Virological, immunological and toxic effects of highly active antiretroviral therapy in adult HIV-1 infection

Ph.D. thesis

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Glossary of abbreviations

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
bid	Twice daily
c/ml	Copies per millilitre of plasma
$CD4^+$ count	CD4 receptor positive T lymphocyte cell fraction
CHIP	Copenhagen HIV Programme
C_{max}	Maximal plasma concentration of drug
DSMB	Independent Data Safety and Monitoring board
EMEA	European Agency for the Evaluation of Medicinal Products
ERC	End point Review Committee
FDA	The United States Food and Drug Administration
HAART	Highly active antiretroviral therapy
HIV-1	Human immunodeficiency virus type 1
IDV/r	Indinavir/ritonavir
IL-2 (rIL-2)	Interleukin-2 (recombinant interleukin-2)
IQR	Inter-quartile range
ITT	Intention to treat analysis
ITT/e	ITT analysis on all subjects that have received at least one dose of the assigned medication. Also termed ITT si (switch included)
ITT/e/s	ITT/e analysis where subjects that switch from the assigned treatment are counted as failures; ITT/e/s = f (switch equals failure)
iv	Intravenous
LLD	Lower level of detection
LPV/r	Lopinavir/ritonavir
Nadir $CD4^+$	Lowest ever $CD4^+$ cell count prior to initiation of treatment /baseline
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
OT	On treatment analysis including all subjects that remain on the assigned trial medication
Phase	Phase of development of drug [1]
PI	Protease inhibitor
pVL	Plasma viral load = HIV-1 RNA level in plasma
qd	Once daily
RCT	Randomised clinical trial
SAQ/r	Saquinavir/ritonavir
sc	Subcutaneous
tid	Three times daily

Original articles

This thesis is based upon three original manuscripts, which are referred to by Roman numerals. In addition, data from three trials are included, which are referred to by capital letters.

I. UB Dragsted, J Gerstoft, C Pedersen, B Peters, A Duran, N Obel, A Castagna, P Cahn, N Clumeck, JN Bruun, J Benetucci, A Hill, I Cassetti, P Vernazza, M Youle, Z Fox, and JD Lundgren for the MaxCmin1 trial group. A randomised trial to evaluate indinavir/ritonavir versus saquinavir/ritonavir in HIV-1 infected patients: The MaxCmin1 Trial. *Submitted to the Journal of Infectious Diseases*

II. UB Dragsted, A Mocroft, S Vella, J-P Viard, AE Hansen, G Panos , D Mercey, L Machala, A Horban, and JD Lundgren for the EuroSIDA study group. Predictors of immunological failure after initial response to HAART in HIV-1 infected adults: a EuroSIDA study. *Under review by the Steering Committee*

III. UB Dragsted, JD Lundgren. Aldesleukin - recombinant interleukin-2. Current Opinion in Anti-infective Investigational Drugs 2000; 2(3): 323-331

A. UB Dragsted, J Gerstoft, M Youle, A Duran, DT Jayaweera, A Rieger, JN Bruun, A Castagna, S Walmsley, Z Fox, A Hill, and JD Lundgren for the MaxCmin2 trial group. The interim analysis of a randomised, open-label, phase IV, multi-centre trial to evaluate efficacy and safety of lopinavir/ritonavir (400/100 mg bid) versus saquinavir/ritonavir (1000/100 mg bid) in adult HIV-1 infection: The MaxCmin2 trial. Oral late breaker presentation at the 6th International Congress on Drug Therapy in HIV Infection. Nov. 2002, Glasgow, UK

B. A phase IV randomised, open-label, multi-centre trial to evaluate the safety and efficacy of continued 3TC twice daily versus discontinuation of 3TC, as part of the new treatment of HIV-1 infection in patients who have shown virological failure on ongoing combination treatments containing 3TC: The COLATE Trial (COntinuation of Lamivudine Treatment in Europe)

C. A Randomized, Open-Label, Phase III, International Study of Subcutaneous Recombinant IL-2 (Proleukin®) in Patients With HIV-1 Infection and CD4$^+$ Cell Counts $\geq$ 300/mm^3: Evaluation of Subcutaneous Proleukin® in a Randomized International Trial (ESPRIT)

Preface

The scientific work underlying this Ph.D.-thesis was conducted from 2000 through 2002 during my employment as clinical research associate at Copenhagen HIV Programme (CHIP), Hvidovre University Hospital, Denmark.

Research is increasingly being performed in scientific networks including multiple sites in many countries [2]. Copenhagen HIV Programme (CHIP) is a research group that along these lines focus on performing clinical, relevant new knowledge on the treatment of HIV-1 infection. I am in deep gratitude to my supervisor, Director of CHIP Jens D. Lundgren, for asking me to join the group in March 1999 and for the way he has paved the scientific road I have been trotting since then. In CHIP, I have been a piece of the puzzle forming an international, scientific collaboration striving to produce, perform and present high-quality, resource- & cost-effective HIV-1 research. This effort has involved many people at CHIP, in Europe, Australia, South and North America. I would like to convey my thanks to all colleagues and collaborators for all the support offered.

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Ulrik Bak Dragsted
Hvidovre, January 2003

Introduction

HIV-1 epidemic

In 1981 the first report of five previously healthy men suffering from Pneumocystis carinii pneumonia (PCP) was published [3]. Two years later the causative agent - human immunodeficiency virus type 1 (HIV-1) had been identified [4, 5]. In the two decades following these important discoveries the scope of the epidemic has been unravelled. It is estimated that worldwide 42 million persons are HIV-1 infected, and HIV-1/AIDS is now the fourth biggest killer globally [6].

Antiretroviral treatment

Antiretroviral medication

Developed as an anti-cancer drug in 1964, zidovudine was the first drug shown to reduce the replicative capacity of HIV-1 *in vitro* and *in vivo* in 1985 - 1986 [7-9]. Seventeen generic antiretroviral drugs are now licensed in Europe and North America. These drugs are grouped in four classes (Table 1):

- Nucleoside & nucleotide reverse transcriptase inhibitors (NRTIs)
- Non-NRTIs (NNRTIs)
- Protease inhibitors (PIs)
- Fusion inhibitors

Some generic drugs are also licensed in combination preparations including two or three drugs from one drug class (Table 2).

Mono- and dual antiretroviral therapy (ART)

In 1987 zidovudine monotherapy was shown to reduce morbidity and mortality in HIV-1 infected patients [10]. A meta-analysis of four randomised clinical trials (RCTs), which had consistently found superior effect of zidovudine/lamivudine compared to zidovudine monotherapy on $CD4^+$ T lymphocyte count ($CD4^+$ count) and HIV-1 RNA in plasma (plasma viral load (pVL)), showed less progression of disease in patients receiving zidovudine/lamivudine [11]. A similar result was found in a RCT, the CAESAR trial, where the addition of lamivudine to zidovudine resulted in significantly less progression of disease including death compared to continued zidovudine monotherapy [12].

Table 1 - List of antiretroviral drugs by date of FDA approval

Brand name	Generic name	ART class	FDA approval date*
Retrovir	Zidovudine, AZT	NRTI	19-03-87
Videx	Didanosine, ddI	NRTI	09-10-91
Hivid	Zalcitabine, ddC	NRTI	**19-06-92**
Zerit	Stavudine, d4T	NRTI	**24-06-94**
Epivir	Lamivudine, 3TC	NRTI	**17-11-95**
Invirase	Saquinavir hard gel	PI	**06-12-95**
Crixivan	Indinavir	PI	**13-03-96**
Viramune	Nevirapine	NNRTI	**21-06-96**
Viracept	Nelfinavir	PI	**14-03-97**
Rescriptor	Delarvidine	NNRTI	**04-04-97**
Fortovase	Saquinavir soft gel	PI	07-11-97
Sustiva	Efavirenz	NNRTI	**17-09-98**
Ziagen	Abacavir	NRTI	**17-12-98**
Agenerase	Amprenavir	PI	**15-04-99**
Norvir	Ritonavir	PI	**29-06-99**
Kaletra[#]	Lopinavir, ABT-378/ritonavir	PI	**15-09-00**
Viread	Tenofovir disoproxil fumarate	NRTI	**26-10-01**
Fuzeon	Enfurvitide, T-20	Fusion inhibitor	**13-03-03**

*) In bold are drugs with accelerated approvals
[#]) Lopinavir is only approved in co-formulation with ritonavir

Table 2 - List of antiretroviral combination preparations by date of FDA approval

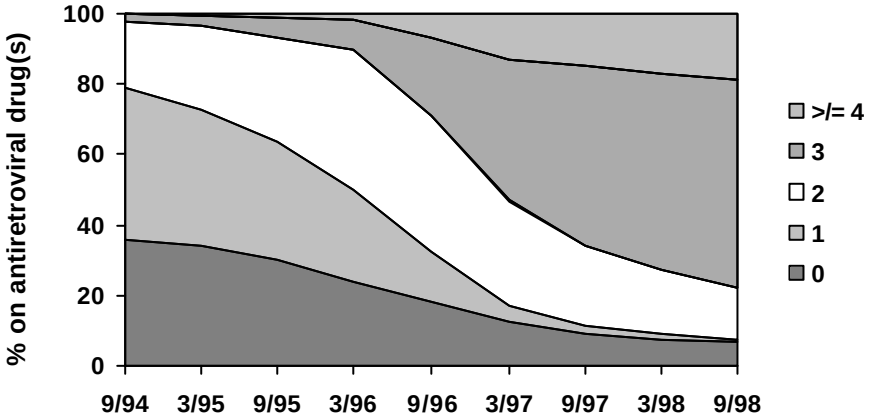
Brand name	Generic name	ART class	FDA approval date
Combivir	Zidovudine & lamivudine	NRTIs	26-09-97
Kaletra	Lopinavir & ritonavir	PIs	**15-09-00**
Trizivir	Abacavir, zidovudine & lamivudine	NRTIs	14-11-00

Highly active antiretroviral therapy

Since the early 1990's an increasing number of HIV-1 infected patients have received combination ART (Figure 1)[13]. From 1997 clinical trials, cohort studies and meta-analyses of RCTs have shown superior effect on morbidity and mortality of three-drug combination therapy - termed highly active antiretroviral therapy (HAART) - compared to mono- or dual therapy [14-22]. In these trials HAART has primarily consisted of two NRTIs and one 1 PI. In 1999 NNRTI (efavirenz) based HAART was shown to have superior virological efficacy when compared to PI (indinavir) based HAART [23]. In a RCT of PI-experienced but NNRTI-naive

patients no difference was found in favour of using four-drug HAART regimens when compared to three-drug HAART regimens after 16 weeks of treatment [24]. On the other hand, in a small non-randomised trial five-drug / three-drug class HAART regimens were shown to have superior efficacy (suppression of pVL at 48 weeks) compared to three-drug / two-drug class HAART regimens [25]. Also, in a placebo-controlled RCT of treatment-experienced patients with advanced immunodeficiency a five-drug HAART regimen was found to better suppress pVL at 24 weeks compared to a four-drug HAART regimen [26]. However, short-term virological efficacy is not a goal in itself and should be weighed against toxicity and future treatment options. Three large ongoing RCTs (ACTG 384, FIRST, INITIO) are addressing the question of which initial and subsequent combination of drugs and drug classes, including three and four drugs from two or three drug classes, are most efficient and safe in the long-term [27-29]. In the meantime four and more drugs HAART regimens are being used increasingly in clinical practise as a "hit HIV-1 hard" strategy, to overcome resistance in patients experiencing treatment failure ("salvage" regimens) and in pharmacologically enhanced regimens (see below) [13, 30, 31]. HAART including three or four antiretroviral drugs is the standard of care in countries with sufficient infrastructure, financial resources, and political will, including Argentina and Brazil [32-34]. Of note, in low- and middle-income countries less than 4 % of HIV-1 infected people with a need for antiretroviral treatment have access to this [35].

Figure 1 HIV-1 treatment across Europe - number of ART drugs used



Adapted from O Kirk et al. AIDS 1998

Ritonavir-boosting

Pharmacological enhancement in anti-HIV-1 treatment is mostly done using ritonavir-boosted PI regimens, i.e. ritonavir in doses of 50 - 200 mg in combination with one or two PI(s), plus (N)NRTIs. Ritonavir inhibits the *P450* CYP3A4 enzyme system in the intestine and liver, and possibly the P-glycoprotein efflux [36, 37]. This results in higher plasma concentrations of the other PI(s) whereas ritonavir in these doses is thought to have little or no antiretroviral effect [38]. Viral suppression has been shown to be dependent on high plasma concentrations of antiretroviral drugs [39-41]. Other benefits from ritonavir-boosting are a reduction in daily intake from three times daily (tid) to twice (bid) or once daily (qd), less food restrictions and often a lower pill count. These factors have been associated with a better treatment outcome [42, 43]. Results from comparative RCTs of the efficacy and safety of different ritonavir-boosted PI HAART regimens have not been published.

Immunostimulatory treatment

Different immune-based therapies have been used as adjunctive treatment to HAART in HIV-1 infected patients (reviewed in [44]). Of these, intermittent subcutaneous (sc) recombinant interleukin-2 (rIL-2) treatment has been shown, in phase I and II trials including more than 1000 patients, to significantly increase the $CD4^+$ count in a dose-dependent manner [45-49]. The CD4+ count increases have not been accompanied by changes in virological outcome during concomitant sc rIL-2 and combination ART, nor when sc rIL-2 has been administered without ART [49-53]. $CD4^+$ cells resulting from rIL-2 treatment have been shown to have similar functionality *in vitro* as other $CD4^+$ cells [54]. In a pooled analysis of three early rIL-2 trials of HIV-1 patients randomised to combination ART ± rIL-2, a higher number of patients experienced progression of disease and death in the ART only group compared to the ART and rIL-2 group [55]. The analysis did, however, not have sufficient statistical power to detect a difference in clinical outcome. Two phase III trials, the SILCAAT and ESPRIT studies, are currently investigating the effect of combination ART with or without intermittent sc rIL-2 on the risk of clinical disease progression and death [56, 57].

Treatment goal

Eradication

The ultimate goal of anti-HIV-1 therapy is to eliminate the risk of excess morbidity and mortality in HIV-1 infected patients preferably by eradication of the virus. Based on mathematical modulations of a three-phased decay rate of HIV-1 in plasma and other body compartments during HAART it has been estimated that eradication will take 7 to 60 years provided continuous viral suppression [58, 59]. This is due to “HIV-1 sanctuaries”, i.e. latently

infected resting cells harbouring HIV-1 in which HAART has little or no effect, which is also the reason why only short-lived virological control is seen following cessation of virologically suppressive HAART [60, 61]. To overcome the limited effect HAART has on the pool of latently infected cells Chun et al. suggested that intermittent rIL-2 treatment could "flush" HIV-1 out of the resting cell pool thereby making the virus susceptible to HAART [62]. However, the same group later demonstrated that this treatment combination does not lead to viral eradication either [63]. Therefore, current HAART regimens with or without sc rIL-2 are unlikely to eradicate HIV-1 in any significant number of patients - if at all.

Viral suppression & $CD4^+$ count increase

In current treatment guidelines the primary treatment goal is maximal and durable suppression of viral replication [32, 33]. In routine clinical care, this is assessed by the ability to lower the pVL to below the lower level of detection of the assay used (LLD, 500-50 copies(c)/ml). This is a rational approach, since viral suppression prevents the evolution of resistance thus is likely to result in a better long-term treatment outcome [39]. Other treatment goals are to restore and preserve immunological function as measured by the $CD4^+$ count, to improve quality of life and to reduce HIV-1 related morbidity and mortality [32]. These guidelines are in part based on data from the Multicenter AIDS Cohort Study (MACS) that showed baseline pVL to be the strongest marker of progression to AIDS and death [64, 65]. The MACS data were, however, limited per design because the analyses included baseline values of markers of disease progression - as opposed to time-updated values - thus not reflecting the dynamics of the treatment response. Others have shown that $CD4^+$ count is a stronger time-updated marker of disease progression than pVL [66]. Therefore, identification of predictors of a sustained immunological response could prove useful in patient management.

End points in clinical trials

The RCTs and cohort study included in this thesis use different end points (virological, immunological, clinical). In consequence, I have felt it important to include the below section about the use of end points in clinical trials in the HIV-1 field.

Regulatory approvals

Prior to the introduction of HAART, the end points used in HIV-1 RCTs were progression of disease and/or death. Following the introduction of HAART the low rate of disease progression and death has made use of "hard" end points, i.e. the comparison of treatment effect(s) on morbidity and mortality, less feasible [67]. In consequence, the United States

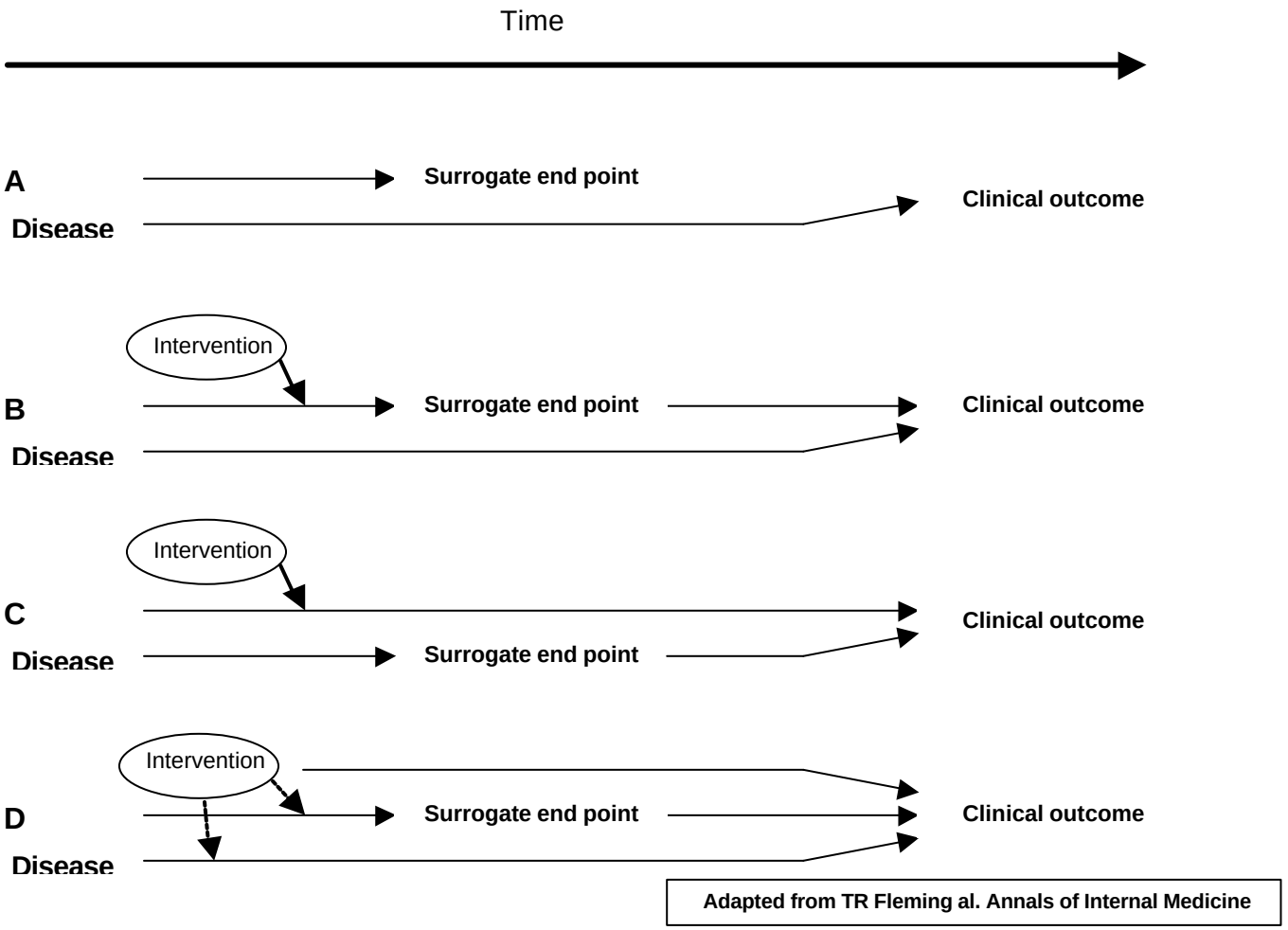
Food and Drug Administration (FDA) has released guidelines for the use of 16 - 24 weeks data with pVL end points for accelerated approval of antiretroviral drugs (latest update Oct. 2002 [68])[69]. Due to the demand for fast release of antiretroviral drugs, most licensed antiretroviral drugs have been approved based on different measures of pVL. In RCTs the primary efficacy outcome is now most commonly viral suppression or time to virological failure although no uniform way of measuring this exists, and is often done in discordance with current guidelines for the approval of drugs [68, 70].

