Interruptions of cART limits CD4 T-cell recovery and increases the risk for opportunistic complications and death

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Background: A major goal of antiretroviral therapy (ART) for HIV-1-infected persons is the recovery of CD4 T lymphocytes, resulting in thorough protection against opportunistic complications. Interruptions of ART are still frequent. The long-term effect on CD4 T-cell recovery and clinical events remains unknown.

Methods: Immunological and clinical endpoints were evaluated in 2491 participants of the Swiss HIV Cohort Study initiating ART during a mean follow-up of 7.1 years. Data were analysed in persons with treatment interruptions ($n = 1271$; group A), continuous ART, but intermittent HIV-1 RNA at least 1000 copies/ml ($n = 469$; group B) and continuous ART and HIV-1 RNA constantly less than 1000 copies/ml ($n = 751$; group C). Risk factors for low CD4 T-cell counts and clinical events were analysed using Cox proportional hazards models.

Results: In groups A–C, CD4 T lymphocytes increased to a median of 427, 525 and 645 cells/μl at 8 years. In group A, 63.0 and 37.2% reached above 350 and 500 CD4 T cells/μl, whereas in group B 76.3 and 55.8% and in group C 87.3 and 68.0% reached these thresholds ($P < 0.001$). CD4 T-cell recovery directly depended on the cumulative duration of treatment interruptions. In addition, participants of group A had more Centers for Disease Control and Prevention B/C events, resulting in an increased risk of death. Major risk factors for not reaching CD4 T cells above 500 cells/μl included lower baseline CD4 T-cell count, higher age and hepatitis C virus co-infection.

Conclusion: In persons receiving continuous ART larger CD4 T-cell recovery and a reduced risk for opportunistic complications and death was observed. CD4 T-cell recovery was smaller in persons with treatment interruptions more than 6 months.

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Introduction

In recent years, antiretroviral therapy (ART) for HIV-1 infection has become more potent [1,2]. Plasma HIV-1 RNA levels are more frequently below the limit of detection and life expectancy has further increased [3]. In addition, ART regimens are now frequently applied once daily due to improved pharmacokinetic properties [4]. Although an extensive number of possible treatment options are available to combine individual antiretroviral drugs, most ART combinations appear to have a similar effect on the recovery of CD4 T lymphocytes [5]. ART has frequently to be discontinued because of drug intolerance, inconvenience or psychological factors [6,7]. In addition, potential interactions are increasing with more complex ART regimens and may result in additional modifications or interruptions of ART [7]. The SMART and the Staccato study have shown that episodic interruptions of ART guided by CD4 T-cell count increase the risk of HIV-1-associated complications and death. In the Staccato study, ART was intermittently discontinued and re-initiated if CD4 T-cell counts declined below 350 cells/μl. The interruption of ART resulted in significantly lower CD4 T-cell counts and in an increased incidence of oral and genital candidiasis [8,9]. In the SMART study, the risk for opportunistic complications and death was significantly higher in persons interrupting ART. It has to be considered that the threshold of 250 CD4 T cells/μl in the SMART study to discontinue ART was very low [8,9]. Both studies had a relatively short median follow-up of 21.9 and 16 months, respectively. The consequences of short and long-term interruptions of ART or discontinuations of ART at high CD4 T-cell counts during a long observation period of several years remain largely unknown.

In this study, we evaluated the effect of interruptions of ART in participants of the Swiss HIV cohort study over a mean observation period of 7.1 ± 3.4 years. We analysed CD4 T-cell recovery, the incidence of opportunistic complications and the mortality in three groups on ART. In the first group, ART was intermittently discontinued at different CD4 T-cell levels, for distinct time intervals and various reasons. In the second group, ART was continuously administered, but participants intermittently showed plasma HIV-1 RNA values above 1000 copies/ml. In the third group, individuals received continuous ART and had plasma HIV-1 RNA values below 1000 copies/ml at all time points 6 months after initiation of ART.

Methods

Study participants
The Swiss HIV Cohort Study (SHCS) is a prospective, observational cohort study of HIV-1–infected adults that

was initiated in 1988 [10]. Enrollment of patients in the SHCS is independent of HIV-1 disease stage and treatment (www.shcs.ch). Participants usually have a clinical and laboratory follow-up every 3–6 months. Laboratory values included CD4, CD8 T-cell count and plasma HIV-1 RNA. In addition, clinical events such as opportunistic complications and death were recorded.

In this study, 2491 participants of the SHCS from January 1996 until July 2008 were included who initiated at least a triple combination of indinavir ($n = 685$), saquinavir ($n = 261$), ritonavir ($n = 463$), amprenavir or fosamprenavir ($n = 19$), lopinavir ($n = 217$), atazanavir ($n = 54$), nelfinavir ($n = 583$), efavirenz ($n = 420$), nevirapine ($n = 83$), abacavir ($n = 90$) in combination with zidovudine ($n = 1386$), didanosine ($n = 262$), dideoxycytidine ($n = 26$), lamivudine ($n = 2021$), stavudine ($n = 886$), emtricitabine ($n = 64$) or tenofovir ($n = 158$). Of all included individuals, 1629 (64.3%) persons were antiretroviral drug naive. The remaining persons ($n = 862$; 34.6%) were treated with one or two antiretroviral drugs before initiating at least triple ART.

Initially, 1988 persons (79.8%) received a protease inhibitor in combination with at least two nucleoside analogues. Four hundred and seventy-five persons (19.1%) received a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) with at least two nucleoside analogues and 28 persons (1.1%) were treated with a protease inhibitor in combination with a NNRTI and at least two nucleoside analogues. None of the participants initially received enfuvirtide, maraviroc or raltegravir as part of ART, but during the study HIV-1 therapy was changed in most persons and new antiretroviral compounds were introduced. Particularly, the proportion of persons receiving boosted protease inhibitor therapy including atazanavir and lopinavir rapidly increased as well as the number of persons treated with tenofovir, abacavir or efavirenz.

