 212-318) per year in the period 1995-2001 (2).

The HIV epidemic is not evenly spread throughout the country, but concentrated to the urban areas, and in particular to the metropolitan area of Copenhagen. Merely 26% of the HIV cases were reported from the region of West Denmark, defined as the counties of Jutland and Funen (2). As this region comprises a population of 2.95 million, or 55% of the entire Danish population, the prevalence of HIV infection is considerably lower in West, than in East Denmark.

An estimated 4500 individuals were living with HIV infection in Denmark in 2000, yielding a prevalence of 0.1% in the total population (3). Despite this low prevalence, the impact of HIV infection on the health care system is considerable due to the chronic course of the disease, combined with the life-long and costly therapy. Furthermore, the impact is only expected to increase in the years to come with an increasing number of HIV-infected patients receiving therapy, and with new and generally more expensive antiretroviral agents entering the market. With the current incidences of new HIV diagnoses and deaths among HIV-infected individuals, the total number of individuals living with diagnosed HIV infection in Denmark increases by about 250 per year.

1.2. HIGHLY ACTIVE ANTIRETROVIRAL THERAPY FOR HIV INFECTION

With the approval in Europe of the first protease inhibitor (PI) in 1996, the era of highly active antiretroviral therapy (HAART) took its start. Several definitions have been proposed for the term "HAART", the commonest being a treatment combination of at least three antiretroviral drugs. By the end of 1997 HAART was widely used in Denmark, primarily in the combination one PI and two NRTIs (paper VII). As opposed to the previous treatment regimens consisting of NRTIs in mono- or double therapy, the introduction of HAART dramatically improved the prognosis for HIV-infected patients, with a decline in rates of AIDS-defining events as well as in mortality (4-7). In Denmark the national surveillance unit reported a decline in absolute numbers of deaths from 237 in 1995 to 16 in 2001, coinciding with the increasing use of HAART (2). The reported annual AIDS incidence showed a similar trend, decreasing from 4.6 to 0.9 per 100.000 from 1993 to 2000 (2, 3).

As reflected by the currently updated treatment recommendations, the standard of antiretroviral treatment has changed continuously since the introduction of HAART, with contributions from the approval of new formulations of existing drugs, new compounds within the classes of antiretroviral drugs, new drug classes, and new treatment paradigms (8-15). Despite these new treatment options, the frequency of therapeutic failure remains high due to factors such as non-adherence to treatment, drug toxicity with subsequent treatment discontinuation, selection of resistant viral strains, and sub-optimal antiretroviral regimens (16-18). With a number of second-line treatment options to choose from after shifting from a first-line HAART regimen, it is not surprising that an immense heterogeneity in treatment history is one of the major problems when evaluating the outcome of subsequent regimens.

1.3. EVALUATING THE OUTCOME OF ANTIRETROVIRAL TREATMENT

1.3.1. Clinical outcome

As the ultimate goal of antiretroviral treatment is to prevent, or at least decrease, morbidity and mortality from HIV infection, data on AIDS defining events and death would obviously be the main outcome measures in any study on treatment efficacy. However, with the low risk of clinical progression following HAART, it has become impractical to use clinical endpoint data (19). Strategies used to comply with this problem include the use of a longer observation period, a larger study population, and/or collapsing the data on morbidity and mortality into one clinical outcome measure, often given the term: "clinical progression". Especially in clinical trials evaluating new or existing treatment options, it is difficult and costly to fulfill these requirements due to the short-term follow-up. Furthermore high rates of drug discontinuations will limit the value of efficacy trials with long observation periods. In many instances it is instead convenient to use surrogate markers for clinical progression.

1.3.2. Surrogate markers

Many clinical and laboratory measures have been evaluated to assess the prognosis of HIV infection (20). Of these, especially two markers have been shown to correlate with disease progression, being useful as surrogate markers when evaluating the natural history of HIV: CD4 cell counts and levels of plasma HIV-1 RNA (21-24). Early reports showed that treatment-induced improvements in these biologic activity measures would also reliably predict clinical progression when treating with NRTIs (25-27). Later studies have found CD4 cell count and plasma HIV-1 RNA to be valuable surrogate markers for clinical progression after starting HAART (28-35). Hence, CD4 cell count and plasma HIV-1 RNA are frequently used as prognostic markers for disease progression, with serial monitoring accepted as the principal means of evaluating outcome. CD4 cell responses and the degree of suppression of plasma HIV-1 RNA are usually related (36, 37), even though discordant responses have been shown to occur in up to 30% of the patients, depending on the definitions used (38-42). Overall, plasma HIV-1 RNA is considered the strongest single predictor for long-term clinical outcome, with the very goal of therapy being achievement of maximal reduction of plasma viral load to undetectable values for as long as possible (11, 15).

1.4. TREATMENT OUTCOME IN RELATION TO STUDY POPULATION AND -DESIGN

In the evaluation of the outcome of HAART, large variations are found between studies in terms of the level of suppression of plasma HIV-1 RNA. In clinical trials on HAART regimens high success rates are generally reported, with viral suppression to undetectable values in up to 90% of the study population at 48 weeks of follow-up (43-46). In contrast, the rates of viral suppression are found to be considerably lower in unselected patients in observational cohort studies, with 40-75% reported to have undetectable viral loads after 48-52 weeks of follow-up (16, 18, 47-52).

The explanations for these immense variabilities in the outcome of HAART are complex. Hence, they are not only a product of treatment-related differences (potency of regimen, daily pill burden, combination of drug classes), but also differences in disease stage in the study population at the time of starting treatment (baseline CD4 cell count, viral load, AIDS), previous antiretroviral experience, demographic characteristics (age, gender, mode of transmission, race/ethnicity, geographic area), viral characteristics (baseline resistance mutations, viral subtypes), and degrees of adherence to therapy (16, 18, 53). Furthermore the studies differ in methods of analysis (intent-to-treat or on-treatment approach), handling of missing data, size of study population, and sensitivity of viral load assays.

Consequently, summarised outcome measures such as the prevalence of undetectable viral load at specified time points during follow-up, are difficult to compare between studies, and especially between randomised trials and observational cohort studies. However, cross-study comparisons are difficult even when the results are obtained from studies of similar design, e.g. observational cohort studies. When evaluating treatment issues and outcome on a national or regional scale, population-based cohorts on the HIV-infected patients receiving treatment in the specific region offers valuable information, which can supplement the findings from single-center, or international cohorts.

1.5. AIMS OF THESIS

The aims of this thesis were:

1. To describe the HIV epidemic in the region of West Denmark.
2. To evaluate the outcome of HAART in West Denmark with emphasis on the use of SQVhgc as the initial PI, and on the influence of the racial/ethnic background of the patients.
3. To describe the incidence of, and reasons for, discontinuation of first-line HAART.
4. To evaluate treatment strategies in patients failing a first-line PI-based HAART regimen, as well as to evaluate treatment for diarrhea as a common side-effect.
5. To describe the mortality in HIV-infected patients starting HAART, compared to the general population.

2. DATA SOURCES AND METHODS

2.1. DATA SOURCES

The studies included in this thesis were based on data from the following sources:

Data from clinics treating HIV-infected patients in Scandinavia (papers I and II)

The data included in these studies were obtained retrospectively from clinical charts on HIV-infected patients followed at the Department of Infectious Diseases, Aarhus University, and from a random subset of HIV-infected patients followed at 5 clinics in Denmark, Norway, and Sweden.

Data from two single-center clinical trials (paper V, VI)

Data included in paper V were obtained prospectively in a single-center, randomised, controlled trial at the Department of Infectious Diseases, Aarhus University. Data included in paper VI were ob-

tained from a single-center, controlled, pilot study trial at the Department of Infectious Diseases, Aarhus University.

The HIV Cohort Study in West Denmark (papers III, IV, and VII-IX)

The HIV Cohort Study in West Denmark is a prospective, population-based cohort study on HIV-infected patients followed at the clinics treating HIV-infection in the counties of Jutland and Funen. These five clinics are located in the cities of Aarhus, Odense, Aalborg, Herning and Kolding. Eligible to the study are all HIV-infected patients seen at least once at one of the centers after January 1, 1995. The patients are followed prospectively with data collection once a year. The study population and variables included in the database are described in detail in paper VII. As of January 1, 2002 a total of 971 patients were enrolled.

The Danish Civil Registration System (paper IX)

The Danish Civil Registration System is storing information of vital status on all persons residing in Denmark after 1 April, 1968. The registry includes information on residency, date of birth, date of immigration or emigration, and date of death.

2.2. METHODOLOGICAL AND STATISTICAL CONSIDERATIONS

2.2.1. Baseline characteristics

In the studies evaluating the outcome of HAART (papers I-III, V, VIII-IX), the study populations were described by their characteristics at the time of starting treatment, defined as baseline characteristics. These characteristics included data on i) demographic factors (gender, age, race, mode of transmission), ii) factors describing the stage of disease (CD4 cell count, viral load, previous AIDS event), and iii) factors describing previous antiretroviral treatment. For CD4 cell count and viral load, these were referred to as baseline values if measured within 6 months prior to starting treatment. In the definition of AIDS events, we used the 1993 clinical definition of AIDS from the US Centers for Disease Control and Prevention (54). A baseline AIDS event was defined as having experienced an event prior to – or within one month of starting treatment. Information on previous antiretroviral treatment was dichotomised into yes or no. When comparing baseline data across groups, we used the following tests when appropriate: Chi-square, Kruskal-Wallis, Student's t, Mann-Whitney, or Fisher's exact test.

2.2.2. Evaluation of clinical outcome

In the evaluation of clinical outcome, we used two different outcome measures in the studies: clinical progression, and death from any cause. Clinical progression is a compound measure of morbidity and mortality, defined as the occurrence of a new AIDS defining event (occurring more than 30 days after starting HAART), or death from any cause. In the analysis of mortality the outcome was death from any cause.

2.2.3. Evaluation of plasma HIV-1 RNA and CD4 cell responses

In the analysis of the response in plasma HIV-1 RNA after starting HAART, we used the following two well-described statistical methods: i) The prevalence of plasma HIV-1 RNA undetectability at specified time points during follow-up, and ii) Time to achieving plasma HIV-1 RNA undetectability after starting treatment (55-58). Both methods are widely used in both clinical trials and observational cohort studies, as they represent clinically relevant notions with plasma HIV-1 RNA undetectability being the goal of HIV treatment. In the analyses we generally used a lower limit of detection (LLOD) of 500 HIV-1 RNA copies/ml, as this represented the least sensitive analysis of plasma HIV-1 RNA in the observation period. In the clinical trial (paper V), however, we used a LLOD of 200 HIV-1 RNA copies/ml, as this was the least sensitive value in the study period. In the analysis of CD4 cell response, we used the me-

dian absolute CD4 cell count, or the median absolute change in CD4 cell count during follow-up.

For both markers, the values were measured at specified time points after starting therapy in the protocolled study. However, due to the more irregular visits in the non-protocolled observational studies, evenly spaced measures of CD4 cell count and plasma HIV-1 RNA were constructed for every 12-week interval after starting therapy. If more measures were available within the 12-week period, the mean value of the CD4 cell counts and of the log-transformed HIV-1 RNA were calculated and used in the analysis.

2.2.4. Missing data

We used different replacement methods depending on the design of the study. In the clinical trial (paper V), the principle of "missing value equals failure" (MV=F) was applied for the dichotomised summary outcome of HIV-1 RNA (undetectable or detectable), representing a conservative test analysis compared to the procedure of "latest observation carried forward" (59). In the observational cohort studies, characterised by more irregular visits, a missing value of the dichotomised viral load was regarded as being undetectable only if the previous and the following values were undetectable; Otherwise a missing value was regarded as detectable. In the analysis of continuous values, a missing value was replaced by the mean of the preceding and the following value.

