

Clinical Predictors of Immune Reconstitution following Combination Antiretroviral Therapy in Patients from the Australian HIV Observational Database

Reena Rajasuriar^{1,2}, Maelenn Gouillou³, Tim Spelman³, Tim Read⁵, Jennifer Hoy^{1,4,6}, Matthew Law⁴, Paul U. Cameron^{1,6,7}, Kathy Petoumenos⁴, Sharon R. Lewin^{1,6,7*}

1 Department of Medicine, Monash University, Melbourne, Victoria, Australia, **2** Faculty of Medicine, University Malaya, Kuala Lumpur, Federal Territory, Malaysia, **3** Centre for Population Health, Burnet Institute, Melbourne, Victoria, Australia, **4** National Centre in HIV Epidemiology and Clinical Research, Sydney, New South Wales, Australia, **5** Melbourne Sexual Health Centre, Melbourne, Victoria, Australia, **6** Infectious Disease Unit, The Alfred Hospital, Melbourne, Victoria, Australia, **7** Centre for Virology, Burnet Institute, Melbourne, Victoria, Australia

Abstract

Background: A small but significant number of patients do not achieve CD4 T-cell counts >500cells/ μ l despite years of suppressive cART. These patients remain at risk of AIDS and non-AIDS defining illnesses. The aim of this study was to identify clinical factors associated with CD4 T-cell recovery following long-term cART.

Methods: Patients with the following inclusion criteria were selected from the Australian HIV Observational Database (AHOD): cART as their first regimen initiated at CD4 T-cell count <500cells/ μ l, HIV RNA<500copies/ml after 6 months of cART and sustained for at least 12 months. The Cox proportional hazards model was used to identify determinants associated with time to achieve CD4 T-cell counts >500cells/ μ l and >200cells/ μ l.

Results: 501 patients were eligible for inclusion from AHOD (n = 2853). The median (IQR) age and baseline CD4 T-cell counts were 39 (32–47) years and 236 (130–350) cells/ μ l, respectively. A major strength of this study is the long follow-up duration, median (IQR) = 6.5(3–10) years. Most patients (80%) achieved CD4 T-cell counts >500cells/ μ l, but in 8%, this took >5 years. Among the patients who failed to reach a CD4 T-cell count >500cells/ μ l, 16% received cART for >10 years. In a multivariate analysis, faster time to achieve a CD4 T-cell count >500cells/ μ l was associated with higher baseline CD4 T-cell counts (p<0.001), younger age (p = 0.019) and treatment initiation with a protease inhibitor (PI)-based regimen (vs. non-nucleoside reverse transcriptase inhibitor, NNRTI; p = 0.043). Factors associated with achieving CD4 T-cell counts >200cells/ μ l included higher baseline CD4 T-cell count (p<0.001), not having a prior AIDS-defining illness (p = 0.018) and higher baseline HIV RNA (p<0.001).

Conclusion: The time taken to achieve a CD4 T-cell count >500cells/ μ l despite long-term cART is prolonged in a subset of patients in AHOD. Starting cART early with a PI-based regimen (vs. NNRTI-based regimen) is associated with more rapid recovery of a CD4 T-cell count >500cells/ μ l.

Citation: Rajasuriar R, Gouillou M, Spelman T, Read T, Hoy J, et al. (2011) Clinical Predictors of Immune Reconstitution following Combination Antiretroviral Therapy in Patients from the Australian HIV Observational Database. PLoS ONE 6(6): e20713. doi:10.1371/journal.pone.0020713

Editor: Geneviève Chêne, Institut National de la Santé et de la Recherche Médicale, France

Received: December 30, 2010; **Accepted:** May 8, 2011; **Published:** June 2, 2011

Copyright: © 2011 Rajasuriar et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The Australian HIV Observational Database is funded as part of the Asia Pacific HIV Observational Database, a program of The Foundation for AIDS Research, amfAR, and is supported in part by a grant from the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases (NIAID) (Grant No. U01-AI069907) and by unconditional grants from Merck Sharp & Dohme; Gilead; Bristol-Myers Squibb; Boehringer Ingelheim; Roche; Pfizer; GlaxoSmithKline; and Janssen-Cilag. The National Centre in HIV Epidemiology and Clinical Research is funded by The Australian Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, The University of New South Wales. RR is a recipient of the King Scholarship from the Malaysian government. SRL is an NHMRC Practitioner Fellow. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Sharon.Lewin@med.monash.edu.au

Introduction

Most patients receiving suppressive cART will experience significant increases in CD4 T-cell counts [1,2]. In most studies, the pattern of change in CD4 T-cells following cART includes a rapid increase in CD4 T-cells in the initial three months [3,4,5] which is then followed by a slower increase in CD4 T-cells in the subsequent 2–3 years [4,6,7,8,9,10,11]. After 2–3 years of cART, changes in CD4 T-cells are less predictable. Some studies have reported sustained

increases in CD4 T-cell numbers up to 4 years following suppressive cART [2,4,12], while others have reported a plateau beyond 3–4 years of cART [6,7,9,10,13]. In most patients, a plateau in CD4 T-cells occurs within the normal range of CD4 T-cells [2], however, in a small but significant number of patients CD4 T-cells plateau below the normal threshold of 500 cells/ μ l [1,14]. There is now growing evidence that patients with CD4 T-cell counts <500 cells/ μ l are at an increased risk of AIDS and non-AIDS defining illnesses, despite achieving complete viral suppression on cART [15,16,17,18].

Multiple cohort studies have assessed factors associated with CD4 T-cell recovery following cART and have found that older age [1,4,6,8,13,19,20], lower baseline CD4 T-cell counts [3,6,8,12,13,21], higher baseline HIV RNA [3,5,6,10,14,22,23,24], reduced thymic function [25,26], increased levels of T-cell activation [27,28] and detectable viremia while on treatment [3,4,6,12,22] are all associated with reduced CD4 T-cell recovery. Many of these studies have followed changes in CD4 T-cells in large cohorts [2,3,4,6,7,9,12,13,21,22,24], but few studies have had prolonged follow-up (>10 years) upon cART. The methodology used in these previous studies was unable to distinguish subgroups of patients who take a longer time to achieve CD4 T cells >500 cells/ μ l including those initiating cART at low baseline CD4 T-cell counts [1], from those who have plateaued at CD4 T-cell counts below 500 cells/ μ l and were unlikely to ever achieve this threshold. In addition, many prior cohort studies included patients who were treatment experienced prior to initiation of an effective cART regimen [1,6,11,21,24] and restricted their analysis to only include patients who have maintained viral suppression (defined differently from <50 to <1000 copies/ml) throughout follow-up [1,8,9,12,13,14]. Though this approach measures the maximal capacity of immune recovery in patients achieving the best possible virologic outcome with cART, the findings from these studies may not be generalisable to clinical practice where treatment responses may be variable and the occurrence of virologic failure is unpredictable.

Given the clinical significance of achieving CD4 T-cell counts >500 cells/ μ l in HIV-infected patients and the relative limitations of some prior studies to identify patients who are unlikely to reach this threshold especially following prolonged treatment, the aim of this study was to identify factors associated with the time taken to reach CD4 T-cell counts >500 cells/ μ l following long-term cART in a large prospective clinic-based cohort with prolonged follow-up.

Methods

Patient selection

The study population consisted of all patients enrolled in the Australian HIV Observational Database (AHOD) at the time of study, $n = 2853$ (data updated March 2009). AHOD is an observational database that collects demographic and HIV treatment-related data from 27 sites consisting of general practitioner services, sexual health clinics and hospitals throughout Australia. The protocol for recruitment to AHOD was approved by the institutional review board of each recruiting site (listed in acknowledgements). All patients provided written informed consent prior to AHOD recruitment and no further consent was required for the conduct of this study. Details of this observational cohort have been described elsewhere [29]. For this study, patients were selected if they fulfilled the following inclusion criteria; men or women aged at least 18 years and were ART naïve at commencement of cART (defined as at least three antiretroviral drugs), treatment was initiated at CD4 T-cell counts <500 cells/ μ l and patients achieved controlled viral suppression (defined as HIV RNA <500 copies/ml) by 6 months of treatment initiation and maintained viral suppression for at least 12 months.