Excluded were 4522 patients because they received a study medication ($n = 1747$), had missing baseline plasma HIV-1 RNA data ($n = 2016$), had missing baseline CD4 T cells ($n = 315$) or CD8 T cells ($n = 20$) or changed ART for a short time period to a single or double ART mostly due to adverse events ($n = 424$).

Statistical analysis and endpoints
Study participants were classified into three groups according to their ART. Group A consisted of 1271 persons (51.0%) who initially received ART, but discontinued intermittently ART for at least 1 month during the follow-up. In group B, 469 persons (18.8%) were included who continued ART without treatment interruption, but had intermittently poorly suppressed plasma HIV-1 RNA values above 1000 copies/ml. Persons of group C ($n = 751$; 30.1%) received continuously ART and showed a good virologic response with

plasma HIV-1 RNA values below 1000 copies/ml at all time points 6 months after initiation of ART.

Baseline characteristics in these three groups are presented as frequencies for categorical variables or medians and interquartile ranges for continuous variables. Baseline characteristics were compared between groups using χ^2 test for categorical variables and Mann–Whitney U test for continuous variables. Cox proportional hazards models were used for the analysis of the response of CD4 T lymphocytes to ART to above 350 and 500 cells/µl, respectively, the incidence of opportunistic complications and the mortality. In the statistical models the following potential predictors were evaluated: sex, age, duration of HIV-1 infection, pretreatment with fewer than three antiretroviral drugs, intravenous drug use, hepatitis C virus (HCV) co-infection, hepatitis B virus (HBV) co-infection, Centers for Disease Control and Prevention (CDC) stage C, baseline HIV-1 RNA, CD4 and CD8 T-cell count, year of initiation of ART, and the three treatment groups A–C. A two-sided P value less than 0.05 was considered statistically significant. All statistical analyses were performed with SPSS release 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics
The mean age of study participants was 38.1 ± 9.7 years. 67.9% of included individuals ($n = 1692$) were men. The mean follow-up was 7.1 ± 3.4 years. Group B had the longest (7.9 ± 3.3 vs. 7.1 ± 3.3 years of group A; $P < 0.001$) and group C had the shortest follow-up (6.5 ± 3.4 vs. 7.1 ± 3.3 years of group A; $P < 0.001$).

A total of 35.7% of patients ($n = 890$) were pretreated with ARTs, not meeting ART criteria. The proportion of 40.6% of pretreated patients of group A ($n = 516$) was similar to 42.0% of group B ($n = 197$), but significantly fewer patients of group C were pretreated (23.6%; $n = 177$; $P < 0.001$).

Median baseline CD4 T-cell count was higher in group A than in group B and C (249 vs. 161 and 199 cells/µl; $P < 0.001$ for both comparisons) and fewer persons were in CDC stage C [18.9% ($n = 241$) vs. 27.5% ($n = 130$) and 20.8% ($n = 156$); $P < 0.001$ for both comparisons]. Similarly, baseline CD8 T-cell count was slightly higher in group A than in group B (788 vs. 722 cells/µl; $P = 0.017$), but did not statistically differ to baseline CD8 T-cell count of group C (788 vs. 744 cells/µl; $P = 0.254$). In contrast, baseline plasma HIV-1 RNA was similar in groups A to C (4.6 vs. 4.7 and 4.8 $\log_{10}$ copies/ml; $P = 0.092$ and $P = 0.336$).

In group A, the proportion of intravenous drug users was higher than in group B and C [28.0% ($n = 356$) vs. 13.0%

($n = 61$) and 14.2% ($n = 107$); $P < 0.001$ for both comparisons] and a higher proportion had HCV co-infection [36.1% ($n = 459$) vs. 25.0% ($n = 117$) and 21.0% ($n = 157$); $P < 0.001$ for both comparisons].

In groups B and C, the proportion of female study participants was slightly smaller [27.7% ($n = 130$) and 29.8% ($n = 224$) vs. 35.0% ($n = 445$); $P < 0.001$ for both comparisons] and mean age was significantly higher (39.2 and 39.6 vs. 36.7 years; $P < 0.001$ for both comparisons) (Table 1).

Virologic response
In all persons median plasma HIV-1 RNA could be reduced from 4.7 to 0.7 $\log_{10}$ copies/ml at 8 years. In group A, viral load declined from 4.6 to 1.6 $\log_{10}$ copies/ml, whereas in groups B and C slightly larger median HIV-1 RNA declines from 4.7 to 0.7 $\log_{10}$ copies/ml and from 4.7 to 0.7 $\log_{10}$ copies/ml were observed ($P < 0.001$ at 8 years for both comparisons). By study definition, only group C had plasma HIV-1 RNA levels below 1000 copies/ml at all time points 6 months after initiation of ART (Fig. 1).

CD4 and CD8 T-cell responses
In all participants, median CD4 T-cell count increased substantially from 210 cells/µl at baseline to 491 cells/µl at 8 years. Similarly, CD8 T-cell count increased slightly in the first months of ART, but thereafter remained stable.

Only 329 of 522 persons (63.0%) of group A reached a CD4 T-cell count of more than 350 cells/µl, whereas a significantly larger number of 190 of 249 persons (76.3%) of group B and 226 of 259 persons (87.3%) of group C reached this goal ($P < 0.001$ for both comparisons).

Similarly, only 194 of 522 persons (37.2%) of group A reached a CD4 T-cell count of more than 500 cells/µl at 8 years despite a higher median baseline CD4 T-cell count, whereas 139 of 249 persons (55.8%) of group B and 176 of 259 persons (68%) of group C reached this goal ($P < 0.001$; Fig. 2a and b).