2.2.5. Comparing treatment outcomes

When evaluating the outcome after starting HAART, we used the "intention-to-treat" (ITT) principle, ignoring treatment changes and interruptions during follow-up. The ITT analysis is the recommended approach in clinical trials, as it avoids potential severe bias due to dropouts and non-compliant patients (60-63), and is also widely used in observational cohort studies (64).

Statistical methods used in the clinical trial (paper V) and in the retrospective studies (papers I, II) included chi-square and Fishers exact test for comparison of the summarised plasma HIV-1 RNA outcomes, and the Students t and Kruskal-Wallis for comparisons of the increase in CD4 cell counts. In the observational cohort studies (papers III, VII, and IX), univariate comparisons were performed across the groups of interest, with subsequent multivariate modeling for controlling for a number of available potential confounders. These models included the Cox proportional hazards regression model for the time-to-event analyses (time to undetectable viral load and time to clinical progression), the logistic regression model for comparisons of the proportions with undetectable viral load during follow-up, and the multivariate linear regression model for comparisons of absolute CD4 cell counts during follow-up. CD4 cell counts were square-root transformed to restore the normal distribution. Additionally, we used stratification as confounder control when estimating mortality according to baseline CD4 cell count and baseline plasma HIV-1 RNA (paper IX). In the confounder control we used the change-in-estimate method, in which variables resulting in changes in the estimated exposure effect of more than 10% were entered into the final model (65).

2.3. ETHICAL CONSIDERATIONS AND DATA SAFETY

The two controlled trials (papers V and VI) were both approved by the local Ethics Committee, and by the Danish Medicines Agency. All participating patients gave written informed consent. As data from the HIV Cohort Study in West Denmark are anonymous, no informed consent from the patients was obtained. The database project was approved by the Danish Data Protection Agency, and was presented to the local Ethics Committee, who had no objections.

3. TREATMENT RESPONSE IN PATIENTS

STARTING SAQUINAVIR HARD-GEL CAPSULE (PAPERS I-III)

3.1. BACKGROUND

In the "early" HAART period (1996-98), combination antiretroviral

therapy including two nucleoside reverse transcriptase inhibitors (NRTIs) and an HIV protease inhibitor became the standard of care for HIV infection (9, 13). These triple combination regimens resulted in substantial reductions in plasma HIV-1 RNA, increases in CD4 cell counts, and reductions in risk of disease progression and death, when compared to dual therapy with two NRTIs (45, 66-70).

The first HIV protease inhibitor used in clinical practice was saquinavir in a hard-gelatin capsule formulation (SQVhgc; Invirase®), which was approved in the US by the US Food and Drug Administration (FDA) in December 1995, and in Europe by The European Agency for the Evaluation of Medicinal Products (EMEA) in October 1996. Early dose-ranging studies found saquinavir to be a potent antiviral agent, with a dose-dependent effect in terms of suppression of plasma HIV-1 RNA and elevation of CD4 cell counts over 16 to 24 weeks of follow-up (71, 72). A dosage of 600 mg three times daily became the standard dose when prescribing SQVhgc (71).

3.2. PHARMACOKINETICS

Saquinavir is a potent inhibitor of the proteases of HIV-1 and HIV-2 in vitro, exerting antiretroviral activity at low concentrations in a wide range of cell types (73-79). However, the clinical antiviral activity of SQVhgc is limited by a low oral bioavailability, due to poor gastrointestinal absorption (80) and an extensive hepatic and enteric first-pass metabolic clearance (81-84). The mean absolute bioavailability of a single 600 mg oral dose in healthy volunteers is reported to be as low as 4% (85). Studies on HIV-infected patients receiving multiple oral doses of SQVhgc (600 mg three times daily), showed a marked intersubject variability, up to 12-fold, in saquinavir pharmacokinetic parameters (86-89). Hence, when treating with the standard dose of SQVhgc, some patients achieve subinhibitory plasma levels of the drug.

3.3. RESISTANCE PROFILE

Phenotypic resistance to saquinavir is primarily associated with mutations at codons 48 (G→V) and 90 (L→M) in the HIV protease gene. The relative distribution of these two mutations seems to differ with the study set-up. The main mutation selected for in vitro appears to be G48V (90-92), whereas L90M is the predominant mutation selected in vivo, with G48V and the double mutant form, G48V/L90M occurring less often (93-97).

An important factor for the development of resistance is the number of antiretroviral components used in the first-line regimen. SQVhgc given as monotherapy or in dual therapy with an NRTI, seems to favor the emergence of resistance mutations compared to SQVhgc given as part of a HAART regimen (93, 98). In early phase clinical studies on first-line treatment with SQVhgc as monotherapy or in combination with zidovudine, about 45% of all patients had provirus with mutations at positions 48 or 90 after 8-12 months of follow-up (98); In the same study, patients treated with a three-drug combination of SQVhgc, zidovudine, and zalcitabine, only 22% carried mutant virus.

PI-induced mutations have been shown to confer cross-resistance to other PIs (99, 100). Early reports suggested that it might be less of a problem when using saquinavir as first-line PI (94, 101), whereas later reports found that first-line saquinavir also selected for virus being cross-resistant to other PIs (100-104). Hence, saquinavir treatment has been demonstrated to select for resistant viral strains, that display cross-resistance to both indinavir (104-107) and nelfinavir (105). In a clinical study on patients pre-treated with SQVhgc, an insufficient virological response to indinavir correlated to mutations at several codons, including codon 90 (102). In addition to the initially selected ("primary") resistance mutations at positions 48 and 90, treatment with saquinavir also selects for a number of secondary mutations, including mutations at positions 10, 36, 46, 63, 71, 82, and 84 (93, 98, 102, 103, 107). Winters et al demonstrated in a group of patients treated with saquinavir who shifted to an alter-

Table 1. Cohort Studies on the effectiveness of SQVhgc-based HAART regimens.

Authors (ref)	Country/region	n	ART exp	Differing outcome variables, SQVhgc vs other PI	*Risk estimate for SQVhgc (95% CI)	Compared to
D'Arminio Monforte et al (17)	Italy	250	100%	Treatment failure (new AIDS, death, or definitive drug discontinuation)	RR 2.46 (1.20-5.03)	IDV
Jensen-Fangel et al (paper III)	Denmark	212	100%	Virological failure (during follow-up)	RR 2.44 (1.79-3.33)	IDV+RTV
Casado et al (51)	Spain	400	91%	Virological failure (at 12 months)	RR 1.55 (1.03-2.27)	IDV+RTV
Kirk et al (112)	Europe	2.708	88%	Clinical progression (new AIDS or death) Virological response (within 12 months) Immunological response (change in CD4 cell count)	RR 1.30 (1.01-1.67) OR 0.55 (0.40-0.75) OR 0.42 (0.33-0.54) p<0.0007	IDV RTV IDV IDV
Fätkenheuer et al (16)	Germany	198	83%	Virological failure (at 6 months)	RR 4.62 (no 95% CIs)	IDV
Paredes et al (113)	Europe	1.469	83%	Virological failure (at 6 months)	RR 1.61 (1.22-2.13)	RTV
Grabar et al (114)	France	1.402	82%	Virological failure (at 12 months)	OR 1.96 (1.48-2.29)	IDV
Wit et al (49)	The Netherlands	271	78%	Virological failure (at 48 weeks)	OR 3.21 (1.75-5.89)	IDV+RTV+ RTV/SQV
Paris et al (116)	Switzerland	274	73%	Virological failure (within 6 months)	OR 3.06 (1.03-9.06)	IDV
Staszewski et al (50)	Germany	901	67%	Virological response (within 24 weeks)	RR 0.61 (0.45-0.81)	IDV
Ledergerber et al (18)	Switzerland	2.674	57%	Virological response (during initial treatment regimen)	RR 0.31 (0.22-0.44)	Other PIs
Easterbrook et al (115)	United Kingdom	690	51%	Virological response (within 6 months)	RR 0.36 (0.24-0.54) RR 0.39 (0.28-0.55) RR 0.41 (0.26-0.66) RR 0.48 (0.30-0.78) RR 0.51 (0.35-0.74)	NFV IDV RTV RTV/SQV NVP
Jensen-Fangel et al (paper I)	Denmark	87	0%	Virological response (at 12 months)	34% vs 73%	IDV+RTV

*) RR=Relative risk, OR=Odds ratio.

IDV=indinavir, RTV=ritonavir, NFV=nelfinavir, NVP=nevirapine.

native PI-based regimen, that all isolates with reduced susceptibilities to more than one PI possessed either the G48V or L90M mutation, along with an average of 6.4 secondary protease gene mutations (106).

The major concern of the insufficient viral suppression along with the emergence of cross-resistant viral strains, is that it may influence the outcome of subsequent PI-containing regimens, leading to treatment failure and limited options for future therapy (108-110).

3.4. CLINICAL STUDIES ON THE OUTCOME OF SQVHGC-BASED HAART

3.4.1. Randomised Trials

In the clinical evaluation of the efficacy of SQVhgc as PI component in HAART regimens, a few randomised trials have reported on the superior effect of SQVhgc in triple combination regimens when compared to mono- or dual therapy with NRTIs (66, 111). No randomised trials have ever been undertaken to compare first-line SQVhgc based HAART regimens with HAART regimens based on other PIs. Two new PIs, ritonavir and indinavir, were approved only few months after SQVhgc. At this time point the low bioavailability and suboptimal viral suppression of SQVhgc was well documented, making it unethical to implement a controlled trial to compare these new and more bioavailable PIs with SQVhgc.

3.4.2. Cohort studies

Whereas the number of clinical trials on SQVhgc are limited, a number of cohort studies have focused on the outcome of SQVhgc containing HAART regimens (Table 1). The majority of these studies were performed on study populations that included both patients pretreated with NRTIs, and patients naïve to antiretroviral therapy (16, 18, 49-51, 112-116). These studies consistently report on the inferior virological outcome of SQVhgc compared to other PIs. In most studies the insufficient suppression of the virus did not translate into a differential increase in CD4 cell count, or a difference in clinical progression. However, in a large study on 2708 patients with a median follow-up of 30 months, the EuroSIDA study

group found that initial treatment with SQVhgc was associated with an increased risk of clinical progression (defined as AIDS-defining event or death) when compared to indinavir-based HAART regimens (112).

From studies evaluating the outcome of first-line PI-based HAART regimens it appears that pre-treatment with NRTIs is a risk factor for insufficient treatment outcome, most likely due to the accumulation of viral strains cross-resistant to other NRTIs (16, 18, 51, 112, 114). Only few studies have focused on the effectiveness of SQVhgc-based HAART regimens in patients naïve to antiretroviral therapy. In a retrospective study (papers I and II), we found that antiretroviral naïve patients starting zidovudine, lamivudine and SQVhgc at short-term (after 6 and 12 months of follow-up) had an insufficient virological outcome when compared to patients starting zidovudine, lamivudine and either ritonavir or indinavir (paper I). With extended follow-up, we found that at long-term (after 24 and 30 months) there were no longer any significant differences in the virological outcomes between the two groups (paper II). Simultaneously, more patients in the SQVhgc group had the initial PI discontinued in favor of new HAART regimens. We found no differences in median increase in CD4 count, or in clinical progression (new AIDS event or death).