Demographic and clinical parameters such as date of birth, sex, hepatitis B and C status, HIV exposure category, diagnosis of AIDS-defining illness (ADI) before cART initiation or at follow-up, initial cART treatment regimen and all CD4 T-cell counts and HIV RNA measures from cART initiation to the most recent data available, were obtained from the March 2009 updated AHOD database. Only CD4 T-cell counts with date matched HIV RNA measures (within 1 month) were included in the analysis. The

closest matched CD4 T-cell count and HIV RNA measure prior (within 1 year) to the date of cART commencement were considered as baseline values. Patients who could not be assigned a baseline CD4 T-cell count or HIV RNA measure by this definition were excluded.

Statistical analysis

Survival analysis was used to identify determinants of CD4 T-cell recovery following cART. Two clinically relevant end-points were used to define the outcome, which were time taken to achieve CD4 T-cells counts >500 cells/ μ l and >200 cells/ μ l [30,31]. HIV RNA for all patients was included as a time-dependent co-variate. This meant patients who were initially aviremic (based on inclusion criteria) but subsequently developed virological failure (two consecutive HIV RNA >500 copies/ml) during follow-up remained in the analysis with appropriate model adjustments for the influence of this parameter on the outcome of interest. The Cox proportional univariate model was used to initially identify candidate predictors of immune reconstitution ($p < 0.2$). These candidate variables were then included in the Cox proportional multivariate model to identify independent predictors of recovery. A p -value of <0.05 in the multivariate model was considered significant. Finally, Schoenfeld residuals were used to assess the model for violations of the proportional hazard assumptions and the model was accepted only after no violations were shown to occur. Baseline CD4 T-cell counts were square transformed in order to meet the proportional hazard assumption in the analysis of time to achieve CD4 T-cell counts >500 cells/ μ l. Comparisons of survival curves of patients achieving CD4 T-cell counts >500 cells/ μ l were done using the Log-rank test. All statistical analyses were performed using STATA (version 10).

Results

Cohort characteristics

Five hundred and forty two (19%) patients fulfilled the inclusion criteria and were selected from a study population of 2853. The remainder of AHOD ($n = 2311$) were excluded for the following reasons; treatment experienced ($n = 1599$; receiving mono or dual therapy prior to cART), initiated treatment at CD4 T-cell counts >500 cells/ μ l ($n = 424$) and did not achieve controlled viremia by 6 months or sustain viral suppression for at least 12 months post-cART ($n = 288$). Of the 542 patients who fulfilled the inclusion criteria, 41 patients were excluded because they had no matched baseline CD4 T-cell/HIV RNA measure recorded in the database or had no recorded baseline HIV RNA or follow up CD4 T-cell counts (Figure 1). The remaining 501 patients were included in the final analysis.

The majority of patients were males (94%) and the median (IQR) age at cART initiation was 39 (32–47) years (Table 1). The median (IQR) baseline CD4 T-cell count was 236 (130–350) cells/ μ l and HIV RNA levels were 88 050 (25 875–250 070) copies/ml. Sixty seven patients (13%) reported a history of ADI prior to the commencement of cART while 23 patients (4.6%) developed 29 episodes of ADI on cART. These episodes mostly occurred soon after cART initiation (median (IQR) = 4.5 (0.9–38.4) months). The median (IQR) follow-up duration for the cohort was 6.5 (3.4–10.2) years and a median of 22 (IQR 12–34) pairs of matched CD4 T-cell and HIV RNA measures per patient were included in the analysis. Nelfinavir and indinavir were the most commonly used PIs (32% and 27% of all PI regimens respectively). Most recruitment to AHOD was before 2005, when these less potent PIs were being widely used

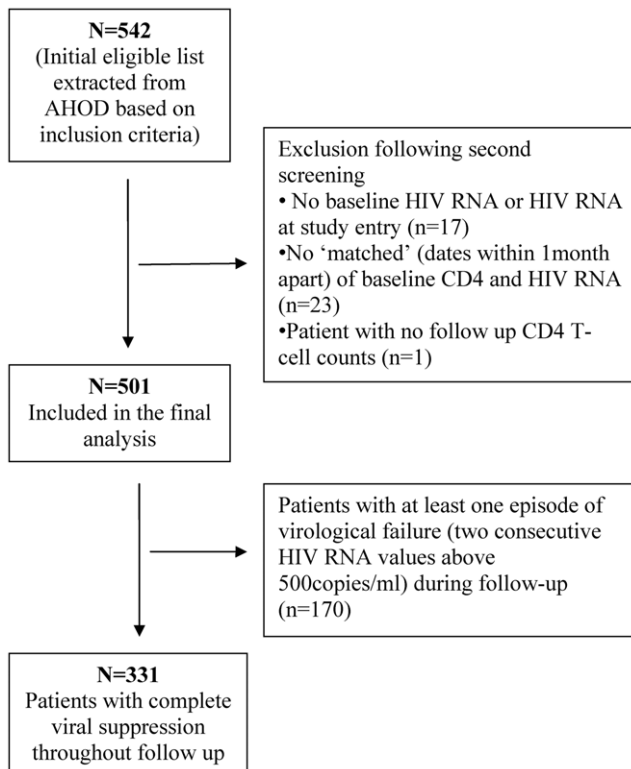


Figure 1. Patient disposition.
doi:10.1371/journal.pone.0020713.g001

in Australia. Nevirapine and efavirenz were used equally (45% and 55% of all NNRTI regimens). Thirty-four percent of patients developed at least one episode of virological failure (VF) during the follow-up period (5 and 10-year cumulative incidence (95% CI) to the first episode of VF was 8.1/100 person-years (100 pyr) (6.9–9.6) and 7.0/100 pyr (6.0–8.1) respectively). Among the patients who developed episodes of VF, the median (IQR) proportion of time spent with HIV RNA >500 copies/ml in relation to the total duration of observation for each patient was 20 (7–38)%. Seventeen (3.4%) patients died during the follow-up period (5 and 10-year cumulative incidence (95% CI) to death was 0.2/100 pyr (0.1–0.6) and 0.5/100 pyr (0.3–0.8) respectively).

Time taken to achieve CD4 T-cell counts >500 cells/ μ l with long-term cART

The majority of patients (80%) eventually reached CD4 T-cell counts >500 cells/ μ l and the median time taken to achieve this was variable 1.2 (IQR = 0.3–2.6) years. The majority of patients (75%) who reached CD4 T-cell counts >500 cells/ μ l in the cohort, achieved this threshold within 3 years of initiating cART (Figure 2A) but a subset of patients took longer. These findings did not change when the analysis was restricted to patients who showed no evidence of virological failure (defined as 2 consecutive HIV RNA >500 copies/ml) during follow-up, indicating that slower reconstitution in these patients was unlikely to be due to loss of virological control. As previously shown, stratification of the time taken to achieve CD4 T-cell counts >500 cells/ μ l by baseline CD4 T-cell counts (Figure 2B) showed that patients starting cART at lower baseline CD4 T-cell categories took the longest times to achieve counts >500 cells/ μ l [1].

A small subset of patients fail to achieve CD4 T-cell counts >500 cells/ μ l despite long-term cART

Twenty percent of patients failed to achieve CD4 T-cell counts >500 cells/ μ l. A similar proportion was found in the subset of patients who achieved complete viral suppression throughout follow-up (n = 331, Figure 2A, characteristics described in Table 1). Of the patients who failed to achieve CD4 T-cells >500 cells/ μ l despite good virological control (n = 67), 25% received at least 5 years of continuous suppressive therapy while 8% had been on therapy for more than 10 years.