Surrogate end points

Surrogate markers of disease progression and death are useful in patient management and early drug development [71]. In clinical practise and for drug applications, measurements of $CD4^+$ count and pVL are and have been used as surrogate markers of HIV-1 disease progression and death [33]. This is based on results from RCTs and cohort studies showing higher $CD4^+$ count, lower pVL or a combination hereof to be correlated with a better clinical outcome during (HA)ART [21, 64, 65, 72-81]. The use of these surrogate end points in HIV-1 RCTs is, however, a matter of debate [82-84]. The reason being that results from RCTs have questioned whether these surrogate markers meet the criteria defined by Prentice [85]: to qualify it is required that the surrogate marker should be a correlate of the clinical outcome and the surrogate marker should fully capture the net effect of an intervention on the clinical outcome (Figure 2).

Even though pVL and $CD4^+$ count have been shown not to fully capture the effect of HAART on HIV-1 disease progression and death, in practise they have been accepted as surrogate markers during HAART [84]. This makes it even more important to caution their use when new drugs and drug classes with different sites of action or toxicity profiles, e.g. fusion inhibitors, are reported as part of HAART. The reason for this being the uncertainty about the net effect of the new drug(s) on the clinical end point that may be different from the effects of current components of HAART regimens. Further, the effect of a treatment on a surrogate marker can often be assessed in multiple ways. Reporting of efficacy can e.g. be reported as a measure of time-to failure or pVL suppression, and for each measure different statistical approaches can be instituted: intention-to-treat (ITT), ITT/exposed, ITT/e/s, on treatment (OT) analysis. This makes it almost impossible for the clinician to compare treatment outcomes, and question whether the reliability of the surrogate end point is maintained [86]. Validation in RCTs of surrogate markers of *long-term* clinical outcome of HAART is missing [71, 87].

Figure 2 Reason for failure of surrogate end points



A. The surrogate marker is not the casual pathway of the disease process
B. Of different pathways, the intervention only affects the pathway mediated through the surrogate marker
C. The surrogate marker is not affected by the intervention
D. The intervention has mechanisms of action independent of the disease process
Dotted lines = mechanisms of action that might exist

Biological markers

Numerous biological markers of disease activity and progression have been correlated with a reduced risk of HIV-1 disease progression and death including neopterin, p24 antigen, β2-microglobulin, tumour necrosis factor & soluble APO-1/Fas, IgA , IgG, phase angel from bioelectrical impedance analysis, soluble urokinase-type I plasminogen activator receptor, CCR5-Δ32 & CCR2-64I alleles, and pVL decay kinetic [72, 88-94]. However, it is important to distinguish between biological markers correlated with disease progression and surrogate markers of disease progression [82]. To date no biological marker has been demonstrated to be a true surrogate marker of clinical HIV-1 disease progression and death including pVL and $CD4^+$ count.

Treatment modification

A decline in morbidity and mortality has been observed in all countries where combination ART including HAART has been introduced [17, 95, 96]. However, even HIV-1 infected patients with optimal response to treatment have excess mortality compared to non-HIV-1 infected subjects [97]. Furthermore, due to toxicity, difficulties in taking medication as prescribed (sub-optimal adherence), and development of (multi-drug) resistant HIV-1 strains, a high proportion of patients modifies or stops taking HAART. In a multi-centre cohort of ART-naïve patients starting their first HAART, 26 % discontinued treatment due to toxicity and 8 % due to failure within one year from starting [98]. Slightly higher percentages were found in a single-site cohort of ART-naïve and experienced patients [99].

Treatment failure

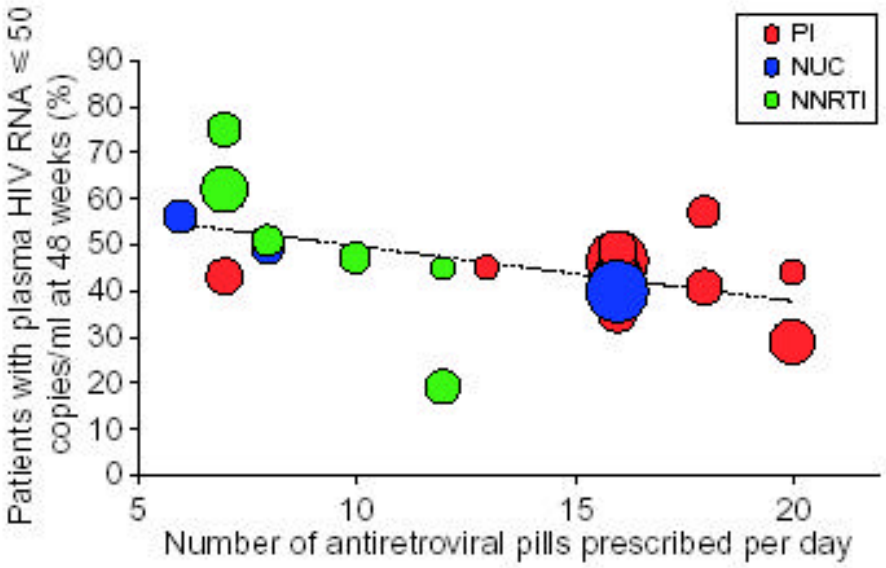
Virological failure, i.e. virological rebound after initial response or the inability to suppress pVL below a certain threshold most often the LLD, has been related to all drug classes. In cohort studies the annual rate of virological failure is 8 % - 25 % among patients starting HAART and achieving suppression of pVL below the LLD [21, 98, 100]. The main reasons for failure of first and subsequent HAART regimens are treatment limiting toxicity, sub-optimal adherence, virological failure, low potency of the drugs and previous drug exposure [98, 101-104]. In a meta-analysis of 23 comparative clinical trials of ART-naïve patients on HAART Bartlett el al. found pill count to be the strongest predictor of virological response (Figure 3) [43]. The authors suggest this to be caused by sub-optimal adherence of patients on more complex regimens. In a single site cohort, the factor most strongly associated with survival was adherence [105]. The challenge to the clinician is to combine the different drugs and drug classes of the initial and subsequent regimens so that the combined treatment failure rate from toxicity, sub-optimal adherence and virological failure is minimised. The theoretical possibilities of such combinations are multiple, and only a limited number of comparative RCTs are available for guidance.

Resistance

Development of resistance to antiretroviral drugs may limit the future ability to suppress viral replication [106]. Development of resistance to NRTIs, NNRTIs and PIs has been observed during mono-, dual, triple and quadruple (HA)ART [107-109]. Resistance development during long-term suppressive HAART, i.e. pVL below LLD (50 - 500 c/ml) for one to three years, has also been observed [110-112]. However, results from clinical trials indicate virological, immunological and clinical benefits of continued treatment despite the presence of resistance mutations to drugs included in the (HA)ART regimen [113-115]. Re-use or continuation of

drugs to which patients are harbouring resistant viral strains may be of benefit, and have not previously been reported from RCTs.

Figure 3 Virological response according to daily pill count in ART-naive patients



Symbol size is directly proportional to weight of the data point in the analysis (r, -0.57; p = 0.0085)

Reprint from JA Bartlett al. AIDS 2001

Interim analyses

Data from three of the trials included in this thesis are from interim analyses. In consequence, I have felt it important to include the below section about the use of interim results in HIV-1 RCTs.

Interim analyses of RCTs are used to limit the risk for patients in one trial group from receiving a significantly inferior or toxic treatment [116]. The extent of any interim analysis should preferably be outlined in the trial protocol, and the result of the analysis be assessed by an independent data monitoring committee also termed Data Safety and Monitoring Board (DSMB) [116]. A DSMB system was made operational in 1987 for HIV-1 RCTs sponsored by the US government (National Institute of Allergy and Infectious Diseases, NIAID) [117]. A good example in HIV-1 research of the usefulness of an interim analysis is the CAESAR trial. The trial was prematurely stopped following the second planned interim analysis that showed a significant survival benefit for patients receiving dual therapy compared to monotherapy [12]. This decision was based on a recommendation from the trial's DSMB.

Interim analyses are not intended for publication because release of premature findings may result in wrong conclusions, e.g. due to multiple statistical comparisons, and/or may harm the integrity of the trial e.g. due to subsequent treatment changes in one trial group [118]. In HIV-1 research it has, however, been customary to present interim results stratified by treatment allocation at the many national and international conferences held each year. No assessment of the clinical impact of these presentations has been performed. The field does, however, seem to be moving away from this practise (not supported by data) towards not presenting interim data or only presenting overall results rather than analyses stratified by treatment group.

Objective of Ph.D. thesis

Based on the current knowledge as presented in the Introduction, the aim of this Ph.D. thesis was to:

1. Assess the efficacy and safety of IDV/r compared to SAQ/r as part of a HAART regimen

2. Assess the efficacy and safety of LPV/r compared to SAQ/r as part of a HAART regimen

3. Assess the efficacy and safety of continued lamivudine treatment in patients failing a lamivudine containing HAART regimen

4. Assess predictors of a sustained immunological response to (HA)ART

5. Describe the patient population enrolled in the ESPRIT trial

6. Assess the $CD4^+$ cell response of patients assigned rIL-2 in the ESPRIT trial

The MaxCmin1 trial

Background & rationale

Both indinavir and saquinavir undergo extensive metabolism in the *P450* CYP3A4 pathway. The CYP3A4 isoenzymes can break down drugs both during first-pass metabolism (in the intestinal wall and liver), or during disposition metabolism from the systemic circulation as the blood passes the liver. However, the effect of ritonavir appears to differ between the two drugs. The main effect of ritonavir on saquinavir metabolism is to prevent first-pass metabolism (and thus increase the maximal plasma concentrations, C_{max}) whereas disposition metabolism is relatively unaffected (and hence plasma half-life). Conversely, ritonavir has minor or no effect on the C_{max} of indinavir, but increases the plasma half-life by inhibition of the disposition metabolism in the liver.

At the time the trial was designed no comparative data from RCTs existed on efficacy and safety of ritonavir-boosted PI regimens thus the MaxCmin1 trial (**I**) was the first head-to-head comparison of ritonavir-boosted regimens.

Design

With the objective of comparing the rate of virological failure between the two trial groups, the trial was designed as a prospective, randomised (1:1), international, multi-centre, open-label, and phase IV (post-marketing) trial with 48 weeks follow-up of all patients. The trial was designed to show equivalence in the rate of virological failure between ritonavir-boosted indinavir (IDV/r) 800/100 mg bid and saquinavir (SAQ/r) 1000/100 mg bid. Further, to assess viral suppression, change in $CD4^+$ count, disease progression, and safety. The trial was open for enrolment in Europe, Argentina and the US between September 2000 and March 2001.

The role in the MaxCmin1 trial of CHIP has been as coordinating office and sponsor according to the ICH-GCP guidelines. In the MaxCmin1 trial I have served as trial physician. This has included but not been limited to site initiation, participation in the protocol development, site initiation, site monitoring, statistical analyses, and presentation of data.

Results

In total 317 patients were randomised of which 306 patients initiated the assigned treatment. The trial population was heterogeneous with 25 % of the patients being ART naïve, 14 % being ART experienced but PI-naïve and 61 % being PI-experienced. Complete follow-up at

Week 48 was available from 93 %, at which time 66 % remained on the assigned treatment. Sixty-seven of the 104 patients, who prematurely switched from the assigned treatment, did so due to clinical non-fatal adverse events (AEs). There was a significantly higher percentage of patients in the IDV/r group (41 %) than in the SAQ/r group (27 %) who prematurely switched from the assigned treatment ($p = 0.013$, Chi-squared).

The primary efficacy measure, the rate of virological failure, was seen in 25 %, with no difference between the trial groups in the ITT/e analysis (log rank test: $p = 0.84$). The difference in the proportion of subjects failing in the two groups was 1.6 % (95 % CI: -8%, 11.4%) with a higher proportion of protocol defined virological failures in the IDV/r group. Using a Farrington-Manning equivalence test we found sufficient evidence at the 5 % level of significance to claim that the difference in success rates between the 2 treatments is less than 15 % ($p < 0.0048$).

The higher discontinuation rate in the IDV/r group resulted in significantly higher rates of virological failure and patients without suppression of pVL (< 50 or 400 c/ml) in this group compared to the SAQ/r group in the ITT/e/s analysis (log rank test: $p = 0.01$). No differences were observed in $CD4^+$ count response between the two treatment groups. The low number of events precluded formal statistical analysis of patients experiencing progression of disease and death.

Of the patients exposed to the trial medication 33 % experienced at least one grade 3/4 AE, 41 % in the IDV/r group versus 24 % in the SAQ/r group ($p = 0.001$, Chi-squared). Significantly larger elevations in fasting total and LDL cholesterol, and in total triglyceride were seen in the IDV/r group compared to the SAQ/r group at Week 4 and 48 (ITT/e, $p < 0.05$ for all comparisons).

Discussion

The MaxCmin1 trial was designed in the early part of year 2000 to assess if equivalence exist in efficacy and safety between IDV/r 800/100 mg bid and SAQ/r 1000/100 mg bid, the primary outcome being incidence of protocol-defined virological failure. Equivalence was observed for efficacy whereas IDV/r lead to an increased risk of treatment-limiting AEs and grade 3/4 AEs. As a consequence of the safety profile of IDV/r, fewer patients remained on this treatment through 48 weeks leading to differences in the efficacy analyses, where

continuation of trial medication influence the outcome. Additionally, IDV/r was found to cause a higher risk of elevating blood levels of lipids and bilirubin.

The trial demonstrated ritonavir-boosted indinavir and saquinavir HAART regimens to have efficacy and safety comparable with other HAART regimens at 48 weeks [119]. The proportion of patients who switched from the assigned treatment due to virological failure was lower but the proportion that switched due to AEs was similar to what has been reported from cohort studies of heterogeneous patient populations [99].

The trial has limitations by design, one being the inclusion of a heterogeneous patient population (reflecting the average out-patient clinic population). The trial would not have sufficient power to describe the outcome within each of the subgroups included, if the outcomes of the treatments were affected by the stage of HIV-1 infection or treatment experience at inclusion. The sample size should at least have been doubled to address this concern, but financial restrictions did not allow for such an approach. To address this limitation, several multivariate models of the key efficacy outcomes were developed to see whether the finding in univariate models was affected by adjusting for variables that may influence the benefit of antiretroviral drugs on HIV-1 replication. The hazard's ratios for the comparison of virological failure in the IDV/r versus SAQ/r group were comparable in univariate and multivariate models adjusting for other variables. To further investigate baseline characteristics that may have influenced the efficacy outcomes, two substudies are currently investigating genotypic resistance mutations at baseline and time of virological failure, and single nucleotide polymorphisms in the multi drug resistance 1 (MDR-1) locus.

Other limitations of the trial are:

- Open-label design
- The difference in pill burden (number, size, food restriction, storage) between the two trial groups
- No measurement of adherence
- The relatively short follow-up period
- The use of a surrogate end point that has not been validated as a marker of HIV-1 disease progression and death in ritonavir-boosted PI HAART

The trial was open-labelled and hence subjective to investigator and patient bias in drug preference and clinical management. It would not have been possible to effectively blind the

trial as indinavir leads to an increase in bilirubin level, as also observed in the present trial. The trial group have maintained equipoise between the two trial groups throughout the trial period and have on numerous occasions encouraged each other to maintain such an attitude in order to maintain the integrity of the trial. If the integrity was compromised, this would affect the scientific validity and interpretations of the trial and hence the entire effort. As an example of this more patients in the SAQ/r group compared to the IDV/R group did not initiate the assigned treatment. However, no differences were observed in the efficacy outcomes when all randomised patients were included in the analyses (ITT population) rather than those who initiated the assigned treatment (ITT/e population). Furthermore, all treatment limiting adverse events were scrutinised by source verification by the monitors. Also, such types of bias appear not to vary between sites since adjustment for location of the site was not an independent predictor of the various efficacy outcomes examined. In addition, no difference was observed in the proportion of patients lost to follow-up between the trial groups.

The number of capsules to be taken per day - twelve in the SAQ/r group versus six in the IDV/r regimen, the markedly larger size of the saquinavir capsules, the difference in food requirements (no food restrictions for IDV/r, but a fluid intake of > 1.5 litre/day recommended, whereas SAQ/r should be taken in conjunction with a regular meal), and the need for refrigerator storage of saquinavir could potentially impact negatively on patients' adherence to SAQ/r. Only two patients did not start on SAQ/r due to expressed inconvenience of the regimen. Patients were allowed to switch from the soft gel formulation (Fortovase®) to the hard gel formulation (Invirase®) of saquinavir, which are smaller capsules, but only 4 patients did. The proportion of patients who prematurely switched from the assigned treatment was higher in the IDV/r group (41 %) compared to the SAQ/r group (27 %; $p = 0.013$, Chi-squared). Hence the data do not suggest a negative impact of the theoretical higher pill burden on the SAQ/r group compared to the IDV/r group.

A golden standard on how to measure adherence has not been identified hence the interpretation of such measurements is difficult and potentially misleading [32, 33]. Furthermore, an important part of the "philosophy" of the trial was to focus on the main objectives of the trial rather than using it as a vehicle for pursuing a variety of questions. The interest of participating investigators to expand the follow-up period to 96 or more weeks was investigated but was too limited to be implemented in a meaningful way, i.e. it would have been prone to selection bias.

Whether or not the chosen surrogate end point is a valid one is a question that could not be addressed within the frame of the trial. Therefore, one should be cautious in extrapolating the trial's short-term results to long-term efficacy and safety.

The MaxCmin2 trial

Background & rationale

Both lopinavir and saquinavir undergo extensive first-pass metabolism, which results in low oral bioavailability. Co-administration of low dose ritonavir significantly enhances the exposure time to both these agents, substantially increasing their C_{max}. This is thought to occur primarily through inhibition of *kP450* CYP3A4 metabolism by ritonavir. The increase in the Area Under the Curve (AUC) is primarily due to an increase in C_{max} whereas the half-life of the respective drug remains relatively unchanged. At the time of design of the trial no comparative data from RCTs existed on efficacy and safety of ritonavir-boosted PI regimens (the MaxCmin1 trial was ongoing). To our knowledge the MaxCmin2 trial (**A**) is the second head-to-head comparison of ritonavir-boosted regimens.

One protocol-defined interim analysis has been performed including efficacy data through Week 24 and safety data as available on 30th of September 2002. In November 2002, the analysis was presented to the DSMB that stated that no changes were warranted in the conduct of the trial, and that no specific concerns regarding safety had been identified. The trial's Steering Committee – which has overall responsibility for publication and presentation of trial data – had planned to make a conference presentation of the interim results. However, the DSMB recommended not presenting the interim efficacy data stratified by treatment group, and in order to preserve the integrity of the trial, the Steering Committee chose to follow this recommendation and only allow for presentation of safety data stratified by treatment group.