In patients pretreated with NRTIs, d'Arminio Monforte et al found that the use of SQVhgc-based regimens correlated with virological treatment failure after a median of 8 months of follow-up (17). Similarly, in a population-based cohort study we found that starting with a SQVhgc-based HAART regimen in patients pretreated with NRTIs, was associated with an overall inferior virological response compared to patients starting with ritonavir or indinavir (paper III). However, after long-term follow-up (median 4.6 years), the differences in treatment outcome between the two groups had decreased, and there were no longer any significant differences in virological outcome. Similar to the findings in the study on NRTI-naïve patients (paper II), an earlier discontinuation of SQVhgc in favor of new and more potent HAART regimens probably explains the equal long-term outcomes.

3.4.3. Observational studies and treatment evaluation

The use of observational data to assess the effect of treatment is controversial and subject to continuous debate (117-126). However, in some situations no data are, or will be, available from randomised trials because these trials would be unethical or not feasible. In these situations observational studies can indeed provide important information (127). Of specific concern when using observational data is the introduction of bias, as each patient's treatment is deliberately chosen rather than randomly assigned. Although baseline characteristics are well balanced, as it is the case in our study (paper I and II), the patients are by no means randomly allocated, and there will be a risk of bias and systematic differences that are not due to the treatment itself. One way to strengthen an observational study is to restrict the cohort, for instance by only including patients who are naïve to antiretroviral therapy (paper I and II). Another way is to adjust for potential confounding factors in the statistical analysis (paper III). However, confounding factors that can not be adjusted for will remain, and may bias the findings in observational studies (121).

The use of the ITT approach in the analysis of treatment outcome produces a conservative estimate of treatment effects. As a result of the insufficient virological effect of SQVhgc, virtually all patients will have changed to alternative regimens after a limited duration of follow-up (papers II and III). By using the ITT approach, the results will therefore reflect the impact of SQVhgc on subsequent treatment outcome, and not the efficacy of the drug per se.

One of the main forces in an observational study on treatment evaluation, is the possibility to achieve a high degree of external validity of the findings. Controlled trials will tend to enroll a selected group of patients, depending on how rigorous the inclusion criteria are (128). Patients included in trials may be healthier, more motivated and possibly more adherent than non-selected patients. The use of population-based data from a well-defined region diminishes the risk of selection bias (paper III).

3.5. OPTIMISING SAQUINAVIR-BASED REGIMENS

The higher plasma levels of SQVhgc achieved when administering higher doses of 3600 and 7200 mg daily, have shown to result in greater suppression of viral load and increases in CD4 cell counts (72). However, instead of increasing the pill burden of SQVhgc, alternative methods have been used to increase the bioavailability of saquinavir.

A soft-gelatin formulation of saquinavir (SQVsgc; Fortovase®) with a better bioavailability increases the plasma levels of saquinavir around eight-fold compared to a standard dose of SQVhgc (129). In a randomised phase II trial, SQVsgc gave significantly more potent suppression of plasma HIV-1 RNA in ART-naïve patients than the hard gel formulation (130). The new formulation of saquinavir seems to be as potent *in vivo* as other protease inhibitors. In the CHEESE study no difference was recorded in antiviral potency between SQVsgc and indinavir after 24 weeks (131).

Another method used to increase the plasma drug level of saquinavir, is by pharmacokinetic enhancement of the drug. Ritonavir is a potent inhibitor of the cytochrome P450 metabolic pathway, and in particular of the isoenzyme CYP3A4 (132, 133). As a result of the inhibition of saquinavir metabolism (83), co-administration of ritonavir and saquinavir has been demonstrated to lead to a more than 20-fold increase in saquinavir plasma levels (86, 134-137). The combination has been shown to produce a considerable and sustained viral suppression in a dose-ranging study in a cohort of predominantly NRTI experienced patients (134, 138). When compared to the potency of single-PI based regimens, several observational studies have demonstrated that the combination of ritonavir and saquinavir results in a similar viral suppression in PI-naïve patients (115, 139, 140). Several controlled trials have compared the outcome of ritonavir/saquinavir based first-line HAART with the outcome of mono-PI-based HAART (141-144). In a Danish ran-

domised multicenter trial, the combination of ritonavir and saquinavir with two NRTIs had a superior antiviral efficacy at short-term (24 weeks), when compared with two NRTIs and either indinavir or ritonavir in ART-naïve patients (141). At long-term (72 weeks), however, there were no differences in virological endpoints between the groups (142). When comparing the efficacy of ritonavir-enhanced saquinavir to other ritonavir-boosted regimens, only one head-to-head comparison has been performed to date. In the MaxCmin1 trial, no significant differences were found between ritonavir/indinavir and ritonavir/saquinavir with respect to virological, or immunological outcome (145). In an ongoing study (MaxCmin2) the efficacy of ritonavir-boosting of lopinavir and saquinavir are being compared (146).

Both formulations of saquinavir (SQVhgc and SQVsgc) have been used in studies on co-administration with ritonavir. Recent findings indicate that ritonavir-boosting of SQVhgc actually produces plasma exposures comparable to boosting of SQVsgc (147, 148). The hard-gel formulation of saquinavir might even be preferable, as it seems to be favorable in terms of gastrointestinal side-effects (148).

3.6. CONCLUSION

Although being the first HIV protease inhibitor to be approved, SQVhgc has never been recommended as first-line PI option (9, 10, 149). No randomised trial has conferred the insufficient effect of the drug, but a number of observational studies have consistently found that the use of SQVhgc in first-line HAART is associated with an inferior virological outcome, when compared to other single-PI regimens. We found this association at short-term both in HIV-infected patients who were NRTI experienced prior to starting SQVhgc, and in patients naïve to antiretroviral therapy. However, at long-term the insufficient virological effect was overcome, probably due to the earlier discontinuation of SQVhgc in favor of new, and probably more potent HAART regimens. In terms of clinical outcome, a large cohort study found an increased risk of clinical progression in patients starting with SQVhgc, when compared to indinavir as the PI component. Whether this will apply to the HIV-infected patients in our region who were previously treated with SQVhgc remains to be demonstrated, as we found no such differences in clinical progression.

Increased plasma concentrations of saquinavir have been achieved by the introduction of a new formulation of saquinavir with a better bioavailability, and by pharmacokinetic enhancement when co-administered with ritonavir. In a boosted strategy the hard-gel formulation of saquinavir might even be preferable, as it seems to be favorable in terms of gastrointestinal side-effects.

4. MODIFICATION OF FIRST-LINE PI-BASED HAART (PAPER IV)

4.1. MODIFICATION OF PI-BASED HAART: RATES

Despite being composed of highly potent antiretroviral drugs, the clinical effectiveness of HAART is compromised by a number of factors such as drug-related toxicities, pharmacokinetic issues, adherence problems, complex treatment regimens, and the emergence of resistant viral strains. Furthermore the steady introduction of new antiretroviral drugs and new treatment paradigms with lower pill burdens and different toxicity profiles offers attractive alternatives to existing treatment regimens.

Accordingly, modification or discontinuation of the initial HAART regimen occurs at high rates, especially in observational studies (150, 151). In a population-based cohort study on patients starting HAART in the period 1996-2000, we found that 45% of the patients had the initial HAART regimen modified during the first year of follow-up (paper IV). Modification was defined as discontinuing at least one of the antiretroviral compounds in the regimen. As it appears from Table 2, rates of drug modification/discontinuation are generally found to be high in studies of first-line HAART

Table 2. Modification/Discontinuation of first-line PI-based HAART – rates and reasons.

Author (ref)	Country	Study period	n	HAART regimen	Risk assessment	Discontinuations or modifications of HAART	Reasons (% of discontinuations)
Dorrucchi et al (155)	Italy	1997-2000	2002	First-line HAART	Probability during follow-up (median 9.7 months) Cumulative probability (12 months)	42.8% ¹ 37.6% ¹	Toxicity (43%), failure (29%), poor adherence (23%)
d'Arminio Monforte et al (151)	Italy	1998-1999	862	First-line HAART	Probability during follow-up (median 45 weeks)	36.2% ¹	Toxicity (58%), failure (14%), poor adherence (20%)
Guardiola et al (156)	Spain	1996-1997	653	First-line PI-based HAART	Cumulative probability (12 months)	11% ²	Toxicity (74%), failure (26.1%)
Mocroft et al (150)	United Kingdom	1996-1999	556	First-line HAART	Probability during follow-up (median 14.2 months) Cumulative probability (6 months)	44.4% ¹ 26.6% ³ 25.6% ¹ 14.8% ³	Toxicity and/or poor adherence (64%), failure (29%)
Jensen-Fangel et al (paper IV)	Denmark	1996-2001	537	First-line HAART	Cumulative probability (12 months)	45.1% ¹	Drug class specific
Wit et al (49)	The Netherlands	1996-1998	271	First-line HAART	Cumulative probability (48 weeks)	53% ¹	Toxicity (42%), failure (24%)
Hänsel et al (153)	Switzerland	1996-1999	252	First-line PI-based HAART	Probability during follow-up (mean 13.6 months)	44.1% ¹	Toxicity (46%), failure (21%), poor adherence (12%)
d'Arminio Monforte et al (17)	Italy	1996-1997	250	First-line PI-based HAART	Probability during follow-up (median 8 months)	25.6% ¹	Toxicity (47%), failure (42%), poor adherence (11%)
Ferrer et al (154)	Spain	1996-1997	230	First-line PI-based HAART	Probability during follow-up (median 6 months)	41.3% ¹	Toxicity (29%), failure (17%), poor adherence (45%)
van Roon et al (152)	The Netherlands	1996-1997	99	First-line PI-based HAART	Probability during follow-up (mean 15 months)	30.3% ¹	Toxicity (20%), failure (70%), poor adherence (10%)

1) Stopping at least one of the antiretrovirals in the regimen.

2) Switch from one PI to another.

3) Simultaneous stopping of all antiretrovirals in the regimen.

(17, 49, 150-156). The range in rates is not surprising, given the diversity in definitions of modification and discontinuation, the different statistical approaches, and the differences in baseline factors when starting treatment. The largest of the studies reported on more than 2000 antiretroviral naïve patients starting HAART, and found a cumulative probability of discontinuing the regimen within the first year at 37.6% (155).

4.2. MODIFICATION OF PI-BASED HAART: REASONS

When determining reasons for discontinuing or modifying therapy, a number of different classifications and definitions have been used. In the HIV Cohort Study in West Denmark we used the following three main categories (paper IV): (i) Treatment failure (virological, immunological, or clinical), (ii) Toxicity, and (iii) Adherence problems/other reasons. As there obviously are considerable overlaps between these categories, some authors prefer an even more rigorous classification, especially when comparing the results obtained in separate studies.

Despite being prone to a number of uncertainties, studies on the reasons of treatment modification/discontinuation show some common features (Table 2). In a review of 10 observational studies, Park-Wyllie et al found that the main reason for stopping antiretroviral therapy was drug toxicity/intolerance (poor adherence included), followed by therapeutic failure (157). However, the composition of the antiretroviral regimens used in the studies are likely to influence the findings. In line with the insufficient virological effect of SQVhgc, discontinuation due to failure is reported to be the most frequent reason for modification of SQVhgc-based HAART (17, 151)(paper IV). In contrast, indinavir- and ritonavir-based regimens were modified predominantly due to toxicity (150, 151 153)(paper IV). As new treatment options with different toxicity

profiles have emerged, the findings from these studies which include outdated HAART regimens can probably not be implemented into the current settings.

