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Combining CD4 recovery and CD4/CD8 ratio restoration in an indicator for evaluating the outcome of continued antiretroviral therapy: an observational cohort study

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3 **Combining CD4 recovery and CD4/CD8 ratio restoration in an indicator for**
4 **evaluating the outcome of continued antiretroviral therapy: an observational**
5 **cohort study**
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ABSTRACT

Objectives. Immune recovery following highly active antiretroviral therapy (HAART) is commonly assessed by the degree of CD4 reconstitution alone. In this study, we aimed to assess immune recovery by incorporating both CD4 count and CD4/CD8 ratio.

Design: Observational cohort study

Setting and participants. Clinical data from Chinese HIV+ patients attending the largest HIV service in Hong Kong and who had been on HAART for ≥ 4 years were accessed.

Main outcome measures. Optimal immune outcome was defined as a combination of a CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 .

Results. A total of 718 patients were included for analysis (6353 person-years). At the end of Year 4, 318 out of 715 patients achieved CD4 $\geq 500/\mu\text{L}$, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥ 0.8 . Patients with a pre-HAART CD8 $\leq 800/\mu\text{L}$ (428 out of 704) were more likely to be optimal immune outcome achievers with CD4 $\geq 500/\mu\text{L}$ and CD4/CD8 ratio ≥ 0.8 , the association of which was stronger after adjusting for pre-HAART CD4 counts. In a multivariable logistic model, optimal immune outcome was positively associated with male gender, younger pre-HAART age and higher pre-HAART CD4 count, longer duration of HAART and pre-HAART CD8 $\leq 800/\mu\text{L}$. Treatment regimen and cumulative viral loads played no significant role in the pattern of immune recovery.

Conclusions. A combination of CD4 count and CD4/CD8 ratio could form an immune marker useful for the characterisation treatment outcome over time, compared to the reliance on CD4 alone.

Strengths and limitations of this study

- The combined use of both CD4 and CD4/CD8 ratio as an outcome measure for immune recovery could prevent overestimation of treatment performance by high CD4 count but low CD4/CD8 ratio.

- As only a small proportion of patients achieved CD4/CD8 ratio ≥ 1 by Year 4 after HAART initiation, we have therefore set the threshold ratio at 0.8 instead.

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INTRODUCTION

Highly active antiretroviral therapy (HAART) forms the cornerstone of modern day treatment of human immunodeficiency virus (HIV) infection. In monitoring treatment outcome, peripheral blood CD4+ lymphocyte (hereafter referred as CD4) count measurement is widely used, the results of which feature a rapid rise in the first 3-6 months followed by a second phase of gradual increase, plateauing 4 to 6 years afterwards.¹ Nadir CD4 counts and advanced age are associated with poorer CD4 recovery, a well-reported phenomenon that has been reviewed in the literature.^{1,2} While high and persistently elevated CD8+ lymphocytes (hereafter referred as CD8) is commonly observed in chronically infected HIV patients, relatively little attention has been paid to its impact on immunological recovery.³ A large cohort study suggested that markedly elevated CD8 count at HAART initiation was associated with a poor increase in CD4 count.⁴ Host factors aside, virus burden exerted by HIV could also impact immunological recovery. In the absence of timely and effective HAART, HIV cumulate over time leading to a state of cumulative viraemia, a predictor of suboptimal immunological outcome in primary HIV infection.⁵ Separate studies have shown that high cumulative viral load was a potential marker for progression to acquired immune deficiency syndrome (AIDS) in chronic HIV infection.⁶ Despite effective therapy, some 20-30% of patients were unable to achieve optimal immunological recovery,^{1,7} an outcome resulting from the interaction of a good range of host and viral factors, as well as co-infection with other pathogens, notably hepatitis C virus.³

Over the last decade, a CD4-guided approach to treatment initiation has gradually

1
2
3 been replaced by early initiation of HAART irrespective of baseline CD4 level.⁸
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5 Achievement of a high CD4 count of, say, over 500/ μ L remains a commonly used
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7 marker of immune restoration. Knowingly, prompt treatment and full viral
8
9 suppression do not imply freedom from co-morbidities, as HIV disease is also
10
11 characterised by a state of immune activation, with the emergence of non-AIDS
12
13 morbidity and mortality.⁹ This morbid state of immune activation cannot be inferred
14
15 from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following
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17 HAART has however been linked to this scenario of immune activation.^{10 11} A high
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19 CD8 count following HAART was shown to be associated with inflammatory
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21 non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction
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23 has been reported.^{4 12} Apparently, a target CD4 count is inadequate for reflecting
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25 effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly
26
27 recognized as an important marker of immune reconstitution.^{10 13}
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34 To better monitor immunological recovery following HAART, new biomarkers are
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36 needed, which should preferably be derived from routinely collected laboratory data.
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38 Optimal outcome could be founded on CD4/CD8 normalization on top of the
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40 regularly monitored CD4 count. In this study, we define HAART associated immune
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42 recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to
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44 examine its predictors by analysing regularly collected viral load and immunological
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46 data, the latter including CD8 count, in a cohort of HIV patients following HAART.
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54 METHODS

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3 Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical
4 service in Hong Kong were accessed for this observational study. Data access
5 approval was granted by Department of Health, Hong Kong Special Administrative
6 Region Government in compliance with the Personal Data (Privacy) Ordinance.
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8 Individual consent for the study was waived following approval of the Joint Chinese
9 University of Hong Kong – New Territories East Cluster Clinical Research Ethics
10 Committee (CREC). HIV patients of age ≥ 18 diagnosed in 1985-2012, on HAART
11 continuously for ≥ 4 years without treatment interruption, with at least 1 CD4
12 measurement during treatment and with viral load fully suppressed (without
13 consecutive viral load > 500 copies/mL in the first 4 years on treatment) were selected.
14
15 We included patients who were treatment naïve or have been on non-standard
16 treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and
17 CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b)
18 viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as
19 appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease
20 inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as
21 other regimens were rarely used for treatment naïve patients
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43 Estimated cumulative viral load was expressed as years $\times \log_{10}$ copies/mL, in
44 accordance with the method reported by Zoufaly et al.¹⁴ with modifications. Patients
45 with available negative HIV testing result within 3 years before HIV diagnosis were
46 included, so that one's seroconversion date could be estimated with confidence.¹⁵ In
47 brief, the products of the \log_{10} viral load were summed from estimated seroconversion
48 to subsequent specified time-point(s), with the computation of the highest viral load
49 for the undiagnosed interval and an upward adjustment by 1 \log_{10} for the presumed
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3 primary infection period. The time of seroconversion was determined as the mid-point
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5 between last negative and first positive HIV antibody testing dates. On the other hand,
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7 optimal immune outcome was defined as the achievement of a CD4 count of $\geq 500/\mu\text{L}$
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9 and a CD4/CD8 ratio ≥ 0.8 while conventional outcome was defined as achieving CD4
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11 count of $\geq 500/\mu\text{L}$ but not CD4/CD8 ratio ≥ 0.8 within specific time. Late HIV
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13 diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis.
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15 The latest CD4 and CD8 measurements ≤ 30 days before HAART initiation were used
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17 as the baseline.
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22 Comparisons between pre-HAART CD8 $> 800/\mu\text{L}$ vs $\leq 800/\mu\text{L}$ were made by odds ratio
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24 (OR) and multivariable logistic regression with pre-HAART CD4 as confounder,
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26 while correlation coefficients were calculated to test the associations between CD4
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28 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking
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30 reference from the criteria of high CD8 count (i.e. over $800/\mu\text{L}$) during primary
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32 infection reported in another study.¹⁶ CD4 (maximum value), CD8 (minimum value)
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34 and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome
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36 and conventional outcome by Year 4 on HAART over time (≤ 12 months, 12.1-24
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38 months, ... , > 96 months) were compared in generalized estimating equations (GEE).
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40 Multivariable logistic regression model (stepwise) was applied to examine the
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42 predictors of optimal immune outcome and conventional outcome. Complete case
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44 analysis was performed. Loss to follow-up and death were data end points. Statistical
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46 tests were performed in SPSS.
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56 RESULTS

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5 As of the end of 2012, data of 2974 diagnosed adults were accessed. Of these, 718
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7 eligible treatment-naïve diagnosed cases who had been on HAART continuously for
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9 ≥ 4 years were included in the study. Their case records contained 18857 clinical
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11 measurements (18693 CD4, 18521 CD8 and/or 17776 viral load measurements) at
12
13 multiple time points spanning over 6353 person-years' follow-up. General
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15 characteristics of the study population are displayed in Table 1. Overall, a majority
16
17 (84%) were male with a median age at diagnosis of 37 years (interquartile range
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19 (IQR): 31 – 45 years). The median interval from diagnosis to the latest assessment
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21 was 100 months (IQR= 74-141 months). Most were infected by either HIV-1 subtype
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23 B (31%) or CRF01_AE (38%), with men who have sex with men (MSM) accounting
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25 for 39% of the study population. The pre-HAART median CD4 and CD8 counts were
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27 109/ μ L and 673/ μ L respectively, which were positively correlated (Pearson
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29 correlation coefficient $r = 0.50$, $p < 0.001$) (See web-only Supplementary Figure 1(d)).
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31 The life-time estimated cumulative viral load at the last assessment increased with the
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33 interval between seroconversion and HAART initiation ($r = 0.94$, $p < 0.001$).
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41 During the study period, a CD4-guided approach was in place, implying that HAART
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43 was recommended when one's CD4 count fell below 350/ μ L. A majority of the
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45 patients (74%) had been started on a PI-based with 25% on NNRTI-based regimen,
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47 and 1 % had been started on non-standard regimen subsequently changed to HAART
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49 within 1 year. Integrase Inhibitors (INSTI) had not been used as a component of one's
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51 first regimen, but 3 patients had changed to raltegravir-based regimen
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53 afterwards. (Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to
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55 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients
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3 (44%) had reached 500/ μ L or above, of which 105 (33%) gave a CD4/CD8 ratio of
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5 ≥ 0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio.
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7 On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients
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9 could not reach the CD4 target. (Table 3) The temporal changes of CD4 count, CD8
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11 count and CD4/CD8 ratio over time is shown in figure 1. Whereas both CD4 count
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13 (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a steady rise from the first
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15 time-point following HAART, the temporal pattern of CD8 counts was inconspicuous
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17 (figure 1(c)). Patients with optimal immune outcome had significantly higher median
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19 CD4 and CD4/CD8 ratio but lower CD8 count than those only with satisfactory CD4
20
21 recovery (conventional outcome) in all time points (GEE model results in
22
23 Supplementary Table 1). The CD4 count at Year 4 was positively correlated with
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25 pre-HAART CD4 ($r=0.38$, $p<0.001$)(See web-only Supplementary Figure 1(a))
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27 Categorised by one's pre-HAART CD8 count, about half ($n=428$, 61%) had a lower
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29 count of $\leq 800/\mu$ L. The 2 groups had similar demographic, cumulative viral load levels
30
31 and had received similar treatment regimens. The CD4 count at Year 4 was positively
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33 correlated with pre-HAART CD8 count ($r=0.18$, $p<0.001$) (See web-only
34
35 Supplementary Figure 1(b)) whereas the latter was also positively correlated with
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37 CD8 at Year 4. ($r=0.35$, $p<0.001$) (See web-only Supplementary Figure 1(c)). After
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39 adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had higher
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41 chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR (aOR)=64.63,
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43 95% C.I.=23.47 to 177.98) (Table 2). Likewise, a low pre-HAART CD8 count of
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45 $\leq 800/\mu$ L was associated with the optimal immune outcome at Year 4, with an
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47 increased odds (aOR=5.07, 95% C.I.=2.74-9.41) after adjusting for pre-HAART CD4.
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49 There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See
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51 web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8
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3 at Year 4 ($r=0.33$, $p<0.001$) could be demonstrated (See web-only Supplementary
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5 Figure 1(f)).
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10 The following independent variables were then tested for their prediction of optimal
11 immune outcome and conventional outcome achieved since treatment initiation
12 throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART
13 age, treatment duration and male gender. In the final model, both high pre-HAART
14 CD4 and low pre-HAART CD8 were significant predictors of optimal immune
15 outcome, while only the former was a significant predictor of conventional outcome
16 (Table 4). Patients who were male and started HAART at younger age were more
17 likely to achieve both outcomes. Patients on treatment for longer time (≥ 97 months)
18 had higher odds to achieve optimal immune outcome (aOR=3.34, 95% C.I.=2.17 to
19 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78,
20 95% C.I.=1.89 to 4.09, 49-72 months as reference). As a substudy (results not shown),
21 cumulative viral load was measured but it did not show any correlation with the
22 pattern of immune response.
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43 **DISCUSSION**