In contrast, CD8 T-cell count did statistically not differ between groups (Fig. 2c).

Impact of the duration of antiretroviral therapy interruptions on CD4 T-cell response
Fifty-one per cent of participants ($n = 1271$) discontinued ART at least once. The median duration of cumulative interruption of ART was 9 months [interquartile range (IQR) 2–26]. Persons interrupting ART had a median CD4 T-cell increase of 133 cells/µl at 8 years (IQR −11 to 333). This value was significantly higher in persons without ART interruption, but intermittently detectable plasma HIV-1 RNA above 1000 copies/ml (360 cells/µl, IQR 186–519, $P < 0.001$) and in individuals with continuous plasma HIV-1 RNA levels below 1000

Table 1. Baseline demographics.

	All patients (n = 2491)	Group A intermittent interruption of ART (n = 1271)	Group B continuous ART and plasma HIV-1 RNA intermittently ≥1000 copies/ml (n = 469)	Group C continuous ART and plasma HIV-1 RNA <1000 copies/ml (n = 751)
Sex (%)				
Male	1692 (67.9)	826 (65.0)	339 (72.3)	527 (70.2)
Female	799 (32.1)	445 (35.0)	130 (27.7)	224 (29.8)
Age (years)	38.1 ± 9.7	36.7 ± 9.1	39.2 ± 9.7	39.6 ± 10.4
Ethnicity (%)				
Caucasian	1931 (77.5)	970 (76.3)	355 (75.7)	606 (80.8)
Black	345 (13.8)	182 (14.3)	75 (16.0)	88 (11.7)
Hispanic	61 (2.4)	36 (2.8)	14 (3.0)	11 (1.5)
Asian	76 (3.1)	24 (1.9)	14 (3.0)	38 (5.1)
Other/no information	78 (3.2)	59 (4.8)	11 (2.3)	8 (1.0)
Antiretroviral drug naive	1601 (64.3)	755 (59.4)	272 (58.0)	574 (76.4)
HIV transmission category (%)				
MSM	788 (31.6)	363 (28.6)	170 (36.2)	255 (34.0)
Heterosexual	1040 (41.8)	504 (39.7)	192 (40.9)	344 (45.8)
IDU	552 (22.2)	356 (28.0)	61 (13.0)	107 (14.2)
Other/unknown	111 (4.5)	48 (3.8)	46 (9.8)	45 (6.0)
Duration of HIV-1 infection (years)	8.8 ± 4.6	8.9 ± 4.8	9.8 ± 4.5	8.0 ± 4.4
CDC stage (%)				
A	1341 (53.8)	692 (54.4)	210 (44.8)	439 (58.5)
B	623 (25.0)	338 (26.6)	129 (27.5)	156 (20.8)
C	527 (21.2)	241 (18.9)	130 (27.5)	156 (20.8)
Hbs-Ag positive	178 (7.1)	83 (6.9)	36 (7.9)	59 (8.1)
HCV-Ab positive	729 (29.3)	455 (36.1)	117 (25.0)	157 (21.0)
Baseline HIV-1 RNA ($\log_{10}$ copies/ml)	4.68 (3.88–5.21)	4.6 (3.9–5.2)	4.7 (3.9–5.3)	4.8 (3.8–5.2)
Baseline CD4 T-cell count (cells/μl)	210.5 (97.3–340)	249 (119–389)	161 (66–273)	199 (92–303)
Baseline CD8 T-cell count (cells/μl)	761 (488–1122)	788 (517–1138)	722 (433–1108)	744 (474–1107)

ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HCV, hepatitis C virus; IDU, intravenous drug use. Age and the duration of HIV-1 infection are shown as means and standard deviations. CD4, CD8 T-cells and plasma HIV-1 RNA are presented as medians and interquartile ranges. All other data are shown as numbers and proportions.

during the observation period (395 cells/μl, IQR 243–573; $P < 0.001$).

The increase in CD4 T-cell count strongly and inversely depended on the cumulative duration of ART interruption ($r = -0.470$; $P < 0.001$). A discontinuation of ART for 3–5 months resulted in a smaller increase of CD4 T cells/μl than in persons receiving continuous ART (Fig. 3a, b). A discontinuation of ART for a median time of 6–14 months further reduced CD4 T-cell recovery to 192 cells/μl (IQR 56–394) and an interruption of ART for 15–31 months led to a modest CD4 T-cell increase of 114 cells/μl (IQR −47 to 181). Finally, an interruption of ART for more than 31 months resulted even in a slight median decay of CD4 T-cell count of −1 cells/μl (IQR −134 to 90).
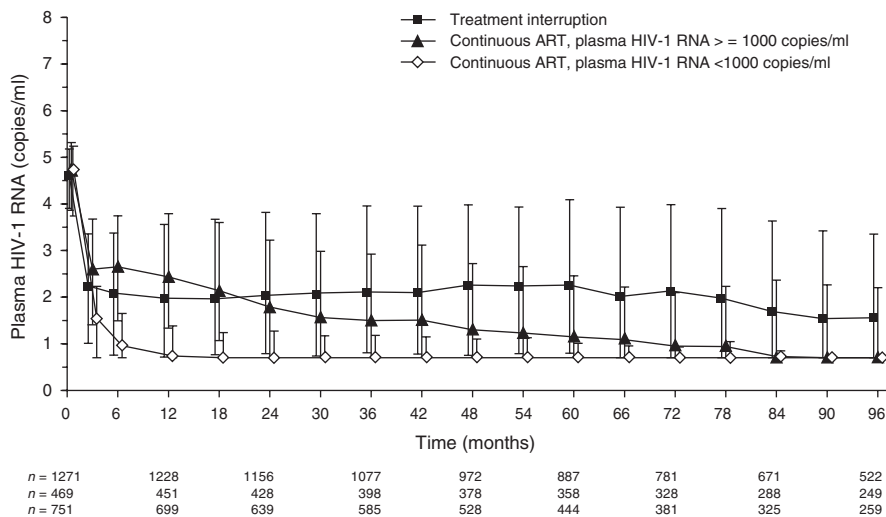