4.3. IMPLICATIONS AND LIMITATIONS

The rates of modification of first-line HAART in observational studies are considerably higher than in controlled trials. In trials on first-line mono-PI based triple therapy, only 7-26% were reported to modify the randomised treatment after 38-52 weeks of follow-up (45, 68-70). This discrepancy in the rates of drug discontinuations between these two study designs illustrates one of the major drawbacks of controlled trials, namely the risk of a low external validity, which may influence the generalisability of the findings. More or less restrictive in- and exclusion criteria will lead to enrollment of a selected group of patients, when compared to population-based studies (128). Moreover, trials may take place in atypical settings (eg. university hospitals), patients might be taken care of by unrepresentative health care professionals (127), and patients in trials will be more intensively followed than in clinical practice.

As it also appears from papers II and III, the fraction of patients remaining on the initial regimen decreased considerably with increasing follow-up time. Consequently, it is difficult to relate long-term treatment outcome to an initial treatment regimen. When using the ITT approach in the analyses, the findings will reflect the outcome of sequential treatment regimens rather than the long-term outcome of a regimen that might be obsolete (eg. SQVhgc-based HAART). However, such analyses will still be of interest, as the sequence of antiretrovirals used might influence the outcome of subsequent regimens.

Obviously, classification of the reasons for drug discontinuations will be prone to a number of uncertainties. In our study, data on the

reasons for drug discontinuation were obtained retrospectively from patient files, which will lead to some degree of inaccuracy. Only one reason was recorded for each discontinuation of a drug; As the physician frequently will have to choose one of several competing reasons, there will inevitably be a risk of misclassification. Adherence to therapy was not routinely assessed. However, when poor adherence was the most probable reason for a treatment discontinuation, this was noted by the physician. Furthermore side-effects related to single drugs are difficult to establish, due to the use of complex treatment regimes and a tendency to shift more than one drug when modifying treatment.

4.4. CONCLUSION

Modification of first-line single-PI based HAART occurs at high rates, mainly due to drug-related toxicities and treatment failure. The reason for modification depends of the PI used; SQVhgc was mainly discontinued due to treatment failure, whereas drug-related toxicity was the main reason for discontinuing indinavir and ritonavir. The high rate of drug discontinuation has implications for the analysis of long-term outcome of a first-line regimen when using the ITT approach.

5. THE ROLE OF NNRTIS AND NELFINAVIR IN SECOND LINE HAART (PAPER V)

5.1. SEQUENTIAL MONO-PI REGIMENS

Before the widespread use of NNRTIs and ritonavir-boosted PIs, only limited treatment options existed for patients failing, or being intolerant to a first-line HAART regimen. PIs were switched for alternative PIs, and/or NRTIs were switched for alternative NRTIs. Due to the emergence of cross-resistant viral strains, the few studies focusing on sequential mono-PI based regimens report only limited and transient effect in terms of viral suppression (48, 102, 158). This was illustrated in the prospective open-label AIDS Clinical Trials Group (ACTG) 333 trial on the effect of switching from SQVhgc to indinavir or SQVsgc (102); As no treatment arms reached the defined endpoint of a 0.7 log reduction in viral load, the trial was terminated prematurely after 8 weeks. In another study conducted by Lawrence et al, salvage therapy with nelfinavir plus two new NRTIs resulted in minimal and transient viral suppression in patients failing on SQVhgc (158). In the same study, rapid failure was associated with baseline presence of the protease gene L90M mutation.

Another strategy used to enhance the effectiveness of salvage therapy is to shift to NRTIs to which the patients were previously naïve. The benefits of this strategy has been shown in a number of observational cohort studies, both in initial and second-line PI-based HAART (18, 50, 113, 159).

5.2. NEVIRAPINE

With the FDA approval in June 1996 of nevirapine, the NNRTIs, representing a new class of antiretroviral drugs, were introduced to the clinical setting. Nevirapine potently inhibits the reverse transcriptase activity (160), but given as monotherapy it causes rapid induction of resistant viral strains (161-164). However, when administered with two NRTIs the combination has been shown to suppress viral load substantially, as demonstrated in a number of controlled trials (165-171). Whether nevirapine is as efficacious in first-line HAART as the other widely used NNRTI, efavirenz, has been subject for much debate in the recent years. Several cohort studies have found differences in treatment outcome between the drugs, consistently favoring the use of efavirenz (64, 172-174). A large-scale comparative randomised study, however, did not indicate superiority of efavirenz over nevirapine (171), underscoring the importance of controlled trials, whenever feasible, in the evaluation of drug efficacy.

5.3. NNRTIS IN SECOND-LINE/SALVAGE THERAPY

The introduction of NNRTIs along with the limited success of sequential mono-PI therapies, made this new drug class an obvious

applicant for salvage therapy (175). Table 3 summarises the findings from a number of studies assessing the outcome of second-line therapy including an NNRTI following a mono-PI based HAART regimen (48, 158, 176-185).

Overall, relatively few randomised trials on this subject have been published. In paper V we report on the findings from a single-center randomised study evaluating the effect of adding nevirapine to a second-line regimen consisting of nelfinavir and two NRTIs. Of the 56 patients enrolled, the majority (77%) were exposed to only one previous PI, and 80% were shifted from a regimen containing SQVhgc as PI component. The main reason for changing the treatment was failure, with 89% of the patients having a viral load above 200 copies/ml, and 75% above 1000 copies/ml at study entry. The addition of nevirapine lead to a favorable virological outcome: at 24 weeks, 55% in the nevirapine/nelfinavir group and 22% in the nelfinavir-only group ($p=0.015$) had an undetectable viral load. No differences were observed in immunological or clinical outcome.

In the ACTG 359 study, Gulick et al randomised 277 patients failing indinavir to six different salvage regimens based on a double PI plus either delavirdine (an NNRTI), adefovir (a nucleotide analogue), or both (177). Overall, the four delavirdine-containing arms had superior virologic effect with 33-47% of the patients having undetectable viral load at 16 weeks compared to 16-20% in the two arms without delavirdine.

The beneficial effect of adding an NNRTI to the second-line regimen after failing a mono-PI based HAART has also been demonstrated in a number of non-randomised, open-label, controlled trials (158, 179-182). In these studies, 52-78% of the patients had undetectable viral loads at follow-up (up to 12 months), compared to 14-19% when an NNRTI was not included. One prospective study found no association between the use of nevirapine in salvage therapy in extensively pretreated patients and virological success; However, as the authors state, this could be due to the small study number and the heterogenous patient population (185). Also a few observational studies have found that successful second-line therapy was associated with the use of NNRTIs in the subsequent regimen (48, 183, 184, 186, 187).

5.4. NELFINAVIR AS PART OF SALVAGE THERAPY

Nelfinavir was approved by the FDA in March 1998, two years after SQVhgc. When used as part of first-line therapy with two NRTIs, early trials indicated that nelfinavir was equivalent to other PIs (70, 188). However, the potency has never been directly compared to other mono-PI regimens in randomised trials. Because of its favorable toxicity profile, nelfinavir was by some clinicians preferred to other PIs, and became widely used as part of HAART regimens. In 1999, 51% of the patients receiving HAART in the region of West Denmark were exposed to nelfinavir (paper IV).

The use of nelfinavir in second-line therapy is more controversial. Very few studies have focused on salvage therapy with nelfinavir and two NRTIs after failing a PI-based regimen. As it appears, we only found that 22% had an undetectable viral load at 24 weeks of follow-up (paper V). In a study by Lawrence et al, salvage therapy with nelfinavir plus two new NRTIs resulted in minimal and transient viral suppression in patients failing on SQVhgc: 3 (19%) patients reached an undetectable viral load, and no patients were undetectable by week 12 (158). In that study failure of salvage therapy was associated with the baseline presence of the protease mutation L90M, indicating cross-resistance to nelfinavir by saquinavir selection (158, 189). We did not perform genotypic resistance testing as part of our study, but would expect a similar association with primary mutations in the protease gene, as 80% of the patients were shifted from a SQVhgc-based regimen (paper V).

A number of studies have focused on the use of nelfinavir with an NNRTI, and/or a second PI as part of salvage therapy. Being highly heterogenous in design, outcome measures, first- and second-line therapies, the success rates in terms of virological response varies

Table 3. Studies on the addition of an NNRTI to salvage HAART.

Author (ref)	Country	Study design	n	Previous therapy	NNRTI-containing salvage regimen	Virological success ¹ , overall	Virological success ¹ , according to use of NNRTIs
Hammer et al (176)	United States	RCT	481	SQV, NFV, IDV, RTV, and/or NNRTI (44%)	ABC+EFV+APV+adefovir+2 nd PI (SQV, IDV, NFV or placebo)	31% (<200, Wk 24) ²	NNRTI-naïve: 43% vs 16%
Gulick et al (177)	United States	RCT	277	IDV	6 different salvage regimens based on SQV/RTV or SQV/NFV +/- DLV, +/- adefovir	30% (<500, Wk 16) ² 27% (<500, Wk 24) ²	Pooled DLV: 40% vs 33%
Jensen-Fangel et al (paper V)	Denmark	RCT	56	PI-based HAART	NFV +/- NVP	39% (<200, Wk 24) ²	NVP: 52% vs 22%
Benson et al (178)	United States	RCT	70	PI-based HAART	RTV/LPV, 2 diff doses Day 15: NVP+ new NRTI	70% (<400, Wk 48) ²	All patients assigned to NNRTI
Lawrence et al (158)	United States	Open-label	16	SQV	NFV, subsequently IDV+NVP	13% (<400, Wk 12) ² 33% (<400, Wk 24) ³	IDV+NVP: 60% vs 19% in initial NFV treatment
Deeks et al (179)	United States	Open-label	20	IDV or RTV	NFV+SQV+ABC+an NRTI or NVP	50% (<500, Wk 24) ³	NVP: 78% vs 14% in NRTI-arm
Gulick et al (180)	United States	Open-label	56	APV (as monotherapy, or in HAART)	IDV, d4T, 3TC, and NVP	73% (<500, Wk 16) ² 59% (<500, Wk 48) ²	All patients assigned to NNRTI
Piketty et al (181)	France	Open-label	32	IDV or RTV	RTV/SQV+EFV	71% (<500, Wk 24) ²	All patients assigned to NNRTI
Casado et al (182)	Spain	Open-label	31	RTV or IDV	SQV+NFV+NVP+NRTI	25% (<50, Wk26) ² 31% (<50, Wk 52) ²	All patients assigned to NNRTI
Deeks et al (48)	United States	Cohort	337	RTV, IDV or NFV	PI +/- NNRTI	22% (<500, Wk 24) ²	PI+NNRTI: 59% vs 8% in PI-only
Moyle et al (183)	United Kingdom	Cohort	31	Mono-PI based HAART	ABC+EFV or NVP	34% (<500, Wk 24) ²	EFV 58% vs 11% in NVP
Sullivan et al (184)	United Kingdom	Cohort	19	PI-based HAART	NFV+NVP	38% (<200, Wk) ³	All patients treated with NNRTI
Fatkenheuer et al (185)	Germany	Cohort	30	PI-based HAART	RTV/SQV+/- NNRTI	20% (<200, Wk 12) ²	No effect of adding an NNRTI

1) Defined as proportion of patients with undetectable viral load.

2) Intention-to-treat analysis (ITT)

3) On-treatment analysis (OT).

NFV=nelfinavir, IDV=indinavir, RTV=ritonavir, ABC=abacavir, EFV=efaviranz, APV=amprenavir, DLV=delavirdine, NVP=nevirapine, LPV=lopinavir.

considerably; However, these studies generally find higher rates than in treatment with nelfinavir and NRTIs alone (176, 177, 182, 184, 190, 191).