Design

With the objective of comparing the rate of virological failure between the two trial groups, the trial was designed as a prospective, randomised (1:1), international, multi-centre, open-label, and phase IV trial with 48 weeks follow-up of all patients. The trial was designed to show equivalence between ritonavir-boosted lopinavir (LPV/r) 400/100 mg and saquinavir (SAQ/r)

1000/100 mg. The trial was open for enrolment in Europe, Argentina, Canada and the US between June 2001 and December 2001, and follow-up is ongoing with the last scheduled visit in May 2003.

For the Week 24 interim analysis presented to the DSMB, the Peto method of repeated significance testing was used to test for treatment difference with a p-value of 0.001 as the significance level, giving a significance level of 0.05 (two-sided) for the final Week 48 analysis.

The role in the MaxCmin2 trial of CHIP has been as coordinating office and sponsor according to the ICH-GCP guidelines. In the MaxCmin2 trial I have served as trial physician. This has included but not been limited to site initiation, participation in the protocol development, site initiation, site monitoring, statistical analyses, and presentation of data. Apart from the members of the DSMB only the trial statistician and I have been unblinded to the results of the interim analysis.

Interim results

In total 339 patients were randomised of which 326 patients initiated the assigned treatment. The trial included a heterogeneous population with 32 % of the patients being ART naïve, 15 % being ART experienced but PI-naïve and 53 % being PI-experienced. No differences were observed between the trial groups in baseline characteristics (Table 3). Complete follow-up at Week 24 was available from 93 %, at which time 83 % remained on the assigned treatment. Twenty-two of the 55 patients, who prematurely switched from the assigned treatment, did so due to clinical non-fatal AEs. There was no significant difference at the p = 0.001 level between the trial groups in patients who prematurely switched from the assigned treatment.

The overall proportion of patients with viral suppression is shown in Figure 4. The median increase in $CD4^+$ count from baseline to Week 24 was 81 cells/µl, and 179 patients had a $CD4^+$ count increase of > 100 cells/µl after a median of 74 days.

Table 3 Baseline characteristics according to treatment group

Parameter	LPV/r n = 163	SAQ/r n = 163	Total N = 326
Gender (No. male, %)	124 (76)	133 (82)	257 (79)
Age (median, IQR)	40 (35-47)	40 (35-50)	40 (35-48)
BMI* (median, IQR)	23 (21-25)	23 (21-26)	23 (21-26)
CDC, cat. C (No., %)	49 (30)	53 (33)	102 (31)
HIV exposure group (No., %)			
Homosexual/bisexual	72 (44)	77 (47)	149 (46)
IVDU	13 (8)	13 (8)	26 (8)
Haemophiliac	1 (1)	3 (2)	4 (1)
Transfusion	3 (2)	0 (0)	3 (1)
Heterosexual	65 (40)	58 (36)	123 (38)
Unknown	9 (6)	12 (7)	21 (6)
Region (No., %)**			
Argentina	27 (17)	27 (17)	54 (17)
Scandinavia	52 (32)	54 (33)	99 (33)
C Europe	13 (8)	10 (6)	23 (7)
S Europe	8 (5)	9 (6)	17 (5)
NW Europe	28 (17)	27 (17)	54 (17)
USA + Canada	35 (21)	36 (22)	70 (22)
HIV-1 RNA (c/ml log_{10})* (IQR)	4.6 (3.4-5.3)	4.4 (3.1-5.1)	4.5 (3.3-5.2)
HIV-1 RNA < 400 c/ml* (No., %)	34 (21)	35 (21)	69 (21)
$CD4^+$ count (10^6/l, median, IQR)*	239 (95-420)	239 (86-393)	239 (94-415)
$CD4^+$ nadir (10^6/l, median, IQR)*	95 (30-195)	100 (31-219)	100 (30-210)
Prior use of NRTI(s) (No., %)	65 ()	69 ()	67 ()
Prior use of NNRTI(s) (No.,%)	29 ()	36 ()	32 ()
Prior use of PI(s) (No.,%)	52 ()	52 ()	52 ()

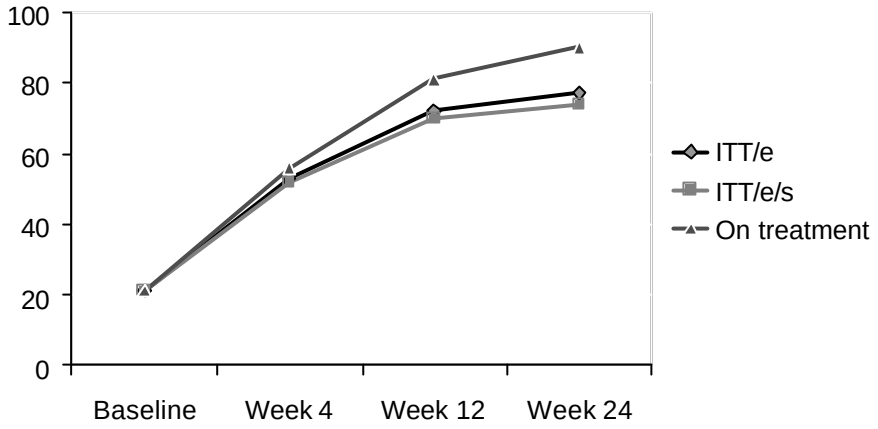
* These variables include missing information therefore the denominator is less than the number of subjects who received treatment.

** Scandinavia includes Denmark, Sweden and Norway, C. Europe includes Switzerland and Austria, S. Europe includes Italy and Spain and NW Europe includes Belgium and the UK.

A total of 90 grade 3/4 AEs was reported as of 30th of September 2002. The time to development of the first grade 3/4 AE was comparable in the two groups (data not shown). There were no statistical significant differences at the p = 0.001 level between the two treatment groups in the number of patients experiencing grade 3/4 AEs. The total number of patients experiencing grade 3/4 AEs was 31 and 27 in the LPV/r group and the SAQ/r group, respectively, and 14 and 10 when only grade 3/4 AEs at least possibly related to the assigned treatment as judged by the treating physician. Further, no differences were seen in the number of grade 3/4 AEs experienced, 53 versus 37 (all) and 19 versus 14 (related) in the LPV/r and SAQ/r group, respectively. More patients in the LPV/r group developed

gastrointestinal grade 3/4 AEs compared to the SAQ/r group both overall and treatment-related, however, these differences were not statistically significant.

Figure 4 % with HIV-1 RNA < 400 c/ml (ITT/e, ITT/e/s and OT analyses)



Discussion

The MaxCmin2 trial was designed in the latter part of year 2000 to assess if equivalence exists in efficacy and safety between LPV/r 800/100 mg bid and SAQ/r 1000/100 mg bid, the primary outcome being incidence of protocol-defined virological failure. Based on the recommendation from the trial's DSMB no presentation of interim analysis of the primary efficacy outcome, rate of virological failure, or other efficacy measures stratified by treatment group has been performed.

The results of the interim analysis showed a well-matched heterogeneous patient population. Overall a high proportion of patients had suppression of pVL in the ITT/e, ITT/e/s and OT analyses, and a high immunological response rate and $CD4^+$ count increase were seen.

Few patients switched from the assigned treatment due to AEs. No difference was observed in the number of patients experiencing grade 3/4 AEs, time to development of grade 3/4 AEs, or difference in type or number of grade 3/4 AEs.

The trial demonstrated ritonavir-boosted lopinavir and saquinavir HAART regimens to have virological and immunological efficacy comparable to other ritonavir-boosted PI HAART

regimens at 24 weeks [109, 120]. The proportion of patients with complete follow-up data at Week 24 was equal to what was observed at Week 48 in the MaxCmin1 trial, seven %, with four % in the LPV/r group versus ten % in the SAQ/r group. A high and/or disproportionate rate of patients lost to follow-up could impact the trials' ability to reach conclusions in the ITT/e analyses. It is, however, our experience that additional (missing) follow-up data are reported during the latter part of the trial and/or identified during final site monitoring. Had efficacy data been presented stratified by treatment group, results from the analyses where continuation of trial medication influence the outcome could potentially be misleading as patients with missing data would have been counted as failures. This would then have been an example of a difference in reporting between the interim and the final results of a trial that could potentially have influenced clinicians' and patients' interpretation of the early trial results [118].

The limitations by design discussed for the MaxCmin1 trial also apply for the MaxCmin2 trial, i.e. the open-label design, the heterogeneous patient population, the difference in pill burden (although both trial PIs were to be taken in conjunction with a meal in the MaxCmin2 trial), the relatively short follow-up period, the choice of surrogate end point, and not measuring adherence. It is the intention to address these issues in the MaxCmin2 trial in much the same way as has been done for the MaxCmin1 trial.

The COLATE trial

Background & rationale

If lamivudine is used as monotherapy, the virus develops high-level resistance (inhibitory concentration-50 (IC_{50}) increased by 100- to 1000-fold) within weeks [121]. The genetic correlate to this resistance is the point mutation M184V (transiently M184I) [122]. Despite the development of high-level resistance, the pVL remains below baseline values, indicating that the resistant virus is less fit than the wildtype [121, 123]. In the setting of HAART, the development of *in vitro* resistance to lamivudine is an indicator of treatment failure and a marker of residual viral replication.

Development of the 184V mutation has not uniformly been associated with a poor response to lamivudine in combination with other antiretroviral drugs. This is unlike other frequently observed mutations e.g. at the codon 215, which consistently have been shown to be

associated with a poor virological and clinical outcome. The reasons for this different nature of the 184V mutation are not fully understood. Possible explanations include: a relative replicative deficiency of the mutant, an enhanced efficacy of other antiretroviral drugs, a higher fidelity of the mutant reverse transcriptase resulting in less risk of generating other essential mutations or a combination of the above [123]. It is not known if continued lamivudine exposure as part of a HAART regimen after development of the 184V mutation is of virological, immunological or clinical significance (reviewed in [115]).

Due to a much slower than expected rate of recruitment to the trial (**B**), the trial's Steering Committee decided to perform a non-protocol driven interim analysis to assess safety and efficacy issues, and the feasibility of continued recruitment. The analysis was performed including all efficacy and safety data as available on 24th of July 2002. The analysis was presented to the DSMB in November 2002 who stated that no changes were warranted in the conduct of the trial, and that no specific concerns regarding safety issues followed their evaluation. Following a recommendation from the DSMB, the Steering Committee decided to prematurely terminate enrolment in the trial in November 2002.

Design

The trial is a prospective, randomised (1:1), international, multi-centre, open-label, and phase IV trial with 48 weeks follow-up of all patients. The trial was designed to assess the safety and efficacy of continuation versus discontinuation of lamivudine in patients experiencing virological failure to a regimen including lamivudine.

The trial was opened for enrolment in April 1999 and enrolment was terminated in December 2002. Follow-up is currently ongoing with the last scheduled visit in May 2002. Initially, only patients with virological failure to their first-line therapy were included (Stratum A). In January 2000 the protocol was amended to allow for inclusion of all patients failing a lamivudine-containing regimen (Stratum B).

The trial was powered to detect a difference in pVL reductions between treatment groups of at least 0.5 $\log_{10}$ within each of the strata with one interim analysis, 90% power and a significance level of 0.05. For the non-protocol driven interim analysis presented to the DSMB, the Peto method of repeated significance testing was used to test for treatment differences with a p-value of 0.001 as the significance level.

The role in the COLATE trial of CHIP has been as coordinating office and sponsor according to the ICH-GCP guidelines. In the COLATE trial I have served as trial physician. This has included but not been limited to participation in the protocol development, site initiation, and statistical analyses. Apart from the members of the DSMB only the trial statistician and I have been unblinded to the results of the interim analysis. The DSMB has recommended that efficacy outcome data from this analysis not be presented in any form. In Appendix I is listed the principal investigators, members of the Steering Committee and DSMB, and the sponsor and coordinator of the trial.

Interim results

The sample size for the COLATE trial is 160 patients of which 136 had been randomised at the time of this analysis, 55 in Stratum A and 74 in Stratum B. Three patients were randomised in error and 4 did not initiate the assigned treatment leaving 129 patients for the ITT/e analysis population (Table 4). The proportion of patients with complete follow-up data at Week 48 was low, 72 %, but with no significant differences at the p = 0.001 level between the two trial groups in the proportion of patients with complete follow-up data.

Patients' baseline characteristics are shown in Table 5. There were no significant differences at the p = 0.001 level between the trial groups in these or other collected baseline parameters.

Table 4 Patient disposition at Week 24 and 48

	Lamivudine **%** (N = 65)	**No lamivudine** **%** (N = 68)	**Total** **No. (%)** (N = 133)
Initiated the assigned treatment¤)	100	94	129 (97)
Never initiated the assigned treatment¤)	0	6	4¤) (3)
Initiated but permanently switched from the assigned treatment¤) by Week 24	15	11	17 (13)
Initiated but permanently switched from the assigned treatment¤) by Week 48	17	16	21 (16)
Subjects with an outcome available at Week 24*)	92	91	118 (91)
Subjects an outcome available at Week 48*)	69	75	93 (72)
Still on assigned treatment¤) at Week 48	84	94	83 of 93 (89)

¤) Patients still on assigned treatment means continuation lamivudine treatment with versus not including lamivudine in HA(ART) regimen.

*) Subjects with an outcome at Week 24 / 48 include subjects who have completed follow-up to that point as well as subjects who died

¤) Of the four patients, two withdrew consent prior to baseline and for two patients information about initiation of assigned treatment is pending

Table 5 Baseline characteristics according to treatment group

	Lamivudine N = 65	No lamivudine N = 64	Total N = 129
Trial stratum A (%)	43	42	43
Gender (% male)	82	83	82
Age (median, IQR)	42 (36.5-50)	40 (36-47.8)	40 (36-48)
HIV factor (%)			
Homo-/bisexual activity	60	56	58
Heterosexual activity	28	30	29
Iv drug use	3	2	2
Other/unknown	9	13	11
pVL (c/ml log_{10}; IQR)	3.9 (3.3-4.4)	4.0 (3.4-4.6)	4.0 (3.3-4.5)
$CD4^+$ count (cells/µl)* (median, IQR)	360 (257-481)	279 (170-372)	310 (199-440)
$CD4^+$ nadir (cells/µl) * (median, IQR)	128 (33-189)	122 (60-200)	123 (45-191)
CDC, clinical category C (%)	35	30	33

* These variables include missing information therefore the denominator is less than the number of subjects who received treatment

No significant differences were seen between the trial groups in the number of drugs or drug classes that patients had been expose to prior to randomisation, were on or initiated at baseline, or were on at Week 48 (Tables 6 - 8). Further, no differences were seen between the trial groups in the number of drugs that were added or removed through Week 48 to (from) the HAART regimen patients were on at baseline (data not shown).

Table 6 Number of drugs patients had been exposed to prior to randomisation

Drug class	Lamivudine			No lamivudine			Total		
	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range
NRTI	65 100)	4	2-5	64 (100)	3	2-6	129 (100)	3	2 - 6
PI	51 78)	1	0-6	52 (81)	2	0-5	103 (83)	2	0 - 6
NNRTI	27 42)	0	0-3	31 48)	0	0-2	58 (45)	0	0 - 3

Table 7 Number of drugs (median) patients was on at baseline

Drug class	Lamivudine			No lamivudine			Total		
	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range
NRTI	65 (100)	3	1-3	52 (81)	2	0-3	117 (91)	2	0 - 3
PI	48 74)	1	0-2	32 (50)	0.5	0-3	80 (62)	1	0 - 3
NNRTI	29 (45)	0	0-1	22 (34)	0	0-1	51 (40)	0	0 - 1

Table 8 Number of drugs (median) patients was on at Week 48

Drug class	Lamivudine			No lamivudine			Total		
	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range	Patients (No. (%))	Drugs	Range
NRTI	40 (91)	3	0-3	42 (91)	2	0-3	82 (91)	2	0 - 3
PI	23 (52)	1	0-2	25 (54)	1	0-2	48 (53)	1	0 - 2
NNRTI	0 (0)	0	-	0 (0)	0	-	0	0	-

This analysis only includes the 90 patients who completed the 48 weeks of follow-up and were alive at that visit

Discussion

The trial has recruited a well-matched patient population with an almost equal number of patients in the two strata, and the proportion of patients on assigned treatment at Week 48 is high with no statistically significant difference between the two trial groups. This may make up for some of the loss of power resulting from the fact that only 129/160 (81 %) of the ITT/e analysis population stipulated in the protocol will be available for final analysis. Despite the lower than stipulated number of patients enrolled, the trial still has more than 80 % power to detect a difference in pVL reductions between treatment groups of at least 0.5 $\log_{10}$. However, the proportion of patients lost to follow-up at Week 48 is higher than anticipated, and if additional data are not identified during final site monitoring - which we expect will be the case - this will further compromise the trials' ability to address the primary objective. As it is the trial continues to have a potential for addressing a clinically important question. It is still the largest RCT of continuation of a drug following development of resistance to this drug in the setting of HAART. Besides addressing the protocol stipulated primary and secondary objectives (albeit with less statistical power), a thorough assessment of development of resistance can be made on stored plasma, which has been collected from all patients at all scheduled trial visits (6 per patient).

The trial has some limitations:

- Open-label design
- Long recruitment period
- No measurement of adherence
- The relatively short follow-up period
- The use of a surrogate end point that has not been validated as a marker of HIV-1 disease progression and death in ritonavir-boosted PI HAART

The pill burden from taking lamivudine is very limited (one small-size tablet bid with no food restrictions) and all patients enrolled very adherent to and tolerated lamivudine at the time of

randomisation hence it was not found feasible to perform blinding of the trial assignment. No difference was observed in the proportion of patients lost to follow-up at Week 24 and 48 between the trial groups, which may indicate that the open design has not biased the data available for analysis.

It is generally difficult to recruit patients to “failure trials“, i.e. trials where inclusion of selected patients must be done at the time of failure. In the case of the COLATE trial, the reasons why recruitment has not been as anticipated are many, but most important was the need for continuous awareness at the site level in combination with the relatively few patients that are failing virologically during (HA)ART after having had viral suppression. In the COLATE trial site awareness has been difficult to maintain over time despite numerous efforts: investigator teleconferences and meetings, newsletters, regular e-mail notification, real-time randomisation assistance, and various tools for easy selection of patients (prints of eligibility criteria and patient selection flow-charts from pocket to poster size).

One limitation of the trial is the evolution of treatment (HAART) during the long recruitment period. The trial has allowed for use of most approved antiretroviral drugs hence patient enrolled in the second millennium A.D. might have received less potent HAART compared to those enrolled in the third millennium. This issue will be addressed in the final analysis by assessing treatment responses by calendar time.

The arguments carried in the MaxCmin1 section for the latter three limitations by design listed above also applies for the COLATE trial.

Non-protocol driven interim analysis should generally not be performed, however, in this case, with recruitment during a period at least three to four times longer than expected, it seems justified - if not even demanded - to investigate if a significant difference in efficacy or safety had developed between the trial groups. Had the trial not had a DSMB the interpretation of the interim results had been in the hands of the Steering Committee with the potential of influence on the conduct of the trial. As it is, the Steering Committee members and other investigators remain blinded to the interim results and no efficacy or safety data originating from the interim analysis will be presented. Therefore, the integrity of the trial will not be harmed due to the interim analysis.