The 5-year cumulative incidence (95% CI) of not achieving CD4 T-cell counts >500 cells/ μ l in patients initiating cART with baseline CD4 T-cell counts of <100, 101–200, 201–350 and >350 cells/ μ l was 7.3/100 pyr (4.9–10.9), 10.2/100 pyr (6.9–15.0), 5.8/100 pyr (3.4–10.1) and 1.5/100 pyr (0.2–10.7) respectively (Figure 2B). The 5-year cumulative incidence (95% CI) of patients not achieving counts >500 cells/ μ l was also higher among those initiating cART with a NNRTI-based regimen (9.1/100 pyr (6.8–12.1)) compared to patients initiating therapy with PI-based regimens (boosted and unboosted; 4.0/100 pyr (2.3–7.0)).

Predictors of time taken to achieve CD4 T-cell counts >500 cells/ μ l and >200 cells/ μ l following long-term cART

In a univariate analysis, factors associated with a more rapid time to achieve CD4 T-cell counts >500 cells/ μ l included higher baseline CD4 T-cells (square-transformed) (HR 1.14, 95% CI 1.13–1.16, p < 0.001), younger age at cART initiation (HR 0.99, 95% CI 0.98–0.99, p = 0.023), no history of ADI prior to cART initiation (HR 0.45, 95% CI 0.33–0.63, p < 0.001) and earlier calendar year of cART initiation (HR 0.77, 95% CI 0.61–0.97, p = 0.024) (Table 2). Although HIV exposure category, HIV RNA measure as a time dependent covariate and treatment regimen were not significant in the univariate model, these parameters were still included in the multivariate model because they were considered candidate predictors (p < 0.2) based on our pre-defined analysis strategy.

In the multivariate model, only higher baseline CD4 T-cell counts (square-transformed) (HR 1.15, 95% CI 1.13–1.16, p < 0.001), younger age (HR 0.99, 95% CI 0.98–0.998, p = 0.019) and initiating treatment with a PI-based regimen (boosted and unboosted) (vs NNRTI-based regimen) (HR 1.24, 95% CI 1.01–1.52, p = 0.043) were significantly associated with time taken to achieve CD4 T-cells >500 cells/ μ l. A prior history of ADI and calendar year of starting cART were no longer significant in the multivariate model.

In a subset of patients initiating cART at CD4 T-cell counts <200 cells/ μ l, we assessed time taken to achieve CD4 T-cells >200 cells/ μ l (n = 196). The median (IQR) baseline CD4 T-cells for these patients was 110 (50–170) cells/ μ l. In this multivariate analysis, more rapid recovery to CD4 T-cell counts >200 cells/ μ l was significantly associated with a history of ADI (HR 1.53, 95% CI 1.07–2.18, p = 0.018), higher baseline CD4 T-cell count (HR 4.53, 95% CI 3.32–6.19, p < 0.001) and higher baseline HIV RNA (HR 1.07, 95% CI 1.05–1.10, p < 0.001) (Table 3).

Discussion

CD4 T-cell recovery following cART is variable and patients who fail to achieve CD4 T-cell counts >500 cells/ μ l remain at risk of AIDS and non-AIDS defining illnesses. In a large prospective clinic-based cohort, we found that 80% of patients who achieved early (12 months post-ART) virological control following cART, achieved a CD4 T-cell count >500 cells/ μ l within a median

Table 1. Demographic and clinical characteristics of patients from AHOD who met the inclusion criteria for this study.

Patient characteristics	All patients (n=501) Median (IQR)	Patients with complete viral suppression (n=331) Median (IQR)
Gender		
- Male, n (%)	470 (93.8%)	310 (93.7%)
- Female, n (%)	30 (6.0%)	21 (6.3%)
- Transgender, n (%)	1 (0.2%)	0
Age at cART initiation (years)	39 (32.0–47.0)	41 (34.0–48.0)
Positive hepatitis C virus antibody, n (%)	41 (8.2%)	23 (6.9%)
Positive hepatitis B surface antigen, n (%)	26 (5.2%)	14 (4.2%)
Baseline CD4+ T-cell count (cells/μl)	238 (130–350)	234 (130–340)
Baseline HIV RNA (copies/ml)	87 800 (25 650–248 450)	85 600 (23 000–220 000)
HIV exposure category		
- MSM	376 (75.1%)	247 (74.8%)
- Heterosexual contact	66 (13.1%)	44 (13.3%)
- Others*	59 (11.8%)	40 (13.0%)
Follow-up duration, years	6.5 (3.4–10.2)	5.0 (2.7–9.3)
History of AIDS defining illness, n (%)	67 (13.3%)	44 (13.3%)
Death during follow-up, n (%)		
- AIDS/HIV-related	4 (0.8%)	2 (0.6%)
- Not HIV-related	9 (1.8%)	6 (1.8%)
- Unknown	4 (0.8%)	1 (0.3%)
cART regimen at baseline, n (%)		
-NNRTI-based	293 (58.4%)	202 (60.8%)
-PI-based	179 (35.7%)	106 (31.9%)
-Triple NRTI-based	20 (4.2%)	15 (4.8%)
-Others	9 (1.8%)	8 (2.4%)

All parameters are median (IQR) unless otherwise stated.

cART-combination Antiretroviral therapy; MSM – men who have sex with men; NNRTI – non-nucleoside reverse transcriptase inhibitor; PI – protease inhibitor (boosted and unboosted); NRTI – nucleoside reverse transcriptase inhibitor.

*Others included injecting drug use, transfusion and unrecorded.

PIs included Atazanavir (boosted and unboosted) (5%), Lopinavir/Ritonavir (11%), Indinavir (boosted and unboosted) (32%), Nelfinavir (27%), Ritonavir (10%), Saquinavir (boosted and unboosted) (13%), Tipranavir (1%), Fosamprenavir (1%); NNRTIs included Delavirdine (1%), Efavirenz (44%), Nevirapine (55%);

doi:10.1371/journal.pone.0020713.t001

follow-up of 1.2 years. However, there was a subset of patients who took significantly longer to eventually reach a CD4 T-cell count of 500 cells/ μ l, independent of whether there was complete viral suppression throughout follow-up. Twenty percent of this clinic-based cohort did not achieve CD4 T-cell counts >500 cells/ μ l after a median follow-up of 6.5 years. In a multivariate analysis, we found faster immune reconstitution to CD4 T-cell counts >500 cells/ μ l was significantly associated with higher baseline CD4 T-cell counts, younger age and initiation of cART with a PI-based (vs NNRTI-based) regimen.

Previous studies have used multiple approaches to quantify CD4 T-cell recovery following cART. Unlike most studies which have assessed CD4 T-cell recovery as the rate of CD4 T-cell increase or an absolute change in CD4 T-cell count, we quantified CD4 T-cell recovery by the time taken to achieve a CD4 T-cell count >500 cells and >200 cells/ μ l. We believe this approach was more robust because it accounted for both the differences in rate, pattern and extent of immune reconstitution that patients receiving cART may experience especially following long-term therapy. Additionally, this approach eliminated the need to establish a consistent pattern of CD4 T-cell increase with time, which is required when more complex regression models are used to assess CD4 T-cell recovery

[9]. Regression models however, do have the advantage of estimating subject specific and average CD4 T-cell gains in the cohort and unlike the Kaplan Meier approach is less affected by the biases that may be inferred due to the loss of patients during follow-up.