5.5. OTHER OPTIONS FOR SALVAGE THERAPY

Apart from the addition of an NNRTI, a large number of other options for salvage therapy after failure of a PI-based regimen have been investigated. These options are concentrated on i) The use of ritonavir boosting of other PIs (185, 192-195), ii) The use of new compounds within existing drug classes (178, 196, 197), iii) The use of drugs with new mechanisms of action, e.g. HIV-1 fusion inhibitors (198, 199), and iv) The concomitant use of a larger number of drugs, the so-called "Mega-HAART" or "Giga-HAART" regimens (181, 200-203).

5.6. LIMITATIONS IN STUDIES ON SALVAGE THERAPY

With the lack of randomised trials, most information on the effectiveness of salvage therapy is derived from observational cohort studies, or from small non-randomised, open-label studies. The main obstacle is that it is difficult to find homogeneous and sufficiently large study populations, as each patient has an individual pre-treatment history, harbouring different combinations of resistance mutations. This, in turn, makes it difficult to evaluate clinical outcome, even in studies with long observation periods. The heterogeneity in both pre-treatment experience and the composition of the salvage regimens renders it difficult to compare the results from one study to another. Furthermore the distinction between the terms "salvage" and "second-line" therapy is not always clear, with

studies often including both patients with a well defined treatment failure, and patients shifting due to other causes.

5.7. CONCLUSION

In the early HAART period treatment was composed of a mono-PI and two NRTIs. In patients failing or being intolerant to the first-line regimen, shifting to a new mono-PI based regimen was associated with a limited treatment response due to the evolution of viral strains with cross-resistance to other PIs. Studies on the outcome of second-line HAART are highly heterogeneous in design, outcome measures, first- and second-line therapies. However, the addition of an NNRTI to the second-line PI-based regimen in patients naïve to NNRTIs is associated with a superior virological outcome. With the approval of new drugs and the use of new treatment paradigms during the recent years, new options for second-line therapy in patients shifting from PI-based regimens have emerged.

6. TREATMENT OF HAART-ASSOCIATED DIARRHEA (PAPER VI)

6.1. BACKGROUND

The use of HAART is associated with a high prevalence of toxic effects. In a large cross-sectional study on 1160 patients who received antiretroviral treatment, Fellay et al found that 47% reported clinical adverse events (204). A considerable number of different short- and long-term toxicities was observed, with the individual antiretroviral drugs having compound-specific associations (204). While the long-term toxicities are subject to intensive research, the common short-term toxicities are generally less so.

6.2. CHANGING ETIOLOGY OF DIARRHEA

Diarrhea is a common clinical manifestation in patients with HIV infection. In the pre-HAART era, lifetime incidences were reported to be as high as 50-70%, depending on the population studied and the case definition of diarrhea (205, 206). The majority of cases were caused by enteric pathogens, which in various prospective studies were isolated in 50%-85% of the cases (207-211).

Despite the widespread use of HAART, diarrhea still constitutes a major clinical problem among HIV-infected patients, being a frequent side-effect associated particularly with the use of PIs (212). In a cross-sectional study on a cohort of HIV-infected patients, Knox et al found that 39% had at least one episode of diarrhea in the month before data-collection, and 28% reported chronic diarrhea (213). The changing etiology of diarrhea in HIV-infected patients was shown by Call and colleagues in a retrospective study in HIV-infected patients with chronic diarrhea and a CD4 cell count of less than $200 \times 10^6/l$ (214). Although there was no change in the incidence of chronic diarrhea, the proportion caused by opportunistic infections decreased from 53% to 10% in the period 1995-97, while the proportion diagnosed with non-infectious causes concomitantly increased from 32% to 70% (214). Several studies have shown a correlation between immune recovery induced by HAART, and a decrease in diarrheal disease caused by entero-pathogenic opportunistic infections. Hence, the use of PI-based HAART has been correlated to considerable response rates in patients with HIV-related chronic diarrhea (215), including patients with intestinal pathogens that notoriously are difficult to treat, such as crypto- and microsporidia (216-219).

6.3. NELFINAVIR AND DIARRHEA

The prevalence of diarrhea in patients treated with HAART varies widely according to the constitution of the regimen. Although most antiretroviral drugs are known to cause diarrhea (212), some compounds are associated with particular high prevalences of diarrhea. In a large observational study, Fellay and colleagues found that the occurrence of diarrhea was most strongly associated with the use of nelfinavir (204). It is well established that mild to moderate diarrhea is the dose-limiting side-effect of nelfinavir. In randomised trials on

the efficacy and safety of the triple combination nelfinavir (750 mg t.i.d.), lamivudine and zidovudine, diarrhea occurred in 20%-45% of study subjects (70, 188). However, other combinations of antiretrovirals seem to result in even higher frequencies of diarrhea. In a randomised study we found that 73% developed diarrhea during 24 weeks of follow-up (paper V). When stratifying on the use of NRTIs, we found that 91% of the patients who received the treatment combination didanosine and nelfinavir reported diarrhea.

6.4. CONSEQUENCES

Apart from the risk of malnutrition and weight loss, the potential consequences of diarrhea in HIV-infected patients include decreased quality of life (220), discontinuation of therapy with limited future treatment options, and non-adherence to therapy that might ultimately lead to treatment failure. Data from the Swiss HIV Cohort Study revealed that the presence of diarrhea was an independent negative predictor of survival (206). In a retrospective study on 181 patients receiving nelfinavir, Kosmyrna et al found that HIV-infected patients without diarrhea had a greater increase in CD4 cell count than patients reporting diarrhea (51 vs -19 cells/mm³, respectively) (206). These data underscore the importance of evaluating effective treatment for PI-associated diarrhea in HIV-infected patients.

6.5. TREATMENT OF NELFINAVIR-ASSOCIATED DIARRHEA

6.5.1. Treatment options

As the underlying mechanism of PI-induced diarrhea is unknown, no specific and curative treatment has been directed against this condition. Data on the treatment of PI-associated diarrhea are limited. The knowledge on this field has mainly been derived from small non-randomised or retrospective studies, published primarily in abstract form. A summary of these studies is shown in Table 4. The majority of these studies have focused on diarrhea associated with the use of nelfinavir as the PI component. Several different agents have been evaluated: loperamide (222, 223), oat bran bars or psyllium (224-226), pancreatic lipase (222, 227), SP-303 (Provir) (228), diphenoxylate/atropine (Lomotil) (222), and calcium carbonate (Paper VI) (223, 229). In most of the studies the authors report im-

Table 4. Studies evaluating the effectiveness of agents used for treatment of PI-associated diarrhea.

Author(ref)	Study design	n	PI used in HAART regimen	Treatment	Results
Perez-Rodriguez et al (229)	Open-label trial	24	NFV	Calcium (various brands)	Decrease in mean diarrhea grade 1.58-0.33. 100% reported "dramatical" improvement of diarrhea, 67% reported normal stools.
Negredo et al (223)	RCT	42	NFV	Diet counselling+Calcium (n=13) Diet counselling+Loperamide(n=14) Diet counselling (n=15)	Improvement: 63%, Normalisation: 28% Improvement: 62%, Normalisation: 38% Improvement: 6%
Jensen-Fangel et al (paper VI)	Open-label cross-over trial	15	NFV	Calcium-carbonate (n=9) Calcium-gluconate/-carbonate (n=6)	No difference in diarrhea grade (+/- calcium) No difference in diarrhea grade (+/- calcium)
Ronagh et al (224)	Open-label trial	14	NFV	Psyllium HUSK (fiber bars)	Decrease in mean diarrhea grade 1.78-0.93 93% reported improvement
Hellinger et al (227)	Open-label trial	22	NFV	Pancrealipase	Improvement in frequency and urgency, large study drop-out (36%) at Wk 12
Razzeca et al (222)	Retrospective	38	NFV	LoperamideLomotil (n=6) Pancrealipase (n=26)	32% responded (frequency) No response in 6 patients 96% responded
Hawkins et al (226)	Telephone survey	77	NFV (87% with diarrhea)	Psyllium	50% reported less frequent stools
Holodniy et al (228)	RCT	51	PI (77%)	SP-303 (plant extract) or placebo	Significant reduction in 1) stool weight, and 2) stool frequency
Hoffman et al (225)	Open-label trial	51	PI (NFV43%)	Oat bran	Decrease in frequency (mean grading score 2-1.04)84% reported improvement

RCT=randomised controlled trial, NFV=nelfinavir.

provement of diarrhea with the administration of these agents. However, the studies are highly heterogeneous with respect to design, treatment duration, and outcome measures, allowing no comparisons between the evaluated agents. A number of different practices have been used to evaluate outcome, ranging from subjective patient opinions, over scales with different methods of diarrheal grading, to objective measures such as stool weighting and observed stool frequency.

Only one of the studies was conducted as a randomised, double-blind, placebo-controlled trial (228). However, not all patients in this study received antiretroviral treatment, and only 77% were treated with a PI. All the other studies are prone to bias due to the open-label design, often without control groups, or control periods.

6.5.2. Calcium carbonate for nelfinavir-associated diarrhea

As constipation is a well documented adverse effect of high-dose oral calcium supplementation, this agent has also been evaluated for use in HIV-infected patients with diarrhea. In 1999, Rodriguez et al reported a beneficial effect of calcium carbonate for nelfinavir-associated diarrhea (229). Although a diarrheal scale was also used, the conclusion of the study focused on patient opinions, where all study participants reported an improvement of diarrhea. In a prospective, open-label cross-over study on 9 patients with nelfinavir-associated diarrhea, we found a minor, but non-significant, improvement in diarrhea score after treatment with high-dose calcium-carbonate for 14 days, compared to a control period of 14 days off calcium (paper VI). In another treatment arm we evaluated the effect of calcium given as calcium-carbonate/calcium-gluconate, used in clinical practice in the prevention of osteoporosis. In this arm we found no improvement in diarrhea score.

In a third study on 13 patients, Negredo et al found calcium supplements given with diet counselling to improve diarrheal symptoms compared to counselling alone (223). Hence, the three studies report different levels of improvement; With the small study populations leading to a considerable risk of type 2 errors, and the differences in design and outcome, the variation in outcome is not surprising.

6.5.3. Pharmacokinetic considerations

Like the other PIs, nelfinavir is metabolised primarily in the liver by the cytochrome P450 system (230). One of the routes of metabolism is catalysed by the isoenzyme CYP2C19, leading to the formation of M8, an active metabolite reported to have an antiretroviral capacity comparable to the parent compound (231). The involvement of the cytochrome P450 system makes nelfinavir prone to pharmacokinetic interactions when administered with a number of other drugs, including other PIs (231).

When introducing new drugs for HIV-infected patients treated with PIs, it is important to assess potential pharmacokinetic interactions. Two studies have focused on the pharmacokinetics of nelfinavir when evaluating new treatment options for nelfinavir-associated diarrhea, both reporting on the use of calcium-carbonate (232) (paper VI). In 9 patients treated with nelfinavir, the intake of calcium carbonate did not significantly alter the median plasma concentrations of nelfinavir+M8 (paper VI). Similarly, Perez-Rodriguez et al found no differences in mean nelfinavir concentrations in patients concomitantly treated with calcium-carbonate, when compared with historic controls (232).

6.6. CONCLUSION

Diarrhea is a common clinical manifestation among patients receiving nelfinavir-based HAART, potentially leading to decreased quality-of-life, adherence problems and risk of treatment failure. Several small-scale studies have evaluated the effect of different constipating agents against PI-based diarrhea, the majority reporting varying degrees of improvement. Among these agents, oral calcium-carbonate has been proposed for the treatment of nelfinavir-associated diar-

rhea. However, the studies on this subject vary considerably in design and outcome measures, and convincing data on the beneficial effect of calcium-carbonate are still lacking.