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47 Pre-HAART CD4 count has long been shown to be a predictor of immunological
48 outcome 3-5 years following antiretroviral therapy.¹ Our previous longitudinal studies
49 in a cohort of Chinese HIV patients have demonstrated positive associations between
50 nidus and maximum CD4 count over 5 years irrespective of the causative virus
51 subtype or the regimens prescribed.^{17 18} In assessing antiretroviral treatment response,
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3 however, CD4 count alone appeared to add little to viral load monitoring.¹⁹ To
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5 account for the potential risk of non-AIDS related comorbidities including metabolic
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7 complications,⁹ parallel CD4/CD8 ratio testing is gaining popularity as it reflects also
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9 the intensity of chronic inflammation implicated.^{9 10} In this study, a CD4 count
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11 $\geq 500/\mu\text{L}$ in conjunction with a ratio of ≥ 0.8 was examined as a target outcome
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13 indicator for chronically infected patients on continued antiretroviral therapy. This
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15 target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year
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17 treatment period. Both pre-HAART CD4 and CD8 count, as well as the treatment
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19 interval were independent predictors of this new outcome target. While CD4 remained
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21 a useful prognostic marker, using it as the sole marker might overestimate treatment
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23 performance by including patients with high CD4 count but high CD8 count and low
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25 CD4/CD8 ratio as achiever (205 out of 715 achieved CD4 target only).
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32 In this study, we have shown that 44% of patients on HAART achieved a CD4 count
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34 $\geq 500/\mu\text{L}$ at the end of 4 years, an outcome slightly poorer than that of 59% reported
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36 by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our
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38 shorter observation period (4 instead of 5 years) and the lower median pre-HAART
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40 CD4 count ($158/\mu\text{L}$ compared to $180/\mu\text{L}$).²⁰ As concluded in the recently published
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42 “START” study examining the benefits of the initiation of antiretroviral therapy in
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44 HIV-positive adults with a CD4 count $>500/\mu\text{L}$, CD4 count *per se* could not capture
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46 all outcome effects arising from immediate HAART in chronic HIV infection.²¹ Our
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48 study confirmed that CD4/CD8 ratio could be a readily available supplementary
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50 marker to monitor immune recovery. Evidently, the ratio may vary with lengths of
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52 observations, demographics, and/or even HAART regimens.²²⁻²⁴ As the CD4/CD8
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54 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio
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3 of 0.8²² to assess the state of immune recovery at 4 years after HAART initiation.
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5 Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7
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7 years, the median observation interval of our cohort.
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11 Pre-HAART CD8 count and its normalisation following antiretroviral treatment is
12 relatively under-investigated.^{25 26} In our study, pre-HAART CD4 and pre-HAART
13 CD8 counts were positively correlated. Over time, CD4 rise went in parallel with
14 slowly falling CD8 until reaching an optimal CD4 level of $\geq 500/\mu\text{L}$ with a
15 near-normalised CD4/CD8 ratio ≥ 0.8 at Year 4. Pre-HAART CD8 was a significant
16 predictor of optimal immune outcome but not conventional outcome. The median
17 CD8 count of former group was lower than latter group of patients in all time points
18 since HAART initiation. Significant expansion of CD8 is known to occur soon after
19 infection and the phenomenon might persist throughout the course of HIV infection.
20 Recent studies suggested that CD8 normalisation was associated with early initiation
21 of HAART during acute infection.¹⁶ HIV-specific CD8 has been proposed to play an
22 important role in effecting functional cure of HIV infection.²⁷ Its relationship with the
23 absolute count of CD8 before and after HAART has not been established. With the
24 growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS
25 morbidity and chronic inflammation,^{9 10 28 29} it is possible that HIV+ patients' clinical
26 outcome could be better explained from both the ratio and CD4 count rather than from
27 the latter alone. From a virological perspective, the estimated cumulative viral load
28 can be viewed as a surrogate of prolonged non-suppression of virus load. It does not
29 however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its
30 immunological impacts could be overtaken by a long interval of HAART, if the
31 pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to
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3 early initiation of HAART in chronic HIV infection to avoid temporal accumulation
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5 of virus, a conclusion similar to that for primary HIV infection.⁵
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9 We acknowledge that our study carries a number of limitations. Foremost, all patients
10 had been on HAART during the time when a CD4-guided approach to treatment
11 initiation was enforced. As the patients had been started on either a PI-based or
12 NNRTI-based regimen, the possible impacts of newer generations of antiretroviral
13 like INSTI could not be ascertained. The results should therefore be interpreted with
14 caution, especially that strong association between INSTI-based regimen and
15 CD4/CD8 normalisation has recently been reported.³⁰ These were selection bias
16 which might have limited the extrapolation of results to the entire HIV population. In
17 addition, our dataset did not include other inflammatory or infectious outcomes and
18 therefore these could not be analysed in perspective. As the main comparative period
19 was 4 years, the minimum treatment duration of study population, the immunological
20 recovery achieved by patients in this study may not necessarily be reflecting the
21 ultimate response to HAART. We have nevertheless evaluated the outcome
22 (comprising both CD4 count and CD4/CD8 ratio) of all enrolled patients with a
23 median duration of treatment of over 6 years in the final analysis. Theoretically,
24 cohorts with patients observed throughout their lifetime would be invaluable to
25 determine the health benefits of HAART. Analyses from such life-long cohorts should
26 become a reality in the coming years or decades.
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54 CONCLUSIONS

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3 Conventionally, CD4 count has been commonly used as the main outcome marker
4 following HAART. In light of the increasing incidence of co-morbidities associated
5 with HIV related chronic inflammations, CD4 count per se appears to carry little
6 prognostic value in predicting HAART-associated immune recovery. Our results
7 suggested that a combination of CD4 count and CD4/CD8 ratio provides a better
8 reflection of immune outcome, compared to the reliance on CD4 alone. In evaluating
9 immune recovery following long-term HIV viral suppression, pre-HAART CD8 count
10 could be as important as nadir CD4 count as the independent predictors of the
11 ultimate immune outcome. As both CD4 and CD8 are often routinely collected in the
12 course of HIV management, an assessment of the temporal trends of CD4, CD8 and
13 CD4/CD8 ratio could conveniently predict the immunological outcome without the
14 need for sophisticated immune markers. Virological impact, as inferred from the
15 estimated cumulative viral load after infection, does not however add to the outcome
16 reflected from viral load suppression. The monitoring of the host immunological
17 responses remains the most important approach in assessing treatment outcome
18 following HAART.
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8 9 **Contributors**

10
11 SSL motivated and designed the study. KHW, BCKW, KCWC contributed the data
12
13 and their interpretation. NSW analysed the data. SSL wrote the article. All authors
14
15 contributed to interpretation of results and critically reviewed and edited the article.
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17

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27 interpretation of data and drafting the manuscript.
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32 33 **Competing interests**

34
35 The authors declare that there is no conflict of interest.
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40 41 **Ethics approval**

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43 Data access approval was granted by Department of Health, Hong Kong Special
44
45 Administrative Region Government in compliance with the Personal Data (Privacy)
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47 Ordinance. Individual consent for the study was waived following approval of the
48
49 Joint Chinese University of Hong Kong – New Territories East Cluster Clinical
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51 Research Ethics Committee (CREC).
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56 57 **Data sharing statement**

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3 No additional data are available
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8 **Disclaimer**

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REFERENCES

1. Battegay M, Nüesch R, Hirschel B, et al. Immunological recovery and antiretroviral therapy in HIV-1 infection. *The Lancet Infectious Diseases* 2006;6:280-87. doi: 10.1016/S1473-3099(06)70463-7
2. Gazzola L, Tincati C, Bellistri GM, et al. The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009;48(3):328-37. doi: 10.1086/595851
3. Tsiara CG, Nikolopoulos GK, Dimou NL, et al. Effect of hepatitis C virus on immunological and virological responses in HIV-infected patients initiating highly active antiretroviral therapy: a meta-analysis. *J Viral Hepat* 2013;20(10):715-24. doi: 10.1111/jvh.12101
4. Helleberg M, Kronborg G, Ullum H, et al. Course and Clinical Significance of CD8+ T-Cell Counts in a Large Cohort of HIV-Infected Individuals. *J Infect Dis* 2015;211(11):1726-34. doi: 10.1093/infdis/jiu669
5. Seng R, Goujard C, Krastinova E, et al. Influence of lifelong cumulative HIV viremia on long-term recovery of CD4+ cell count and CD4+/CD8+ ratio among patients on combination antiretroviral therapy. *AIDS (London, England)* 2015;29:595-607. doi: 10.1097/QAD.0000000000000571
6. Marconi VC, Grandits G, Okulicz JF, et al. Cumulative viral load and virologic decay patterns after antiretroviral therapy in HIV-infected subjects influence CD4 recovery and AIDS. *PLoS One* 2011;6(5):e17956. doi: 10.1371/journal.pone.0017956
7. Gaardbo JC, Hartling HJ, Gerstoft J, et al. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. *Clin Dev Immunol* 2012;2012:670957. doi: 10.1155/2012/670957
8. World Health Organization. WHO | Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV 2015 [updated 2015-10-09 06:58:51].
9. Saracino A, Bruno G, Scudeller L, et al. Chronic inflammation in a long-term cohort of HIV-infected patients according to the normalization of the CD4:CD8 ratio. *AIDS research and human retroviruses* 2014;30:1178-84. doi: 10.1089/aid.2014.0080
10. Mussini C, Lorenzini P, Cozzi-Lepri A, et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. *Lancet HIV* 2015;2(3):e98-106. doi: 10.1016/S2352-3018(15)00006-5
11. Lu W, Mehraj V, Vyboh K, et al. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc* 2015;18:20052. doi: 10.7448/IAS.18.1.20052
12. Badejo OA, Chang CC, So-Armah KA, et al. CD8+ T-cells count in acute myocardial infarction in HIV disease in a predominantly male cohort. *Biomed Res Int* 2015;2015:246870. doi: 10.1155/2015/246870
13. Serrano-Villar S, Deeks SG. CD4/CD8 ratio: an emerging biomarker for HIV. *Lancet*