We found that the time taken to achieve CD4 T-cell counts >500cells/ μ l was highly variable, with a small but significant subset of patients experiencing prolonged periods (>5 years) with a CD4 T-cell count <500 cells/ μ l. As expected this was most commonly observed in patients who initiated cART at CD4 T-cell counts <100 cells/ μ l. Many other studies have demonstrated poor CD4 T-cell recovery in patients initiating cART at low CD4 T-cell counts [1,3,13,21] or those experiencing poor virological control [6,22,32,33] following cART. We surprisingly found that in our cohort, loss of virological control during treatment did not significantly alter the distribution of time taken to achieve counts >500cells/ μ l, nor did it influence the proportion of patients achieving this threshold. This observation may have been influenced by the fact that our study excluded patients with poor virological response in the first 12 months following cART, a time period when CD4 T-cell increases are greatest and the occurrence of virological failure at this early phase may have the strongest

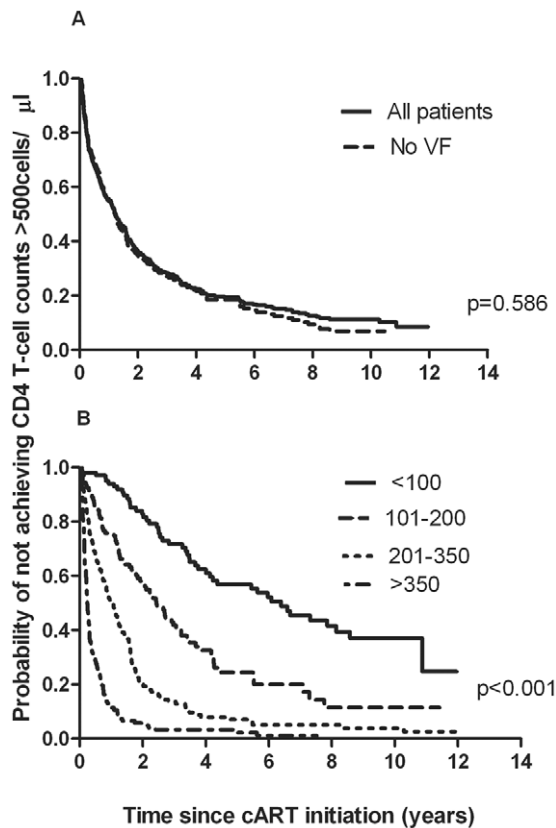


Figure 2. Kaplan-Meier plots showing time taken to achieve CD4 T-cell counts >500 cells/μl following cART initiation. Kaplan-Meier plots showing time taken to achieve CD4 counts >500cells/μl among (A) patients in the total cohort (n=501) and patients with no evidence of virological failure (VF) (2 consecutive HIV RNA>500copies/ml) throughout follow-up (n=331) and (B) all patients (n=501) stratified by baseline CD4 T-cell counts (<100cells/μl, n=99; 101–200cells/μl, n=107; 201–350cells/μl, n=172; >350cells/μl, n=123). Comparisons of survival curves were done using the Log-rank test (STATA 10.0). doi:10.1371/journal.pone.0020713.g002

negative impact on subsequent rates of CD4 T-cell increase [32]. Additionally, the median proportion of time spent with HIV RNA >500 copies/ml over the entire observation period among patients experiencing virological failure was short in this study. Other studies have described higher thresholds of detectable viremia before a significant negative influence on CD4 T-cell recovery was found to occur [6].

Even among patients who experienced continued viral suppression throughout follow-up, there was a subset of patients who took significantly longer to achieve CD4 T-cells >500 cells/μl. Similar to that described in other studies [1,13,14], we also found a small subset of patients failed to achieve counts >500 cells/μl despite receiving long-term suppressive cART.

We found baseline CD4 T-cell count was independently associated with the time taken to reach both CD4 counts >500 cells/μl and >200 cells/μl in both multivariate analyses. Numerous studies with long-term follow-up have also reported similar findings [3,4,6,8,13,21]. Patients starting therapy at low CD4 T-cell nadir may experience immunologic dysfunction that may impede their capacity to achieve robust CD4 T-cell increases with cART. Longitudinal studies have shown that patients starting therapy at low CD4 T-cell nadir experience a skewed distribution

of CD4 T cell subsets with significantly lower naïve and higher effector cell subsets compared to patients starting treatment at higher CD4 T-cell nadir (counts >350cells/μl) [34,35]. This abnormality persists following CD4 T-cell recovery even after prolonged (median 6 years) suppressive therapy [35]. The greater turnover of effector cells compared to the longer-lived naïve cells may in part contribute to the slower net increase in CD4 T-cell numbers experienced by these patients following treatment. Additionally numerous studies have also found that poor CD4 T-cell recovery following suppressive cART is associated with increased immune activation (measured by soluble and T-cell activation markers) and T-cell apoptosis [36,37,38,39,40]. Taken together, these data imply that in patients starting cART at low baseline CD4 T-cell counts, numerous pathogenic mechanisms may collectively work to slow their increase in CD4 T-cell numbers and prolong their risk of acquiring AIDS and non-AIDS defining illnesses.

Current HIV treatment guidelines are mixed on when to initiate cART with some guidelines recommending initiation at <350 cells/μl [41,42] and others recommending initiation at <500 cells/μl [43,44]. Our data demonstrated that a significantly higher proportion of patients initiating treatment at counts >350 cells/μl achieved counts >500 cells/μl and that these patients spent shorter periods below this threshold following cART compared to patients starting therapy at CD4 T-cell counts between 200–350 cells/μl. These data provide further support for earlier initiation of cART.

As previously reported [1,4,6,8,13,19,20], we found younger age to be a significant predictor of faster reconstitution to counts >500 cells/μl. The favourable immune response associated with starting cART at a younger age is probably related to the T-cell regenerative capacity of these patients compared to older patients in whom physiological involution of the thymus may limit their ability to produce naïve T-cells [45,46,47,48]. Younger age has also been associated with better CD4 T-cell recovery in other lymphopenic conditions including following hematopoietic stem cell transplantation and chemotherapy [49,50,51]. In this study, age was only significantly associated with time to reach a CD4 T-cell count >500 cells/μl and not >200 cells/μl. This was consistent with the findings from other studies [3,12,23,45] where age positively influenced long-term immune reconstitution and not early recovery (<3 months) where increases in CD4 T-cell numbers immediately following cART initiation was generally associated with the redistribution of T-cells from lymphoid tissue [34,52] rather than de novo T-cell production.

We surprisingly found that patients initiating cART with a PI-based regimen (boosted and unboosted) achieved faster reconstitution to CD4 T-cell counts >500 cells/μl when compared to patients receiving NNRTI-based regimens. This association remained significant even when calendar year was included in the multivariate model to adjust for the historical preference of initiating patients with PI-based regimens during the early cART era (data not shown). We also explored if treatment switches from the anchor regimen during follow-up had an influence on the time to reach CD4 T-cell counts >500 cells/μl by including treatment regimen as a time dependent co-variate in the model but did not find a significant association (data not shown), consistent with another recent study [53]. The lack of association between subsequent treatment switch and CD4 T-cell recovery may be because the majority of patients (86.5%) who achieved counts >500 cells/μl in this cohort did so while receiving their initial treatment regimen. The influence of treatment regimen on the capacity of long-term CD4 T-cell reconstitution is still unclear. Multiple studies have reported that patients receiving PI-based

Table 2. Predictors of time taken to achieve CD4 T-cells >500 cells/ μ l (n = 501).