Fig. 1. Plasma HIV-1 RNA in participants with either treatment intermittent interruptions, continuous ART and plasma HIV-1 RNA levels below 1000 copies/ml or in persons with continuous ART, but intermittent plasma HIV-1 RNA levels above 1000 copies/ml.
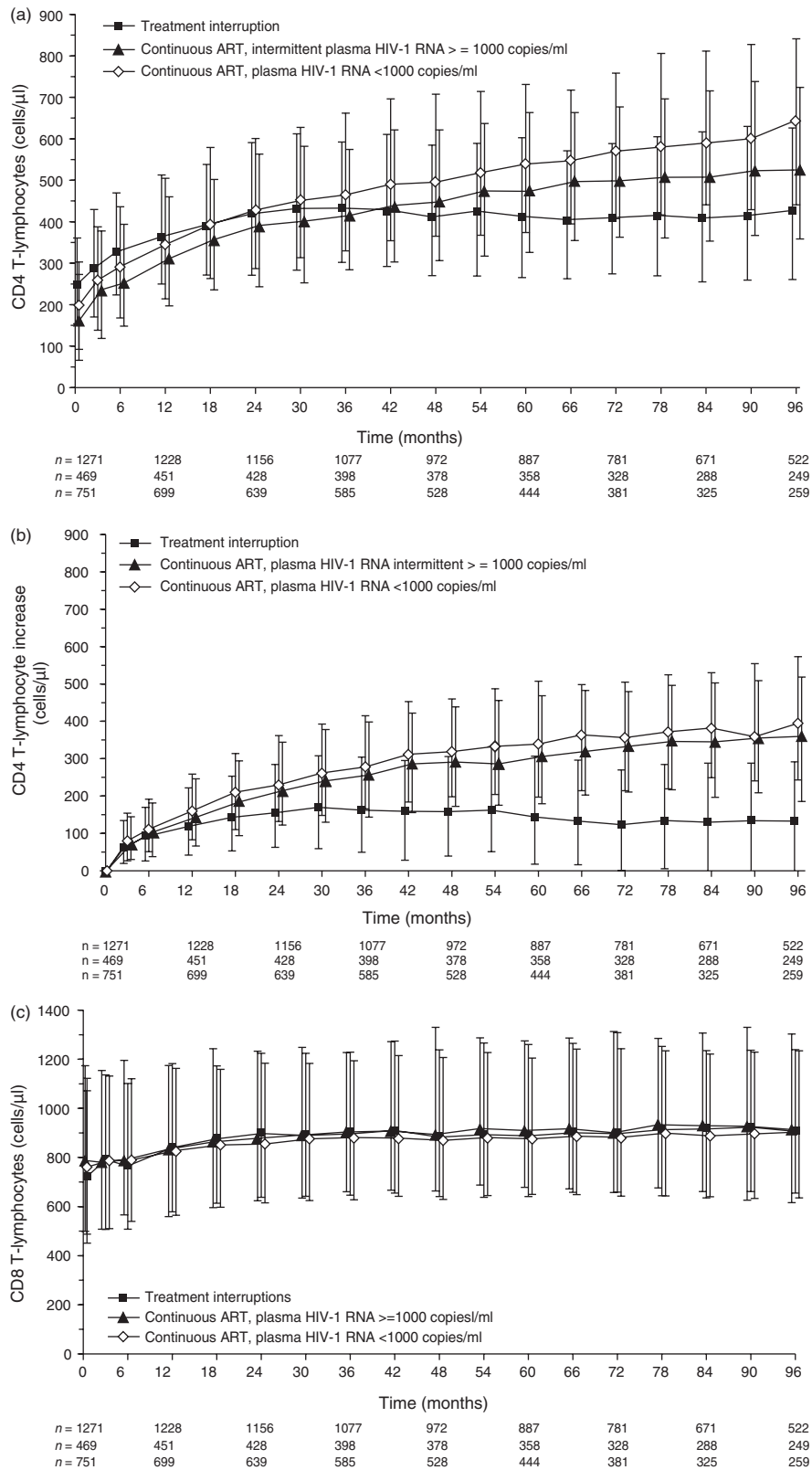
Fig. 2. (a) Median absolute CD4 T-cell counts, (b) CD4 T-cell changes and (c) time course of CD8 T-cell count in persons with either treatment intermittent interruptions, continuous ART and plasma HIV-1 RNA levels below 1000 copies/ml or in persons with continuous ART, but intermittent plasma HIV-1 RNA levels above 1000 copies/ml.
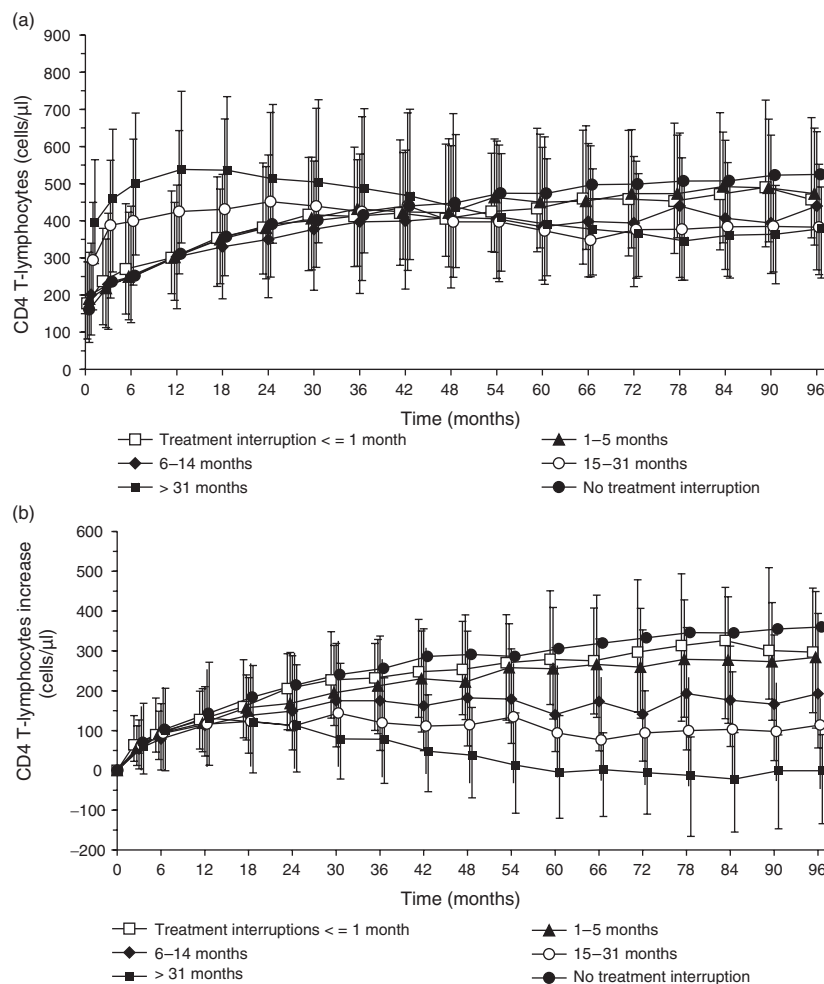
Fig. 3. (a) Median absolute CD4 T-cell counts and (b) median absolute CD4 T-cell changes in persons with different durations of treatment interruptions. The duration of treatment interruptions is directly associated with the level of CD4 T-cell recovery.