- 1
2
3 HIV 2015;2(3):e76-7. doi: 10.1016/S2352-3018(15)00018-1
- 4 14. Zoufaly A, Stellbrink H-J, Heiden MAd, et al. Cumulative HIV viremia during highly
5 active antiretroviral therapy is a strong predictor of AIDS-related lymphoma.
6 *The Journal of Infectious Diseases* 2009;200:79-87. doi: 10.1086/599313
- 7 15. Pantazis N, Porter K, Costagliola D, et al. Temporal trends in prognostic markers of
8 HIV-1 virulence and transmissibility: an observational cohort study. *Lancet*
9 *HIV* 2014;1(3):e119-26. doi: 10.1016/S2352-3018(14)00002-2
- 10 16. Cao W, Mehraj V, Trottier B, et al. Early Initiation Rather Than Prolonged Duration
11 of Antiretroviral Therapy in HIV Infection Contributes to the Normalization of
12 CD8 T-Cell Counts. *Clinical infectious diseases : an official publication of the*
13 *Infectious Diseases Society of America* 2016;62(2):250-7. doi:
14 10.1093/cid/civ809
- 15 17. Naftalin CM, Wong NS, Chan DP, et al. Three different patterns of CD4 recovery in
16 a cohort of Chinese HIV patients following antiretroviral therapy - a five-year
17 observational study. *International journal of STD & AIDS* 2015;26:803-09. doi:
18 10.1177/0956462414553826
- 19 18. Wong NS, Reidpath DD, Wong KH, et al. A multilevel approach to assessing
20 temporal change of CD4 recovery following HAART initiation in a cohort of
21 Chinese HIV positive patients. *J Infect* 2015;70(6):676-8. doi:
22 10.1016/j.jinf.2014.10.012
- 23 19. Ford N, Meintjes G, Pozniak A, et al. The future role of CD4 cell count for
24 monitoring antiretroviral therapy. *Lancet Infect Dis* 2015;15(2):241-7. doi:
25 10.1016/S1473-3099(14)70896-5
- 26 20. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and
27 clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type
28 1-infected individuals receiving potent antiretroviral therapy. *Clinical*
29 *infectious diseases : an official publication of the Infectious Diseases Society of*
30 *America* 2005;41(3):361-72. doi: 10.1086/431484
- 31 21. INSIGHT START Study Group, Lundgren JD, Babiker AG, et al. Initiation of
32 Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med*
33 2015;373(9):795-807. doi: 10.1056/NEJMoa1506816
- 34 22. Menozzi M, Zona S, Santoro A, et al. CD4/CD8 ratio is not predictive of
35 multi-morbidity prevalence in HIV-infected patients but identify patients with
36 higher CVD risk. *J Int AIDS Soc* 2014;17(4 Suppl 3):19709. doi:
37 10.7448/IAS.17.4.19709
- 38 23. Leung V, Gillis J, Raboud J, et al. Predictors of CD4:CD8 ratio normalization and its
39 effect on health outcomes in the era of combination antiretroviral therapy.
40 *PLoS One* 2013;8(10):e77665. doi: 10.1371/journal.pone.0077665
- 41 24. Lichtenstein KA, Armon C, Nagabhushanam V, et al. A pilot study to assess
42 inflammatory biomarker changes when raltegravir is added to a virologically
43 suppressive HAART regimen in HIV-1-infected patients with limited
44 immunological responses. *Antiviral Therapy* 2012;17:1301-09. doi:
45 10.3851/IMP2350
- 46 25. Cao W, Mehraj V, Kaufmann DE, et al. Elevation and persistence of CD8 T-cells in
47 HIV infection: the Achilles heel in the ART era. *J Int AIDS Soc* 2016;19(1):20697.
48 doi: 10.7448/IAS.19.1.20697
- 49 26. Mudd JC, Lederman MM. CD8 T cell persistence in treated HIV infection. *Curr*
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3 *Opin HIV AIDS* 2014;9(5):500-5. doi: 10.1097/COH.0000000000000086
4 27. Jones RB, Walker BD. HIV-specific CD8(+) T cells and HIV eradication. *J Clin Invest*
5 2016;126(2):455-63. doi: 10.1172/JCI80566
6 28. Serrano-Villar S, Perez-Elias MJ, Dronda F, et al. Increased risk of serious
7 non-AIDS-related events in HIV-infected subjects on antiretroviral therapy
8 associated with a low CD4/CD8 ratio. *PLoS One* 2014;9(1):e85798. doi:
9 10.1371/journal.pone.0085798
10 29. Sainz T, Serrano-Villar S, Diaz L, et al. The CD4/CD8 ratio as a marker T-cell
11 activation, senescence and activation/exhaustion in treated HIV-infected
12 children and young adults. *AIDS* 2013;27(9):1513-6. doi:
13 10.1097/QAD.0b013e32835faa72
14 30. De Salvador-Guillouet F, Sakarovitch C, Durant J, et al. Antiretroviral Regimens
15 and CD4/CD8 Ratio Normalization in HIV-Infected Patients during the Initial
16 Year of Treatment: A Cohort Study. *PLoS One* 2015;10(10):e0140519. doi:
17 10.1371/journal.pone.0140519
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Table 1. General characteristics of study population (n=718)

	frequency	%
	<u>median</u>	<u>(IQR)</u>
(a) Demographics		
Male gender	605	84%
Ethnicity		
Chinese	581	81%
Asian (Asian other than Chinese)	87	12%
White	47	7%
African	3	0.4%
Age (yrs, at HIV diagnosis)	<u>37</u>	(31 to 45)
(b) HIV infection and diagnosis		
Mode of transmission		
heterosexual	394	55%
man-to-man sex	280	39%
injection drug use	34	5%
contaminated blood transfusion	6	1%
undetermined	4	1%
HIV-1 Subtype		
CRF01_AE	270	38%
B	224	31%
C	8	1%
Others	31	4%
unavailable	185	26%
AIDS diagnosis before treatment	239	33%
Late HIV diagnosis*	192	27%

	frequency	%
	<u>median</u>	<u>(IQR)</u>
estimated cumulative viral load [#] from seroconversion to diagnosis (n=199)	<u>8</u>	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	<u>39</u>	(33 to 46)
Months from diagnosis to treatment initiation	<u>8.67</u>	(2.75 to 33.13)
CD4 count (cells/ μ L)	<u>109</u>	(29 to 190)
CD4/CD8 ratio ^a	<u>0.14</u>	(0.06 to 0.23)
CD8 count (cells/ μ L) ^a	<u>673</u>	(441 to 966)
Viral load (log ₁₀ copies/mL) ^b	<u>5.15</u>	(4.62 to 5.58)
Estimated cumulative viral load [#] from seroconversion to treatment initiation (n=199)	<u>18</u>	(11 to 29)
(d) Antiretroviral treatment and clinical outcomes		
First HAART regimen		
NNRTI-based	182	25%
PI-based	131	18%
PI-based with booster	397	55%
non-standard	8	1%
Total treatment duration (months)	<u>85.38</u>	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years ^c	<u>476</u>	(354 to 630)
Highest CD4/CD8 ratio within 4 years ^d	<u>0.55</u>	(0.39 to 0.76)

	frequency	%
	<u>median</u>	(IQR)
CD4 count $\geq 500/\mu\text{L}$ within 4 years ^c	318	44%
CD4/CD8 ratio ≥ 0.8 within 4 years ^e	145	20%
Deceased	39	5%

*Late HIV diagnosis refers to the diagnosis of AIDS within 3 months of HIV

diagnosis

Estimated cumulative viral load expressed as years*log₁₀ viral load copies/mL

^a 14 missing values; ^b 18 missing values; ^c 2 missing values; ^d 8 missing values; ^e 3

missing values

Table 2: Comparison between patients with high (>800/ μ L) and low (\leq 800/ μ L) pre-HAART CD8 counts

Variables included in the analyses were (a) general baseline characteristics, (b) pre-HAART virological status, (c) antiretroviral therapy, and (d) outcome at year 4.

	pre-HAART CD8 >800 (n=276)		pre-HAART CD8 \leq 800 (n=428)		Univariate analysis	adjusted by pre-HAART CD4		
	median/IQR/ <u>no.</u>	%	median/IQR/ <u>no.</u>	%	OR	95% CI	aOR	95% CI
(a) Baseline characteristics								
Male gender	242	87.7%	352	82.2%	0.65	0.42 to 1.01	1.82	1.12 to 2.96*
Chinese ethnicity	222	80.4%	351	82.0%	1.11	0.75 to 1.63	1.10	0.71 to 1.71
Mode of transmission	(n=372)		(n=427)					
MSM	120	44.0%	153	35.8%	<i>ref</i>		<i>ref</i>	
Heterosexual	140	51.3%	249	58.3%	1.39	1.02 to 1.91*	0.93	0.65 to 1.33
injection drug user	13	4.8%	19	4.4%	1.15	0.54 to 2.41	0.47	0.21 to 1.08
contaminated blood transfusion	0	0.0%	6	1.4%	/		/	
Subtype	(n=206)		(n=322)					
CRF01_AE	95	46.1%	171	53.1%	<i>ref</i>		<i>ref</i>	
B	94	45.6%	129	40.1%	0.76	0.53 to 1.1	1.35	0.88 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4				
	median/IQR/ <u>no.</u>	% <u>no.</u>	median/IQR/ <u>no.</u>	%	OR	95% CI	aOR	95% CI
								2.06
C	4	1.9%	4	1.2%	0.56	0.14 to	1.13	0.25 to
						2.27		5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to	1.37	0.6 to 3.17
						1.64		
Age at diagnosis (yrs)	36.80	31.74 to	37.46	30.27 to	1.00	0.98 to	0.99	0.98 to
		43.54		45.17		1.01		1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to	0.98	0.64 to
						3.28*		1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to	0.94	0.63 to
						2.88*		1.41
(b) Pre-HAART virological status								
viral load (log ₁₀ copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to	5.20	4.69 to	1.23	1.03-1.47*	0.80	0.64-0.99*
		5.52		5.58				
Viral load log ₁₀ > 145	52.9%		259	61.7%	1.43	1.05 to	0.71	0.49 to
5						1.95*		1.02
Estimated cumulative viral load #	(n=96)		(n=101)					
	17.74	10.00 to	18.53	10.88 to	1.004	0.98 to	1.004	0.98 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4				
	median/IQR/ <u>no.</u>	median/IQR/ <u>no.</u>	OR	95% CI	aOR	95% CI		
	29.61	27.73	1.03		1.03			
(c) Antiretroviral treatment								
Months from diagnosis to HAART initiation	12.80 3.87 to 5.60	35.52 2.44 to 5.60	1.00	0.99 to 1	1.01	1 to 1.01*		
NNRTI-based initial regimen	70 25.4%	105 24.5%	0.96	0.67 to 1.36	1.84	1.22 to 2.78*		
(d) Outcome at Year 4								
CD4 count /μL	(n=246)	(n=370)						
	488 386 to 625	437 332 to 589	0.999	0.998 to 1	1.001	0.9997 to 1.002		
CD4>500/μL	117 47.6%	141 38.1%	0.68	0.49 to 0.94*	1.29	0.88 to 1.91		
CD4/CD8 ratio	(n=246)	(n=370)						
	0.49 0.36 to 0.68	0.57 0.41 to 0.79	3.61	1.93 to 6.75*	64.63	23.47 to 177.98*		
Viral load (log ₁₀ copies/mL)	(n=245)	(n=366)						
	1.88 1.88 to 2.6	1.88 1.88 to 2.6	1.18	0.73 to 1.91	0.83	0.48 to 1.44		
Suppressed viral	245 100.0%	364 99.5%	/	/	/	/		

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4
	median/IQR/ <u>no.</u> %	median/IQR/ <u>no.</u> %	OR	95% CI
			aOR	95% CI
load (≤500 copies/mL)				
CD4>500/μL & CD4/CD8 ratio>0.8	(n=243)	(n=370)		
	24 9.9%	59 15.9%	1.73	1.04 to 5.07
			2.87*	2.74 to 9.41*
Treatment (months)	83.83 62.13 to 117.42	85.05 64.17 to 116.75	1.000	0.997 to 0.999
			1.004	1.003

Note: all analyses were performed in logistic regression: simple logistic regression for univariate analyses, and multivariable logistic regression with selected confounders for multivariable analyses.