Variable	Univariate Hazard Ratio (95% CI)	p-value	Multivariate Hazard Ratio (95% CI)	p-value
CD4 T-cell baseline (square-transformed), per 100 units[#]	1.14 (1.13–1.16)	<0.001	1.15 (1.13–1.16)	<0.001
Baseline HIV RNA, per 100 000 copies/ml	0.99 (0.97–1.02)	0.606		
HIV RNA per 100 000 copies/ml (time dependent covariate)	0.33 (0.09–1.12)	0.074		
Age at cART initiation, years	0.99 (0.98–0.99)	0.023	0.99 (0.98–0.998)	0.019
Initial cART regimen				
-NNRTI regimen*	-		-	
-PI regimen	1.16 (0.94–1.42)	0.158	1.24 (1.01–1.52)	0.043
-Triple NRTI regimen	1.10 (0.66–1.83)	0.716	1.08 (0.65–1.81)	0.763
-Others	1.40 (0.69–2.84)	0.349	0.77 (0.38–1.57)	0.476
Calendar year of starting cART				
-1997–1999*	-			
-2000–2004	0.77 (0.61–0.97)	0.024		
- \geq 2005	0.93 (0.70–1.23)	0.607		
HIV exposure category				
-MSM*	-			
-Heterosexual contact	0.74 (0.54–1.00)	0.051		
-Other**	0.92 (0.67–1.27)	0.610		
HBsAg positive				
-Yes	0.88 (0.56–1.38)	0.563		
-No*	-			
HCV Ab positive				
-Yes	0.81 (0.56–1.17)	0.256		
-No*	-			
Gender				
-Male	0.90 (0.61–1.33)	0.604		
-Female*	-			
History of ADI				
-Yes	0.45 (0.33–0.63)	<0.001		
-No*	-			

*Reference category.

**Others included injecting drug use, transfusion and unrecorded.

[#]For every 100-unit increase in square transformed baseline CD4 T-cell counts, the hazard of achieving a CD4 T-cell threshold of >500cells/ μ l increased by 15%.
cART-combination Antiretroviral therapy; MSM – men who have sex with men; NNRTI – non-nucleoside reverse transcriptase inhibitor; PI – protease inhibitor (boosted and unboosted) ; NRTI – nucleoside reverse transcriptase inhibitor; ADI – AIDS-defining illness; HBsAg – hepatitis B surface antigen; HCV Ab – hepatitis C antibody.
doi:10.1371/journal.pone.0020713.t002

regimens have better CD4 T-cell recovery [32,54,55,56,57,58, 59,60] while others have not confirmed these observations [61,62,63,64,65,66]. Protease inhibitors have been described to exert anti-apoptotic [67] and restore T-cell proliferative responses [68] independent of their antiretroviral activity, however these findings have also been inconsistent [69]. The positive association of PI-based regimen and more rapid CD4 T-cell recovery in our study should be interpreted with caution because this is an observational study of a clinic-based cohort where the choice of treatment offered to patients may not have been random and may have been influenced by multiple factors including degree of immunosuppression at the time of initiation of cART and viral mutation patterns that has not been adjusted for in our statistical analysis.

Apart from baseline CD4 T-cell counts, the factors associated with time to reach CD4 T-cell counts >200 cells/ μ l were found to be different from those associated with recovery to >500 cells/ μ l.

We found that patients with a history of an ADI and higher baseline HIV RNA were associated with faster recovery to counts >200 cells/ μ l consistent with other reports [3,5,6,12,14,22,23,24]. HIV-infected patients with profound immune-suppression may experience greater sequestration of CD4 T-cells into lymphoid tissues which are then released into the peripheral circulation following cART-induced viral suppression [45,52,70,71]. This might potentially explain the faster time taken to achieve CD4 T-cell counts >200 cells/ μ l among patients with higher baseline HIV RNA and ADI in this study.

We did not find an association between hepatitis C serostatus and CD4 T-cell recovery as has been previously described in some [22,61,72,73] but not all [74,75,76] studies. This could be due to the small number of patients (<10%) with HCV in this study. Additionally, HCV seropositivity is strongly associated with active injecting drug use [72] which in this study only accounted for a minority in the HIV-risk transmission group.

Table 3. Predictors of time taken to achieve CD4 T-cells >200 cells/ μ l (n = 196).

Variable	Univariate Hazard Ratio (95% CI)	p-value	Multivariate Hazard Ratio (95% CI)	p-value
CD4 T-cell baseline, per 100 cells/μl	3.35 (2.55–4.40)	<0.001	4.53 (3.32–6.19)	<0.001
Baseline HIV RNA, per 100 000 copies/ml	1.04 (1.01–1.07)	0.012	1.07 (1.05–1.10)	<0.001
HIV RNA, per 100 000 copies/ml (time dependent covariate)	0.80 (0.46–1.39)	0.425		
Age at cART initiation, years	0.99 (0.98–1.01)	0.271		
Initial cART regimen				
-NNRTI regimen*	-			
-PI regimen	1.07 (0.79–1.44)	0.666		
-Triple NRTI regimen	0.51 (0.23–1.12)	0.093		
Calendar year of starting cART				
-1997–1999*	-			
-2000–2004	0.96 (0.70–1.32)	0.802		
- \geq 2005	1.24 (0.84–1.83)	0.275		
HIV exposure category				
-MSM*	-			
-Heterosexual contact	0.71 (0.47–1.06)	0.091		
-Others**	0.64 (0.41–1.01)	0.055		
HBsAg positive				
-Yes	1.11 (0.61–2.00)	0.738		
-No*	-			
HCV Ab positive				
-Yes	1.04 (0.64–1.68)	0.868		
-No*	-			
Gender				
-Male	0.95 (0.53–1.71)	0.865		
-Female*	-			
History of ADI				
-Yes	0.79 (0.58–1.08)	0.138	1.53 (1.08–2.18)	0.018
-No*	-			

*Reference category.

**Others included injecting drug use, transfusion and unrecorded.

cART-combination Antiretroviral therapy; MSM – men who have sex with men; NNRTI – non-nucleoside reverse transcriptase inhibitor; PI – protease inhibitor (boosted and unboosted); NRTI – nucleoside reverse transcriptase inhibitor; ADI – AIDS-defining illness; HBsAg – hepatitis B surface antigen; HCV Ab – hepatitis C antibody.
doi:10.1371/journal.pone.0020713.t003

The major strength of our study is the long duration of patient follow-up and this characteristic of the cohort allowed us to distinguish patients with a slow recovery to CD4 T-cell counts >500 cells/ μ l from those who are unlikely to ever achieve this threshold. However, there were several important limitations too. First, we defined viral suppression as an HIV RNA <500 copies/ml instead of the more sensitive cut-off of <50 copies/ml [2,77]. We did this because not all patients had testing with the more sensitive HIV RNA assays in the early cART era. Second, we used the time to reach a defined event of CD4 T-cells >500 cells/ μ l or >200 cells/ μ l which could potentially overestimate the number of patients achieving these events given the wide fluctuations in CD4 T-cell counts that may occur in a patient and it is possible that these patients may not consistently maintain CD4 T-cell counts above these thresholds over the long term. As previously reported [1,53], we found that the likelihood of a patient achieving a threshold of >500 cells/ μ l or >200 cells/ μ l and then experiencing a decline in CD4 T-cell count was low. The majority of patients who reached these thresholds maintained these levels of CD4 T-

cell reconstitution unless they experienced episodes of virological failure. In patients without episodes of virological failure, only 4 patients (1.5%) who reached counts >500 cells/ μ l experienced a subsequent decline in CD4 T-cell numbers without any evidence of increase in HIV RNA while all patients who reached CD4 T-cell counts >200 cells/ μ l subsequently maintained counts above this threshold. Finally, this was an observational clinic-based cohort and therefore our results may have been biased towards patients who reliably attend physician appointments.

In summary, we found that the majority of patients in a clinic-based observational cohort eventually achieved CD4 T-cell counts >500 cells/ μ l but in 8% of patients this took over 5 years. Our findings support the initiation of cART early as both younger age and higher baseline CD4 T-cell counts were associated with faster CD4 T-cell recovery to counts >500 cells/ μ l. The small number of patients who failed to achieve counts >500 cells/ μ l despite receiving long-term suppressive cART suggest that current standard cART regimens alone may not be sufficient to achieve complete CD4 T-cell recovery in some patients. In these patients,

other alternative immune-based therapies may need to be explored.