7. TREATMENT RESPONSE ACCORDING TO RACIAL/ETHNIC BACKGROUND (PAPER VII AND VIII)

7.1. THE CHANGING HIV EPIDEMIC IN DENMARK

Similar to other countries in Western Europe, the demographics of the HIV epidemic in Denmark has changed during the recent years, with an increasing proportion of newly diagnosed HIV-infected patients being women, being infected through heterosexual contact, and being immigrants (3, 233, 234) (paper VII). Data from the HIV case surveillance in Denmark show that immigrants represented 18% of the newly diagnosed HIV-infected individuals in 1991, increasing to 37% in 2000 (3). The majority of the HIV-infected immigrants in Denmark derive from areas with high prevalences of HIV infection (235). Cumulative data (1999) from the HIV Cohort Study in West Denmark showed that 71% of the cohort were ethnic Danes, with immigrants from Sub-Saharan Africa constituting 19% (or 66% of the immigrants in the cohort) (paper VII).

In immigrants starting HAART in our region, we found that the median time spent in the Denmark before an established HIV diagnosis was only 1.6 years (paper VIII). The relatively few patients with a diagnosis of HIV infection prior to arrival in the country were not included in this analysis. This shift in the epidemic in Denmark has brought in a group of patients with potentially very different social and cultural backgrounds, factors that may have an impact on the outcome of treatment for HIV infection.

7.2. DEFINING RACE AND ETHNICITY

The definition and use of the terms "race" and "ethnicity" in medical research has been subject for debate for several years (236-239). In general, "race" applies to the biological inheritance of an individual, whereas "ethnicity" is a broader construct that takes into consideration cultural tradition, common history, religion, and often a shared genetic inheritance (240). In practice the terms are often used interchangeably or collapsed into a single dimension as race/ethnicity. This may be problematic in multiracial and multicultural societies with a long-standing tradition of immigration. However, in terms of racial and ethnic composition, Denmark is a relatively homogenous country with immigration from countries outside Europe occurring only during the last few decades. In this situation the two terms apply almost to the same population, making it reasonable to use the aggregated term "race/ethnicity" (paper VIII).

7.3. RACE/ETHNICITY AS PREDICTOR OF REVERSE DISEASE OUTCOME

In epidemiological and clinical research, racial/ethnic categorisation is useful for exploring hypotheses about environmental and/or genetic risk factors (238). In medical research racial/ethnic variations have been reported on several levels: clinical presentation, access to treatment and disease outcome. In the evaluation of overall and cause-specific mortality, a large register-based study conducted in the US found the number of potential life-years lost for all causes of death being 35% greater for blacks than for whites, with a few conditions accounting for most of these disparities: hypertension, HIV infection, diabetes mellitus, and trauma (241). Of notice, data on mortality in the study extended only to 1997, i.e. before the dramatic improvements in mortality rates among HIV-infected patients. Racial/ethnic disparities with a poorer clinical outcome among black people have been reported for a number of diseases, e.g. breast cancer (242, 243), colorectal cancer (244, 245), late-stage diabetic complications (246, 247), stroke (248), congestive heart failure (249), and chronic hepatitis C virus infection (250). Most of these studies reporting racial/ethnic differences in outcome of disease are performed in the US, where socioeconomic status and ac-

Table 5. Race/ethnicity, distribution of baseline characteristics (demographic and markers of disease progression)

Authors(ref)	Country/region	n	Definition of baseline	Categorisation (% of study population)	Markers of disease progression (baseline)		
					CD4 cell count ($\times 10^6/l$)	VL (copies/ml)	AIDS
Jensen-Fangel et al (Paper VIII)	Denmark	524	Start of HAART	Whites (74%)	203	4.76	23.1%
				Non-Whites (26%)	168	4.81	17%
Blaxhult et al (257)	Europe	7230	Recruitment to cohort	European origin (87%)	202	–	30.4%
				African origin (6%)	214	–	25.4%
				Asian origin (1%)	252	–	27.1%
Del Amo et al (254)	United Kingdom	2048	HIV-diagnosis	Non-African (48%)	371	–	15% *
				African (52%)	238	–	9%
Smith et al (258)	United Kingdom	537	Recruitment	Caucasians (75%)	390	4.7	7.5%
				Blacks (25%)	286	4.5	15.4%
Saul et al (255)	United Kingdom	450	HIV-diagnosis	White (50%)	340	–	No diff
				Black African (38%)	200	–	No diff
Barry et al (234)	United Kingdom	390	HIV-diagnosis	White (1997: 72%, 2000: 48%)	475 (1997)	No consistency	12% (1997)
					286 (2000)		26% (2000)
				Black African (1997: 24%, 2000: 45%)	240 (1997)		41% (1997)
					230 (2000)		27% (2000)
Saul et al (256)	United Kingdom	322	HIV-diagnosis and first vl	White (54%)	360	Lower in Blacks	Overall 19.6%
				Black African (35%)	220		

*) CDC-B symptoms.

cess to medical care is correlated with race and ethnic background (252, 253). Whereas these factors in part account for the racial/ethnic disparities in outcome of disease, the full explanation is complex (253), and will vary from one society to another.

7.4. RACIAL/ETHNIC DISPARITIES IN BASELINE CHARACTERISTICS WHEN STARTING HAART

When evaluating the outcome of HAART across racial/ethnic categories, baseline characteristics are often found to differ considerably between the groups. In patients starting HAART in our region, Caucasians and non-Caucasians differed in demographic characteristics, and to a lesser degree in markers of disease progression. The group of non-Caucasians was younger, with a higher proportion being women, and a higher proportion reporting heterosexual contact as the primary mode of infection (paper VIII). Similar demographic variations have been reported in other European studies on baseline characteristics at HIV diagnosis (234, 254-256), or at recruitment to the study cohort (Table 5) (257, 258).

We found only minor differences in markers of disease progression at the time of starting HAART (paper VIII). Non-Caucasians were found to have lower CD4 cell counts and slightly higher viral loads than Caucasians, while fewer had a previous AIDS event when starting therapy. These findings are, however, difficult to compare and to interpret in public health terms, as they are biased due to a cohort effect: the group of Caucasians had a longer follow-up, and a longer duration of diagnosed HIV-infection. The resulting larger exposure to antiretroviral drugs before starting HAART will affect the baseline surrogate markers.

Variations in disease markers by race have been reported in other studies on baseline characteristics at HIV diagnosis or study recruitment. Greatest consistency has been found in the distribution of the baseline CD4 cell counts, that are generally reported to be lower in Blacks/Black Africans than in Whites/Caucasians at HIV diagnosis (234, 254-256), or at recruitment (258). One study found the median CD4 cell counts to be slightly higher among patients born in Africa at study recruitment (257). However, this was not surprising as more Africans had started HAART prior to study recruitment compared to Europeans, and actually the median CD4 cell count at the time of starting a PI (indinavir) was found to be lower among Africans. Hence, from these European studies it appears that Black Africans present at a more advanced clinical stage. More conflicting results have been reported with regard to baseline HIV-RNA and the

proportions having an AIDS defining event at baseline (234, 255, 256, 258, 259).

Altogether, the observed disparities by race/ethnicity in demographic characteristics and in disease markers at HIV diagnosis and at the time of starting HAART are important in the evaluation treatment outcome, and might furthermore have important public health implications.

7.5. RACE/ETHNICITY AND THE OUTCOME OF HAART

HIV/AIDS surveillance data from the US show racial disparities in AIDS incidence, with Blacks accounting for 48.7% of new AIDS cases in 2001 (260), though comprising only 13% of the population (261). Whereas a disproportionally high HIV prevalence and differences in utilisation of health care services and access to antiretroviral treatment and prophylaxis against opportunistic infections seem to be major contributions (262-267), differences in the outcome of HAART once treatment is initiated is a matter of debate. Several US-based studies on adherence to antiretroviral therapy find African American race to be a predictor of non-adherence (268-271). One study by Paterson et al did not find an association between race and degree of adherence (272); However, the conclusion was based on significance testing, ignoring a high point estimate (OR 8.4) after confounder adjustment for the relation between white race and high adherence. Despite these reports on lower adherence, the findings in several observational cohort studies do not generally show differences in treatment outcome, neither clinical (273-275), nor immunological or virological (276, 277). However, a large register-based study on nearly 25,000 HIV positive subjects (20% on HAART) found a higher relative hazard for death in Blacks compared to Whites, even when adjusting for differences in antiretroviral treatment (278).

In Europe, only few studies have focused on racial/ethnic issues in the access to, and outcome of HAART. We found no major differences between Caucasians and non-Caucasians in the region of West Denmark, neither in the proportion starting HAART once diagnosed with HIV infection and fulfilling the criteria for starting therapy, nor in the analyses of immunological, virological or clinical response to HAART (paper VIII). In a large prospective European cohort study of more than 7000 patients, no differences were observed in survival after a median follow-up of 12 months between patients born in Europe, and patients born in Africa or Asia (257); The study cohort consisted of both antiretroviral treated, and untreated patients.

7.6. UNDERLYING DIFFERENCES

Given the fact that the majority of HIV-infected non-Caucasians in our region are first-generation immigrants to Denmark with different social and cultural backgrounds, the lack of major racial/ethnic disparities in treatment initiation and outcome is an important and reassuring finding. However, non-Caucasians appear to start therapy at a later stage of disease, as indicated by the lower CD4 cell counts. Whether this is due to patient or health care related factors remains to be clarified. In a study by Erwin and Peters on treatment issues among HIV positive Africans in UK, a number of particular treatment concerns were characteristic for this group (279). Among these were fears of discrimination, lack of confidence in the drugs and the health care system, and fear of experimentation. These concerns could in turn affect important treatment issues such as the motivation for starting treatment, adherence to therapy, and participation in clinical drug trials. A US-based cross-sectional survey found disparities in participation by race in AIDS clinical trials (280). No similar studies have been performed in European settings.

Apart from these potential social and cultural differences, several genetic and virological factors have been shown to vary according to race and origin, including viral groups and subtypes (281), and host genetic factors such as the CCR5 delta 32-bp deletion (282, 283) and MDR1 gene polymorphisms (284, 285). Whether these factors affect the outcome of HAART still is a matter of controversy (286-293); However, though these factors are likely to vary between the two groups in our region, they do not appear to affect the treatment outcome (paper VIII).

7.7. CONCLUSION

An increasing proportion of individuals living with HIV infection in Denmark are non-Caucasians, the majority being first-generation immigrants from Sub-Saharan Africa with potentially very different social and cultural backgrounds. Apart from the different racial/ethnic background, the group also differs in a number of demographic characteristics such as gender distribution, age at the time of starting HAART, and mode of transmission. A number of US-based studies report on racial/ethnic disparities in health outcome, including the outcome of HIV infection. Studies in European settings, including a population-based cohort in West Denmark, found no major disparities between the racial/ethnic background and the access to, and outcome of HAART.

8. MORTALITY IN HIV-INFECTED PATIENTS STARTING HAART (PAPER IX)

8.1. BACKGROUND

In the early 1990's, HIV infection had become one of the leading causes of premature morbidity and mortality, especially among younger males, in both the US (294) and in Europe (295, 296). In a Danish register-based study AIDS was found to be the 5th leading cause of death among men 25-49 years of age in 1993, and the major cause of death in the municipality of the City of Copenhagen in the period 1990-93 (297).