Further factors affecting CD4 T-cell responses

In a first multivariate analysis evaluating CD4 T-cell recovery of more than 350 cells/μl, persons with higher age ($P = 0.001$), a positive HCV serology ($P < 0.001$) and with treatment interruptions ($P = 0.001$) had a higher risk of CD4 T cells remaining below 350 cells/μl. In contrast, patients starting ART with higher CD4 T cells showed an improved CD4 T-cell recovery ($P < 0.001$; Table 2).

In a second multivariate analysis, evaluating CD4 T-cell recovery more than 500 cells/μl persons with older age ($P < 0.001$), a positive HCV serology ($P < 0.003$) or a detectable HBs antigen ($P = 0.020$), and treatment interruptions ($P < 0.001$) had a significantly higher risk that CD4 T-cell count remained below 500 cells/μl during the follow-up (Table 3), but patients initiating ART with higher CD4 T cells had a significantly higher chance of reaching 500 CD4 T cells/μl ($P < 0.001$).

Opportunistic infections

A total of 11.9 individuals/1000 person-years showed a CDC stage B event during the observation period and 11.3/1000 person-years individuals a CDC stage C event (Tables 4 and 5 and Fig. 4a, b).

In group A, 15 individuals/1000 person-years experienced CDC B events. These events were significantly more frequent than in group B [8.1/1000 person-years; hazard ratio 1.74 (1.13–2.67); $P = 0.013$] and C (9.2/1000 person-years; hazard ratio 1.46 (1.03–2.06); $P = 0.034$]. Particularly, certain CDC B events such as oral hairy leucoplakia, candida infections and herpes zoster infections showed a higher incidence rate in group A. Similarly, the incidence rate of CDC category C events was significantly higher in group A (14.8/1000 person-years) than in group B [8.1/1000 person-years; hazard ratio 1.70 (1.14–2.55); $P = 0.009$] and group C [7.4/1000 person-years; hazard ratio 2.11 (1.46–3.05); $P < 0.001$]. Particularly non-Hodgkin lymphoma,

Table 2. Predictors of CD4 T-lymphocyte count above 350 cells/µl.

Parameter	Univariate analysis Hazard ratio (95% CI)	P value	Multivariate analysis Hazard ratio (95% CI)	P value
Female sex	1.22 (1.09–1.38)	0.001	1.17 (1.02–1.33)	0.023
Age[a]	0.91 (0.86–0.97)	0.002	0.89 (0.84–0.96)	0.001
Previous treatment with <3 antiretroviral drugs	0.80 (0.71–0.90)	<0.001	0.96 (0.84–1.10)	0.536
Duration of HIV-1 infection[a]	0.98 (0.96–0.99)	<0.001	0.99 (0.97–1.01)	0.189
Intravenous drug use	0.76 (0.66–0.86)	<0.001	0.88 (0.68–1.14)	0.343
HCV antibody positive	0.74 (0.66–0.84)	<0.001	0.73 (0.64–0.84)	<0.001
HBs antigen positive	0.85 (0.68–1.06)	0.149	0.81 (0.64–1.04)	0.100
CDC stage C	0.70 (0.62–0.80)	<0.001	1.09 (0.93–1.27)	0.276
Baseline HIV-1 RNA[b]	0.94 (0.90–0.99)	0.020	1.07 (1.01–1.13)	0.027
Baseline CD4 T-cell count[c]	1.72 (1.62–1.83)	<0.001	1.73 (1.62–1.85)	<0.001
Baseline CD8 T-cell count[c]	1.03 (1.02–1.04)	<0.001	0.99 (0.98–1.00)	0.105
Treatment interruption	0.84 (0.75–0.94)	0.002	0.80 (0.70–0.91)	0.001
Intermittent plasma HIV-1 RNA >1000 copies/mL	1.04 (0.91–1.19)	0.609	1.02 (0.86–1.21)	0.839

Excluded were all patients who had baseline CD4 T-cell counts >349 cells/µl (*n* = 890). CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus. A Cox proportional hazards model was used for this analysis.
[a]Per 10 years higher.
[b]Per 1 log plasma HIV-1 RNA higher.
[c]Per 100 cells higher. Adjusted for the year of initiation of ART.