Predictors of immunological failure after initial response to HAART in HIV-1 infected adults: A EuroSIDA study

Background & rationale

Current treatment guidelines acknowledge the role of $CD4^+$ count in predicting HIV-1 disease progression but current treatment guidelines focus on obtaining maximal and durable suppression of pVL [32, 33]. However, no comparative data exist of the risk of HIV-1 disease progression and death between patients treated according to current treatment guidelines compared to patients treated according to immunological status, i.e. maintaining the $CD4^+$ count above a certain threshold, which is above the level where patients are at significantly increased risk of disease progression. One ongoing trial (A Large, Simple Trial Comparing Two Strategies for Management of Anti-Retroviral Therapy, The SMART study (CPCRA 065)), investigating this will be finalised within the next 6 - 7 years. While awaiting the results of this trial, identification of predictors of a sustained immunological response during HAART may have implications on patient management, and have not been reported from a large international HIV-1 cohort.

Design

The study (**II**) was designed to investigate if predictors of a sustained immunological response during (HA)ART could be identified from parameters collected in the EuroSIDA cohort study. Patients included in the analysis had available follow-up data in the EuroSIDA database, which have been obtained between May 1994 and autumn 2002.

My role in the EuroSIDA study has been limited to the development of this study and in assisting the statistician with the analyses.

Results

Follow-up data were available on 9803 patients of which 2347 patients were eligible for this analysis. Included patients were from 26 European countries and Argentina, with 85 % being Caucasians, 78 % being men, 48 % being homo-/bisexual, and 27 % having had an AIDS-defining illness. Prior to starting HAART the median $CD4^+$ count was 200 cells/µl (IQR 82 - 317 cells/µl), and the median pVL was 4.54 $\log_{10}$ (IQR 3.75 - 5.18 $\log_{10}$).

Of the 2347 immunological responders, 515 (22 %) subsequently experienced immunological failure, i.e. had at least one $CD4^+$ count that was = than the pre-HAART $CD4^+$ count. The

median $CD4^+$ count at the time of immunological failure, for those who failed was 230 cells/µl (IQR 120 – 340 cells/µl).

In a multivariate Cox regression model four factors were found to be significantly associated with an increased risk of immunological failure: pre-HAART $CD4^+$ count per 50 % higher (RH 2.11, 95 % CI 1.87 - 2.37, $p < 0.0001$), time-updated pVL per 1 $\log_{10}$ higher (RH 1.74, 95 % CI 1.61 - 1.89, $p < 0.0001$), ≥ 5 drugs in HAART regimen compared to 3 drugs (RH 1.83, 95 % CI 1.14 - 2.93, $p = 0.012$), and risk group being intravenous drug use compared to male homosexuality (RH 1.55, 95 % CI 1.19 - 2.02, $p = 0.0011$).

Discussion

$CD4^+$ count and pVL are markers of HIV-1 disease progression and death during HAART [32, 33]. Discordant $CD4^+$ count and pVL responses during HAART suggest that HAART affects these parameters differently hence predictors of immunological failure may be different from predictors identified of a virological failure during HAART. In this analysis we identified four predictors of immunological failure after an immunological response in patients initiating their first HAART regimen. Among these, baseline pVL was the only parameter that has also been identified as a predictor of virological failure [100, 124]. An explanation of why predictors of immunological and virological failure are not identical could simply be because a factual difference exists. This would be in agreement with observations of discordant immunological and virological responses during HAART [79, 125, 126]. Another possible explanation could be that different patient populations have been selected for analysis. Findings from cohort studies are hypothesis generating and should be interpreted with caution until the results have been reproduced in other cohorts or, preferably, been tested in a RCT. Results from the SMART study will be relevant to the question tested in this study.

The EuroSIDA cohort is the largest international observational HIV-1 study. A clear strength of the study is the size itself, which makes it possible to analyse rare events. The study of immunological failures during HAART is an example of this. Another strength is the diversity of the study in terms of the patient population, the clinics, and the geographical area included. The study does, however, also have limitations by design. Although a system is in place for data validation, on-site monitoring, and data entry, due to financial and other resource limitations, monitoring procedures including the amount of data that are source verified, and data entry are less strict compared to RCTs. Single entry of data into the EuroSIDA database compared to double entry by two persons followed by a computer-based

comparison function and final approval by a trained monitor in the MaxCmin trials is an example of this. Also, $CD4^+$ count measurements are performed locally without being standardised. This could potentially influence on the results of this study due to intra-assay variability. We do not believe such variability had any significant impact on the outcome of this study as we found similar results when a more strict definition of failure where patients were required to have a confirmatory $CD4^+$ count was used. Further, it is impossible or not feasible to collect detailed information about some aspects of HIV-1 infection from such a large cohort hence some analyses may be less detailed than whished to assess various parameters' influence on the outcome under investigation, e.g. regarding patients' adherence to treatment.

In conclusion, in a large international HIV-1 cohort we identified predictors of immunological failure, which were different from predictors of virological failure. This finding may have implications for the clinical management of HIV-1 infected patients.

The ESPRIT study

Background & rationale

IL-2, a substance naturally produced by lymphocytes to enhance growth and maturation of T-cells upon stimulation with antigens, is an essential component in the establishment of an adequate immunological host response upon microbial challenge. During HIV-1 infection the number and repertoire of $CD4^+$ cells are diminished and IL-2 production decreased. Complete immune restoration, including normalization of $CD4^+$ count and IL-2 production, does not result from (HA)ART alone emphasizing the need for other treatment strategies to restore immune function. Concomitant HAART and immunostimulatory treatment may prove to be efficacious in HIV-1 infected patients [44]. One option is the use of rIL-2, which acts similarly to natural IL-2 in all functional assays examined. Clinical trials have demonstrated that intermittent 5-days cycles of rIL-2 is capable of increasing the $CD4^+$ count to normal levels in a dose-dependent way in HIV-1 infected patients (reviewed in **III**)[127]. However, no data exist on rIL-2 treatment in children, pregnant and lactating women.

Design

With the objective of comparing the effects of sc rIL-2 and no sc rIL-2 on disease progression and death in HIV-1 infected patients with $CD4^+$ counts of = 300 cells/µl who are taking

combination antiretroviral therapy, the trial was designed as a prospective, randomised (1:1), international, multi-centre, open-label, and phase III trial with an average follow-up of five years. The trial enrolled the first patient in February 2000 and is currently enrolling. Enrolment will end when the recruitment goal of 4000 patients have been reached which is estimated to occur in the spring of 2003.

Patients included in the trial are to receive combination ART, i.e. at least two antiretroviral drugs. In addition, patients assigned rIL-2 will receive three five-days rIL-2 cycles of 7.5 million international units (MIU) bid eight weeks apart (induction phase). The induction phase will be followed by a "maintenance phase" where additional rIL-2 cycles will be provided on an individual basis to reach and/or maintain the $CD4^+$ count above the $CD4^+$ count goal, i.e. twice the baseline $CD4^+$ count for patients with a baseline $CD4^+$ count between 300 and 499 cells/µl and = 1000 cells/µl for patients with a baseline $CD4^+$ count = 500 cells/µl. The starting dose of 7.5 MIU bid could be lowered in decrements of 1.5 or 3 MIU bid due to treatment-limiting adverse events. Dose escalation in increments of 1.5 and 3.0 MIU was allowed for patients tolerating their current dose.

In anticipation of the ESPRIT trial four phase II (Vanguard) studies were set up in Argentina, Thailand and the US. All Vanguard patients were eligible for ESPRIT, and following completion of these studies patients from sites also participating in ESPRIT were given the opportunity to "roll-over" into ESPRIT. Follow-up data - starting on the day of enrolment in the Vanguard study - on patients from sites were at least 90 % of surviving Vanguard patients consented to ESPRIT will be followed for the primary end point of ESPRIT [57].

The Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), sponsor ESPRIT. In accordance with guidelines for HIV-1 RCTs sponsored by NIAID, a DSMB has been set up for ESPRIT. The ESPRIT DSMB reviews trial data including enrolment progress at least annually. An open part of the DSMB reports is being distributed to site investigators. In these reports the DSMB has expressed concerns about the publication of trial data stratified by treatment group including $CD4^+$ count responses and recommended that this should not be done without careful consideration. The highest deciding body for ESPRIT, the Executive Committee, which remains blinded to the treatment outcome, concurs with this recommendation and only allowed for presentation of response to treatment in the rIL-2 group. In Appendix II is listed the members of the Executive Committee and DSMB, and the sponsor of the trial.

The role of CHIP in ESPRIT is as one of four regional coordinating offices and as national coordinating office. CHIP has the responsibility for 47 sites participating in Austria, Belgium, Denmark, Germany, Norway, Poland, Portugal, Spain, and Sweden. I have served as trial physician, which has included but not been limited to site initiation, membership of the trial's Toxicity Management Group and extended Executive Committee. In addition, I have been responsible for the development and implementation of two intervention substudies: FLUVAC and TEPVAC (for protocols see Appendix III). The primary objective of FLUVAC is to assess if a difference exists between ESPRIT patients in the IL-2 group versus the Non IL-2 group in the antibody response to influenza vaccination at one month post-vaccination. The primary objective of TEPVAC is to assess if a difference exists between ESPRIT patients in the IL-2 group versus the Non IL-2 group in the antibody response to tetanus and pneumococcal vaccination at one month post-vaccination. Both these substudies are currently enrolling patients; in FLUVAC 98 of the planned 400 patients have been enrolled, and in TEPVAC 58 of the planned 450 patients have been enrolled.

Interim results

From the 5th of February 2000 to the 27th of January 2003, 3666 (92 %) of the planned 4000 patients were enrolled of which 3538 patients have available baseline data (Table 9). Of these, 638 were patients rolled-over from the Vanguard studies. Patients were recruited from 244 of the 270 participating sites in 24 countries in Austral-Asia, Europe, and the Americas. Patients are primarily Caucasian men with homo/bisexual risk behaviour and in an early stage of HIV-1 disease.

By the 7th of January 2003, 1832 patients had been randomised to the IL-2 group of which 1670 (91 %) had taken at least one dose of rIL-2. Approximately 82 % of patients assigned rIL-2 had completed the three protocol-stipulated rIL-2 cycles by Month 8. In Figure 5 is shown the overall rIL-2 cycle status among patients who initiated rIL-2 treatment. The median increase in $CD4^+$ count between baseline and Month 8 was 246 cells/µl (IQR 90 - 432). This number remained stable through Month 24 (Table 10). By Month 8, 33 % had reached their $CD4^+$ count goal and an additional 25 % had had an increase of = 200 cells/µl. The current $CD4^+$ count distribution is shown in Table 11.

Table 9 Baseline characteristics of the ESPRIT population (N = 3525)

Parameter	Result
Age (mean)	41
Gender (% female)	18
Weight (mean kg)	73
Race	
Asian	10
Black	9
Caucasian	77
Other	4
Risk factors *	
Homo-/bisexual activity (%)	57
Heterosexual activity (%)	36
Iv drug use (%)	11
Blood products (%)	2
$CD4^+$ (median cells/µl)	460
Nadir $CD4^+$ (median cells/µl)	203
pVL (% undetectable)	64
ART	
NRTI(s) only (%)	16.1
PI(s) only (%)	0.5
NNRTI(s) only (%)	0.6
NRTI(s) + NNRTI(s) (%)	36.9
NRTI(s) + PI(s) (%)	37.9
PI(s) + NNRTI(s) (%)	1.0
NRTI(s) + PI(s) + NNRTI(s) (%)	7.1
Stage of HIV-1 disease	
Asymptomatic (CDC category A) (%)	55
Symptomatic (CDC category B) (%)	18
Symptomatic (ESPRIT Disease Progression) (%)	27

***) More than one factor could be indicated

Figure 5 IL-2 cycle status for patients with at least 8 months of follow-up (as of 7th of January 2003)

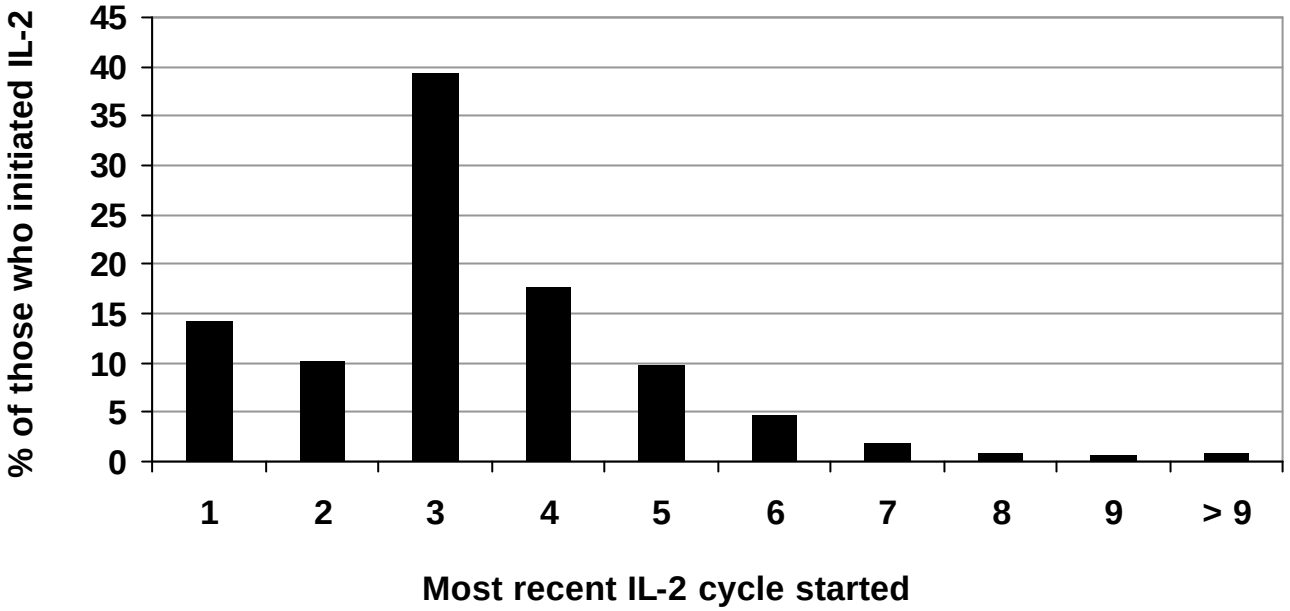


Table 10 Change in $CD4^+$ counts from Baseline for patients assigned IL-2 (as of 7th of January 2003)

ESPRIT visit (post-randomisation)	N	Mean (standard error)	Median change (IQR)
Month 4	1504	292 (9.1)	226 (78 - 424)
Month 8	1293	291 (8.8)	246 (90 - 432)
Month 12	1080	272 (9.1)	232 (70 - 413)
Month 16	812	284 (11.5)	231 (84 - 441)
Month 20	546	282 (14.3)	245 (60 - 474)
Month 24	367	282 (18.7)	252 (59 - 454)

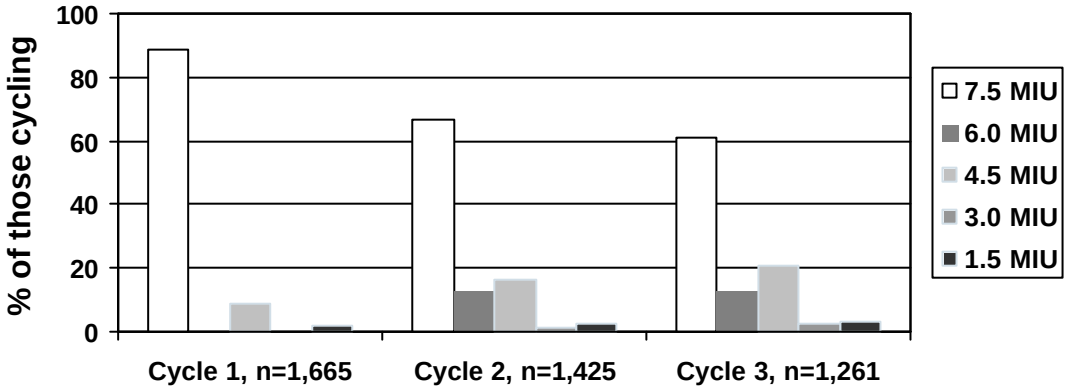
Table 11 Current $CD4^+$ count status for patients assigned IL-2 (as of 7th of January 2003)

$CD4^+$ count status	N (1293)	%
$CD4^+$ count < 300 cells/µl	51	3.9
$CD4^+$ count < baseline but > 300 cells/µl	206	15.9
$CD4^+$ count increase < 50 cells/µl and less than goal	87	6.7
$CD4^+$ count increase of 51 - 200 cells/µl but less than goal	235	18.2
$CD4^+$ count increase of = 200 cells/µl but less than goal	330	25.5
$CD4^+$ count increase of at least goal	384	29.7

* Includes patients with at least 8 months of follow-up

The percent of patients with treatment-limiting toxicities leading to dose reductions or cycle interruption were 21 %, 15 % and 13 % in cycles 1 to 3. Overall, 451 (27 %) of the 1670 patients who initiating rIL-2 treatment experienced one or more treatment limiting AEs leading to dose modification(s) during the first three rIL-2 cycles, 234 (14 %) experienced one or more treatment limiting AEs of grade 3/4. The most common toxicities observed were constitutional/systemic. The starting dose of rIL-2 decreased during the first three rIL-2 cycles. Approximately 89 % initiated the first rIL-2 cycle at the 7.5 MIU dose (some Vanguard patients were randomised to a starting dose of 4.5 or 1.5 MIU). In cycles two and three the number was 67 % and 61 %. The distribution of rIL-2 doses in the first three cycles is shown in Figure 6. The number total number of injections per rIL-2 cycle is 10 if no dose interruption occurs. The number (mean) of injections observed was 9.6, 9.7 and 9.6 in the first to third rIL-2 cycle.

Figure 6 % of starting dose of IL-2 for the first three cycles



Discussion

ESPRIT is the largest ever RCT in the HIV-1 field, and the trial is very close to having achieved the recruitment goal. It was estimated that recruitment could be finalised within two years but due delays for primarily administrative reasons the recruitment period has been prolonged and is likely to be finalised with a one years delay in the spring of 2003. The trial will be terminated when 320 primary events have been reported which is expected to occur after approximately five years of follow-up from the enrolment of the last patient.

The trial has enrolled patients from all risk groups and from 24 countries on five continents. Unless data from the trial suggest that some sub-groups benefit or fare substantially different from the remainder of the trial population, the diversity of the trial is likely to ensure that the findings of the trial can be generalised to all HIV-1 infected patients (except children,

pregnant and/or lactating women that have been excluded from the trial population). As an example no Sub-Saharan countries are participating in the trial, however, about nine % of patients are of black African origin.

In order to preserve the power of the trial it is essential to assure that patients in the IL-2 group are at or above their $CD4^+$ count goal and that the rate of patients lost to follow-up is kept low. Currently, approximately 80 % in the IL-2 group complete the three protocol-stipulated rIL-2 cycles within Month 8. Of these, only one third were at or above their $CD4^+$ count goal. These numbers are, however, close to the assumptions in the sample size calculation, and the DSMB has consistently stated that there are no indications in the trial data to suggest that the trial should not be able to address the primary objective. Even so, several measures have been implemented at the regional, national and site level to ensure compliance with the protocol including long-term retention of patients. This includes updated information on site performance regarding rIL-2 recipients at $CD4^+$ count goal, data reporting, and follow-up rates, and in addition regular feedback of trial progress to investigators and participants (weekly e-mails to investigators, newsletters, meetings, conference presentations, public homepage). Another measure is continuous education of investigators in management of rIL-2 toxicities. In order to retain patients in the IL-2 group and having them adhere to the protocol regarding rIL-2 re-cycling in the maintenance phase of the trial it is important that the dose of rIL-2 used is acceptable and the managing of toxicities adequate. During the induction phase a decreasing proportion of patients experienced treatment-limiting AEs. Further, the starting dose of rIL-2 was reduced during the first three rIL-2 cycles with no change in the number of injections provided. This indicates that adjustment of rIL-2 dosing was performed to allow for completion of rIL-2 dosing cycles with tolerable toxicity.