8.2. COMPARING MORTALITY ACROSS CALENDAR PERIODS

The introduction of HAART was strongly associated with an improvement of the clinical outcome of HIV infection, with a decline in overall death rates (4-6, 298, 299), an increase in survival time after an AIDS diagnosis (300-303), and a decline in incidence rates of first AIDS defining events (304-307). An overview of the major studies on mortality among HIV-infected patients in the pre- and post-HAART period appears from Table 6. In one of the earliest and most heavily cited reports on this issue, Palella et al found a dramatic reduction in the mortality rate in severely immunosuppressed HIV-infected patients from 29.4 per 100 person-years in 1995 to 8.8 per 100 person-years in the second quarter of 1997 (5). This clinical improvement co-incided with an increased use of antiretroviral therapy, and especially the use of PIs. Similar results were found in a

large European study, in which the overall mortality rate declined from 23.3 to 4.1 per 100 person-years in the period 1995 to 1998 (6). In a later study the same group found that the initial drop in mortality after the introduction of HAART has been sustained, with a further decline in incidence rates of all deaths in the late HAART-period (1998 onwards), compared to the early HAART period (1996-97) (308).

Most studies on the clinical effectiveness of HAART in terms of mortality have focused on the incidence rates of death across calendar periods, with the cohorts being exposed to different levels of antiretroviral therapy. The use of these historical cohorts is obvious, as the access to HAART occurred concomitantly in the countries in the developed world, and as the use of combination antiretroviral therapy became widespread during a very short period. A major drawback in measuring effectiveness of HIV treatment in a population across calendar periods, is that patients are observed at later stages of disease with increasing follow-up.

One way to adjust for this survival bias is by knowing the duration of infection, i.e. the time of seroconversion, as performed by the CASCADE collaboration (306, 309). However, in most cohort studies on HIV-infected patients the date of seroconversion remains unknown for the vast majority of the enrolled individuals. In our cohort on HIV-infected patients in West Denmark, only 60 (6%) reported a last negative antibody test within one year, and 117 (12%) within three years of the first positive antibody test.

Another way is to adjust by markers of disease progression, in particular CD4 cell count. Tarwater et al, using data from the Multi-center AIDS Cohort Study (MACS), demonstrated that almost identical estimates for progression to AIDS were achieved by adjusting for CD4 cell count level in patients whose date of seroconversion was not known, compared to adjustment by date of seroconversion (310). A similar conclusion was found in a study by the CASCADE Collaboration (311).

8.3. COMPARING MORTALITY TO THE GENERAL POPULATION

Only few studies have focused on the mortality in HIV-infected patients starting HAART compared to the mortality in the general population. A french study on 1157 patients starting a PI-containing regimen, found that overall mortality was 7.8 times higher than in the general population (312). The comparison was performed by indirect standardisation using the french national mortality rates, stratified by age and gender. Even in complete responders (defined as stable CD4 cell higher than $500 \times 10^6/l$ and a suppressed viral load below 500 copies/ml from 4 months after starting therapy to end of follow-up) overall mortality remained 5.1 times higher.

In a study on 647 patients starting HAART with a median follow-up time of 3.5 years, we compared the mortality rates with the rates in a sample of matched (gender and age) population controls (paper IX). We found an overall mortality rate of 26.9 per 1000 person-years among HIV-infected patients, compared with 3.8 per 1000 person-years among population controls. However, as the overall mortality rate obviously is a compound estimate that is highly dependent of the composition of the cohort, we estimated the excess mortality as the mortality rate ratio according to different strata of specific prognostic variables. Not surprisingly, the mortality rate ratios (MRR), with population controls as the reference, differed substantially with the CD4 cell count at the time of starting HAART, declining from 15.3 in the lowest CD4 stratum ($<50 \times 10^6/l$), to 3.6 in the highest ($\geq 200 \times 10^6/l$).

8.4. HIV INFECTION – A CHRONIC MEDICAL DISEASE?

From these studies on the effectiveness of HAART it appears that HIV-infected patients still have an excess mortality when compared to the general population. However, as demonstrated in a number of prognostic studies, the CD4 cell count at the time of starting HAART is an important prognostic factor for death (53, 313, 314),

Table 6. Studies on mortality in HIV patients pre-, and post-HAART.

Author(Ref)	Country/region	Patients (deaths)	Period	Aim of study	Measures	Estimates
Egger et al (53)	Europe/ North America	12,574 (344)	HAART-era (years?)	Prognostic modeling	Hazard ratio probability (at 3 years)	CD4 count the dominant prognostic factor
Mocroft et al (6)	Europe	4,270 (1,215)	1994-1998	Death rates pre- and post-HAART	Mortality rates	MR 23.3/100 pyrs (1995) MR 4.1/100 pyrs (1998)
Palella et al (5)	Unites States	1,255	1994-1997	Death rates pre- and post-HAART	Mortality rates	MR 29.4/100 pyrs (1995) MR 8.8/100 pyrs (1997)
Lee et al (300)	Unites States	394,705	1984-1998	Survival time <i>after AIDS</i> pre- and post-HAART	Probability of surviving 24 months	49% (OI diagnosed 1993) 80% (OI diagnosed 1997)
Hogg et al (4)	Canada	– (604)	1994-1996	Death rates pre- and post-HAART	Death rates (per 1000 individuals)	18.4 (1994) to 5.7 (1996) per 1000 pyrs
Pezzotti et al (301)	Italy	2,118 (1,683)	1985-1997	Survival probability <i>after AIDS</i> pre- and post-HAART	Survival probability (KM, Cox)	RH 0.73 first half 1997 compared to 1884
Fordyce et al (302)	Unites States	70,878	1990-1998	Survival time <i>after AIDS</i> pre- and post-HAART	Probability of surviving 24 months	1990-1995: 43% 1996-1998: 76%
Egger et al (7)	Switzerland	5,176 (1,903)	1988-1996	Survival probability pre- and post-HAART	Progression to death (KM, Cox)	1988-1990: 1 1993-1994: 0.74 1991-1992: 0.81 1995-1996: 0.38
Detels et al (307)	Unites States	536 (200)	1990-1997	Relative hazard of death pre- and post-HAART	Relative hazard of death	1990-1992: 1 1993-1995: 0.87 1995-1997: 0.62
Li et al (303)	Australia	4,814 (3,193)	1991-1996	Survival <i>after AIDS</i> pre- and post-HAART	Median survival Hazard ratio	1991: 16.0 months 1996: 27.7 months
Louie et al (332)	Unites States	– (5,234)	1994-1998	Mortality rates pre- and post-HAART Causes of death	Mortality rates SMR	1994: MR 17.1%/year 1998: MR 5.1%/year
Mocroft et al (333)	Europe	8,556 (1,826)	1994-2001	Causes of death Mortality rates pre- and post-HAART	Mortality rates Proportion of causes over calendar time	1994: MR 15.6 per 100 PYFU 2001: MR 2.7 per 100 PYFU
Various (299)	Europe	5,893 (1,613)	<1989-1999	Risk of death within calendar periods	Relative Risk	<1989: 1.22 1995-1996: 1.00 1989-1994: 1.00 1997-1999: 0.35

thus also influencing the degree of excess mortality. It is important to note that estimates derived from these studies will under-estimate the efficacy of the treatment. As we did not use time-updated CD4 cell counts, patients were stratified according to the CD4 cell count at the time of initiation of treatment, ignoring subsequent changes in immunologic status (using the ITT approach from clinical trials). However, patients who discontinue therapy or who experience treatment failure will have a decline in CD4 cell count, and thus a higher risk of clinical progression. Accordingly we found (paper IX) that 4 of 11 patients who started HAART with a high CD4 cell count ($\geq 200 \times 10^6/l$) and who died, had a decline in CD4 cell count to less than $200 \times 10^6/l$ at the latest follow-up.

With access to HAART and initiation of treatment before severe immunosuppression, the prognosis of HIV infection has improved considerably, and is now approaching the diagnosis of another chronic medical disorder, diabetes. Cohort studies on mortality in insulin-treated diabetic patients find an excess mortality compared with the general population, but with large variations (overall standardized mortality ratio, (SMR) ranging from 1.9 to 7.4), probably due to differences in design, location, study populations, gender and age (315-319). In a large British cohort study, Laing et al focused on the mortality in 23,000 insulin-treated young diabetic patients (diagnosed under the age of 30 years). An excess mortality was found in all age groups with an overall SMR of 4.0 for females, and 2.7 for males, reaching a peak of 5.7 in females aged 20-29, and of 4.0 in males aged 40-49 (315).

With the MRR also being an estimate of excess mortality compared to a general population, an overall MRR of 3.6 in patients starting HAART with a high baseline CD4 cell count seems comparable to the estimates of excess mortality in these studies on diabetes in younger adults. However, it is important to bear in mind that SMRs derived in different studies are not directly comparable to

each other, unless they use the same standard, i.e. the same reference population.

8.5. LIMITATIONS

The main limitation in studies using data from population-based registers is the inability to adjust for a number of potential confounders. When comparing mortality in HIV-infected patients receiving HAART to the general population, the two study groups will differ in other factors affecting mortality than the HIV infection itself. One of these co-factors which is associated with a higher risk of premature death, and which is unevenly distributed between the two groups, is intravenous drug use (IDU). A higher prevalence of IDU in the cohort of HIV-infected patients will lead to an over-estimation of the excess mortality. Accordingly we found that the MRR decreased from 3.6 to 3.0 in the patients with a high baseline CD4 cell count (paper IX) when using restriction, i.e. excluding patients infected through IDU. Other factors that might potentially introduce bias include differences in socio-economic status (320-322), prevalence of smoking (323, 324), alcohol consumption (325), and co-infection with hepatitis C (326, 327). Finally, long-term toxicities associated with the use of antiretroviral treatment (hepato-toxicity (328, 329), the metabolic syndrome (330, 331), and probably an increased risk of cardio-vascular disease) might contribute to the excess mortality.

8.6. CAUSES OF DEATH IN THE HAART-ERA

With the decline in mortality rates overall after the introduction of HAART, the proportion of patients dying due to causes not directly related to HIV is increasing. In a register-based study, Louie et al reported on 5234 deaths in the period 1994-1998 among patients with AIDS. Whilst HIV/AIDS-related annual mortality rates declined from 15.1% in 1994 to 3.1% in 1998, annual non-HIV/AIDS related

rates remained stable at 2% and 2.1%, respectively (332). The EuroSIDA study group found that only 16.7% of deaths in 2000-2001 in the cohort were HIV-related, as opposed to 51.6% deaths due to other causes (333). In 1994 the corresponding figures were 54% and 22.6%. In West Denmark, we found that the causes of death in patients starting HAART were HIV-related in 62.3% of the cases, due to other causes in 26.4%, and unknown in 11.3% in the study period 1995-2001 (paper IX). However, the number of deaths was too small to reveal any trend in causes of death with calendar time.

8.7. CONCLUSION

Overall mortality rates have decreased considerably after the introduction of HAART. Although HIV-infected patients starting HAART still have an excess mortality when compared to the general population, this is highly dependent on the CD4 cell count at the time of starting treatment. Hence, patients starting HAART before severe immunosuppression (CD4 cell count $\geq 200 \times 10^6/l$) only have a moderate excess mortality, that can be compared to the mortality of other chronic medical conditions.

9. PERSPECTIVES

With a fairly constant number of newly diagnosed HIV-infected patients and only few deaths among HIV-infected individuals following HAART, the total number of individuals living with HIV infection in Denmark is expected to rise steadily in the years to come. With new treatment options being introduced, the emergence of unforeseen long-term toxicities related to treatment, and the considerable changes in the HIV epidemic in Denmark, a coordinated collection of data on HIV-infected individuals in Denmark would result in a valuable data source. Only part of these issues can ever be clarified by use of controlled trials, strengthening the need of data from observational cohort studies. The HIV Cohort Study in West Denmark was initiated in 1999, and is prospectively collecting data on both demographic, prognostic, and treatment-related factors on the HIV-infected individuals attached to the clinics in the region. Data are anonymous and only used for scientific purposes. This has not at any point been a restriction to the studies performed using data from the cohort.