Pneumocystis jiroveci pneumonia and candida infections occurred more frequently in group A.

There was a clear relationship between the duration of treatment interruptions and the incidence of new CDC C events. A total of 6% of individuals discontinuing ART for less than 1 month, 10.4% discontinuing ART for 1–5 months, 10.7% discontinuing ART for 6–14 months, 13.9% discontinuing ART for 15–31 months and 13.7% discontinuing ART for more than 31 months showed new CDC C events.

Cardiovascular events
A total of 4.7 individuals/1000 person-years showed a cardiovascular event. In group A, 4.6 cardiovascular events/1000 person-years were noted. In group B, a slightly smaller number of 3.5 cardiovascular events/1000 person-years were found and in group C, 5.9 individuals/1000 person-years showed a cardiovascular event. Hence, the incidence rate of cardiovascular events was similar in groups A–C. A multivariate analysis data using all potential predictors did not reveal a significant specific risk factor for cardiovascular events (data not shown).

Mortality after initiation of antiretroviral therapy
A total of 14.3 individuals/1000 person-years of death were documented during the observation period, that is 3.3/1000 person-years from HIV-1-associated diseases, 0.6/1000 person-years from an overdose of illicit drugs, 0.6/1000 person-years from suicide, 0.2/1000

Table 3. Predictors of CD4 T-lymphocyte count above 500 cells/µl.

Parameter	Univariate analysis Hazard ratio (95% CI)	P value	Multivariate analysis Hazard ratio (95% CI)	P value
Female sex	1.20 (1.06–1.35)	0.005	1.08 (0.94–1.25)	0.277
Age[a]	0.90 (0.84–0.96)	0.001	0.85 (0.80–0.91)	<0.001
Previous treatment with <3 antiretroviral drugs	0.91 (0.80–1.03)	0.124	1.04 (0.90–1.20)	0.633
Duration of HIV-1 infection [a]	0.98 (0.97–0.99)	0.002	0.99 (0.97–1.01)	0.205
Intravenous drug use	0.76 (0.66–0.87)	<0.001	0.91 (0.69–1.21)	0.512
HCV antibody positive	0.74 (0.65–0.85)	<0.001	0.80 (0.69–0.92)	0.003
HBs antigen positive	0.78 (0.61–0.99)	0.044	0.73 (0.56–0.95)	0.020
CDC stage C	0.73 (0.63–0.84)	<0.001	1.20 (1.01–1.42)	0.037
Baseline HIV-1 RNA[b]	0.95 (0.91–1.01)	0.075	1.10 (1.03–1.17)	0.003
Baseline CD4 T-cell count[c]	1.60 (1.53–1.68)	<0.001	1.70 (1.60–1.81)	<0.001
Baseline CD8 T-cell count[c]	1.03 (1.02–1.04)	<0.001	0.98 (0.97–0.99)	0.016
Treatment interruption	0.89 (0.79–1.00)	0.059	0.72 (0.63–0.83)	<0.001
Intermittent plasma HIV-1 RNA >1000 copies/ml	1.03 (0.90–1.19)	0.644	1.13 (0.94–1.35)	0.211

Excluded were all patients with baseline CD4 T-cell counts above 499 cells/µl (*n* = 610). CDC, Centers for Disease Control and Prevention; HBV, hepatitis B virus; HCV, hepatitis C virus. A Cox proportional hazards model was used for this analysis.
[a]Per 10 years higher.
[b]Per 1 log plasma HIV-1 RNA higher.
[c]Per 100 cells higher. Adjusted for the year of initiation of ART.

Table 4. CDC B events after initiation of ART.

Events	Group A	Group B	Group C
Cervical dysplasia	15 (1.2%)	5 (1.1%)	9 (1.2%)
Vulvovaginal candidiasis	5 (0.4%)	0 (0%)	4 (0.5%)
Herpes zoster	33 (2.6%)	3 (0.6%)	6 (0.8%)
Hodgkin's disease	1 (0.1%)	2 (0.4%)	1 (0.1%)
Nocardiosis	1 (0.1%)	0 (0%)	0 (0%)
Oral hairy leukoplakia	15 (1.2%)	3 (0.6%)	4 (0.5%)
Peripheral neuropathy	4 (0.3%)	1 (0.2%)	0 (0%)
Hypertension, primary pulmonary	1 (0.1%)	0 (0%)	1 (0.1%)
Candidiasis oral	43 (3.4%)	11 (2.3%)	9 (1.2%)
Weight loss	1 (0.1%)	1 (0.2%)	2 (0.3%)
Fever	0 (0%)	1 (0.2%)	1 (0.1%)
Thrombocytopenia	16 (1.3%)	2 (0.4%)	6 (0.8%)
HIV-related myopathia	0 (0%)	1 (0.2%)	1 (0.1%)
Lymphoid interstitial pneumonia	0 (0%)	0 (0%)	1 (0.1%)
Total	135 (10.8%)	30 (6.2%)	45 (5.8)

person-years through an accident, one death from homicide, and 9.4/1000 person-years from other or unknown causes. Hence, 23% of all individuals dying during follow-up died from HIV-1-related infections or malignancies.