Estimated cumulative viral load from seroconversion expressed as years*log₁₀ viral load copies/mL

*p<0.05

^a 1 missing value; ^b 2 missing value

Table 3 The profiles of immunological outcomes of patients by achievement of none, one or both of the 2 target immunological markers (CD4 \geq 500, CD4/CD8 ratio \geq 0.8) before the end of a 4-year observation period#

	no.	median peak or highest CD4 count (/μL) (IQR)	median months to CD4 target (IQR)	median peak or highest CD4:CD8 ratio (IQR)	median months to target CD4:CD8 ratio (IQR)
CD4 \geq 500/μL and CD4:CD8 ratio \geq 0.8	105	741 (618 to 876)	20.63 (12.6 to 30.53)	0.98 (0.86 to 1.2)	28.90 (14.43 to 42.95)
Concurrent achievement of both targets	15	694 (569 to 1182)	20.27 (13.07 to 28.17)	1.05 (0.9 to 1.49)	20.27 (13.07 to 28.17)
CD4 target before ratio target	57	788 (660 to 921)	15.13 (8.7 to 22.88)	0.89 (0.83 to 0.99)	39.23 (30.78 to 45.98)
Ratio target before CD4 target	33	650 (547 to 764)	31.13 (22.3 to 39.4)	1.17 (1.02 to 1.56)	14.40 (8.68 to 24.08)
CD4 \geq 500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio \geq 0.8 only	32	431 (369 to 475)	/	1.05 (0.89 to 1.17)	29.32 (18.48 to 40.33)
CD4 target then changed to ratio target	4	588 (519 to 660)	20.02 (12.23 to 35.36)	0.86 (0.81 to 0.95)	36.83 (20.68 to 49.72)
Ratio target then changed to CD4 target	4	583 (521 to 636)	29.68 (20.52 to 40.38)	0.87 (0.86 to 1.01)	13.87 (5.48 to 25.45)
Failure to achieve both targets	365	362 (253 to 432)	/	0.43 (0.31 to 0.55)	/

#Equivalent to a maximum of <52 months with the inclusion of a 3-month buffer period;

Table 4. Multivariable logistic regression for evaluating variables associated with a) an optimal immunological outcome and b) conventional outcome

An optimal immunological outcome was defined as achieved CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 , and conventional outcome was defined as only achieved CD4 count $\geq 500/\mu\text{L}$ by study end-point

	a) optimal immune outcome		b) conventional outcome	
	aOR	95% C.I.	aOR	95% C.I.
Male gender	2.23	1.4 to 3.53*	1.81	1.11 to 2.96*
Age at HAART initiation	0.98	0.97 to 0.9996*	0.96	0.94 to 0.97*
Pre-HAART CD4 (μL)				
<=100	<i>ref</i>		<i>ref</i>	
101-200	2.91	1.83 to 4.62*	2.30	1.57 to 3.37*
201-300	4.61	2.53 to 8.39*	3.52	2.1 to 5.9*
>300	20.36	7.51 to 55.17*	12.84	3.6 to 45.75*
Months on treatment				
49-72	<i>ref</i>		<i>ref</i>	
73-96	1.58	0.93 to 2.67	1.67	1.08 to 2.57*
≥ 97	3.34	2.17 to 5.15*	2.78	1.89 to 4.09*
Pre-HAART CD8 $\leq 800/\mu\text{L}$	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

* $p < 0.05$

FIGURE LEGENDS

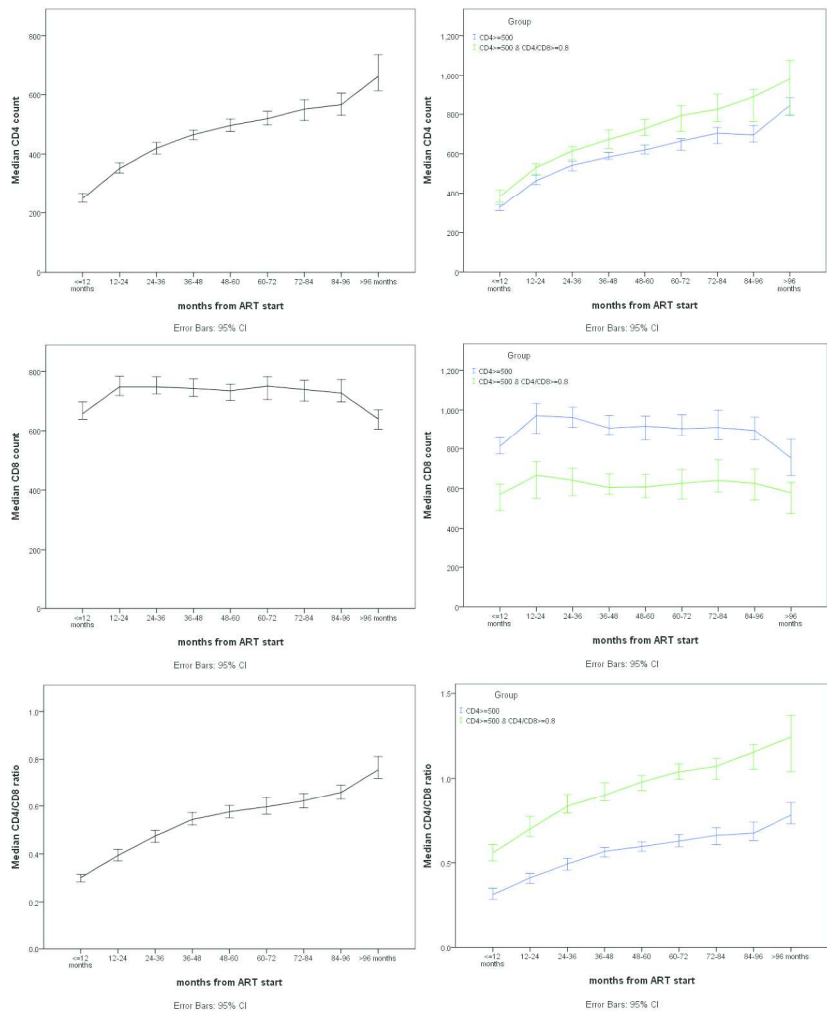
Figure 1. Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards.

Supplementary material

Supplementary Figure 1. Correlations between immunological markers of the study population in scattered plots with fitting line and 95% confidence interval (dotted lines): (a) pre-HAART CD8 versus pre-HAART CD4; (b) CD4 at year 4 versus pre-HAART CD4; (c) CD8 at year 4 versus pre-HAART CD4; (d) CD8 at year 4 versus CD4 at year 4; (e) CD8 at year 4 versus pre-HAART CD8; (f) CD4 at year 4 versus pre-HAART CD8.

Supplementary Table 1. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

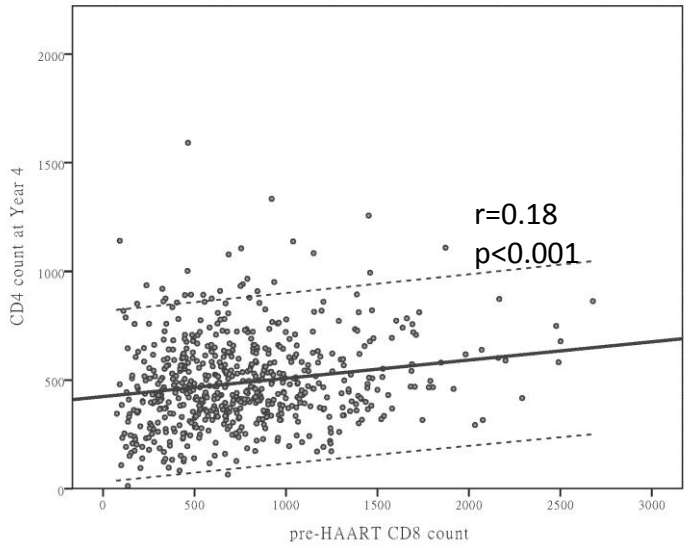
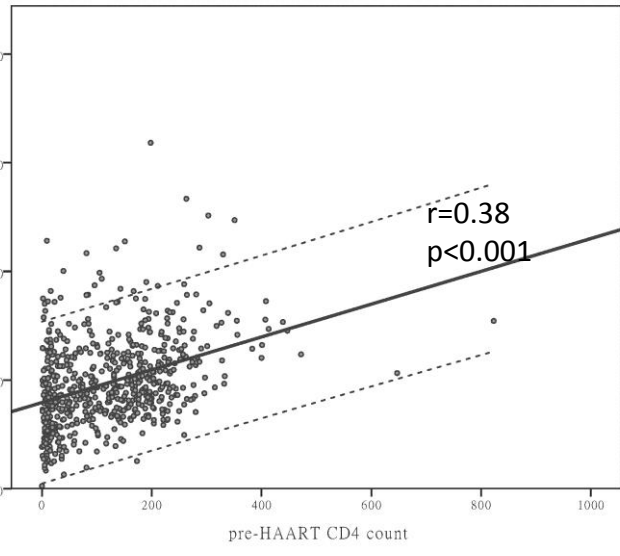
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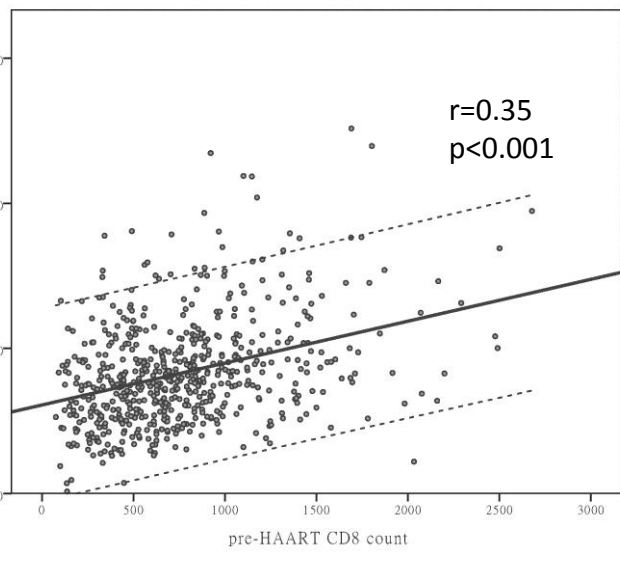
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

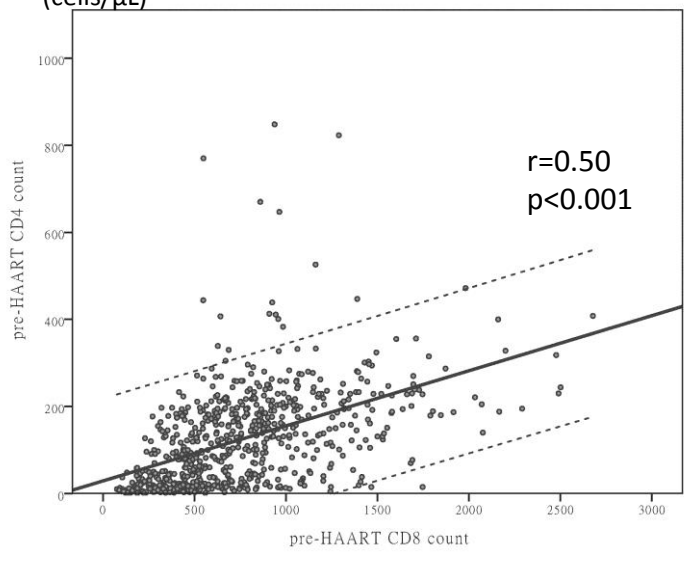
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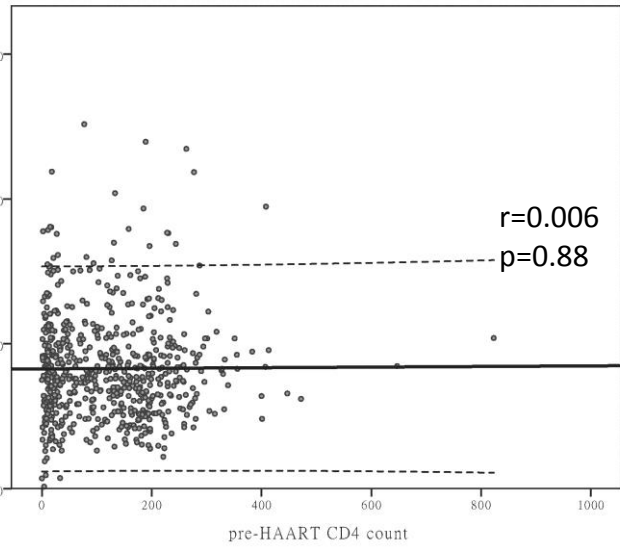
(c) pre-HAART CD8 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