Acknowledgments

We would like to thank Sadaf Marashi Pour and Christina Chang for helping with the data extraction and formatting the dataset, and all the patients and AHOD sites (see below for details) for participating in the study.

Australian HIV Observational Database contributors

Asterisks indicate steering committee members

New South Wales: D Ellis, General Medical Practice, Coffs Harbour; M Bloch, T Franic*, S Agrawal, L McCann, N Cunningham, Holdsworth House General Practice, Darlinghurst; D Allen, JL Little, Holden Street Clinic, Gosford; D Smith, C Gray, Lismore Sexual Health & AIDS Services, Lismore; D Baker*, R Vale, East Sydney Doctors, Surry Hills; DJ Templeton*, CG O'Connor, Chloe Dijanosic, RPA Sexual Health Clinic, Royal Prince Alfred Hospital, Camperdown; E Jackson, J Shakeshaft, K McCallum, Blue Mountains Sexual Health and HIV Clinic, Katoomba; M Grotowski, S Taylor, Tamworth Sexual Health Service, Tamworth; D Cooper, A Carr, K Hesse, K Sinn, R Norris, St Vincent's Hospital, Darlinghurst; R Finlayson, I Prone, Taylor Square Private Clinic, Darlinghurst; E Jackson, J Shakeshaft, K McCallum, Nepean Sexual Health and HIV Clinic, Penrith; K Brown, V McGrath, Illawarra Sexual Health Service, Warrawang; L Wray, P Read, H Lu, Sydney Sexual Health Centre, Sydney; Dubbo Sexual Health Centre, Dubbo; P Canavan*, J Watson*, National Association of People living with HIV/AIDS; C Lawrence*, National Aboriginal Community Controlled Health Organisation; B Mulhall*, School of Public Health, University of Sydney; M Law*, K Petoumenos*, S Marashi Pour*, S Wright*, H McManus*, C Bendall*, M Boyd*, National Centre in HIV Epidemiology and Clinical Research, University of NSW.

Northern Territory: A Kulatunga, P Knibbs, Communicable Disease Centre, Royal Darwin Hospital, Darwin.

Queensland: J Chuah*, M Ngieng, B Dickson, Gold Coast Sexual Health Clinic, Miami; D Russell, S Downing, Cairns Sexual Health Service, Cairns; D Sowden, J Broom, C Johnson, K McGill, Clinic 87, Sunshine Coast-Wide Bay Health Service District, Nambour; D Orth, D Youds, Gladstone Road Medical Centre, Highgate Hill; M Kelly, A Gibson, H Magon, AIDS Medical Unit, Brisbane.

South Australia: W Donohue, The Care and Prevention Programme, Adelaide University, Adelaide.

Victoria: R Moore, S Edwards, R Liddle, P Locke, Northside Clinic, North Fitzroy; NJ Roth*, J Nicolson*, Pahrnan Market Clinic, South Yarra; T Read, J Silvers*, W Zeng, Melbourne Sexual Health Centre, Melbourne; J Hoy*, K Watson*, M Bryant, S Price, The Alfred Hospital, Melbourne; I Woolley, M Giles, T Korman, M Salehin, Monash Medical Centre, Clayton.

Western Australia: D Nolan, J Skett, Department of Clinical Immunology, Royal Perth Hospital, Perth.

CoDe reviewers:

AHOD reviewers: D Sowden, DJ Templeton, J Hoy, L Wray, J Chuah, K Morwood, T Read, N Roth, I Woolley, M Kelly, J Broom.

TAHOD reviewers: PCK Li, MP Lee, S Vanar, S Faridah, A Kamarulzaman, JY Choi, B Vannary, R Ditangco, K Tsukada, SH Han, S Pujari, A Makane, YMA Chen, N Kumarasay, OT Ng, AJ Sasisopin.

Independent reviewers: F Drummond, M Boyd.

Author Contributions

Conceived and designed the experiments: RR SL KP. Performed the experiments: RR KP. Analyzed the data: RR MG TS. Wrote the paper: RR SL. Reviewed the manuscript: MG TS TR JH ML PC KP.

References

- Kelley CF, Kitchen CM, Hunt PW, Rodriguez B, Hecht FM, et al. (2009) Incomplete Peripheral CD4(+) Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment. *Clin Infect Dis* 48: 787–794.
- Mocroft A, Phillips AN, Gatell J, Ledergerber B, Fisher M, et al. (2007) Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. *Lancet* 370: 407–413.
- Smith CJ, Sabin CA, Youle MS, Kinloch-de Loes S, Lampe FC, et al. (2004) Factors influencing increases in CD4 cell counts of HIV-positive persons receiving long-term highly active antiretroviral therapy. *J Infect Dis* 190: 1860–1868.
- Gras L, Kesselring AM, Griffin JT, van Sighem AI, Fraser C, et al. (2007) CD4 cell counts of 800 cells/mm³ or greater after 7 years of highly active antiretroviral therapy are feasible in most patients starting with 350 cells/mm³ or greater. *J Acquir Immune Defic Syndr* 45: 183–192.
- Bosch RJ, Wang R, Vaida F, Lederman MM, Albrecht MA, et al. (2006) Changes in the Slope of the CD4 Cell Count Increase After Initiation of Potent Antiretroviral Treatment. *J Acquir Immune Defic Syndr* 43: 433–435.
- Kaufmann GR, Perrin L, Pantaleo G, Opravil M, Furrer H, et al. (2003) CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. *Arch Intern Med* 163: 2187–2195.
- Garcia F, de Lazzari E, Plana M, Castro P, Mestre G, et al. (2004) Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. *J Acquir Immune Defic Syndr* 36: 702–713.
- Kaufmann GR, Bloch M, Finlayson R, Zaunders J, Smith D, et al. (2002) The extent of HIV-1-related immunodeficiency and age predict the long-term CD4 T lymphocyte response to potent antiretroviral therapy. *AIDS* 16: 359–367.
- Le Moing V, Thiebaut R, Chene G, Sobel A, Massip P, et al. (2007) Long-term evolution of CD4 count in patients with a plasma HIV RNA persistently <500 copies/mL during treatment with antiretroviral drugs. *HIV Med* 8: 156–163.
- Tarwater PM, Margolick JB, Jin J, Phair JP, Detels R, et al. (2001) Increase and plateau of CD4 T-cell counts in the 3(1/2) years after initiation of potent antiretroviral therapy. *J Acquir Immune Defic Syndr* 27: 168–175.
- Smith CJ, Sabin CA, Lampe FC, Kinloch-de-Loes S, Gumley H, et al. (2003) The potential for CD4 cell increases in HIV-positive individuals who control viraemia with highly active antiretroviral therapy. *AIDS* 17: 963–969.
- Hunt PW, Deeks SG, Rodriguez B, Valdez H, Shade SB, et al. (2003) Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. *AIDS* 17: 1907–1915.
- Moore RD, Keruly JC (2007) CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. *Clin Infect Dis* 44: 441–446.
- Kaufmann GR, Furrer H, Ledergerber B, Perrin L, Opravil M, et al. (2005) Characteristics, Determinants, and Clinical Relevance of CD4 T Cell Recovery to <500 Cells/ μ L in HIV Type 1-Infected Individuals Receiving Potent Antiretroviral Therapy. *Clin Infect Dis* 41: 361–372.
- Baker JV, Peng G, Rapkin J, Abrams DI, Silverberg MJ, et al. (2008) CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. *AIDS* 22: 841–848.
- Baker JV, Peng G, Rapkin J, Krason D, Reilly C, et al. (2008) Poor Initial CD4+ Recovery With Antiretroviral Therapy Prolongs Immune Depletion and Increases Risk for AIDS and Non-AIDS Diseases. *J Acquir Immune Defic Syndr* 48: 541–546.
- Lichtenstein K, Armon C, Buchacz K, Chmiel J, Buckner K, et al. (2010) Low CD4+ T Cell Count Is a Risk Factor for Cardiovascular Disease Events in the HIV Outpatient Study. *Clin Infect Dis* 51: 435–447.
- Reekie J, Kosa C, Engsig F, Monforte A, Wiercinska-Drapalo A, et al. (2010) Relationship between current level of immunodeficiency and non-acquired immunodeficiency syndrome-defining malignancies. *Cancer* 116: 5306–5315.
- Althoff KN, Justice AC, Gange SJ, Deeks SG, Saag MS, et al. (2010) Virologic and immunologic response to HAART, by age and regimen class. *AIDS* 24: 2469–2479.
- Viard JP, Mocroft A, Chiesi A, Kirk O, Roge B, et al. (2001) Influence of Age on CD4 Cell Recovery in Human Immunodeficiency Virus-Infected Patients Receiving Highly Active Antiretroviral Therapy: Evidence from the EuroSIDA Study. *J Infect Dis* 183: 1290–1294.
- Falster K, Petoumenos K, Chuah J, Mijch A, Mulhall B, et al. (2009) Poor baseline immune function predicts an incomplete immune response to combination antiretroviral treatment despite sustained viral suppression. *J Acquir Immune Defic Syndr* 50: 307–313.
- Egger S, Petoumenos K, Kamarulzaman A, Hoy J, Sungkanuparph S, et al. (2009) Long-term patterns in CD4 response are determined by an interaction between baseline CD4 cell count, viral load, and time: The Asia Pacific HIV Observational Database (APHOD). *J Acquir Immune Defic Syndr* 50: 513–520.
- Castagna A, Galli L, Torti C, D'Arminio Monforte A, Mussini C, et al. (2010) Predicting the magnitude of short-term CD4+ T-cell recovery in HIV-infected patients during first-line highly active antiretroviral therapy. *Antivir Ther* 15: 165–175.
- Florence E, Lundgren J, Dreezen C, Fisher M, Kirk O, et al. (2003) Factors associated with a reduced CD4 lymphocyte count response to HAART despite full viral suppression in the EuroSIDA study. *HIV Med* 4: 255–262.
- Smith K, Valdez H, Landay A, Spritzler J, Kessler HA, et al. (2000) Thymic Size and Lymphocyte Restoration in Patients with Human Immunodeficiency Virus