In a previous analysis, including 396 patients that had reached ESPRIT Month 8 and had completed three cycles of IL-2, higher nadir $CD4^+$ and shorter duration of (HA)ART was predictive of a better $CD4^+$ count outcome [128]. The analysis included a limited and less advanced HIV-1 population, i.e. at baseline patients were younger, had higher (nadir) $CD4^+$ count, more patients had suppression of pVL below LLD, and fewer patients had progression of disease, compared to the current patients population enrolled in ESPRIT. Therefore, the analysis should be repeated when all patients assigned rIL-2 have completed the induction phase.

The trial is open-label because it is impractical to use placebo injections and impossible to maintain a blind due to the very common side effects of rIL-2. To reduce potential bias associated with the ascertainment and diagnosis of major end points, patients in both treatment groups will be seen at least every four months. During the first year patients assigned sc rIL-2 will be seen more frequently because each will receive at least three cycles of treatment during the induction phase. In subsequent years, the difference between treatment groups in the number of protocol-required visits will be less since fewer rIL-2 cycles will be required on average to maintain the $CD4^+$ count at goal. The difference in visit frequency in early follow-up is unlikely to result in bias in end point ascertainment because very few events are expected to occur until the third year of follow-up. Furthermore, an end point review committee (ERC) will review documentation of disease progression events. The ERC will review events blinded to treatment group and $CD4^+$ count level to determine whether they qualify as end points.

By necessity an end point trial like ESPRIT has taken and will take a vast amount of resources in terms of patients, personnel, time, finance etc. to complete. However, to thoroughly investigate whether sc rIL-2 in HIV-1 infection is of clinical benefit, rather than rely on surrogate markers, there is no other way this can be done than in a RCT. On 18th of October 2002, the producer of rIL-2, Chiron Corporation, announced that the "sister" trial to ESPRIT, the SILCAAT study, enrolling patients with $CD4^+$ count > 50 and < 300 cells/µl, would be prematurely terminated for non-scientific reasons ("business decision"). SILCAAT had enrolled 1975 of the stipulated 2000 patients and "only" the expected four years follow-up period remained. On the 14th of January 2003 a principle agreement was reached between the ESPRIT Executive Committee, SILCAAT investigators and Chiron Corp. about continued follow-up of patients in SILCAAT using the network set up for ESPRIT. This example of a company's misconduct of a trial is unprecedented in the HIV-1 field, and emphasises the need for public financing of independent research activities.

Conclusion

- Ritonavir-boosted indinavir and saquinavir as part of HAART regimens result in virological and immunological efficacy at least comparable with other HAART regimens

- Overall, the toxicity profile of ritonavir-boosted indinavir and saquinavir as part of HAART regimens is comparable with that of other HAART regimens

- Ritonavir-boosted indinavir as part of HAART regimens result in more treatment limiting adverse events compared to ritonavir-boosted saquinavir

- Overall, ritonavir-boosted lopinavir and saquinavir as part of HAART regimens result in virological and immunological efficacy, and toxicity at least comparable with that of other HAART regimens

- Enrolment of a lower than expected sample size in the COLATE trial will limit the ability (power) to assess the efficacy and safety of continued lamivudine treatment. However, currently available data indicate that trial remain conclusive

- Predictors of immunological failure after an immunological response during HAART can be done using a large observational cohort, and are different from predictors of virological failure

- A diverse patient population from around the world has been enrolled in ESPRIT. This will enhance the ability to generalise the findings of the trial

- Clinical end point RCTs are necessary to investigate if surrogate markers, e.g. $CD4^+$ count, are valid as predictors of HIV-1 progression of disease and death for new drugs used for the treatment of HIV-1 infection

- Interim analyses of RCTs are important to assess potential detrimental differences between trial groups

- Independent DSMBs are important entities in the assessment of the results of interim analyses in RCTs and in recommending which data should or should not be presented to the public

Perspectives

In the third decade after the discovery of HIV-1 and more than 15 years after the first antiretroviral drug was demonstrated to reduce morbidity and mortality there is still plenty of room for improvement of our understanding of how best to use the antiretroviral armamentarium currently available for the treatment of HIV-1 infection. Several important questions, which are subject to ongoing investigations, have so far only been partially answered or remain unanswered:

- How many and which combinations of drugs and drug classes in a patients initial HAART regimen will provide the best long-term clinical outcome ?

- Which combination of drugs and drug classes should a patient experiencing virological, immunological or clinical failure be switched to ?

- In adherent patients experiencing virological failure, which option should be preferred, continuation, intensification, switch or discontinuation of treatment ?

- Does adjunctive immunostimulatory treatment add to the clinical benefits of HAART

- Should treatment be guided by pVL or $CD4^+$ count ?

It has neither been the intention of the finalised trials and studies included in this thesis nor of the thesis itself to provide answers to the above questions. Even so, data included in this thesis have added to the current knowledge of how best to treat HIV-1 infected patients in accordance with current treatment guidelines and using surrogate end points. Hopefully, data from the MaxCmin2 trial, substudies to the MaxCmin1 & 2 trials, the COLATE trial, and ESPRIT will further supplement this knowledge. Further, data from the EuroSIDA study will hopefully be useful in clinical management and future RCTs.

Clinical treatment practices are much to often driven by *in vitro* data, cross-study comparisons and data from non-randomised trials rather than comparative data. It is, therefore, encouraging to CHIP and other clinical trial networks that well performed investigator-driven RCTs continue to have influence on HIV-1 treatment practices.

However, uniformity in measuring and presenting short-term efficacy and safety data from RCTs is warranted, not only to comply with regulatory requirements for drug applications but also to lend a hand to clinicians when comparing data from the numerous trials presented at conferences and in scientific journals. Data from RCTs with hard end points will determine if pVL and $CD4^+$ count are valid as surrogate markers of disease progression and death during HAART. Due to the low incidence of AIDS-defining diseases and death, end point trials must necessarily be large and have long-term follow-up. Such trials are ideal for international scientific collaboration, but such endeavours can only be undertaken with sufficient public financial support.

Summary

This Ph.D. thesis is based on work performed during my employment as a clinical research associate at the Copenhagen HIV Programme. The primary aim of the thesis was to assess the effects of highly active antiretroviral therapy (HAART) of HIV-1 infection.

There is a need for new treatment modalities with the ability to lower the risk of developing side effects from and increase adherence to HAART, and at the same time maintain or even increase the efficacy of first and subsequent HAART regimens. The MaxCmin1 & 2 trials were the first comparative trials of pharmacologically enhanced (ritonavir-boosted) protease inhibitor treatment. Overall the trials showed virological and immunological efficacy, and safety profiles at least comparable with current HAART regimens. Ritonavir-boosting of indinavir resulted in more treatment-limiting adverse events and more lipid elevations when compared to ritonavir-boosted saquinavir.

The COLATE trial terminated recruitment with a lower than stipulated patient population. The sample population is, however, reasonable large, well matched, and with sufficient follow-up. This is likely to render the trial with an ability to reach a conclusion of whether continued treatment with an antiretroviral drug to which the patient is harbouring resistant virus is of virological benefit.

In the EuroSIDA study predictors of immunological failure after initial response were identified. These predictors were different from established predictors of virological failure thus indicating that HAART affect these markers differently. This underscores the caution that should be taken when translating results of short-term studies of effect markers into long-term clinical efficacy.

ESPRIT has enrolled more than 90 % of its 4000 patients target and is likely to complete enrolment in the spring 2003. Patients in the IL-2 group that have completed the three rIL-2 cycle induction phase have experienced the expected marked $CD4^+$ count increase. The study is expected to answer if this immunological response is of clinical benefit in 5 - 6 years. The size and diversity of the trial population is likely to ensure that the findings of the trial can be generalised to all HIV-1 infected patients.

Danish resumé

Denne Ph.D.-afhandling er udført under min ansættelse som klinisk assistent i Copenhagen HIV Programme (CHIP). Det primære formål med afhandlingen var undersøge effekten og sikkerheden af høj-effektiv antiretroviral kombinationsbehandling (HAART) af HIV-1 infektion. Afhandlingen er baseret på internationale randomiserede kliniske undersøgelser og et observationsstudie.

Der er behov for nye behandlingsmodaliteter, der kan reducere risikoen for udvikling af bivirkninger, øge komplians og bevare eller øge effekten såvel af første som efterfølgende HAART behandlinger. MaxCmin1- og 2-studierne er de første sammenlignende studier af farmakologisk proteasehæmmer-forstærkning. Studierne viste samlet set en virologisk og immunologisk effekt, og en bivirkningsprofil mindst på linie med andre HAART behandlinger. Farmakologisk forstærkning af indinavir medførte flere behandlingsbegrænsende bivirkninger end ved forstærkning af saquinavir, og en mere udtalt forhøjelse af kolesterol og triglycerider. COLATE-studiets inklusion af patienter blev afsluttet med en mindre end forventet studiepopulation, der dog har en acceptabel størrelse, er velbalanceret, og med rimelig opfølgningsrate. Studiet forventes fortsat at kunne afklare om fortsat behandling med et antiretroviralt præparat som patienten huser resistent virus medfører en virologisk fordel.
I EuroSIDA-studiet er prædiktorer for immunologisk svigt efter initial effekt blevet identificeret. Disse adskiller sig fra prædiktorer for virologisk svigt af HAART. Dette indikerer at HAART influerer forskelligt på disse effektmarkører, hvilket understreger at forudsigelser om den kliniske langtidseffekt af HAART på baggrund af korttidsstudier af effekten på disse effektmarkører er behæftet med usikkerhed.
ESPRIT-studiet har indtil videre inkluderet mere en 90 % af de planlagte 4000 HIV-1 smittede patienter og forventes at fuldende denne del af studiet i foråret 2003. Patienter i IL-2 gruppen, der har gennemgået studiets induktionsfase, har samlet set opnået en markant $CD4^+$ tals stigning. Den kliniske effekt af dette immunologiske respons forventes at kunne opgøres om 5 - 6 år. Studiepopulationens størrelse og heterogenitet øger muligheden for at generalisere studiets resultater.

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Article I

A randomised trial to evaluate indinavir/ritonavir versus saquinavir/ritonavir in HIV-1 infected patients: The MaxCmin1 Trial.

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A randomised trial to evaluate indinavir/ritonavir versus saquinavir/ritonavir in HIV-1 infected patients: The MaxCmin1 Trial

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Abstract

The trial assessed the rate of virological failure at 48 weeks in adult HIV-1 infected patients assigned indinavir/ritonavir (IDV/r) 800/100 mg bid vs. saquinavir/ritonavir (SAQ/r) 1000/100 mg bid in an open-label, randomised (1:1), multi-centre, phase IV design. 306 patients initiated the assigned treatment. At 48 weeks virological failure was seen in 43/158 (27 %) and 37/148 (25 %) in the IDV/r and SAQ/r arms respectively. The time to virological failure did not differ between arms (p = 0.76). When switch from randomised treatment was counted as failure this was seen in 78/158 (IDV/r) vs. 51/148 (SAQ/r) (p = 0.009). Switch from the randomised treatment occurred in 64/158 (41 %) patients in the IDV/r vs. 40/148 (27 %) in the SAQ/r arm (p = 0.013). Sixty-four % of the switches were due to adverse events. In conclusion, this first head-to-head comparison of ritonavir-boosted regimens found SAQ/r to have comparable antiretroviral effects to IDV/r in the doses studied. A greater number of treatment-limiting adverse events were observed in the IDV/r arm relative to the SAQ/r arm.

Introduction

Cohort studies have shown that among patients starting on highly active antiretroviral therapy (HAART) and achieving suppression of HIV-1 RNA to levels below detection, the annual rate of virological rebound is 15 % [1,2]. The main reasons for failure of HAART are treatment limiting toxicity, adherence problems, virological failure and low potency of the drugs [3-5]. Other studies have shown long-term viral suppression to be dependent on safety, good adherence and high plasma concentrations of antiretroviral drugs [6-10]. Ritonavir-boosting, i.e. ritonavir in doses of 50 - 200 mg, in combination with other protease inhibitors (PIs) results in higher plasma concentration of these other PIs [11]. This is due to inhibition of the *P450* CYP3A4 enzyme system in the intestine and liver and possibly of P-glycoprotein efflux [12,13]. Other benefits from ritonavir-boosting are a reduction in number of doses from three times daily (tid) to twice daily (bid), less restrictions on food intake and a lower pill burden, which is associated with better adherence. All these factors have been associated with a better treatment outcome [14,15].

IDV/r 800/100 mg bid was among the most commonly used ritonavir-boosted protease inhibitor within antiretroviral regimens in 2000 when this trial was initiated. Switch from the recommended dose of indinavir (800 mg tid) to the ritonavir-boosted bid regimen was driven by relatively poor adherence to the tid regimen and pharmacokinetic data suggesting that the dosing frequency could be diminished and the fasting requirement lifted [16]. However, recently, it was shown that this switch lead to an accelerated risk of treatment-limiting adverse events among patients on a stable regimen that included indinavir 800 mg tid [17].

Relatively extensive studies had been made using SAQ/r, but mainly at a 400/400 mg bid dosing schedule. The SAQ/r 400/400 mg bid regimen is associated with gastro-intestinal adverse events in most subjects [18]. Some concern existed that in SAQ/r at a dose of

1000/100 mg bid, only the saquinavir element could be expected to have virological activity [19], whereas within the dose of 400/400 mg bid regimen, both drugs had virological activity.

Previous comparative studies of antiretroviral therapy (ART) including a ritonavir-boosted regimen have shown a better virological outcome of the ritonavir-boosted regimen (lopinavir/ritonavir versus nelfinavir) [20]. However, in clinical practise it is important to establish if ritonavir-boosted regimens are comparable with regard to efficacy and safety. The MaxCmin1 trial is the first direct comparison of two ritonavir-boosted PI regimens.

Methods

A randomised (1:1), phase IV, open-label, and multi-centre trial involving 28 sites in 13 countries. The trial was conducted in accordance with the Helsinki II Declaration, the *Good Clinical Practice* guidelines (ICH-GCP Guideline (CPMP/ICH/135/95)) and local Institutional Review Boards or Independent Ethics Committees approved the protocol. Patients were assessed for eligibility at a screening visit and provided written informed consent prior to the conduct of any trial specific procedure. Eligible patients were 18 years or older, had documented HIV-1 infection (ELISA), were not pregnant or breastfeeding, and did not have a serious medical condition at the time of screening. Further, all laboratory values had to be without clinical significance as per the treating physician's judgement. A heterogeneous population was enrolled including patients who were either protease inhibitor (PI) naïve, PI-failing or PI-intolerant. PI-experienced patients with prior use of either of the study drugs were not precluded from participation, however, only patients with an equal chance of benefit from and/or risk of development of treatment-related side effects to the two study PIs at time of screening could be randomised. This assessment was made by site physicians and final decision was made by the trial physician at the Copenhagen HIV Programme (CHIP) based on antiretroviral treatment history, prior virological and clinical failure, and available

resistance tests. Prior to randomisation the treating physician decided the concomitant use of at least two nucleoside reverse transcriptase inhibitors (NRTIs) and/or non-NRTIs (NNRTIs). Computerised block randomisation was done at CHIP. The randomisation was stratified according to geographical region of site and viral load. The countries were grouped in the following regions: South America (Argentina, *Brazil)*, North America (USA), Scandinavia (Denmark, Norway, *Sweden*), Central Europe (Germany, Switzerland, Austria), North-West Europe (Belgium, *France,* United Kingdom, the Netherlands) and Southern Europe (*Greece*, Italy, Portugal, Spain). Sites from countries shown in italics did not enrol patients. In the statistical analysis, the USA (3 patients) was grouped with North-West Europe.

Randomised patients, irrespective of whether they started on or switched from the assigned treatment, were followed up at baseline (first day of intake of assigned treatment), Week 4, 12, 24, 36, and 48. During follow-up visits the following procedures were performed: clinical evaluation, safety analyses (haemoglobin, white blood cell count, lymphocytes, platelets, creatinine, AST and/or ALT, bilirubin, amylase), and viral load and CD4 count measurements. At baseline, at Week 4 and 48 fasting total cholesterol, low-density lipoprotein (LDL) cholesterol and total triglyceride levels were measured. A case report form (CRF) was completed for each study visit and faxed to CHIP where real-time monitoring was performed by trained monitors (licensed nurses). In addition, CHIP monitors performed on-site monitoring at least twice at all participating sites.

Patients randomised to receive SAQ/r were allowed to change from the saquinavir soft gel formulation (Fortovase®) to the hard gel formulation (Invirase®) without this being considered switch from the assigned treatment. During the trial modification of the randomised treatment was allowed in case of virological failure or treatment-limiting

toxicities. If available, dose-reduction was performed based on therapeutic drug monitoring (TDM). Of note, patients experiencing virological failure according to the protocol's definition were allowed to continue on the assigned treatment at the discretion of the treating physician.

Definition of virological, immunological and clinical failure

Virological failure: For patients entering the study with a viral load (VL) of < 200 c/ml, virological failure was a HIV-1 RNA ≥ 200 c/ml. For patients entering the study with a VL ≥ 200 c/ml, virological failure was defined as any rise in HIV-1 RNA of ≥ 0.5 logs and/or a VL of ≥ 50,000 c/ml at the Week 4 visit, ≥ 5,000 c/ml at the Week 12 visit, or ≥ 200 c/ml at the Week 24 visit or thereafter. All cases of suspected virological failure were confirmed by a second VL determination performed at after 2 or more weeks. Once reconfirmed, the time of virological failure was defined as the time of the first measurement that met the failure criteria.

Immunological failure: Immunological failure was defined as a decrease in the CD4 count of more than 50% compared with baseline, providing that the baseline CD4 count was more than 150 cells/µl. For patients with baseline CD4 count in the range of 100-150 cells/µl, immunological failure was a CD4 count < 50 cells/µl and for patients with baseline CD4 count < 100 cells/µl, a CD4 count < 25 cells/µl. All cases of suspected immunological failure was confirmed by a second CD4 count measurement performed at least 1 week later. Once reconfirmed, the time of immunological failure was defined as the time of the first measurement that met the failure criteria.

Clinical failure: Clinical failure was defined as development of a new AIDS-defining disease or relapse of a previously successfully treated AIDS-defining disease.

Power calculation and statistics

The trial was powered to show equivalence between the study arms with 80% chance that the 95% confidence interval for the difference in virological failure rates would exclude a difference greater than 15% in either direction. This was based on a sample size of 150 per arm and an underlying failure rate of 20 % in both arms.

Per protocol the primary population for analysis was the intention-to-treat /exposed (ITT/e) population including all randomised patients that had taken at least one dose of the assigned treatment. This analysis is also termed “ITT switch included” analysis. Further, the protocol stipulated ITT/e analyses where switch from the assigned treatment constituted failure (ITT/e/s; ITT/e/switch = failure). In both analyses, patients who withdrew consent, were lost to follow-up or died constituted failure, and the time of failure was the time of event (whichever came first). Some patients withdrew their consent during follow-up but permitted reporting of laboratory data measured as part of their routine care. For these patients, withdrawn consent did not constitute (virological) failure. Exploratory on-treatment efficacy and toxicity analyses were performed in accordance with CPMP guidelines regarding analysis of equivalence trials [21]. ITT analysis including all patients was done for the primary efficacy analysis based on recommendations from the Data Safety and Monitoring Board (DSMB).