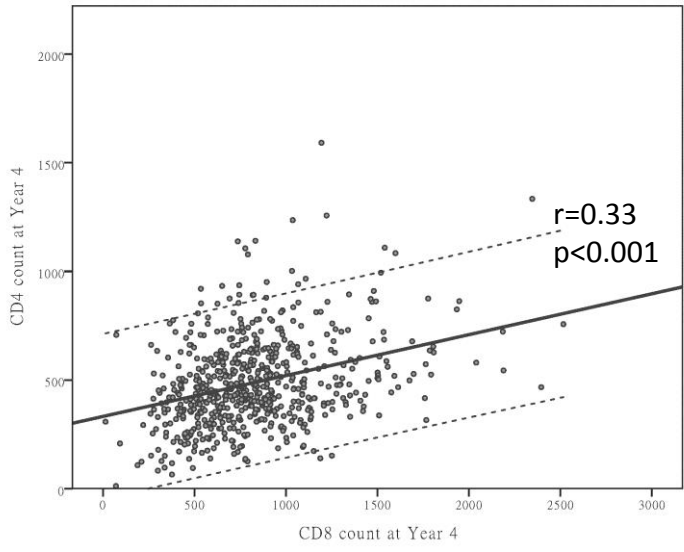
(d) pre-HAART CD8 (cells/ μ L) vs pre-HAART CD4 (cells/ μ L)



(e) pre-HAART CD4 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



(f) year 4 CD8 (cells/ μ L) vs year 4 CD4 (cells/ μ L)



Supplementary Table 1. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

Model:	a. CD4 (cells/ μ L)		b. CD8 (cells/ μ L)		c. CD4/CD8 ratio	
	B	95% C.I.	B	95% C.I.	B	95% C.I.
(Intercept)	282.86	273.99 to	1142.76	1105.97 to	0.24	0.22 to 0.25*
		291.74*		1179.55*		
Months from HAART initiation						
>96 months	421.75	400.9 to 442.59*	-54.91	-95.36 to -14.47*	0.49	0.46 to 0.52*
84-96	388.40	366.41 to 410.39*	-18.28	-61.83 to 25.27	0.44	0.41 to 0.47*
72-84	375.13	358.62 to 391.64*	-18.79	-58.14 to 20.57	0.41	0.39 to 0.43*
60-72	328.63	314.74 to 342.52*	-50.54	-85.11 to -15.98*	0.37	0.35 to 0.39*
48-60	297.14	283.97 to 310.3*	-19.01	-48.33 to 10.3	0.34	0.31 to 0.37*
36-48	267.56	256.63 to 278.49*	-14.72	-42.71 to 13.28	0.28	0.26 to 0.3*
24-36	207.20	196.89 to 217.52*	19.10	-7.03 to 45.24	0.20	0.19 to 0.22*
12-24	119.10	111.79 to 126.42*	61.80	37.11 to 86.48*	0.10	0.08 to 0.12*
≤ 12 months	0 ^a		0 ^a		0 ^a	
Achievement by year 4						
optimal	97.55	80.71 to 114.39*	-401.33	-437.93 to	0.41	0.37 to 0.45*
immune				-364.74*		

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7 conventional 0^a 0^a 0^a

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13 **(Scale)** 31443 151917 0.06

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15 *p<0.05

16 optimal immune outcome – achievement of CD4≥500/μL and CD4/CD8 ratio ≥0.8 by
17 Year 4;

18 conventional outcome – achievement of only CD4≥500/μL by Year 4;

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	14-15
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7-8
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	Table 1,2
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 3
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(b) Report category boundaries when continuous variables were categorized	Table 1,2
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Combining CD4 recovery and CD4/CD8 ratio restoration as an indicator for evaluating the outcome of continued antiretroviral therapy: an observational cohort study

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Keywords:	antiretroviral therapy, CD4, CD8, CD4/CD8 ratio, immune outcome

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3 **Combining CD4 recovery and CD4/CD8 ratio restoration as an indicator for**
4 **evaluating the outcome of continued antiretroviral therapy: an observational**
5 **cohort study**
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9 **Running title:** Immune outcome following antiretroviral therapy
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ABSTRACT

Objectives. Immune recovery following highly active antiretroviral therapy (HAART) is commonly assessed by the degree of CD4 reconstitution alone. In this study, we aimed to assess immune recovery by incorporating both CD4 count and CD4/CD8 ratio.

Design: Observational cohort study

Setting and participants. Clinical data from Chinese HIV+ patients attending the largest HIV service in Hong Kong and who had been on HAART for ≥ 4 years were accessed.

Main outcome measures. Optimal immune outcome was defined as a combination of a CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 .

Results. A total of 718 patients were included for analysis (6353 person-years). At the end of Year 4, 318 out of 715 patients achieved CD4 $\geq 500/\mu\text{L}$, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥ 0.8 . Patients with a pre-HAART CD8 $\leq 800/\mu\text{L}$ (428 out of 704) were more likely to be optimal immune outcome achievers with CD4 $\geq 500/\mu\text{L}$ and CD4/CD8 ratio ≥ 0.8 , the association of which was stronger after adjusting for pre-HAART CD4 counts. In a multivariable logistic model, optimal immune outcome was positively associated with male gender, younger pre-HAART age and higher pre-HAART CD4 count, longer duration of HAART and pre-HAART CD8 $\leq 800/\mu\text{L}$. Treatment regimen and cumulative viral loads played no significant role in the pattern of immune recovery.

Conclusions. A combination of CD4 count and CD4/CD8 ratio could be a useful approach for the characterisation of treatment outcome over time, on top of monitoring CD4 count alone.

Strengths and limitations of this study

- The combined use of CD4 and CD4/CD8 ratio as an outcome measure offers a new perspective for measuring immune recovery following antiretroviral therapy.
- The combined marker could avoid overestimation of treatment performance in patients with CD4 count but low CD4/CD8 ratio.
- The study was limited by not having included clinical events in the analysis, a gap which should be filled in larger scale cohort studies.

INTRODUCTION

Highly active antiretroviral therapy (HAART) forms the cornerstone of modern day treatment of human immunodeficiency virus (HIV) infection. In monitoring treatment outcome, peripheral blood CD4+ lymphocyte (hereafter referred as CD4) count measurement is widely used, the results of which feature a rapid rise in the first 3-6 months followed by a second phase of gradual increase, plateauing 4 to 6 years afterwards.¹ Nadir CD4 counts and advanced age are associated with poorer CD4 recovery, a well-reported phenomenon that has been reviewed in the literature.^{1,2}

While high and persistently elevated CD8+ lymphocytes (hereafter referred as CD8) is commonly observed in chronically infected HIV patients, relatively little attention has been paid to its impact on immunological recovery.³ A large cohort study suggested that markedly elevated CD8 count at HAART initiation was associated with a poor increase in CD4 count.⁴ Host factors aside, virus burden exerted by HIV could also impact immunological recovery. In the absence of timely and effective HAART, HIV cumulate over time leading to a state of cumulative viraemia, a predictor of suboptimal immunological outcome in primary HIV infection.⁵ Separate studies have shown that high cumulative viral load was a potential marker for progression to acquired immune deficiency syndrome (AIDS) in chronic HIV infection.⁶ Despite effective therapy, some 20-30% of patients were unable to achieve optimal immunological recovery,^{1,7} an outcome resulting from the interaction of a good range of host and viral factors, as well as co-infection with other pathogens, notably hepatitis C virus.³

Over the last decade, a CD4-guided approach to treatment initiation has gradually

1
2
3 been replaced by early initiation of HAART irrespective of baseline CD4 level.⁸
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5 Achievement of a high CD4 count of, say, over 500/ μ L remains a commonly used
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7 marker of immune restoration. Knowingly, prompt treatment and full viral
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9 suppression do not imply freedom from co-morbidities, as HIV disease is also
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11 characterised by a state of immune activation, with the emergence of non-AIDS
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13 morbidity and mortality.⁹ This morbid state of immune activation cannot be inferred
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15 from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following
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17 HAART has however been linked to this scenario of immune activation.^{10 11} Low
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19 CD4/CD8 ratio was observed in patients despite high CD4 level (>500/ μ L).¹² A high
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21 CD8 count following HAART was shown to be associated with inflammatory
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23 non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction
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25 has been reported.^{4 13} Apparently, a target CD4 count is inadequate for reflecting
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27 effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly
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29 recognized as an important marker of immune reconstitution.^{10 14}
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36 To better monitor immunological recovery following HAART, new biomarkers are
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38 needed, which should preferably be derived from routinely collected laboratory data.
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40 Optimal outcome could be founded on CD4/CD8 normalization on top of the
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42 regularly monitored CD4 count. In this study, we define HAART associated immune
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44 recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to
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46 examine its predictors by analysing regularly collected viral load and immunological
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48 data, the latter including CD8 count, in a cohort of HIV patients following HAART.
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56 **METHODS**

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5 Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical
6 service in Hong Kong were accessed for this observational study. Data access
7 approval was granted by Department of Health, Hong Kong Special Administrative
8 Region Government in compliance with the Personal Data (Privacy) Ordinance.
9
10 Individual consent for the study was waived following approval of the Joint Chinese
11 University of Hong Kong – New Territories East Cluster Clinical Research Ethics
12 Committee (CREC). HIV patients of age ≥ 18 diagnosed in 1985-2012, on HAART
13 continuously for ≥ 4 years without treatment interruption, with at least 1 CD4
14 measurement during treatment and with viral load fully suppressed (without
15 consecutive viral load > 500 copies/mL in the first 4 years on treatment) were selected.
16
17 We included patients who were treatment naïve or have been on non-standard
18 treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and
19 CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b)
20 viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as
21 appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease
22 inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as
23 other regimens were rarely used for treatment naïve patients
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45 Estimated cumulative viral load was expressed as $\text{years} * \log_{10} \text{copies/mL}$, in
46 accordance with the method reported by Zoufaly et al.¹⁵ with modifications. Patients
47 with available negative HIV testing result within 3 years before HIV diagnosis were
48 included, so that one's seroconversion date could be estimated with confidence.¹⁶ In
49 brief, the products of the \log_{10} viral load were summed from estimated seroconversion
50 to subsequent specified time-point(s), with the computation of the highest viral load
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3 for the undiagnosed interval and an upward adjustment by $1 \log_{10}$ for the presumed
4 primary infection period. The time of seroconversion was determined as the mid-point
5 between last negative and first positive HIV antibody testing dates. On the other hand,
6 optimal immune outcome was defined as the achievement of a CD4 count of $\geq 500/\mu\text{L}$
7 and a CD4/CD8 ratio ≥ 0.8 ¹⁷ while conventional outcome was defined as achieving
8 CD4 count of $\geq 500/\mu\text{L}$ but not CD4/CD8 ratio ≥ 0.8 within specific time. Late HIV
9 diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis.
10 The latest CD4 and CD8 measurements ≤ 30 days before HAART initiation were used
11 as the baseline.
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25 Comparisons between pre-HAART CD8 $> 800/\mu\text{L}$ vs $\leq 800/\mu\text{L}$ were made by odds ratio
26 (OR) and multivariable logistic regression with pre-HAART CD4 as confounder,
27 while correlation coefficients were calculated to test the associations between CD4
28 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking
29 reference from the criteria of high CD8 count (i.e. over $800/\mu\text{L}$) during primary
30 infection reported in another study.¹⁸ CD4 (maximum value), CD8 (minimum value)
31 and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome
32 and conventional outcome by Year 4 on HAART over time (≤ 12 months, 12.1-24
33 months, ... , > 96 months) were compared in generalized estimating equations (GEE).
34 Multivariable logistic regression model (stepwise) was applied to examine the
35 predictors of optimal immune outcome and conventional outcome. Complete case
36 analysis was performed. Loss to follow-up and death were data end points. Statistical
37 tests were performed in SPSS.
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RESULTS

As of the end of 2012, data of 2974 diagnosed adults were accessed. Of these, 718 eligible treatment-naïve diagnosed cases who had been on HAART continuously for ≥ 4 years were included in the study. Their case records contained 18857 clinical measurements (18693 CD4, 18521 CD8 and/or 17776 viral load measurements) at multiple time points spanning over 6353 person-years' follow-up. General characteristics of the study population are displayed in Table 1. Overall, a majority (84%) were male with a median age at diagnosis of 37 years (interquartile range (IQR): 31 – 45 years). The median interval from diagnosis to the latest assessment was 100 months (IQR= 74-141 months). Most were infected by either HIV-1 subtype B (31%) or CRF01_AE (38%), with men who have sex with men (MSM) accounting for 39% of the study population. The pre-HAART median CD4 and CD8 counts were 109/ μL and 673/ μL respectively, which were positively correlated (Pearson correlation coefficient $r = 0.50$, $p < 0.001$) (See web-only Supplementary Figure 1(d)). The distribution of CD4 and CD4/CD8 ratio at baseline before initiation of HAART is shown in Supplementary Table 1(a). The life-time estimated cumulative viral load at the last assessment increased with the interval between seroconversion and HAART initiation ($r = 0.94$, $p < 0.001$).