- Infection after 48 Weeks of Zidovudine, Lamivudine, and Ritonavir Therapy. *J Infect Dis* 181: 141–147.
26. Ruiz-Mateos E, Rubio A, Vallejo A, De La Rosa R, Sanchez-Quijano A, et al. (2004) Thymic volume is associated independently with the magnitude of short- and long-term repopulation of CD4+ T cells in HIV-infected adults after highly active antiretroviral therapy (HAART). *Clin Exp Immunol* 136: 501–506.
 27. Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, et al. (2003) T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J Infect Dis* 187: 1534–1543.
 28. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, et al. (2009) Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis* 199: 1177–1185.
 29. The Australian HIV Observational Database (2002) Rates of combination antiretroviral treatment change in Australia, 1997–2000. *HIV Med* 3: 28–36.
 30. Lewden C, Chane G, Morlat P, Raffi Fo, Dupon M, et al. (2007) HIV-Infected Adults With a CD4 Cell Count Greater Than 500 Cells/mm³ on Long-Term Combination Antiretroviral Therapy Reach Same Mortality Rates as the General Population. *J Acquir Immune Defic Syndr* 46: 72–77.
 31. Lewden C, COHERE MWGo (2010) Time with CD4 Cell Count above 500cells/mm³ Allows HIV-infected Men, but Not Women, to Reach Similar Mortality Rates to Those of the General Population: A 7-year Analysis. 17th Conference on Retroviruses and Opportunistic Infections, 16–19 Feb 2010, San Francisco: paper #527.
 32. Trotta Maria P, Cozzi-Lepri A, Ammassari A, Vecchiet J, Cassola G, et al. (2010) Rate of CD4+ Cell Count Increase over Periods of Viral Load Suppression: Relationship with the Number of Previous Virological Failures. *Clin Infect Dis* 51: 456–464.
 33. Kaufmann GR, Bloch M, Zaunders JJ, Smith D, Cooper DA (2000) Long-term immunological response in HIV-1-infected subjects receiving potent antiretroviral therapy. *AIDS* 14: 959–969.
 34. Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, et al. (2009) Incomplete Reconstitution of T Cell Subsets on Combination Antiretroviral Therapy in the AIDS Clinical Trials Group Protocol 384. *Clin Infect Dis* 48: 350–361.
 35. Sakai K, Gatanaga H, Takata H, Oka S, Takiguchi M (2010) Comparison of CD4+ T-cell subset distribution in chronically infected HIV+ patients with various CD4 nadir counts. *Microbes Infect* 12: 374–381.
 36. Massanella Ma, Negro Eb, Perez-Alvarez Nbc, Puig Jb, Ruiz-Hernandez Ra, et al. (2010) CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. *AIDS* 24: 959–968.
 37. Piconi S, Trabattini D, Gori A, Parisotto S, Magni C, et al. (2010) Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. *AIDS* 24: 1991–2000.
 38. Negro E, Massanella M, Puig J, Perez-Álvarez N, Gallego-Escuredo JM, et al. (2010) Nadir CD4 T Cell Count as Predictor and High CD4 T Cell Intrinsic Apoptosis as Final Mechanism of Poor CD4 T Cell Recovery in Virologically Suppressed HIV-Infected Patients: Clinical Implications. *Clin Infect Dis* 50: 1300–1308.
 39. Hansjee N, Kaufmann GR, Strub C, Weber R, Bategay M, et al. (2004) Persistent Apoptosis in HIV-1-Infected Individuals Receiving Potent Antiretroviral Therapy Is Associated With Poor Recovery of CD4 T Lymphocytes. *J Acquir Immune Defic Syndr* 36: 671–677.
 40. Nakanjako D, Ssewanyana I, Mayanja-Kizza H, Kiragga A, Colebunders R, et al. (2011) High T-cell immune activation and immune exhaustion among individuals with suboptimal CD4 recovery after 4 years of antiretroviral therapy in an African cohort. *BMC Infect Dis* 11: 43.
 41. Gazzard BG, Anderson J, Babiker A, Boffito M, Brook G, et al. (2008) British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Med* 9: 563–608.
 42. Australasian Society for HIV Medicine (2010) US Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents incorporating commentary to adapt the guidelines to the Australian setting. Available: <http://ashm.org.au/guidelines>. Accessed 2010 Dec 5.
 43. Thompson MA, Aberg JA, Cahn P, Montaner JS, Rizzardini G, et al. (2010) Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. *JAMA* 304: 321–333.
 44. Panel on Antiretroviral Guidelines for Adults and Adolescents Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services (2009) Available <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. Accessed 2010 Dec 5.
 45. Lederman MM, McKinnis R, Kelleher D, Cutrell A, Mellors J, et al. (2000) Cellular restoration in HIV infected persons treated with abacavir and a protease inhibitor: age inversely predicts naive CD4 cell count increase. *AIDS* 14: 2635–2642.
 46. Teixeira L, Valdez H, McCune JM, Koup RA, Badley AD, et al. (2001) Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS* 15: 1749–1756.
 47. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, et al. (1998) Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396: 690–695.
 48. Zhang L, Lewin SR, Markowitz M, Lin HH, Skulsky E, et al. (1999) Measuring recent thymic emigrants in blood of normal and HIV-1-infected individuals before and after effective therapy. *J Exp Med* 190: 725–732.
 49. Sfrikakis PP, Gourgoulis GM, Mouloupoulos LA, Kouvatseas G, Theofilopoulos AN, et al. (2005) Age-related thymic activity in adults following chemotherapy-induced lymphopenia. *Eur J Clin Invest* 35: 380–387.
 50. Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, et al. (1995) Age, Thymopoiesis, and CD4+ T-Lymphocyte Regeneration after Intensive Chemotherapy. *N Engl J Med* 332: 143–149.
 51. Douek DC, Vescio RA, Betts MR, Brenchley JM, Hill BJ, et al. (2000) Assessment of thymic output in adults after haematopoietic stemcell transplantation and prediction of T-cell reconstitution. *Lancet* 355: 1875–1881.
 52. Bucy RP, Hockett RD, Derdeyn CA, Saag MS, Squires K, et al. (1999) Initial increase in blood CD4+ lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *J Clin Invest* 103: 1391–1398.