Deaths were more frequently observed in group A, in which 19.6 individuals/1000 person-years were observed; 3.9 individuals/1000 person-years in this group were noted from an HIV-1-related cause.

In group B, a significantly smaller death rate of 9.7 individuals/1000 person-years was observed [hazard ratio 0.53 (0.37–0.76); $P = 0.001$]. However, a relatively large mortality rate due to an HIV-1-related illness of 3.5/1000 person-years was observed which was not significantly different to that of group A [hazard ratio 1.01 (0.53–1.90); $P = 0.985$].

In group C, 8.2 individuals/1000 person-years were documented [hazard ratio 0.37 (0.26–0.52); $P < 0.001$] of 2.0/1000 person-years were from an HIV-1-related death [hazard ratio 0.48 (0.24–0.98); $P = 0.042$].

Hence, the mortality rate declined from 19.6/1000 person-years in group A to 8.2/1000 person-years in group C. Similarly, the HIV-1-associated mortality rate declined from 3.9/1000 person-years in group A to 2.0/1000 person-years in group C.

Discussion

We analysed long-term CD4 T-cell responses and HIV-1-associated clinical events in 2491 persons receiving ART during a mean observation period of 7.1 years. The results

Table 5. CDC C events after initiation of ARTs.

| Events	Group A	Group B	Group C
Cryptococcosis	1 (0.1%)	1 (0.2%)	1 (0.1%)
Cryptococcal meningitis	3 (0.2%)	2 (0.4%)	1 (0.1%)
HIV-related encephalopathy	7 (0.6%)	2 (0.4%)	3 (0.4%)
Candidiasis, oesophageal	38 (3.0%)	5 (1.1%)	4 (0.5%)
Herpes simplex ulceration, chronic	3 (0.2%)	1 (0.2%)	2 (0.3%)
M. genavense disease	1 (0.1%)	0 (0%)	1 (0.1%)
Kaposi sarcoma	10 (0.8%)	2 (0.4%)	8 (1.1%)
Lymphoma, primary, cerebral	0 (0%)	1 (0.2%)	1 (0.1%)
M. avium intracellulare disseminated	3 (0.2%)	1 (0.2%)	1 (0.1%)
Non-Hodgkin's lymphoma	18 (1.4%)	3 (0.6%)	4 (0.5%)
Pneumocystis jiroveci pneumonia	19 (1.5%)	3 (0.6%)	6 (0.8%)
CMV-retinitis	0 (0%)	1 (0.2%)	1 (0.1%)
Cryptosporidiosis, diarrhea > 1 month	0 (0%)	2 (0.4%)	1 (0.1%)
Tuberculosis, pulmonary	8 (0.6%)	1 (0.2%)	1 (0.1%)
Toxoplasmosis, cerebral	2 (0.2%)	3 (0.6%)	1 (0.1%)
Tuberculosis, extrapulmonary	5 (0.4%)	2 (0.4%)	0 (0%)
Cryptosporidiosis, diarrhea > 1 month	0 (0%)	2 (0.4%)	1 (0.1%)
Bacterial pneumonia, recurrent	8 (0.6%)	0 (0%)	0 (0%)
Invasive cervical carcinoma	2 (0.2%)	0 (0%)	0 (0%)
Leukencephalopathy, progressive, multifocal	1 (0.1%)	0 (0%)	0 (0%)
Wasting syndrome	4 (0.3%)	0 (0%)	0 (0%)
Total	133 (10.5%)	30 (6.1%)	36 (4.5%)
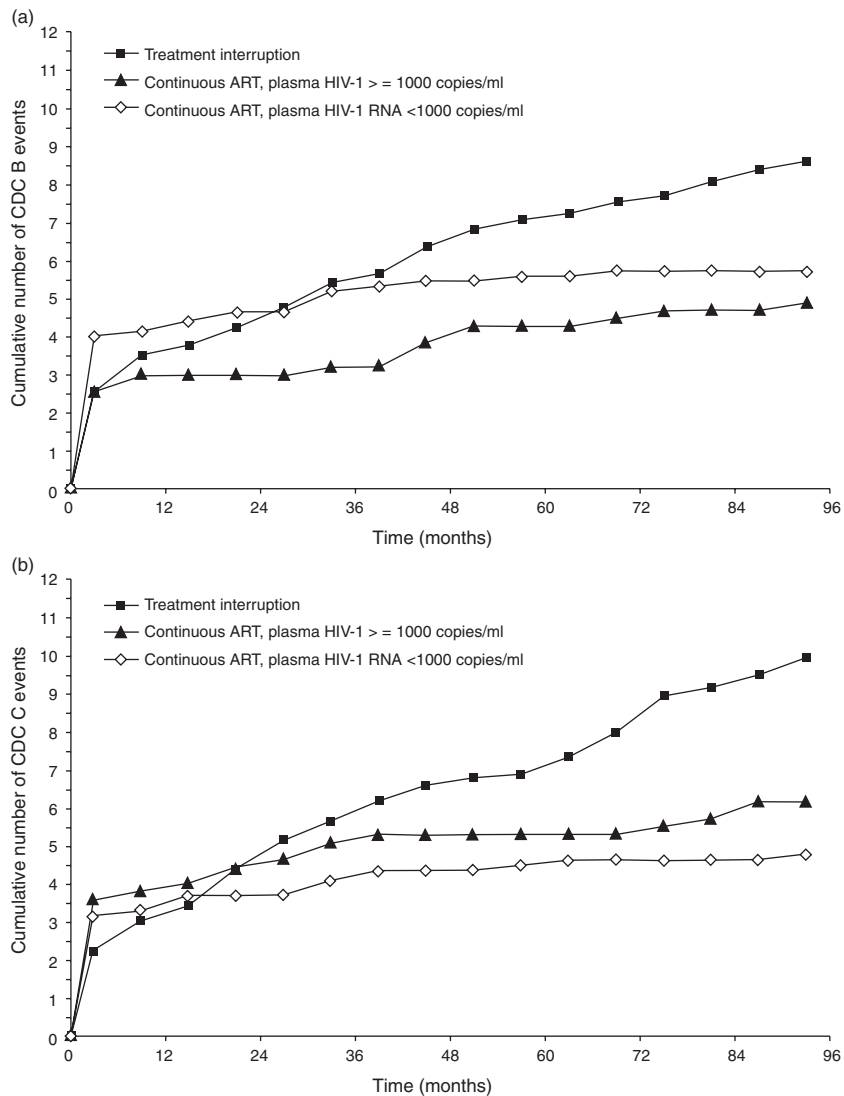
Fig. 4. Cumulative number of (a) CDC B and (b) CDC C events during the observation period.