During the study period, a CD4-guided approach was in place, implying that HAART was recommended when one's CD4 count fell below 350/ μL . A majority of the patients (74%) had been started on a PI-based with 25% on NNRTI-based regimen, and 1 % had been started on non-standard regimen subsequently changed to HAART within 1 year. Integrase Inhibitors (INSTI) had not been used as a component of one's

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3 first regimen, but 3 patients had changed to raltegravir-based regimen
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5 afterwards.(Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to
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7 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients
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9 (44%) had reached 500/ μ L or above, of which 105 (33%) gave a CD4/CD8 ratio of
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11 ≥ 0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio.
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13 On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients
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15 could not reach the CD4 target. (Table 2) The temporal changes of CD4 count, CD8
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17 count and CD4/CD8 ratio over time is shown in figure 1, while distribution of CD4
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19 and CD4/CD8 ratio at the end of Year 4 is shown in Supplementary Table 1(b).
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21 Whereas both CD4 count (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a
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23 steady rise from the first time-point following HAART, the temporal pattern of CD8
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25 counts was inconspicuous (figure 1(c)). Patients with optimal immune outcome had
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27 significantly higher median CD4 and CD4/CD8 ratio but lower CD8 count than those
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29 only with satisfactory CD4 recovery (conventional outcome) in all time points (GEE
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31 model results in Supplementary Table 2). The CD4 count at Year 4 was positively
32
33 correlated with pre-HAART CD4 ($r=0.38$, $p<0.001$)(See web-only Supplementary
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35 Figure 1(a)) Categorised by one's pre-HAART CD8 count, about half ($n=428$, 61%)
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37 had a lower count of $\leq 800/\mu$ L. The 2 groups had similar demographic, cumulative
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39 viral load levels and had received similar treatment regimens. The CD4 count at Year
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41 4 was positively correlated with pre-HAART CD8 count ($r=0.18$, $p<0.001$) (See
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43 web-only Supplementary Figure 1(b)) whereas the latter was also positively correlated
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45 with CD8 at Year 4.($r=0.35$, $p<0.001$) (See web-only Supplementary Figure 1(c)).
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47 After adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had
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49 higher chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR
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51 (aOR)=64.63, 95%C.I.=23.47 to 177.98) (Table 3). Likewise, a low pre-HAART CD8
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3 count of $\leq 800/\mu\text{L}$ was associated with the optimal immune outcome at Year 4, with an
4 increased odds (aOR=5.07, 95%C.I.=2.74-9.41) after adjusting for pre-HAART CD4.
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7 There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See
8 web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8
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10 web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8
11 at Year 4 ($r=0.33$, $p<0.001$) could be demonstrated (See web-only Supplementary
12 Figure 1(f)).
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20 The following independent variables were then tested for their prediction of optimal
21 immune outcome and conventional outcome achieved since treatment initiation
22 throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART
23 age, treatment duration and male gender. In the final model, both high pre-HAART
24 CD4 and low pre-HAART CD8 were significant predictors of optimal immune
25 outcome, while only the former was a significant predictor of conventional outcome
26 (Table 4). Patients who were male and started HAART at younger age were more
27 likely to achieve both outcomes. Patients on treatment for longer time (≥ 97 months)
28 had higher odds to achieve optimal immune outcome (aOR=3.34, 95%C.I.=2.17 to
29 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78,
30 95%C.I.=1.89 to 4.09, 49-72 months as reference). As a sub-study (results not shown),
31 we have performed another set of GEE models with cumulative viral load as an
32 independent variable (results not shown). The results did not support it as a significant
33 predictor of an optimal immune outcome both in CD4 count and CD4/CD8 ratio,
34 though the number of patients eligible for the analyses was only 187.
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56 DISCUSSION

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5 Pre-HAART CD4 count has long been shown to be a predictor of immunological
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7 outcome 3-5 years following antiretroviral therapy.¹ Our previous longitudinal studies
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9 in a cohort of Chinese HIV patients have demonstrated positive associations between
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11 nidus and maximum CD4 count over 5 years irrespective of the causative virus
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13 subtype or the regimens prescribed.^{19 20} In assessing antiretroviral treatment response,
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15 however, CD4 count alone appeared to add little to viral load monitoring.²¹ To
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17 account for the potential risk of non-AIDS related comorbidities including metabolic
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19 complications,⁹ parallel CD4/CD8 ratio testing is gaining popularity as it reflects also
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21 the intensity of chronic inflammation implicated.^{9 10} In this study, a CD4 count
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23 $\geq 500/\mu\text{L}$ in conjunction with a ratio of ≥ 0.8 was examined as a target outcome
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25 indicator for chronically infected patients on continued antiretroviral therapy. This
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27 target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year
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29 treatment period. Both pre-HAART CD4 and CD8 count, as well as the treatment
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31 interval were independent predictors of this new outcome target. While CD4 remained
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33 a useful prognostic marker, using it as the sole marker might overestimate treatment
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35 performance by including patients with high CD4 count but high CD8 count and low
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37 CD4/CD8 ratio as achiever (205 out of 715 achieved CD4 target only).
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45 In this study, we have shown that 44% of patients on HAART achieved a CD4 count
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47 $\geq 500/\mu\text{L}$ at the end of 4 years, an outcome slightly poorer than that of 59% reported
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49 by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our
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51 shorter observation period (4 instead of 5 years) and the lower median pre-HAART
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53 CD4 count (158/ μL compared to 180/ μL).²² As concluded in the recently published
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55 “START” study examining the benefits of the initiation of antiretroviral therapy in
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3 HIV-positive adults with a CD4 count $>500/\mu\text{L}$, CD4 count *per se* could not capture
4 all outcome effects arising from immediate HAART in chronic HIV infection.²³ Our
5 study confirmed that CD4/CD8 ratio could be a readily available supplementary
6 marker to monitor immune recovery. Evidently, the ratio may vary with lengths of
7 observations, demographics, and/or even HAART regimens.^{17 24 25} As the CD4/CD8
8 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio
9 of 0.8¹⁷ to assess the state of immune recovery at 4 years after HAART initiation.
10 Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7
11 years, the median observation interval of our cohort.
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25 Pre-HAART CD8 count and its normalisation following antiretroviral treatment is
26 relatively under-investigated.^{26 27} In our study, pre-HAART CD4 and pre-HAART
27 CD8 counts were positively correlated. It was noted that heterosexuals gave a lower
28 pre-HAART CD8 (Table 3) compared to MSM but the difference became insignificant
29 after the adjustment of pre-HAART CD4. Over time, CD4 rise went in parallel with
30 slowly falling CD8 until reaching an optimal CD4 level of $\geq 500/\mu\text{L}$ with a
31 near-normalised CD4/CD8 ratio ≥ 0.8 at Year 4. Pre-HAART CD8 was a significant
32 predictor of optimal immune outcome but not conventional outcome. The median
33 CD8 count of former group was lower than latter group of patients in all time points
34 since HAART initiation. Significant expansion of CD8 is known to occur soon after
35 infection and the phenomenon might persist throughout the course of HIV infection.
36 Recent studies suggested that CD8 normalisation was associated with early initiation
37 of HAART during acute infection.¹⁸ HIV-specific CD8 has been proposed to play an
38 important role in effecting functional cure of HIV infection.²⁸ Its relationship with the
39 absolute count of CD8 before and after HAART has not been established. With the
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3 growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS
4 morbidity and chronic inflammation,^{9 10 29 30} it is possible that HIV+ patients' clinical
5 outcome could be better explained from both the ratio and CD4 count rather than from
6 the latter alone. From a virological perspective, the estimated cumulative viral load
7 can be viewed as a surrogate of prolonged non-suppression of virus load. It does not
8 however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its
9 immunological impacts could be overtaken by a long interval of HAART, if the
10 pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to
11 early initiation of HAART in chronic HIV infection to avoid temporal accumulation
12 of virus, a conclusion similar to that for primary HIV infection.⁵

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27 We acknowledge that our study carries a number of limitations. Foremost, all patients
28 had been on HAART during the time when a CD4-guided approach to treatment
29 initiation was enforced. As the patients had been started on either a PI-based or
30 NNRTI-based regimen, the possible impacts of newer generations of antiretroviral
31 like INSTI could not be ascertained. The results should therefore be interpreted with
32 caution, especially that strong association between INSTI-based regimen and
33 CD4/CD8 normalisation has recently been reported.³¹ These were selection bias
34 which might have limited the extrapolation of results to the entire HIV population. In
35 addition, our dataset did not include other inflammatory or infectious outcomes (e.g.
36 HCV and/or cytomegalovirus co-infections³²⁻³⁵) and therefore these could not be
37 analysed in perspective. As the main comparative period was 4 years, the minimum
38 treatment duration of study population, the immunological recovery achieved by
39 patients in this study may not necessarily be reflecting the ultimate response to
40 HAART. We have nevertheless evaluated the outcome (comprising both CD4 count
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3 and CD4/CD8 ratio) of all enrolled patients with a median duration of treatment of
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5 over 6 years in the final analysis. Theoretically, cohorts with patients observed
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7 throughout their lifetime would be invaluable to determine the health benefits of
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9 HAART. Analyses from such life-long cohorts should become a reality in the coming
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11 years or decades.
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13 14 15 16 17 18 **CONCLUSIONS** 19

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22 Conventionally, CD4 count has been commonly used as the main outcome marker
23 following HAART. In light of the increasing incidence of co-morbidities associated
24 with HIV related chronic inflammations, CD4 count per se appears to carry little
25 prognostic value in predicting HAART-associated immune recovery. Our results
26 suggested that a combination of CD4 count and CD4/CD8 ratio offers another
27 potentially useful approach to assessing immune outcome, compared to the use of
28 CD4 alone. In evaluating immune recovery following long-term HIV viral
29 suppression, pre-HAART CD8 count could be as important as nadir CD4 count as the
30 independent predictors of the ultimate immune outcome. As both CD4 and CD8 are
31 often routinely collected in the course of HIV management, an assessment of the
32 temporal trends of CD4, CD8 and CD4/CD8 ratio could conveniently predict the
33 immunological outcome without the need for sophisticated immune markers.
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35 Virological impact, as inferred from the estimated cumulative viral load after infection,
36 does not however add to the outcome reflected from viral load suppression. The
37 monitoring of the host immunological responses remains the most important approach
38 in assessing treatment outcome following HAART.
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Contributors

SSL motivated and designed the study. KHW, BCKW, KCWC contributed the data and their interpretation. NSW analysed the data. SSL wrote the article. All authors contributed to interpretation of results and critically reviewed and edited the article.

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Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Data access approval was granted by Department of Health, Hong Kong Special Administrative Region Government in compliance with the Personal Data (Privacy) Ordinance. Individual consent for the study was waived following approval of the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC).

Data sharing statement

No additional data are available

Disclaimer

The opinions and assertions contained herein are private views of the authors and do not necessarily reflect those of the Centre for Health Protection, Hong Kong Special Administrative Region Government Department of Health, or the other affiliating institutions.