 53. Torti C, d'Arminio-Monforte A, Pozniak AL, Lapadula G, Cologni G, et al. (2011) Long-term CD4+ T-cell count evolution after switching from regimens including HIV nucleoside reverse transcriptase inhibitors (NRTI) plus protease inhibitors to regimens containing NRTI plus non-NRTI or only NRTI. *BMC Infect Dis* 11: 23.
 54. Bartlett JA, Fath MJ, DeMasi R, Hermes A, Quinn J, et al. (2006) An updated systematic overview of triple combination therapy in antiretroviral-naïve HIV-infected adults. *AIDS* 20: 2051–2064.
 55. van Leeuwen R, Katlama C, Murphy RL, Squires K, Gatell J, et al. (2003) A randomized trial to study first-line combination therapy with or without a protease inhibitor in HIV-1-infected patients. *AIDS* 17: 987–999.
 56. Yazdanpanah Y, Sissoko D, Egger M, Mouton Y, Zwahlen M, et al. (2004) Clinical efficacy of antiretroviral combination therapy based on protease inhibitors or non-nucleoside analogue reverse transcriptase inhibitors: indirect comparison of controlled trials. *BMJ* 328: 249.
 57. Torti C, Maggiolo F, Patroni A, Suter F, Ladisa N, et al. (2005) Exploratory analysis for the evaluation of lopinavir/ritonavir-versus efavirenz-based HAART regimens in antiretroviral-naïve HIV-positive patients: results from the Italian MASTER Cohort. *J Antimicrob Chemother* 56: 190–195.
 58. Dronda F, Moreno S, Moreno A, Casado JL, Perez-Elias MJ, et al. (2002) Long-term outcomes among antiretroviral-naïve human immunodeficiency virus-infected patients with small increases in CD4+ cell counts after successful virologic suppression. *Clin Infect Dis* 35: 1005–1009.
 59. Van Leth F, Wit F, Reiss P, Schattnerkerk J, Van Der Ende ME, et al. (2004) Differential CD4 T-cell response in HIV-1-infected patients using protease inhibitor-based or nevirapine-based highly active antiretroviral therapy. *HIV Med* 5: 74–81.
 60. Riddler SA, Haubrich R, DiRienzo AG, Peoples L, Powderly WG, et al. (2008) Class-sparing regimens for initial treatment of HIV-1 infection. *N Engl J Med* 358: 2095–2106.
 61. Khanna N, Opravil M, Furrer H, Cavassini M, Vernazza P, et al. (2008) CD4+ T Cell Count Recovery in HIV Type 1-Infected Patients Is Independent of Class of Antiretroviral Therapy. *Clin Infect Dis* 47: 1093–1101.
 62. Friedl AeC, Ledergerber B, Flepp M, Hirschel B, Telenti A, et al. (2001) Response to first protease inhibitor- and efavirenz-containing antiretroviral combination therapy The Swiss HIV Cohort Study. *AIDS* 15: 1793–1800.
 63. De Luca A, Cozzi-Lepri A, Antinori A, Zaccarelli M, Bongiovanni M, et al. (2006) Lopinavir/ritonavir or efavirenz plus two nucleoside analogues as first-line antiretroviral therapy: a non-randomized comparison. *Antivir Ther* 11: 609–618.
 64. AVANTI and INCAS Study Groups (2000) Highly active antiretroviral therapy including protease inhibitors does not confer a unique CD4 cell benefit. *AIDS* 14: 1383–1388.
 65. Giordano TP, Wright JA, Hasan MQ, White AC, Jr., Graviss EA, et al. (2003) Do sex and race/ethnicity influence CD4 cell response in patients who achieve virologic suppression during antiretroviral therapy? *Clin Infect Dis* 37: 433–437.
 66. Young J, Bucher HC, Guenthard HF, Rickenbach M, Fux CA, et al. (2009) Virological and immunological responses to efavirenz or boosted lopinavir as first-line therapy for patients with HIV. *Antivir Ther* 14: 771–779.
 67. Sloand EM, Kumar PN, Kim S, Chaudhuri A, Weichold FF, et al. (1999) Human Immunodeficiency Virus Type 1 Protease Inhibitor Modulates Activation of Peripheral Blood CD4+ T Cells and Decreases Their Susceptibility to Apoptosis In Vitro and In Vivo. *Blood* 94: 1021–1027.
 68. Lu W, Andrieu J-M (2000) HIV protease inhibitors restore impaired T-cell proliferative response in vivo and in vitro: a viral-suppression-independent mechanism. *Blood* 96: 250–258.
 69. Benito JM, Lopez M, Martin JC, Lozano S, Martinez P, et al. (2002) Differences in cellular activation and apoptosis in HIV-infected patients receiving protease inhibitors or nonnucleoside reverse transcriptase inhibitors. *AIDS Res Hum Retroviruses* 18: 1379–1388.
 70. Nokta MA, Li X-D, Al-Harhi L, Nichols J, Pou A, et al. (2002) Entrapment of recent thymic emigrants in lymphoid tissues from HIV-infected patients: association with HIV cellular viral load. *AIDS* 16: 2119–2127.
 71. Diaz M, Douek DC, Valdez H, Hill BJ, Peterson D, et al. (2003) T cells containing T cell receptor excision circles are inversely related to HIV replication and are selectively and rapidly released into circulation with antiretroviral treatment. *AIDS* 17: 1145–1149.
 72. Greub G, Ledergerber B, Bategay M, Grob P, Perrin L, et al. (2000) Clinical progression, survival, and immune recovery during antiretroviral therapy in

- patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet* 356: 1800–1805.
73. Potter M, Oduyungbo A, Yang H, Saeed S, Klein MB (2010) Impact of hepatitis C viral replication on CD4+ T-lymphocyte progression in HIV-HCV coinfection before and after antiretroviral therapy. *AIDS* 24: 1857–1865.
 74. Sulkowski MS, Moore RD, Mehta SH, Chaisson RE, Thomas DL (2002) Hepatitis C and progression of HIV disease. *JAMA* 288: 199–206.
 75. Sullivan PS, Hanson DL, Teshale EH, Wotring LL, Brooks JT (2006) Effect of hepatitis C infection on progression of HIV disease and early response to initial antiretroviral therapy. *AIDS* 20: 1171–1179.
 76. Rockstroh JK, Mocroft A, Soriano V, Tural C, Losso MH, et al. (2005) Influence of hepatitis C virus infection on HIV-1 disease progression and response to highly active antiretroviral therapy. *J Infect Dis* 192: 992–1002.
 77. Mocroft A, Phillips AN, Ledergerber B, Katlama C, Chiesi A, et al. (2006) Relationship between antiretrovirals used as part of a cART regimen and CD4 cell count increases in patients with suppressed viremia. *AIDS* 20: 1141–1150.