All statistical analyses were performed using STATA software (StataCorp. 2001. Stata Statistical Software: Version 7, College Station, Texas, USA). Chi-square test and Fisher’s exact test were used for the comparison of categorical variables between treatment arms. Continuous variables were analysed using Student’s *t*-tests and Kruskall-Wallis test depending on the distribution. Cox analysis was performed and Kaplan-Meier plots were produced for the “time to event” analyses containing sufficient numbers (n > 25). Multivariate

models were developed to identify possible independent predictors of failure and development of AEs. For the Week 24 interim analysis presented to the DSMB, the Peto method of repeated significance testing was used to test for treatment difference with a p-value of 0.001 as the significance level, giving a significance level of 0.05 (two-sided) for the final Week 48 analysis.

Role of sponsor

CHIP developed the protocol and served as sponsor of the trial. Roche Pharmaceuticals Ltd. provided financial support for the conduct of the trial. The conditions for this support were outlined in a contract between the two parties. Among other issues, this contract stipulated that the database will remain at CHIP at all times, and only analyses approved by the trial Steering Committee (see appendix) were to be conducted and such analysis would be performed by CHIP. Furthermore, the contract stipulates that Roche cannot veto the public presentation of any results from the trial.

Results

Baseline parameters & follow-up

From September 2000 to March 2001, 317 patients were enrolled, of whom 306 initiated the randomised treatment. More patients in the SAQ/r arm than in the IDV/r arm did not initiate the assigned treatment, 10 versus 1. Of the 10, 4 knew and 4 did not know the result of the randomisation, 1 was given the wrong treatment, and for 1 this information was not available. Patients who did not initiate the assigned treatment had lower VL and higher CD4 cell count compared to patients who initiated the assigned treatment (data not shown).

No differences were observed at baseline in medical history, demographic, clinical and laboratory parameters or in exposure to ART prior to baseline (Table 1). Patients were primarily white (84 %), males (78 %) with homo/bisexual risk behaviour (49 %) and a median age of 39 years. Median CD4 nadir was 110 cells/µl (IQR 40-205), CD4 count 277 cells/µl (IQR 137-450), VL 3.9 $\log_{10}$ (IQR 1.7-5.1), 39 % had a baseline viral load < 400 c/ml, and 30 % had had a prior clinical AIDS-defining event. At enrolment, 25 % of patients were ART naïve, 14 % were ART experienced but PI-naïve and 61 % were PI-experienced.

The disposition of patients at Week 48 is shown in Table 2. Complete Week 48 follow-up data was available for 285 of the 306 (93 %) patients who initiated the assigned treatment, of which 202 (66 %) remained on the assigned treatment. No difference was seen between the two study arms in the rate of patients lost to follow-up (7 %). The 104 patients, who prematurely switched from the assigned treatment, did so primarily due to clinical non-fatal AEs (n = 67). Among the 104 patients, no significant differences at the 0.05 level were observed between the study arms in the proportion of patients who switched to or at Week 48 received a mono or dual PI-, NNRTI or abacavir-based HAART regimen or who went off treatment for any reason. Nine patients switched from IDV/r to SAQ/r and 4 patients switched from SAQ/r to IDV/r. There was a significantly higher percentage of patients in the IDV/r arm (41 %) than in the SAQ/r arm (27 %) who prematurely switched from the assigned treatment ($p = 0.013$, Chi-squared). This difference was driven by patients who discontinued the randomised treatment due to a non-fatal clinical adverse event, 28 % of patients assigned to IDV/r arm versus 15 % in the SAQ/r arm ($p = 0.004$, Chi-squared). Of the non-fatal clinical AEs leading to switch from the assigned treatment, 66 % were of grade 1 or 2. More renal, skin & hair and gastro-intestinal AEs were observed in the IDV/r arm (data not shown). Twenty-two patients reduced the dose of the assigned treatment during follow-up, 21 in the IDV/r arm and 1 in the SAQ/r arm.

Virological, immunological & clinical outcome

The primary efficacy outcome, rate of virological failure, was seen in 77/306 (25 %) patients, with no difference between the study arms (log rank test: p = 0.76; Figure 1a). The median VL at time of failure was 2,279 c/ml, slightly higher in the IDV/r arm than in the SAQ/r arm, 3,857 and 881 c/ml, respectively (p = 0.40). The difference in the proportion of subjects failing in the two arms was 2.2 % (95 % CI: -2.8 %, 7.2 %) with a higher proportion of protocol defined virological failures in the IDV/r arm. Using a Farrington-Manning equivalence test we found sufficient evidence at the 5 % level of significance claim that the difference in success rates between the 2 treatments is less than 15 % (p < 0.0048).

The higher discontinuation rate in the IDV/r arm resulted in a significantly higher virological failure rate in the SAQ/r arm in the ITT/e/s analysis (log rank test: p = 0.009; Figure 1b). No difference was seen between the study arms in the on-treatment analysis (log rank test: p = 0.24). In the adjusted multivariate Cox models patients with a baseline VL ≥ 400 c/ml had a higher hazard ratio of experiencing virological failure in the ITT/e, ITT/e/s and on treatment analyses (p < 0.001 for all comparisons), whereas antiretroviral naivity and PI naivity failed to independently predict the risk of virological failure. Importantly, the hazard ratio for the comparison of IDV/r versus SAQ/r was not affected by adjusting for other risk factors. Similar trends were found when all randomised patients (ITT population, N = 317) were included in the analyses rather than the ITT/e population (N = 306)(data not shown).

Figure 2 shows the proportion of patients with a plasma VL < 50 c/ml during follow-up using different analytic approaches. At Week 48 203/306 (68 %), 155/306 (51 %) and 186/201 (93 %) had a VL < 50 c/ml in the ITT/e, ITT/e/s and on-treatment analysis, respectively. Only when switch from the assigned treatment was counted as having a VL > 50 c/ml (ITT/e/s)

was a significant difference observed with more patients having a suppressed VL in the SAQ/r arm at Week 48.

Only six patients experienced immunological failure, four in the IDV/r and two in the SAQ/r arm. A rise of ≥ 100 CD4 cells/µl at any time during follow-up was seen in 181 patients at a median of 98 days. There was no significant difference between the study arms in the number of patients (chi-square, p = 0.29) or time to ≥ 100 CD4 cell/µl increase (log rank, p = 0.47). PI-naïve patients were more likely to experience an increase of ≥ 100 cells/µl than PI-experienced patients (relative hazard = 0.50, 95 % CI: 0.4 - 0.7, p < 0.0001).

Clinical failure, including deaths, was seen in 23 patients after a median of 80 days, 13 CDC category B, 7 category C and 3 deaths, of these 14 (4 CDC category C and 1 death) were observed in the IDV/r arm and 9 (3 CDC category C and 2 deaths) in the SAQ/r arm. The low number of events precluded formal statistical analysis. In none of the fatal cases was the death directly related to the assigned treatment, the patient in the IDV/r arm died from Castleman's disease and in the SAQ/r arm death was due to *Pneumocystis carinii* pneumonia in one and hepatitis C end stage liver failure in one patient.

Adverse events

Of the patients exposed to the study medication 100/306 (33 %) experienced at least one adverse event of grade 3 and/or 4, 65 (41 %) in the IDV/r arm versus 35 (24 %) in the SAQ/r arm (p = 0.001, Chi-squared). Of these, the treating physician assessed the relationship to the assigned treatment as being at least possible in 46 (29 %) in the IDV/r arm versus 19 (13 %) in the SAQ/r arm (p = 0.001, Chi-squared). There was a significant difference in the distribution by organ system of AEs grade 3 and 4 between the two study groups with a

higher number of renal, dermatological and gastro-intestinal side effects in the IDV/r arm (data not shown).

Laboratory results

The median fasting baseline lipid values were 4.7, 3.1, 1.6 and 4.8, 3.2, 1.7 mmol/l for total cholesterol (normal range 3.4 - 6.2 mmol/l), LDL-cholesterol (normal range 1.7 - 3.2 mmol/l) and total triglyceride (normal range 0.5 - 2.3 mmol/l) in the IDV/r and SAQ/r arms, respectively. The median percentage change from baseline in fasting total cholesterol, LDL-cholesterol and total triglyceride is shown in Figure 3. Significantly larger lipid elevations were seen in the IDV/r arm compared to the SAQ/r arm at Week 4 and 48 (ITT/e). These differences were even more pronounced when the actual median changes from baseline were considered rather than the median percentage change (data not shown). Similar differences were seen when restricting the analysis to patients that remained on their trial medication (data not shown).

No difference between the study groups was seen in haematological, renal or hepatic laboratory parameters except for bilirubin that were 10 and 11 µmol/l at baseline in the IDV/r arm and the SAQ/r arm, respectively (normal range 4 - 22 µmol/l). In the SAQ/r arm the bilirubin level did not change over time whereas in the IDV/r arm it increased to 20 µmol/l at Week 4 followed by a decline to 15 µmol/l at Week 48.

Discussion

The MaxCmin1 trial was designed in the early part of year 2000 to assess if equivalence exist in efficacy and safety between IDV/r 800/100 mg bid and SAQ/r 1000/100 mg bid, in combination with at least two non-PI drugs, the primary outcome being incidence of protocol-defined virological failure. Equivalence was observed for efficacy whereas IDV/r lead to an increased risk of treatment-limiting adverse events and adverse events of grade 3 and/or 4.

As a consequence of the safety profile of IDV/r, fewer patients remained on this treatment through 48 weeks leading to differences in the efficacy analyses, where continuation on study medication influence the outcome. Additionally, IDV/r was found to cause a higher risk of elevating blood levels of lipids and bilirubin.

The heterogeneous study population included introduces a serious limitation, as the trial would not have sufficient power to describe the outcome within each of the subgroups included, if the outcomes of the treatments were affected by the stage of HIV-infection/treatment. To address this limitation, multivariate models of the key efficacy outcomes were developed. Importantly, subjects entering the trial with a viral load > 400 c/ml had a significantly increased risk of experiencing protocol-defined virological failure and not achieving virological suppression (< 50 / < 400 c/ml) at Week 48 compared to subjects being virologically suppressed at baseline. However, being antiretroviral naïve or being PI naïve at the time of enrolment did not independently affect the risk of a poor virological outcome. The hazards ratios for the comparison of virological failure in the IDV/r versus SAQ/r arm were comparable in univariate and multivariate models adjusting for other variables. In order to further elucidate if baseline characteristics that may have influenced the efficacy outcomes two substudies are currently investigating genotypic resistance mutations at baseline and time of virological failure, and single nucleotide polymorphisms in the multi drug resistance 1 (MDR-1) locus of stored peripheral blood mononuclear cells. In addition, one substudy is investigating efficacy and safety in relation to trough levels (Cmin) of the study PIs at Week 4 and 48.

In the analysis where switch from the assigned treatment is equal to virological failure or lack of virological suppression, SAQ/r tended to have a superior virological activity compared to IDV/r. This was to be expected as a higher proportion of subjects in the IDV/r switched from the randomised treatment. The trial was not designed and did not have the statistical power

to test whether there were differences in risk of protocol-defined immunological and clinical failures between the two study arms. No formal statistical analysis of these efficacy parameters was appropriate due to the low number of such failures observed.

Finally, the efficacy and safety outcome of subjects randomised to receive IDV/r is comparable with data from the recently completed BEST trial [17]. The BEST trial randomised subjects on a stable regimen including IDV 800 mg tid, to either continue this regimen or switch to IDV/r 800/100 mg bid. In the present trial few patients were on IDV tid at time of screening hence the patients who received IDV/r in the two trials are not directly comparable.

In the present trial, 21/158 subjects (13%) in the IDV/r arm reduced the IDV dose. The present trial was not designed to evaluate whether this strategy lowered the risk of adverse events or affected the efficacy of the treatment nor was the sample sufficiently large for formal testing of these important questions. A randomised trial should be done to evaluate whether IDV/r in lower dosing has a more favourable adverse event profile and maintained virological efficacy compared to either IDV/r 800/100 mg bid or other commonly used ritonavir boosted PI regimens prior to the introduction of other IDV/r dosing regimens in routine care.

Compared to the SAQ/r arm subjects in the IDV/r arm had significant increases from baseline in total cholesterol, LDL-cholesterol and triglycerides at Week 4 and 48. Other drugs (NNRTIs and stavudine) that could potentially influence these parameters were well balanced between the two groups at baseline. Therefore, these findings suggest that IDV/r relative to SAQ/r affects the lipid metabolism adversely. Since the same ritonavir dosing was used in both arms, it is likely that the indinavir component that causes the lipids to increase.

However, another possibility is that the ritonavir metabolism is affected differently by indinavir compared with saquinavir. These mechanisms will be explored further by correlating drug levels at Week 4 and 48 with lipid changes. Differences between PI's boosted by the same ritonavir dosing has not previously been observed, whereas it was recently reported that lopinavir/ritonavir lead to more lipid elevation compared to nelfinavir [20].

In conclusion we found that in this open label study of a heterogeneous patient population - reflecting the real life situation - SAQ/r has comparable antiretroviral effects to IDV/r in the doses studied. We observed more treatment-limiting adverse events in the IDV/r arm relative to the SAQ/r arm, and found more patients in the SAQ/r arm remained virologically suppressed on study drug at Week 48 - probably because of a better toxicity profile.

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Appendix A

INVESTIGATORS OF THE MAXCMIN1 TRIAL GROUP

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Appendix A

DATA SAFETY & MONITORING BOARD (DSMB)

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Karoline B. Jensen (trial responsible monitor)

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Table 1

Baseline parameter		IDV/r N=158	SAQ/r N=148	Total N=306
Gender	**(no. male (%))**	117 (74)	122 (82)	239 (78)
Age	**(median (IQR*))**	40 (34-46)	39 (34-48)	39 (34-47)
HIV exposure group	**(no. (%))**			
Homosexual/bisexual		74 (47)	76 (51)	150 (49)
IVDU		16 (10)	19 (13)	35 (11)
Haemophiliac		6 (4)	2 (1)	8 (3)
Transfusion		0 (0)	4 (3)	4 (1)
Heterosexual		55 (35)	47 (32)	102 (33)
Unknown		7 (4)	0 (0)	7 (2)
Race	**(No., %)**			
White		129 (82)	127 (86)	256 (84)
Black		19 (12)	14 (9)	33 (11)
Asian		6 (4)	1 (1)	7 (2)
Other		4 (3)	6 (4)	10 (3)
CDC, category C	**(no. (%))**	45 (28)	48 (32)	93 (30)
PI-naïve	**(no. (%))**	59 (38)	61 (41)	120 (39)
PI-experienced				
Failure	**(VL ³ 400 c/ml; no. (%))**	39 (25)	35 (24)	74 (25)
Intolerance	**(VL < 400 c/ml; no. (%))**	59 (38)	52 (35)	111 (36)
HIV-1 RNA	**(c/ml $\log_{10}$ (IQR*))**	3.9 (1.7-5.2)	4.0 (1.7-5.1)	3.9 (1.7-5.1)
HIV-1 RNA < 400 c/ml	**(no. (%))**	62 (39)	56 (38)	118 (39)
CD4 count	**(10^6/l; median (IQR*))**	280 (139-453)	272 (135-420)	277 (137-450)
CD4+ nadir count	**(10^6/l; median (IQR*))**	119 (47-225)	107 (33-195)	110 (40-205)

*) IQR = Inter quartile range

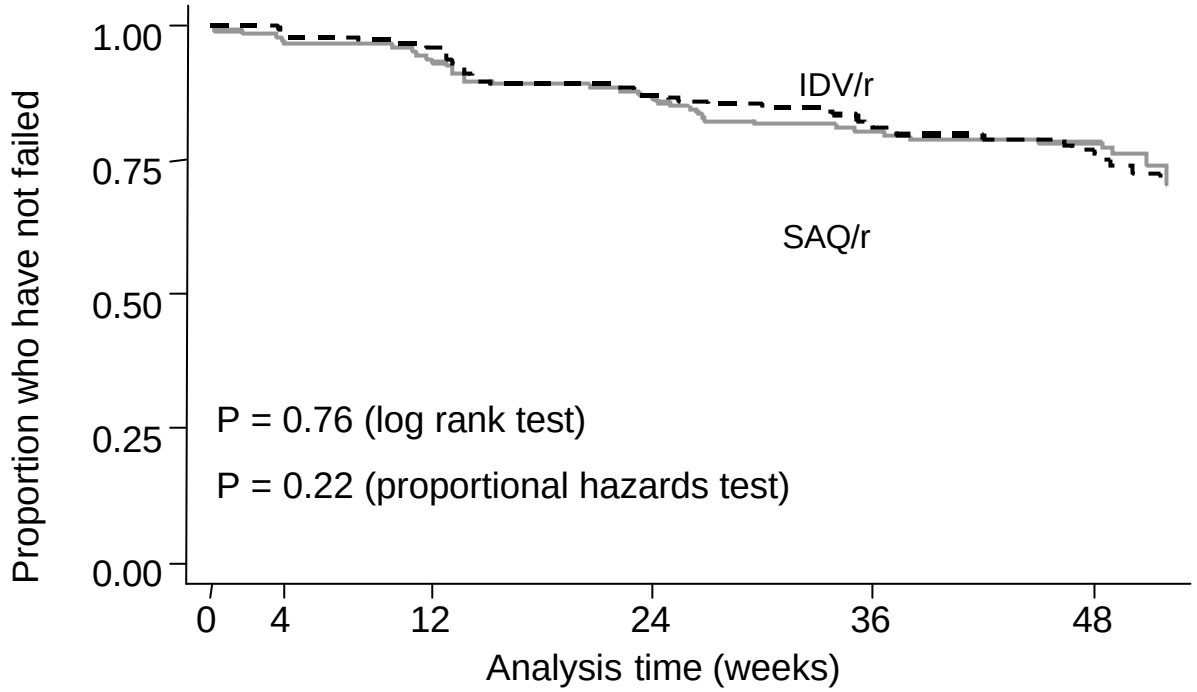
Table 2

Status	IDV/r No. (%)	SAQ/r No. (%)	Total No. (%)
Randomised	159	158	317
Initiated assigned Tx.*	158 (99)	148 (94)	306 (97)
Never initiated assigned Tx.*	1 (1)	10 (6)	11 (3)
Permanently switched from assigned Tx.*	64 (41)	40 (27)	104 (34)
Reason			
Virological failure	3 (5)	2 (5)	5 (5)
Death	1 (2)	1 (3)	2 (2)
Clinical non-fatal AE	45 (70)	22 (55)	67 (64)
Laboratory AE	4 (6)	2 (5)	6 (6)
Patient choice	3 (5)	5 (13)	8 (8)
Lost to follow-up	5 (8)	3 (8)	8 (8)
Other	3 (5)	5 (13)	8 (8)
Completed 48 weeks of assigned Tx.*	94 (59)	108 (73)	202 (66)
Patients with an outcome at Week 48	148 (94)	137 (93)	285 (93)