A large part of this thesis included studies using data from The HIV Cohort Study in West Denmark. The primary limitation of these studies has been the relatively small size of the cohort. However, as the study will continue in the years to come, a larger study population, and a larger observation period will strengthen the results. Furthermore the cohort is currently expanding with the inclusion of HIV-infected patients from the two larger clinics in East Denmark treating HIV infection, Hvidovre Hospital and Rigshospitalet. As this initiative will increase the size of the cohort with at least a factor three, sufficient data will be generated for a number of further studies.

10. SUMMARY

The prognosis of HIV infection has improved dramatically after the introduction of HAART in 1996. Despite potent treatment options, it appears that in unselected study populations only 40-75% achieve satisfactory treatment outcomes in terms of viral suppression to undetectable levels one year after starting therapy. The explanation for this is multifactorial, including factors such as non-adherence to treatment, drug toxicity with subsequent treatment discontinuation, selection of resistant viral strains, suboptimal antiretroviral regimens, socioeconomic factors etc. The relative influence of these factors is likely to vary from one region to another, among others depending on the demographic characteristics of the HIV-infected population, the organisation of the health care system, and the tradition of antiretroviral treatment.

The earliest protease inhibitor to be approved was saquinavir in a hard gel capsule formulation (SQVhgc). In clinical practice the drug appeared to be related to inferior treatment responses compared to

the use of indinavir and ritonavir, two protease inhibitors introduced shortly after SQVhgc. SQVhgc was widely used in the region of West Denmark in 1997 and 1998. In a retrospective multicenter study we confirmed the insufficient effectiveness of SQVhgc in antiretroviral treatment naïve HIV-infected patients 6 and 12 months after starting HAART. With longer follow-up, we found that patients starting with a SQVhgc-based HAART regimen had virological outcomes that were equal to patients starting with indinavir or ritonavir (24 and 30 months of follow-up). This occurred concomitantly with a shift from a SQVhgc-based regimen to an alternative HAART regimen. We found a similar trend in patients experienced to NRTIs prior to starting HAART in a population-based cohort study in West Denmark. Despite a long follow-up (median 4.5 years) we observed no differences in clinical outcome (new AIDS event or death) between patients starting with SQVhgc, and patients starting with either indinavir or ritonavir.

Modification of the initial HAART regimen occurs at a high rate. We found that 45% of the patients starting HAART in the region of West Denmark had the initial regimen modified (defined as stopping at least one of the antiretroviral drugs) during the first year of follow-up. The main reasons for treatment modification were drug-related toxicities and treatment failure, varying according to the antiretroviral compounds used.

When shifting from a single protease-inhibitor based regimen in the early HAART period, only limited treatment options existed. The outcome of sequential protease inhibitor based regimens was severely compromised by the evolution of cross-resistant viral strains. With the introduction of the non-nucleoside reverse transcriptase inhibitors, a new option for second-line treatment emerged. In a randomised controlled single-center trial we found that adding nevirapine when shifting from a SQVhgc, indinavir, or ritonavir-based to a nelfinavir-based HAART regimen was associated with a superior virological response at 24 and 36 weeks of follow-up.

The dose-limiting side-effect of nelfinavir is diarrhea. In the above mentioned study, 73% of the study population had diarrhea during the first 24 weeks of follow-up. Among other agents, calcium-carbonate has been reported to improve nelfinavir-associated diarrhea. In a small controlled cross-over trial we found no overall improvement when administering calcium-carbonate, using a daily diarrheal scale to measure the outcome. Neither did we find that calcium-carbonate altered the plasma levels of nelfinavir and its active metabolite, M8.

The demographics of the HIV-infected population in Denmark has changed during the past decade with an increasing proportion of newly diagnosed HIV-infected patients being women, being infected through heterosexual contact, and being immigrants from areas with a high prevalence of HIV infection. In the region of West Denmark, first-generation immigrants from countries in the sub-Saharan Africa constitute 19% of the HIV-infected population. The potentially very different social and cultural background does not have a major impact on the outcome of HAART in the non-Caucasian group; Population-based data from the region revealed no major racial/ethnic disparities in either virological, immunological and clinical outcome, or in the proportion of patients starting HAART once fulfilling the criteria for initiation of therapy.

The effect of HAART on the prognosis of HIV infection is well established. Most studies on mortality in HIV-infected patients with access to HAART are concentrated on the decline in mortality rates during the recent years, or on the evaluation of prognostic markers of disease progression. The mortality in HIV-infected patients compared to the mortality in the general population remains to be clarified. In the region of West Denmark, we compared mortality rates in HIV-infected patients starting HAART with that in population controls matched by age and gender, using data from Danish Civil Registration System. When starting HAART with CD4 cell counts of more than $200 \times 10^6/l$, HIV-infected patients only had a moderate ex-

cess mortality with a mortality rate ratio compared with the general population of 3.6. In comparison, severely immunosuppressed patients with a baseline CD4 cell count of less than $50 \times 10^6/l$ had a mortality rate ratio of 15.3. Hence, mortality rate ratios were strongly dependent on the CD4 cell count at the time of start of HAART.

11. DANSK RESUMÉ

Afhandlingen omhandler aspekter vedrørende de demografiske forhold omkring HIV-epidemien samt vedrørende udfaldet af antiretroviral kombinations-behandling i Vest-Danmark, og er baseret på ni internationalt publicerede originalarbejder. I oversigten placeres resultaterne fra vore studier i sammenhæng med de væsentligste fund på området.

Prognosen for HIV-infektion er forbedret betydeligt efter indførelsen af antiretroviral kombinationsbehandling i 1996. I Danmark har dette betydet et eklatant fald i såvel incidensen af nye AIDS definerende tilstande, som i dødeligheden blandt HIV-inficerede patienter. Ikke desto mindre viser kohortestudier at kun ca. 40-75% af patienterne opnår et tilfredsstillende behandlingsresultat efter ca. 1 års behandling, vurderet ved fraktionen af patienterne med upåviselig HIV i plasma. Årsagerne her til er mange og varierer fra kohorte til kohorte. Suboptimale eller ubekvemme behandlingssregimer, dårlig kompliance, selektion af resistent virus, bivirkninger med behandlingsophør til følge, graden af immundefekt ved behandlingsstart, socioøkonomiske forhold m.fl. indvirker alle i vekslende grad på behandlingsudfaldet.

Den første godkendte hæmmer af HIV's protease, saquinavir hard gel capsule (SQVhgc), viste sig ved kliniske brug at være forbundet med suboptimalt behandlingsrespons. Trods godkendelse af nye og mere potente protease hæmmere kort tid efter, var SQVhgc den mest anvendte protease-hæmmer i Vest-Danmark i 1997, og den næst mest anvendte i 1998. I et retrospektivt multicenter-studie fandt vi at brugen af SQVhgc hos patienter, som ikke tidligere havde modtaget nogen form for antiretroviral behandling, var forbundet med væsentligt ringere behandlingsudfald 6 og 12 måneder efter behandlingsstart, sammenlignet med brugen af indinavir eller ritonavir som protease-hæmmer komponent. I et opfølgende studie på samme patientmateriale fandt vi, at denne forskel var ophævet efter 24 og 30 måneder i takt med at alle SQVhgc behandlede patienter var skiftet til en ny antiretroviral kombinationsbehandling. Også hos patienter, der før start af antiretroviral kombinationsbehandling havde modtaget behandling med én eller to nukleosid-analoger, fandt vi i et prospektivt kohorte-studie denne tendens. Trods lang opfølgningstid (median ca. 4 år) fandt vi ingen større forskel i klinisk progression (ny AIDS definerende diagnose eller død) sammenlignet med brugen af indinavir eller ritonavir som protease-hæmmer.

En stor del af de HIV-patienter der starter antiretroviral kombinationsbehandling, oplever efterfølgende skift i behandlingen. Vi fandt, at ca 45% af patienterne fik behandlingen modificeret (defineret som ophør af mindst ét antiretroviralt middel) i løbet af det første år efter behandlingsstart, væsentligst pga. bivirkninger eller behandlingssvigt.

Evalueringen af effekten af skift til nye behandlingsregimer vanskeliggøres af de mange muligheder for at kombinere komponenterne i det nye regime, samt af at patientgruppen hurtigt bliver meget heterogen, hvad angår tidligere anvendte behandlinger. I et randomiseret studie evaluerede vi effekten af at supplere med nevirapin, en non-nukleosid revers transkriptase hæmmer, ved skift i antiretroviral kombinationsbehandling fra et SQVhgc, indinavir, eller ritonavir-holdigt regimen, til nelfinavir som protease-hæmmer. Gruppen der fik supplerende nevirapin havde et væsentligt bedre primært behandlingsrespons (umåleligt plasma HIV-1 RNA) efter 24 og 36 ugers observationstid. I overensstemmelse med tidligere studier fandt vi, at vandige diarréer var den hyppigste bivirkning til behandling med nelfinavir. Blandt en række midler er kalcium-karbonat fundet

at have effekt på nelfinavir-induceret diarré. I et kontrolleret pilot-studie efterprøvede vi dette og fandt ingen tilsvarende effekt af kalcium-karbonat. Studierne på dette område er på mange måder forskellige; B.l.a. anvendelsen af væsentligt forskellige scoringsmetoder for graden af diarré, kan være en årsag til de afvigende fund. I sideløbende farmakokinetiske undersøgelser af nelfinavir fandt vi ikke tegn til væsentlige interaktioner med kalcium-karbonat.

I overensstemmelse med rapporter fra den nationale HIV/AIDS-overvågning fandt vi, at der op gennem 1990'erne er sket et skift i HIV-epidemien i Vest-Danmark; En stigende andel af de ny-diagnosticerede HIV-inficerede patienter er af ikke-dansk oprindelse, hvoraf mange kommer fra områder med en høj prævalens af HIV-infektion, f.eks. det sydlige og østlige Afrika. Specielt i amerikanske kohorte- og registerbaserede studier har man fundet racemæssige forskelle i brugen, og i enkelte studier også udfaldet af antiretroviral kombinationsbehandling mod HIV-infektion. Gruppen af non-kaukasere med HIV-infektion i Vest-Danmark adskiller sig ved langt overvejende at være første-generations indvandrere med potentielt væsentligt forskellige sociale og kulturelle baggrunde. Trods dette fandt vi i Den Vestdanske HIV Kohorte ingen racemæssige forskelle i hverken initieringen, eller udfaldet af antiretroviral kombinationsbehandling.

Effekten af antiretroviral kombinationsbehandling på dødeligheden hos HIV-patienter er veldokumenteret. De fleste studier på dette område belyser faldet i mortalitets-rater i de senere år, ændringen i dødsårsager (HIV-relaterede eller ikke HIV-relaterede), eller prognostiske markører for progression til død. Dødeligheden blandt HIV-patienter i forhold til dødeligheden i baggrundsbefolkningen eller blandt patienter med andre kroniske sygdomme er kun sparsomt belyst. Ved at anvende data fra den populationsbaserede Vestdanske HIV-Kohorte sammenlignede vi mortalitetsraterne for HIV-patienter, der startede behandling med antiretroviral kombinationsbehandling, med mortalitetsraterne fra et køns- og aldersmatchet udtræk fra CPR-registret. Vi fandt, at patienter der startede behandling inden de udviklede svær immundefekt ($CD4$ celletal $200 \times 10^6/l$) kun havde en moderat overdødelighed i forhold til baggrundsbefolkningen, samt at denne synes at være sammenlignelig med overdødeligheden hos f.eks. yngre insulinbehandlede diabetes-patienter.

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