strongly support the concept that patients should be discouraged to discontinue ART. A large proportion of persons interrupted ART for a variable time with the long-term consequence that CD4 T-cell count did not recover to the same level as in persons continuing ART without treatment interruption. Importantly, the number of opportunistic complications in this group was significantly higher than in persons without ART interruptions. In addition, more persons with ART interruptions died during the observation period from all causes as well as from HIV-1-specific related reasons.

Our findings are consistent with three other large prospective, randomized studies. In the Staccato study, the follow-up time was much shorter, but the interruption of ART already resulted in significantly lower CD4 T-cell counts, suggesting that after a longer observation time, more serious clinical events might occur [8]. In the

SMART study, the risk of opportunistic infections and death in the group interrupting ART was elevated [9]. However, the threshold of restarting ART of 250 CD4 T cells/μl was very low. In a third investigation, the TRIVACAN study, ART was discontinued and reinitiated when CD4 T-cell count reached the thresholds of 350 and 250 cells/μl, respectively [11]. Similar to the previous study, a 2.5-fold higher morbidity was observed in the ART interruption arm. In summary, all these studies suggest that the interruption may result in a higher frequency of opportunistic infections or a higher death rate. However, the follow-up was short. In addition, our analysis was based on an observational cohort of patients who discontinued ART not systematically in the framework of a prospective, randomized trial. In fact, interruptions of ART in the setting of an observational study may be more common than expected. Nevertheless, our study confirms the results of former three

investigations, but also adds important new information on the long-term clinical consequences of treatment interruptions and the effect of the duration of treatment interruptions to former investigations.

The best CD4 T-cell recovery and clinical results were found in persons responding optimally to ART with continuous plasma HIV-1 RNA levels below 1000 copies/ml. Apart from the negative impact of any treatment interruption we found that a discontinuation of ART of 6 months upwards was associated with a particularly poor recovery of CD4 T cells. Hence, if any treatment interruption is required, it should be as short as possible to avoid poor clinical outcomes.

Only two-thirds of persons reached at least 500 cells/µl CD4 T cells despite long-term ART and favourable virologic responses. An incomplete CD4 T-cell recovery has been observed in several studies [12−17] and strongly suggests that ART should be initiated earlier [18].

It still remains unclear to which level CD4 T cells will ultimately recover in older HIV-1-infected patients. Age was a significant predictor for CD4 T-cell recovery above 350 and 500 cells/µl. This is consistent with a number of published studies, showing that CD4 T-cell regeneration is more complete in younger patients [13,19−23]. One explanation may be that the contribution of the thymus to the regeneration of CD4 T lymphocytes in younger persons may be substantially better. Other possibilities for a poor CD4 T-cell recovery despite favourable virologic responses may include persistent apoptosis of CD4 T cells, increased fibrosis of the T-cell zone of lymphoid tissue [24−26], persistent immune activation [27−30] or persistently elevated inflammatory responses despite ART [31].

Interestingly, non-HIV-1-associated events leading to death were significantly more frequent than HIV-1-associated events. This finding was reported previously [32,33]. However, larger studies are required in order to further characterize risk factors for these events in more detail.

Hepatitis C virus co-infection led to a significantly poorer recovery of CD4 T cells. The effect of HCV co-infection, limiting CD4 T-cell recovery has been observed in several cohorts, but not in all studies [33,34]. One reason for the discrepant results may be that the effect of HCV co-infection on CD4 T-cell recovery is relatively small. Hence, only long-term studies over several years may reveal the true impact of HCV infection on CD4 T-cell recovery.

The absolute risk for cardiovascular events remained low despite a considerable number of study participants and a long mean follow-up of 7.1 years [35,36]. In addition, the incidence of cardiovascular events was not different in the three study groups.

Our study has several limitations. First, there were obvious differences in baseline characteristics in the three study groups which probably could not be completely corrected by multivariate analyses. Secondly, a small bias may have originated from the subsequent selection of different ART regimens. The choice of ART was the decision of the acting physician and was not influenced by the observational study design. However, we assumed that different drug regimens may have only minor effects on CD4 T-cell recovery and the incidence of clinical events [5].

The strength of this study was the analysis of laboratory markers and clinical endpoints in patients on ART for an extended observation period. A further strength of this study is the estimation of the effect of the duration of treatment interruptions on CD4 T-cell recovery.

In summary, we could show that an interruption of ART for 6 months or more resulted in sub-optimum recovery of CD4 T lymphocytes and increased risk for opportunistic complications or death. Particularly with regard to these clinical endpoints, it appears to be essential to initiate ART early, avoid treatment interruptions and suppress plasma HIV-1 RNA to values as low as possible.

Acknowledgements

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