*) Assigned Tx. = the PI treatment patients were randomised to receive

Figure 1a

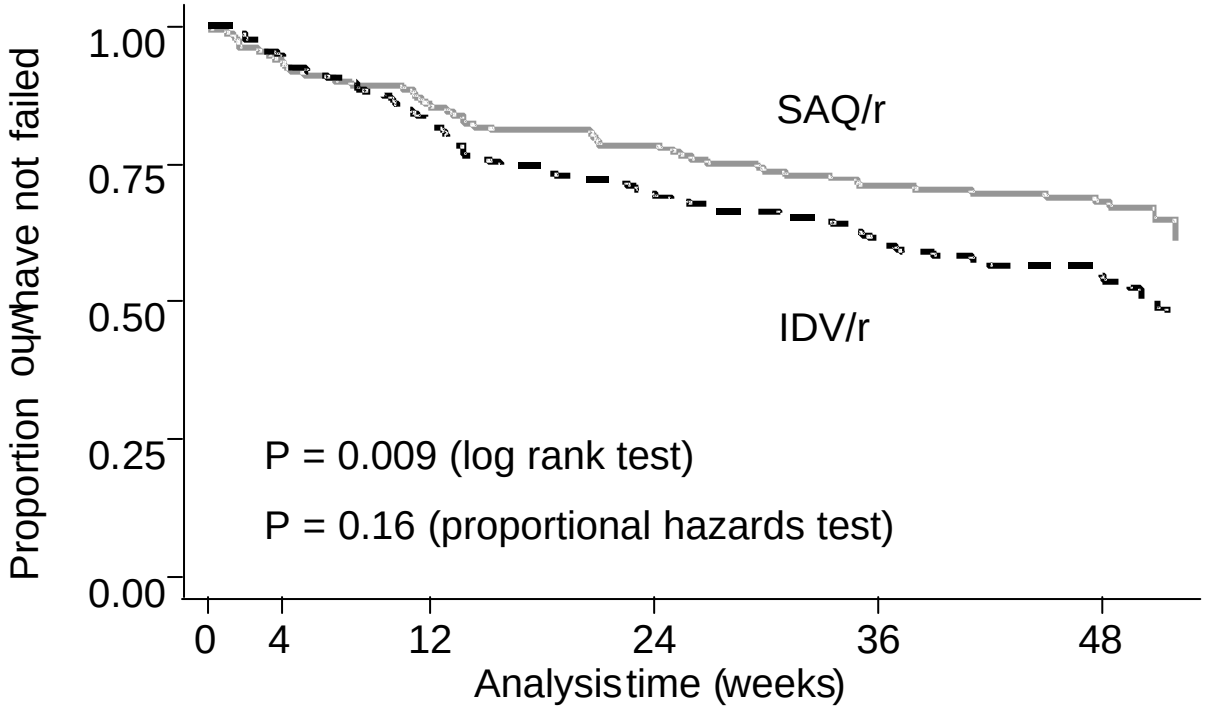
Virological failure - ITT/exposed



IDV/r, N =	**158**	**157**	**150**	**136**	**128**	**88**
SAQ/r, N =	**148**	**144**	**139**	**127**	**117**	**90**

Figure 1b

Virological failure - ITT/e/switch = failure



IDV/r	**158**	**150**	**1290**	**109**	**97**	**60**
SAQ/r	**148**	**139**	**127**	**116**	**104**	**82**

Figure 2

Proportion of patients with VL < 50 c/ml (ITT/e [si], ITT/e/s [s= f] and on treatment [OT] analyses)

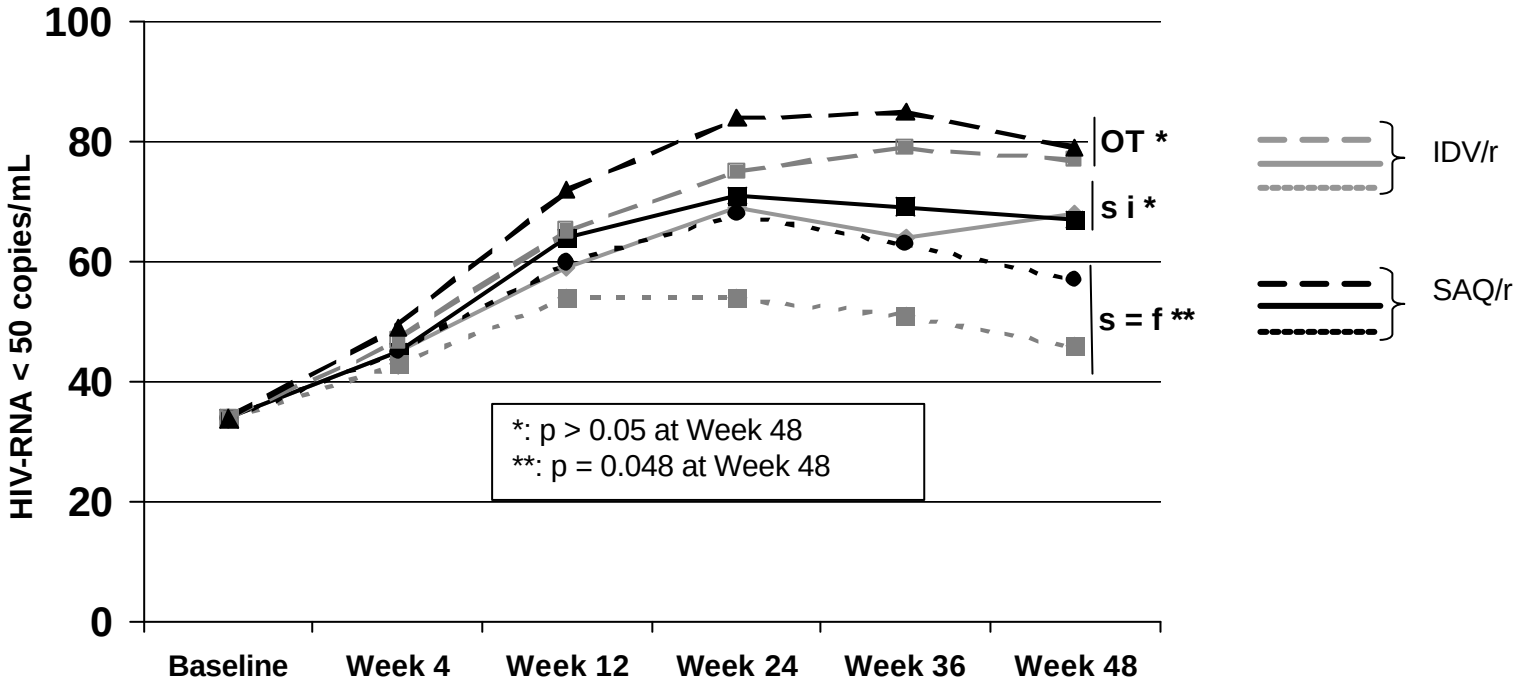
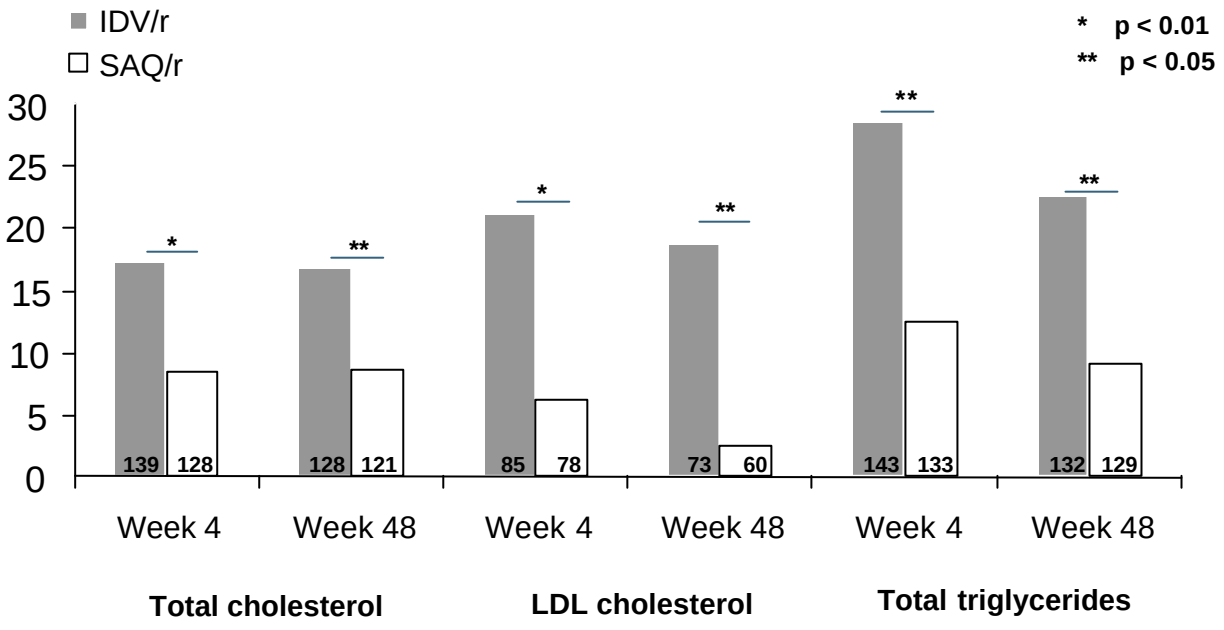


Figure 3

Median % change from Baseline in fasting lipid levels (ITT/e)



(Indication in bars of number of measurements (patients) at each time point)

Article II

Predictors of immunological failure after initial response to HAART in HIV-1 infected adults: a EuroSIDA study.

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Predictors of immunological failure after initial response to HAART in HIV-1 infected adults: a EuroSIDA study

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Abstract

BACKGROUND: The duration of benefit of antiretroviral combination treatment (HAART) is poorly understood. Factors that determine the duration of immunological response are poorly defined.

OBJECTIVE: To investigate factors predictive of immunological failure after initial $CD4^+$ response.

DESIGN: Data was from the EuroSIDA, a prospective, international, observational HIV-1 cohort.

RESULTS: 2347 patients had a $CD4^+$ increase of $\geq 100/mm^3$ within 6-12 months of initiating HAART. Among these, 515 (22 %) subsequently experienced immunological failure, defined as a loss in $CD4^+$ count to or below the pre-HAART value. The rate of failure decreased significantly over time ($p < 0.0001$) with incidences of 11.9 per 100 PYFU (95 % CI 10.4 - 13.4) in the first 12 months and 5.1 per 100 PYFU (95% CI 3.9 - 6.3) at 24 - 36 months after initial immunological response. In a multivariate Cox model the factors found to be independently predicting immunological failure were: pre-HAART $CD4^+$ count per 50 % higher (RH 2.11, 95 % CI 1.87 - 2.37, $p < 0.0001$), time-updated viral load per 1 $\log_{10}$ higher (RH 1.74, 95 % CI 1.61 - 1.89, $p < 0.0001$), and risk group being intravenous drug use vs. homo-/bisexual (RH 1.55, 95 % CI 1.19 - 2.02, $p = 0.0011$).

CONCLUSION: The risk of immunological failure in patients with immunological response to HAART diminishes with more extended time on treatment. Immunological failure was associated with pre-treatment $CD4^+$ level, the rate of ongoing viral replication and intravenous drug use.

Introduction

In patients infected with HIV-1 the primary treatment goal of highly active antiretroviral therapy (HAART) is to suppress the HIV-1 RNA level in plasma (plasma viral load, pVL) to below the level of detection [1,2]. In both cohort studies and randomised clinical trials (RCTs) lower pVL has been associated with reduction in HIV-1 related morbidity and mortality [3-5]. Recent cohort studies and RCTs do, however, suggest that baseline and time updated $CD4^+$ counts are better predictors of HIV-1 disease progression than pVL [6-8].

Discordant virological and immunological responses have been observed during HAART [9-11]. Similarly, virological and immunological failure has been observed to occur independently during HAART [12]. This suggests a complex interaction between the virological response to HAART and the resulting change in the $CD4^+$ count. In a previous study from the EuroSIDA cohort older age and being antiretroviral (ART) naïve was identified as predictors of a sustained virological response during HAART [13]. Similarly, younger age, being ART-naïve, and lower baseline pVL and $CD4^+$ count have been shown to be independent predictors of virological failure in the APROCO cohort [14]. Identification of predictors of immunological failure after initial response to HAART, which may be different from predictors of a virological failure, may have implications on patient management. To our knowledge such data have not previously been reported from a large international HIV-1 cohort.

Methods

PATIENTS

The EuroSIDA study is a prospective observational study of HIV-1 infected patients from 70 centres in 27 countries across Europe including Israel, and Argentina (see appendix). For analysis countries have been grouped into the following regions: Central (Austria, Belgium,

France, Germany (South), Luxembourg, Switzerland), East (Hungary, Poland, Czech Republic), North (Denmark, Finland, Germany (North), Ireland, Norway, Sweden, The Netherlands, UK), South (Greece, Israel, Italy, Portugal, Spain), and Argentina. Details of the study have previously been published [15]. In brief, centres provided data on consecutive patients seen in the outpatient clinic from 2nd of May 1994 until a predefined number of patients was enrolled from each centre. This cohort of 3116 patients was defined as the EuroSIDA I cohort. In December of 1995 enrolment of EuroSIDA II Cohort of began (N = 1365). In April 1997 a further 2839 patients were recruited (EuroSIDA III Cohort). EuroSIDA IV Cohort, consisting of 1225 patients, was enrolled from April 1999, and EuroSIDA V Cohort, including 1256 patients, was recruited from September 2001. For Cohort I-III, eligible patients were those with a $CD4^+$ count of < 500 cells/l in the previous four months, a scheduled clinic appointment and age older than 16 years at the time of enrolment. The $CD4^+$ count restriction was removed for Cohorts IV and V. Information was provided on a standardised data collection form at baseline and every six months thereafter. Follow-up is to Autumn 2002 with information from up to 16 forms available for Cohort I, 11 for Cohort II, 8 for Cohort III, 5 for Cohort IV and 1 for Cohort V. At each follow-up visit, details on all $CD4^+$ counts measured since last follow-up and pVL measurements were collected. For each patient, the date of starting and stopping each antiretroviral drug was recorded. Dates of diagnosis of all AIDS-defining diseases have also been recorded, including those diagnoses made subsequent to the initial diagnosis, using the 1993 clinical definition of AIDS from the Centers for Disease Control and Prevention [16]. An extensive quality assurance programme has been established including data control at the coordinating centre as well as site visits to check patient selection and to perform source verification. Information used in this analysis included demographic (date of birth, ethnic origin, gender, country of origin, risk group) and clinical factors (haemoglobin, $CD4^+$ count, pVL, start date of each antiretroviral therapy, dates and type of AIDS-defining diseases).

Patients were included in the analysis if they initiated HAART and had at least one $CD4^+$ count within 6 months prior to starting HAART, i.e. any combination of ≥ 3 antiretroviral drugs including at least one protease inhibitor (PI), one non-nucleoside reverse transcriptase inhibitor (NNRTI) or abacavir. The last available $CD4^+$ count before staring HAART was termed the "pre-HAART $CD4^+$ count". Patients with an increase from the pre-HAART $CD4^+$ count of at least 100 cell/µl between 6 and 12 months after initiation of HAART were identified (immunological responders). Patients without this $CD4^+$ increase were excluded from further analysis (non-responders). The level and date of the first $CD4^+$ count that was ≥ 100 cell/µl higher than the pre-HAART $CD4^+$ count was chosen as the baseline $CD4^+$ count and date. Patients were followed from baseline until they experienced immunological failure or until the last recorded $CD4^+$ count, if they did not fail immunologically. Patients whose $CD4^+$ count dropped to or below the pre-HAART $CD4^+$ count were considered immunological failures in the analyses. To qualify as an immunological response or failure only one $CD4^+$ count measurement that met the failure criteria was necessary.

STATISTICAL METHODS

Changes in CD4+ count and pVL between starting HAART and baseline were estimated using linear interpolation and expressed relative to pre-HAART levels. After this date, changes in the markers were estimated relative to baseline at monthly intervals using linear interpolation.

Kaplan Meier survival curves were used to describe the median time to modification of therapy or to immunological failure. Person-years of follow-up and Poisson regression were used to determine if the rate of immunological failure changed over time. Cox proportional hazards models were used to determine the factors associated with immunological failure; all Cox models were stratified by centre. Variables fitted in Cox models included all available

demographic variables, previous AIDS diagnosis, and date of starting HAART. Antiretroviral treatment history included ARV naïve, number of ARVs in regimen, total number of prior ARVs, HAART regimen started, number of new ARVs started etc. The models were also adjusted for immunological and virological factors, including $CD4^+$ nadir, $CD4^+$ delta, pre-HAART $CD4^+$ count, time from $CD4^+$ nadir, change in $CD4^+$ count and pVL (included as a time-dependent covariate). Variables that were not significant in univariate analyses ($p > 0.10$) were excluded from multivariate analyses. Various additional analyses were performed to determine the sensitivity of the results to small changes in the assumptions.

All analyses were performed using Statistical Analysis Software (version 6.12); all significance tests were two-sided.

Results

As of autumn 2002, the EuroSIDA database contained follow-up data on 9803 patients of which 6522 had initiated HAART. A $CD4^+$ count obtained in the six months prior to initiation of HAART (pre-HAART $CD4^+$ count) was available from 5516 patients. Of these, 2347 patients experienced a $CD4^+$ count increase of ≥ 100 cells/µl between 6 and 12 months after initiation of HAART (baseline $CD4^+$ count) thus were eligible for this analysis (Figure 1).

PATIENT CHARACTERISTICS

Eighty-five percent of patients were Caucasians, 78 % were men, 48 % had male homosexual HIV-1 risk behaviour, and the median age was 37.5 years (inter-quartile range (IQR) 32.8 – 44.8) (Table 1). Among 629 patients who had an AIDS diagnosis at or before baseline, 416 patients (66 %) had one new AIDS-defining disease, 145 had 2 (23 %) and 68 patients had 3 or more (11 %). The median pre-HAART $CD4^+$ count was 200 cells/µl (IQR 82 - 317 cells/µl), and the median pVL was 4.54 $\log_{10}$ (IQR 3.75 - 5.18 $\log_{10}$). The median time

between the pre-HAART $CD4^+$ count and starting HAART was 1 month (IQR 0 – 2 months). Most patients, 83 %, initiated a PI-based HAART regimen, and 11 % initiated a NNRTI-based HAART regimen. Six percent initiated a mixed regimen of which only 34 patients (1 % of total) started a triple-nucleoside regimen containing abacavir.

RESPONSE TO HAART

The median pVL dropped by approximately 2 $\log_{10}$ within 4 months of initiation of HAART and dropped a further 0.4 $\log_{10}$ between month 4 and 12. Four and 12 months after initiation of HAART the median $CD4^+$ count had increased by 110 cells/µl and 160 cells/µl, respectively (Figure 2). After baseline, the pVL remained very stable, with little change from baseline. In contrast, the CD4+ count initially decreased by a small amount and then slowly began to increase again. The median $CD4^+$ count increase from pre-HAART to baseline $CD4^+$ count was 170 cells/µl (IQR 130 – 240). Significant differences in $CD4^+$ count increase from the pre-HAART level to baseline according to patient characteristics were seen, of which the most pronounced was the difference between patients with $CD4^+$ nadir < 50 versus ≥ 250 cells/µl who had increases of 152 and 202 cells/µl, respectively ($p < 0.0001$, Wilcoxon test).

The median baseline pVL was 2.60 $\log_{10}$ (IQR 1.70 - 3.00 $\log_{10}$), the median baseline $CD4^+$ count was 390 cells/µl (IQR 256 - 543 cells/µl) and the median $CD4^+$ nadir was 150 cells/µl (IQR 54 - 251 cells/µl). The median delta $CD4^+$ count (i.e., baseline $CD4^+$ count minus nadir $CD4^+$) was 216 cells/µl (IQR 152 - 310); this variable was very strongly correlated with the baseline $CD4^+$ count (correlation coefficient 0.94, $p < 0.00001$). The median time between the $CD4^+$ nadir and starting HAART was 4 months (IQR 1 - 14 months), and the median follow-up time from baseline was 38 months (IQR 16 - 52 months), with a total of 6714 person-years of follow-up (PYFU).

MODIFICATION TO HAART

Modification to the HAART regimen during follow-up was seen in 1744 (74 %) of patients. Between initiation of HAART and baseline, 551 (24 %) started at least one new drug, and a further 548 (23 %) patients started at least one new drug after baseline. In the same follow-up periods, 495 (21 %) and 658 (28 %) stopped taking at least one drug. The median time to any modification of HAART was 18 months (IQR 17 - 19 months), and was significantly shorter among patients with both lower baseline $CD4^+$ count ($p < 0.0001$, log rank test) and patients with higher pVL ($p < 0.0001$, log rank test).