REFERENCES

1. Battegay M, Nüesch R, Hirschel B, et al. Immunological recovery and antiretroviral therapy in HIV-1 infection. *The Lancet Infectious Diseases* 2006;6:280-87. doi: 10.1016/S1473-3099(06)70463-7
2. Gazzola L, Tincati C, Bellistri GM, et al. The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009;48(3):328-37. doi: 10.1086/595851
3. Tsiara CG, Nikolopoulos GK, Dimou NL, et al. Effect of hepatitis C virus on immunological and virological responses in HIV-infected patients initiating highly active antiretroviral therapy: a meta-analysis. *J Viral Hepat* 2013;20(10):715-24. doi: 10.1111/jvh.12101
4. Helleberg M, Kronborg G, Ullum H, et al. Course and Clinical Significance of CD8+ T-Cell Counts in a Large Cohort of HIV-Infected Individuals. *J Infect Dis* 2015;211(11):1726-34. doi: 10.1093/infdis/jiu669
5. Seng R, Goujard C, Krastinova E, et al. Influence of lifelong cumulative HIV viremia on long-term recovery of CD4+ cell count and CD4+/CD8+ ratio among patients on combination antiretroviral therapy. *AIDS (London, England)* 2015;29:595-607. doi: 10.1097/QAD.0000000000000571
6. Marconi VC, Grandits G, Okulicz JF, et al. Cumulative viral load and virologic decay patterns after antiretroviral therapy in HIV-infected subjects influence CD4 recovery and AIDS. *PLoS One* 2011;6(5):e17956. doi: 10.1371/journal.pone.0017956
7. Gaardbo JC, Hartling HJ, Gerstoft J, et al. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. *Clin Dev Immunol* 2012;2012:670957. doi: 10.1155/2012/670957
8. World Health Organization. WHO | Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV 2015 [updated 2015-10-09 06:58:51].
9. Saracino A, Bruno G, Scudeller L, et al. Chronic inflammation in a long-term cohort of HIV-infected patients according to the normalization of the CD4:CD8 ratio. *AIDS research and human retroviruses* 2014;30:1178-84. doi: 10.1089/aid.2014.0080
10. Mussini C, Lorenzini P, Cozzi-Lepri A, et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. *Lancet HIV* 2015;2(3):e98-106. doi: 10.1016/S2352-3018(15)00006-5
11. Lu W, Mehraj V, Vyboh K, et al. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc* 2015;18:20052. doi: 10.7448/IAS.18.1.20052
12. Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog* 2014;10(5):e1004078. doi:

- 10.1371/journal.ppat.1004078
13. Badejo OA, Chang CC, So-Armah KA, et al. CD8+ T-cells count in acute myocardial infarction in HIV disease in a predominantly male cohort. *Biomed Res Int* 2015;2015:246870. doi: 10.1155/2015/246870
 14. Serrano-Villar S, Deeks SG. CD4/CD8 ratio: an emerging biomarker for HIV. *Lancet HIV* 2015;2(3):e76-7. doi: 10.1016/S2352-3018(15)00018-1
 15. Zoufaly A, Stellbrink H-J, Heiden MAd, et al. Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. *The Journal of Infectious Diseases* 2009;200:79-87. doi: 10.1086/599313
 16. Pantazis N, Porter K, Costagliola D, et al. Temporal trends in prognostic markers of HIV-1 virulence and transmissibility: an observational cohort study. *Lancet HIV* 2014;1(3):e119-26. doi: 10.1016/S2352-3018(14)00002-2
 17. Menozzi M, Zona S, Santoro A, et al. CD4/CD8 ratio is not predictive of multi-morbidity prevalence in HIV-infected patients but identify patients with higher CVD risk. *J Int AIDS Soc* 2014;17(4 Suppl 3):19709. doi: 10.7448/IAS.17.4.19709
 18. Cao W, Mehraj V, Trottier B, et al. Early Initiation Rather Than Prolonged Duration of Antiretroviral Therapy in HIV Infection Contributes to the Normalization of CD8 T-Cell Counts. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016;62(2):250-7. doi: 10.1093/cid/civ809
 19. Naftalin CM, Wong NS, Chan DP, et al. Three different patterns of CD4 recovery in a cohort of Chinese HIV patients following antiretroviral therapy - a five-year observational study. *International journal of STD & AIDS* 2015;26:803-09. doi: 10.1177/0956462414553826
 20. Wong NS, Reidpath DD, Wong KH, et al. A multilevel approach to assessing temporal change of CD4 recovery following HAART initiation in a cohort of Chinese HIV positive patients. *J Infect* 2015;70(6):676-8. doi: 10.1016/j.jinf.2014.10.012
 21. Ford N, Meintjes G, Pozniak A, et al. The future role of CD4 cell count for monitoring antiretroviral therapy. *Lancet Infect Dis* 2015;15(2):241-7. doi: 10.1016/S1473-3099(14)70896-5
 22. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2005;41(3):361-72. doi: 10.1086/431484
 23. INSIGHT START Study Group, Lundgren JD, Babiker AG, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* 2015;373(9):795-807. doi: 10.1056/NEJMoa1506816
 24. Leung V, Gillis J, Raboud J, et al. Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. *PLoS One* 2013;8(10):e77665. doi: 10.1371/journal.pone.0077665
 25. Lichtenstein KA, Armon C, Nagabhushanam V, et al. A pilot study to assess inflammatory biomarker changes when raltegravir is added to a virologically suppressive HAART regimen in HIV-1-infected patients with limited immunological responses. *Antiviral Therapy* 2012;17:1301-09. doi:

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2
3 10.3851/IMP2350
4 26. Cao W, Mehraj V, Kaufmann DE, et al. Elevation and persistence of CD8 T-cells in
5 HIV infection: the Achilles heel in the ART era. *J Int AIDS Soc* 2016;19(1):20697.
6 doi: 10.7448/IAS.19.1.20697
7
8 27. Mudd JC, Lederman MM. CD8 T cell persistence in treated HIV infection. *Curr*
9 *Opin HIV AIDS* 2014;9(5):500-5. doi: 10.1097/COH.0000000000000086
10 28. Jones RB, Walker BD. HIV-specific CD8(+) T cells and HIV eradication. *J Clin Invest*
11 2016;126(2):455-63. doi: 10.1172/JCI80566
12 29. Serrano-Villar S, Perez-Elias MJ, Dronza F, et al. Increased risk of serious
13 non-AIDS-related events in HIV-infected subjects on antiretroviral therapy
14 associated with a low CD4/CD8 ratio. *PLoS One* 2014;9(1):e85798. doi:
15 10.1371/journal.pone.0085798
16 30. Sainz T, Serrano-Villar S, Diaz L, et al. The CD4/CD8 ratio as a marker T-cell
17 activation, senescence and activation/exhaustion in treated HIV-infected
18 children and young adults. *AIDS* 2013;27(9):1513-6. doi:
19 10.1097/QAD.0b013e32835faa72
20 31. De Salvador-Guillouet F, Sakarovitch C, Durant J, et al. Antiretroviral Regimens
21 and CD4/CD8 Ratio Normalization in HIV-Infected Patients during the Initial
22 Year of Treatment: A Cohort Study. *PLoS One* 2015;10(10):e0140519. doi:
23 10.1371/journal.pone.0140519
24 32. Saracino A, Bruno G, Scudeller L, et al. CD4 and CD4/CD8 ratio progression in
25 HIV-HCV infected patients after achievement of SVR. *J Clin Virol* 2016;81:94-9.
26 doi: 10.1016/j.jcv.2016.05.019
27 33. Brites-Alves C, Netto EM, Brites C. Coinfection by Hepatitis C Is Strongly
28 Associated with Abnormal CD4/CD8 Ratio in HIV Patients under Stable ART in
29 Salvador, Brazil. *J Immunol Res* 2015;2015:174215. doi: 10.1155/2015/174215
30 34. Freeman ML, Mudd JC, Shive CL, et al. CD8 T-Cell Expansion and Inflammation
31 Linked to CMV Coinfection in ART-treated HIV Infection. *Clinical infectious*
32 *diseases : an official publication of the Infectious Diseases Society of America*
33 2016;62(3):392-6. doi: 10.1093/cid/civ840
34 35. Smith DM, Nakazawa M, Freeman ML, et al. Asymptomatic CMV Replication
35 During Early Human Immunodeficiency Virus (HIV) Infection Is Associated
36 With Lower CD4/CD8 Ratio During HIV Treatment. *Clinical infectious diseases :*
37 *an official publication of the Infectious Diseases Society of America*
38 2016;63(11):1517-24. doi: 10.1093/cid/ciw612
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Table 1. General characteristics of study population (n=718)

	frequency	%
	<u>median</u>	<u>(IQR)</u>
(a) Demographics		
Male gender	605	84%
Ethnicity		
Chinese	581	81%
Asian (Asian other than Chinese)	87	12%
White	47	7%
African	3	0.4%
Age (yrs, at HIV diagnosis)	<u>37</u>	(31 to 45)
(b) HIV infection and diagnosis		
Mode of transmission		
heterosexual	394	55%
man-to-man sex	280	39%
injection drug use	34	5%
contaminated blood transfusion	6	1%
undetermined	4	1%
HIV-1 Subtype		
CRF01_AE	270	38%
B	224	31%
C	8	1%
Others	31	4%
unavailable	185	26%
AIDS diagnosis before treatment	239	33%
Late HIV diagnosis*	192	27%

	frequency	%
	<u>median</u>	<u>(IQR)</u>
estimated cumulative viral load [#] from seroconversion to diagnosis (n=199)	<u>8</u>	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	<u>39</u>	(33 to 46)
Months from diagnosis to treatment initiation	<u>8.67</u>	(2.75 to 33.13)
CD4 count (cells/ μ L)	<u>109</u>	(29 to 190)
CD4/CD8 ratio ^a	<u>0.14</u>	(0.06 to 0.23)
CD8 count (cells/ μ L) ^a	<u>673</u>	(441 to 966)
Viral load (log ₁₀ copies/mL) ^b	<u>5.15</u>	(4.62 to 5.58)
Estimated cumulative viral load [#] from seroconversion to treatment initiation (n=199)	<u>18</u>	(11 to 29)
(d) Antiretroviral treatment and clinical outcomes		
First HAART regimen		
NNRTI-based	182	25%
PI-based	131	18%
PI-based with booster	397	55%
non-standard	8	1%
Total treatment duration (months)	<u>85.38</u>	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years ^c	<u>476</u>	(354 to 630)
Highest CD4/CD8 ratio within 4 years ^d	<u>0.55</u>	(0.39 to 0.76)

	frequency	%
	<u>median</u>	<u>(IQR)</u>
CD4 count $\geq 500/\mu\text{L}$ within 4 years ^c	318	44%
CD4/CD8 ratio ≥ 0.8 within 4 years ^e	145	20%
Deceased	39	5%

*Late HIV diagnosis refers to the diagnosis of AIDS within 3 months of HIV diagnosis

Estimated cumulative viral load expressed as years*log₁₀ viral load copies/mL

^a 14 missing values; ^b 18 missing values; ^c 2 missing values; ^d 8 missing values; ^e 3 missing values