IMMUNOLOGICAL FAILURE

Of the 2347 immunological responders, 515 (22 %) subsequently experienced immunological failure, i.e. had at least one $CD4^+$ count that was less than or equal to the pre-HAART $CD4^+$ count. At 12 months after initial immunological success, 11.3% of patients were estimated to have failed immunologically (95% CI 10.0 - 12.6, Kaplan-Meier estimate). In addition, there was a gradual and significant decrease in the rate of immunological failure over time (Table 2). For example, in the first 12 months after the initial response to HAART, the incidence of failure was 11.9 per 100 PYFU (95 % CI 10.4 - 13.4); this decreased to 5.1 per 100 PYFU at 24 - 36 months after initial immunological response (95% CI 3.9 - 6.3). The test for trend over time showed a 31 % decrease in the rate of immunological failure with each additional year since initial immunological success (95 % CI 25 - 37 %, $p < 0.0001$, Poisson regression). The median $CD4^+$ count at the time of immunological failure, for those who failed, was 230 cells/µl (IQR 120 - 340 cells/µl).

PREDICTORS OF IMMUNOLOGICAL FAILURE

Cox models were constructed to investigate factors associated with immunological failure. All models were stratified by centre (Table 3). Factors associated with immunological failure in the univariate model were HIV-1 risk behaviour being intravenous drug use (compared to male homosexuality), initiation of HAART with 5 = drugs (compared to 3 drugs), $CD4^+$ nadir (per 50 % higher), time from $CD4^+$ nadir (per 6 months more), pre-HAART $CD4^+$ count (per 50 % higher), drugs changed in HAART (yes / no), number of drugs the patient had ever been exposed to (per drug), and most recent pVL (per 1 $\log_{10}$ higher). Compared to ART experienced patients, ART-naïve patients were less likely to fail. In the multivariate model four factors remained significantly associated with an increased risk of immunological failure: pre-HAART $CD4^+$ count (RH 2.11, 95 % CI 1.87 - 2.37, $p < 0.0001$), time-updated pVL (RH 1.74, 95 % CI 1.61 - 1.89, $p < 0.0001$), risk group being intravenous drug use (RH 1.55, 95 % CI 1.19 - 2.02, $p = 0.0011$), and ≥ 5 drugs in HAART regimen (RH 1.83, 95 % CI 1.14 - 2.93, $p = 0.012$).

The analyses were repeated using baseline pVL and similar results were found, although the relationship between pVL and risk of immunological failure was much stronger when the latest value of pVL was used (data not shown), suggesting that it is more changes in pVL rather than initial pVL which may drive immunological response.

The analyses were also repeated using a more strict definition of failure where patients were required to have a confirmatory $CD4^+$ count at or below the pre-HAART $CD4^+$ count. This reduced the number of events to 218 (9.3 %), but the results were almost identical to those of Table 3. In addition, the analyses were repeated using a definition of failure of a 100 cells/µl drop from the baseline $CD4^+$ count (thus both the inclusion and failure criterion were defined using absolute $CD4^+$ counts). In this analysis, 10.6 % failed, but the results were

almost identical to the previous finding, except that the association between higher pre-HAART $CD4^+$ count and failure was even stronger. In addition, the analyses were repeated using an inclusion criteria of a 25 % rise in $CD4^+$ counts after initiation of HAART and a 25 % drop to below the pre-HAART $CD4^+$ count (thus both the inclusion and exclusion criteria were defined using %). In this analysis patients with pre-HAART CD4+ cell counts of below 100/µl were excluded. The results were again very similar.

Discussion

In this study of immunological failure in HIV-1 infected patients initiating HAART, 2347 patients from the EuroSIDA cohort were included, of whom 11.3 % experienced immunological failure within 12 months of initial immunological response to HAART. Higher pre-HAART $CD4^+$ count, higher time-updated pVL, and intravenous drug use were found to be independent predictors of immunological failure.

The study confirmed the findings by Deeks et al. that found patients with high pre-HAART $CD4^+$ count to be at increased risk of immunological failure [12]. One reason for this finding could lie in the difference in actual $CD4^+$ numbers at time of failure between patients with high and low pre-HAART $CD4^+$ counts. For instance using our definition of immunological failure ($CD4^+$ count drop to or below the pre-HAART $CD4^+$ count), for a patient with a pre-HAART $CD4^+$ count of 500 cells/µl and a rise to 600 cells/µl (immunological response), immunological failure would be a $CD4^+$ count of 500 cells/µl or less (17 % drop); in comparison for a patient with a pre-HAART $CD4^+$ count of 50 cells/µl and a rise to 150 cells/µl, immunological failure would be at a $CD4^+$ count of 50 cells/µl or less (67 % drop). However, we found similar results when both immunological response and failure were defined as a rise and fall in actual numbers, and in percentages. Furthermore, we do not think that clinical practise is guided by changes in percentages. We hypothesise that the

reason why patients with higher pre-HAART $CD4^+$ counts were found to be at increased risk of immunological failure compared to those with lower pre-HAART $CD4^+$ is that the latter group of patients are at increased risk of developing ODs thus clinicians have a lower threshold for modification of therapy in these patients, thereby preventing or delaying development of immunological failure. This is supported by the significantly shorter time to modification of treatment seen in patients with lower pre-HAART $CD4^+$ counts and higher pVL. Another explanation could be that patients with higher $CD4^+$ counts had lower adherence to or went off therapy at a time where the risk of developing an OD was less. In the example given above, even though the patient with high $CD4^+$ count experiences a substantial numerical drop in $CD4^+$ count, the $CD4^+$ count at the time of immunological failure is still well above any critical level from a clinical point of view. Hence despite immunological failure patients with higher pre-HAART CD^+ counts would be at less immediate risk of disease progression. In support of this, the median $CD4^+$ count at the time of immunological failure was found to 230 cells/µl. Also, if patients with higher baseline CD^+ counts were more likely to be off treatment at time of immunological failure one would expect the patients to experience rebound of pVL. However, we found higher baseline $CD4^+$ count to be predictive of failure independently of time-updated pVL.

Variability in $CD4^+$ count measurements could potentially influence the rate of immunological failure. Such variability is, however, not likely to have had any significant impact on the outcome of this study as we found similar results when a more strict definition of failure where patients were required to have a confirmatory $CD4^+$ count was used.

Time-updated pVL was also found to predict immunological failure. This finding is in accordance with previous studies. In a cohort of patients starting a PI who subsequently experienced virological failure Deeks et al. found change in pVL from pre-PI therapy levels, and high pVL to be associated with immunological failure [12]. Further, we identified intravenous drug use as a predictor of immunological failure. This is in accordance with previous results from cohort studies showing this risk group to have less increases in $CD4^+$

count and an excess rate of progression of disease and death during HAART [7,17]. Finally, patients receiving five or more drugs appear to have an increased risk of immunological failure. This may be an artefact of the methods of data collection, and the results should be interpreted with caution. Another possible explanation for this finding could be that patients receiving complex multi-drug regimens are less adherent to their regimen than patients receiving less complex regimens as found by others [18]. Work is ongoing looking into patients on multi-drug regimens.

Previous cohort studies have shown use of NRTIs prior to HAART to be predictive of failure [19,20]. In this study being ART-naive was found to be significantly associated with a diminished risk of immunological failure in the univariate but not in the multivariate model. In a previous study from the EuroSIDA cohort, Paredes et al. found virological failure in approximately 37 % of patients 12 months after achieving viral suppression [13]. In the present study approximately 11 % experienced immunological failure 12 months after initial response to HAART. This difference is to be expected, if patients are treated according to current treatment guidelines aiming at suppressing pVL rather than guided by $CD4^+$ counts, because treatment is then being changed shortly after virological failure rather than after immunological failure has occurred. No data exists about which treatment strategy is the better, maximal and durable suppression of pVL as stated in current treatment guidelines, or a strategy to maintain the $CD4^+$ count above a certain level where development of ODs is infrequent [1,2]. A randomised international clinical end point trial (the SMART study) is currently investigating this question. Should the SMART study show superiority of a treatment strategy driven by $CD4^+$ count, the identification of predictors of a sustained immunological response could prove to be of clinical significance.

CONCLUSION

In a large international HIV-1 cohort we found that the risk of immunological failure in patients with immunological response to HAART diminishes with more extended time on treatment. Immunological failure was associated with pre-treatment $CD4^+$ level, the rate of ongoing viral replication and intravenous drug use. This finding may have implications for the clinical management of HIV-1 infected patients.

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Figure 1

Selection of patients for analysis

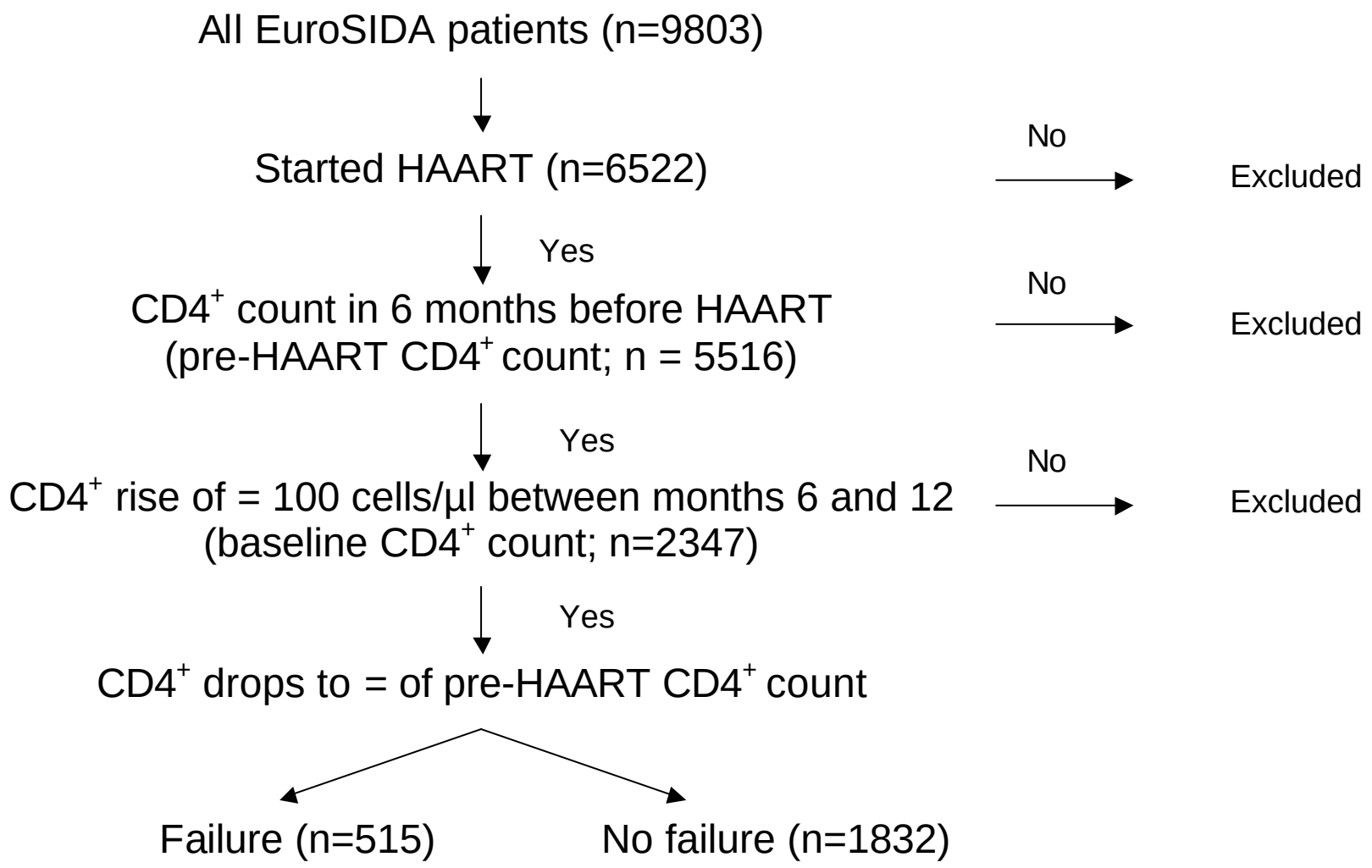

Figure 2

Changes in $CD4^+$ count and plasma viral load after initiation of HAART

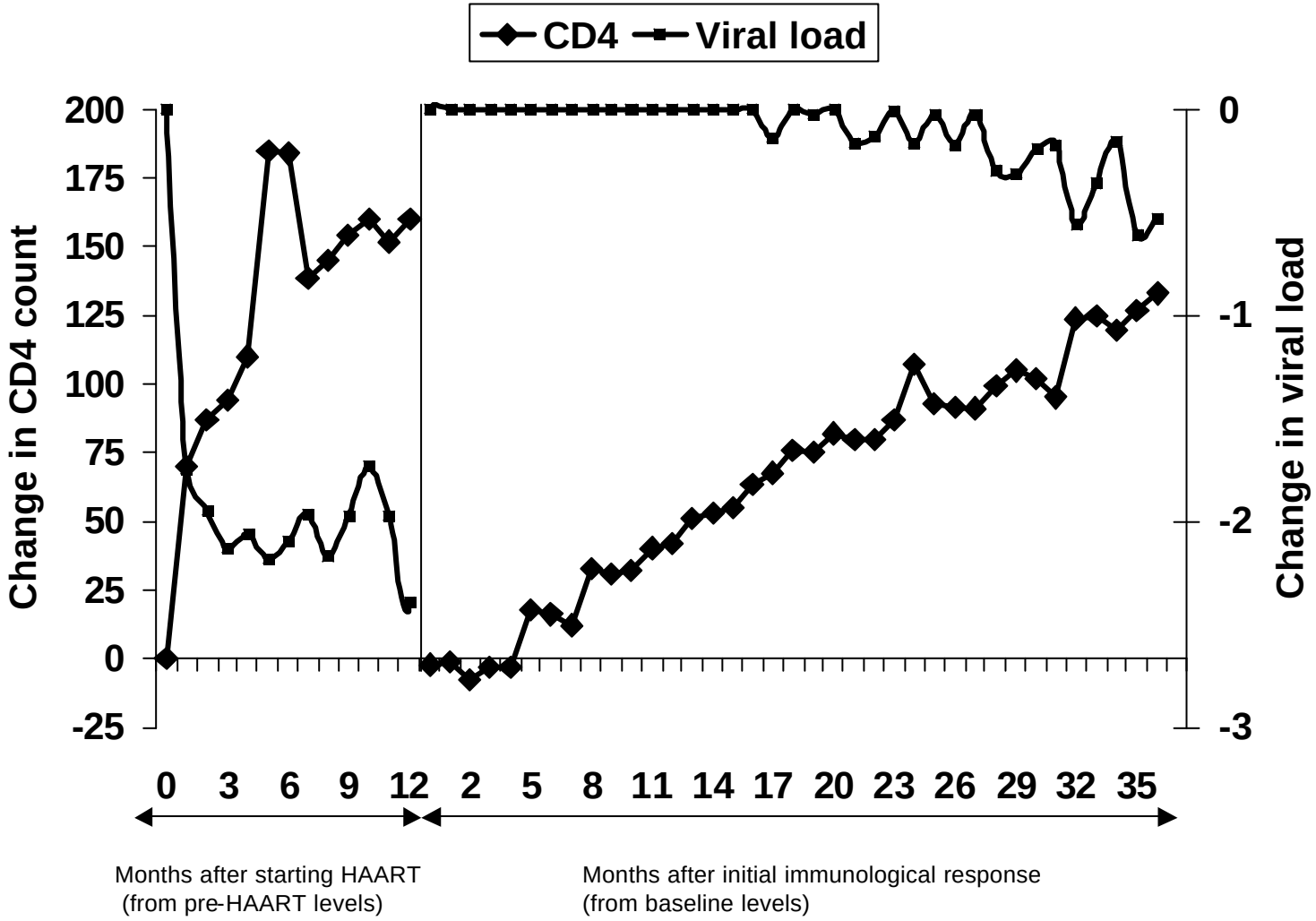

Table 1

Patient characteristics at time of inclusion

Parameter		% (N = 2347)
Gender		
	Female	21.9
	Male	78.1
Risk behaviour		
	Heterosexual contact	25.7
	Homosexual contact	47.9
	Intravenous drug use	19.4
	Other	6.9
Region		
	Central	27.8
	East	8.6
	North	34.6
	South	28.1
	Argentina	0.9
Age (years)		
	< 30	13.4
	30–39	46.6
	= 40	40.0
$CD4^+$ nadir (cells/µl)		
	< 50	23.0
	50-149	26.9
	150-249	24.5
	= 250	25.7
Prior AIDS		
	No	73.2
	Yes	26.8
ARV naïve		
	No	64.6
	Yes	35.4
HAART		
	Single PI-based	75.2
	Dual PI -based	7.3
	NNRTI-based	11.4
	Mixed	6.1

Table 2

Rate of immunological failure over time

Months of follow-up	PYFU	Immunological failures	Incidence	95 % CI
= 12	2109.5	250	11.9	10.4 – 13.4
12 - 24	1686.8	131	7.8	6.5 – 9.1
24 - 36	1381.7	71	5.1	3.9 – 6.3
> 36	1536.2	63	4.1	3.1 – 5.1

Table 3

Factors associated with immunological failure

		Univariate			Multivariate		
		RH	**95 % CI**	**P**	**RH**	**95 % CI**	**P**
Risk behaviour							
	Homosexual contact	1.00	-	-	1.00	-	-
	Intravenous drug use	1.27	0.98 – 1.64	0.0708	1.55	1.19 – 2.02	0.0011
	Heterosexual contact	1.13	0.91 – 1.42	0.27	1.13	0.89 – 1.42	0.31
	Other	0.74	0.47 – 1.15	0.18	0.89	0.57 – 1.39	0.89
Antiretroviral naïve							
	No	1.00	-	-	1.00	-	-
	Yes	0.71	0.62 – 0.96	0.023	0.93	0.69 – 1.24	0.93
No. of drugs in HAART							
	3	1.00	-	-	1.00	-	-
	4	0.94	0.73 – 1.21	0.65	1.12	0.85 – 1.48	0.43
	≥ 5	1.73	1.13 – 2.64	0.011	1.83	1.14 – 2.93	0.012
$CD4^+$ nadir	Per 50 % higher	1.22	1.15 – 1.29	<0.0001	0.93	0.69 – 1.24	0.60
Pre-HAART $CD4^+$ count	Per 50 % higher	1.75	1.60 – 1.92	< 0.0001	2.11	1.87 – 2.37	< 0.0001
Plasma viral load (tdc) [1]	Per 1 $\log_{10}$ higher	1.48	1.38 – 1.59	< 0.0001	1.74	1.61 – 1.89	< 0.0001
Change to HAART		1.23	1.01 – 1.51	0.045	1.12	0.90 – 1.39	0.30
Total no. ARVs [2]	Per ARV	1.10	1.02 – 1.19	0.039	0.96	0.85 – 1.08	0.96
Time from $CD4^+$ nadir	Per 6 months	1.29	1.18 – 1.42	<0.0001	1.03	0.99 – 1.06	0.080

1 tdc = time dependent co-variate
[2] Total number of antiretroviral drugs (ARVs) ever exposed to

Appendix

The EuroSIDA Study Group

The multi-centre study group on EuroSIDA (national coordinators in parenthesis).

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Article III

Aldesleukin - recombinant interleukin-2.

Current Opinion in Anti-infective Investigational Drugs 2000; 2(3):323-331

Appendix I

The COLATE trial

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Appendix II

The ESPRIT study

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Pharmaceutical support

Chiron Corporation

Investigational New Drug (IND) holder

Division of AIDS, NIAID, NIH, Bethesda, USA

Appendix III

Protocols for the ESPRIT substudies: FLUVAC & TEPVAC
available upon request to the author