Table 2 The profiles of immunological outcomes of patients by achievement of none, one or both of the 2 target immunological markers (CD4 \geq 500, CD4/CD8 ratio \geq 0.8) before the end of a 4-year observation period#

	no.	median peak or highest CD4 count (/μL) (IQR)	median months to CD4 target (IQR)	median peak or highest CD4:CD8 ratio (IQR)	median months to target CD4:CD8 ratio (IQR)
CD4 \geq 500/μL and CD4:CD8 ratio \geq 0.8	105	741 (618 to 876)	20.63 (12.6 to 30.53)	0.98 (0.86 to 1.2)	28.90 (14.43 to 42.95)
Concurrent achievement of both targets	15	694 (569 to 1182)	20.27 (13.07 to 28.17)	1.05 (0.9 to 1.49)	20.27 (13.07 to 28.17)
CD4 target before ratio target	57	788 (660 to 921)	15.13 (8.7 to 22.88)	0.89 (0.83 to 0.99)	39.23 (30.78 to 45.98)
Ratio target before CD4 target	33	650 (547 to 764)	31.13 (22.3 to 39.4)	1.17 (1.02 to 1.56)	14.40 (8.68 to 24.08)
CD4 \geq 500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio \geq 0.8 only	32	431 (369 to 475)	/	1.05 (0.89 to 1.17)	29.32 (18.48 to 40.33)
CD4 target then changed to ratio target	4	588 (519 to 660)	20.02 (12.23 to 35.36)	0.86 (0.81 to 0.95)	36.83 (20.68 to 49.72)
Ratio target then changed to CD4 target	4	583 (521 to 636)	29.68 (20.52 to 40.38)	0.87 (0.86 to 1.01)	13.87 (5.48 to 25.45)
Failure to achieve both targets	365	362 (253 to 432)	/	0.43 (0.31 to 0.55)	/

#Equivalent to a maximum of <52 months with the inclusion of a 3-month buffer period;

Table 3. Comparison between patients with high (>800/ μ L) and low (\leq 800/ μ L) pre-HAART CD8 counts

Variables included in the analyses were (a) general baseline characteristics, (b) pre-HAART virological status, (c) antiretroviral therapy, and (d) outcome at year 4.

	pre-HAART CD8 >800 (n=276)		pre-HAART CD8 \leq 800 (n=428)		Univariate analysis		adjusted by pre-HAART CD4	
	median/ <u>no.</u>	IQR/ <u>%</u>	median/ <u>no.</u>	IQR/ <u>%</u>	OR	95% CI	aOR	95% CI
(a) Baseline characteristics								
Male gender	242	87.7%	352	82.2%	0.65	0.42 to 1.01	1.82	1.12 to 2.96*
Chinese ethnicity	222	80.4%	351	82.0%	1.11	0.75 to 1.63	1.10	0.71 to 1.71
Mode of transmission	(n=372)		(n=427)					
MSM	120	44.0%	153	35.8%	<i>ref</i>		<i>ref</i>	
Heterosexual	140	51.3%	249	58.3%	1.39	1.02 to 1.91*	0.93	0.65 to 1.33
injection drug user	13	4.8%	19	4.4%	1.15	0.54 to 2.41	0.47	0.21 to 1.08
contaminated blood transfusion	0	0.0%	6	1.4%	/		/	
Subtype	(n=206)		(n=322)					
CRF01_AE	95	46.1%	171	53.1%	<i>ref</i>		<i>ref</i>	
B	94	45.6%	129	40.1%	0.76	0.53 to 1.1	1.35	0.88 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4				
	median/IQR/ <u>no.</u>	% <u>no.</u>	median/IQR/ <u>no.</u>	%	OR	95% CI	aOR	95% CI
								2.06
C	4	1.9%	4	1.2%	0.56	0.14 to	1.13	0.25 to
						2.27		5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to	1.37	0.6 to 3.17
						1.64		
Age at diagnosis (yrs)	36.80	31.74 to	37.46	30.27 to	1.00	0.98 to	0.99	0.98 to
		43.54		45.17		1.01		1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to	0.98	0.64 to
						3.28*		1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to	0.94	0.63 to
						2.88*		1.41
(b) Pre-HAART virological status								
viral load (log ₁₀ copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to	5.20	4.69 to	1.23	1.03-1.47*	0.80	0.64-0.99*
		5.52		5.58				
Viral load log ₁₀ > 145	52.9%		259	61.7%	1.43	1.05 to	0.71	0.49 to
5						1.95*		1.02
Estimated cumulative viral load #	(n=96)		(n=101)					
	17.74	10.00 to	18.53	10.88 to	1.004	0.98 to	1.004	0.98 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4
	median/IQR/ <u>no.</u> %	median/IQR/ <u>no.</u> %	OR	95% CI
			aOR	95% CI
	29.61	27.73	1.03	1.03
(c) Antiretroviral treatment				
Months from diagnosis to HAART initiation	12.80 3.87 to 5.60	5.60 2.44 to 30.58	1.00	0.99 to 1.01
NNRTI-based initial regimen	70 25.4%	105 24.5%	0.96	0.67 to 1.36
				1.84 1.22 to 2.78*
(d) Outcome at Year 4				
CD4 count /μL	(n=246)	(n=370)		
	488 386 to 625	437 332 to 589	0.999	0.998 to 1.001
CD4>500/μL	117 47.6%	141 38.1%	0.68	0.49 to 0.94*
CD4/CD8 ratio	(n=246)	(n=370)		
	0.49 0.36 to 0.68	0.57 0.41 to 0.79	3.61	1.93 to 64.63
				23.47 to 177.98*
Viral load (log ₁₀ copies/mL)	(n=245)	(n=366)		
	1.88 1.88 to 2.6	1.88 1.88 to 2.6	1.18	0.73 to 1.91
Suppressed viral	245 100.0%	364 99.5%	/	/

	pre-HAART CD8 >800 (n=276)		pre-HAART CD8 ≤800 (n=428)		Univariate analysis	adjusted by pre-HAART CD4		
	median/ IQR/ % <u>no.</u>	median/ IQR/ % <u>no.</u>	OR	95% CI	aOR	95% CI		
load (≤500 copies/mL)								
CD4>500/μL & CD4/CD8 ratio>0.8	(n=243)	(n=370)						
	24	9.9%	59	15.9%	1.73	1.04 to	5.07	
						2.87*	2.74 to	
							9.41*	
Treatment (months)	83.83	62.13 to	85.05	64.17 to	1.000	0.997 to	0.999	
		117.42		116.75		1.004	1.003	

Note: all analyses were performed in logistic regression: simple logistic regression for univariate analyses, and multivariable logistic regression with selected confounders for multivariable analyses.

Estimated cumulative viral load from seroconversion expressed as years*log₁₀ viral load copies/mL

*p<0.05

^a 1 missing value; ^b 2 missing value

Table 4. Multivariable logistic regression for evaluating variables associated with a) an optimal immunological outcome and b) conventional outcome

An optimal immunological outcome was defined as achieved CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 , and conventional outcome was defined as only achieved CD4 count $\geq 500/\mu\text{L}$ by study end-point

	a) optimal immune outcome		b) conventional outcome	
	aOR	95% C.I.	aOR	95% C.I.
Male gender	2.23	1.4 to 3.53*	1.81	1.11 to 2.96*
Age at HAART initiation	0.98	0.97 to 0.9996*	0.96	0.94 to 0.97*
Pre-HAART CD4 (μL)				
<=100	<i>ref</i>		<i>ref</i>	
101-200	2.91	1.83 to 4.62*	2.30	1.57 to 3.37*
201-300	4.61	2.53 to 8.39*	3.52	2.1 to 5.9*
>300	20.36	7.51 to 55.17*	12.84	3.6 to 45.75*
Months on treatment				
49-72	<i>ref</i>		<i>ref</i>	
73-96	1.58	0.93 to 2.67	1.67	1.08 to 2.57*
≥ 97	3.34	2.17 to 5.15*	2.78	1.89 to 4.09*
Pre-HAART CD8 $\leq 800/\mu\text{L}$	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

* $p < 0.05$

FIGURE LEGENDS

Figure 1. Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards.

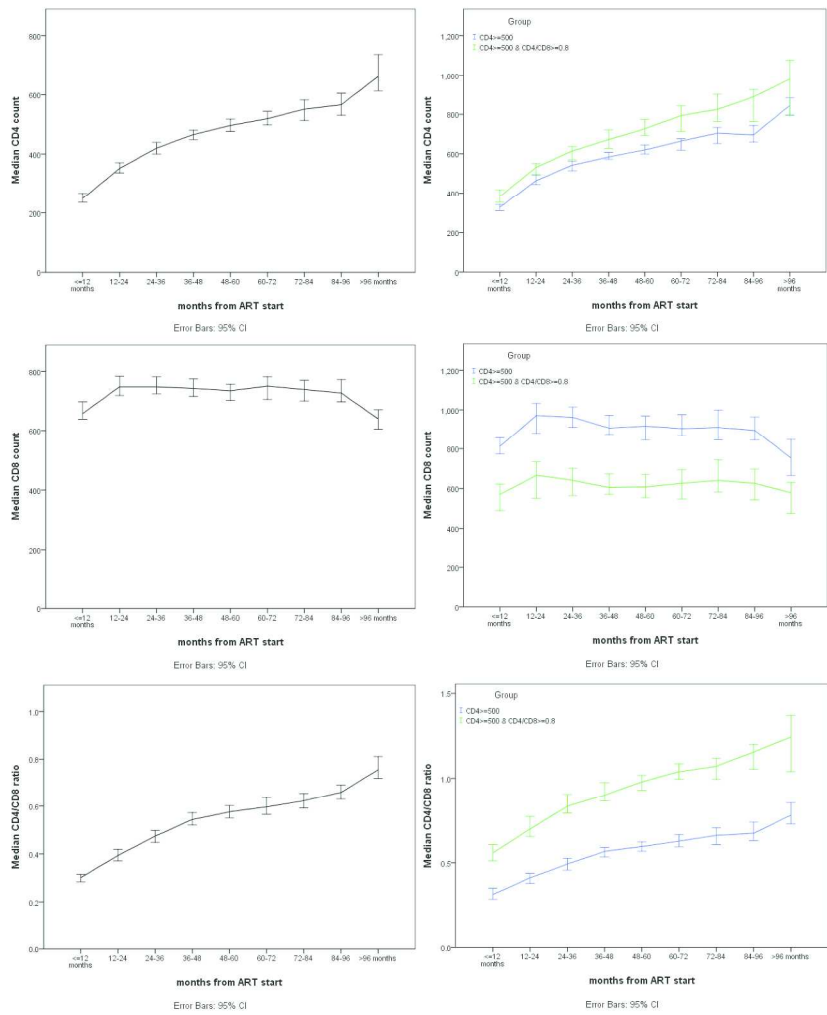
Supplementary material

Supplementary Figure 1. Correlations between immunological markers of the study population in scattered plots with fitting line and 95% confidence interval (dotted lines): (a) pre-HAART CD8 versus pre-HAART CD4; (b) CD4 at year 4 versus pre-HAART CD4; (c) CD8 at year 4 versus pre-HAART CD4; (d) CD8 at year 4 versus CD4 at year 4; (e) CD8 at year 4 versus pre-HAART CD8; (f) CD4 at year 4 versus pre-HAART CD8.

Supplementary Table 1. Relationship between CD4 count and corresponding CD4/CD8 ratio at (a) baseline before initiation of highly active antiretroviral therapy (pre-HAART) and (b) outcome at the end of Year 4 following HAART

Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

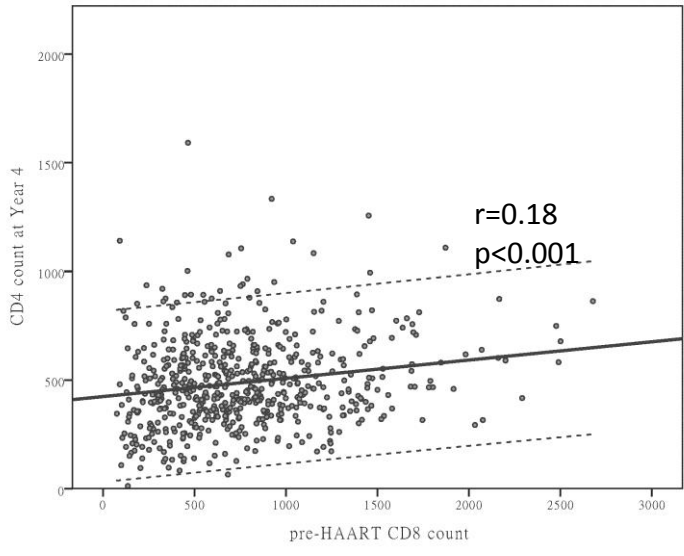
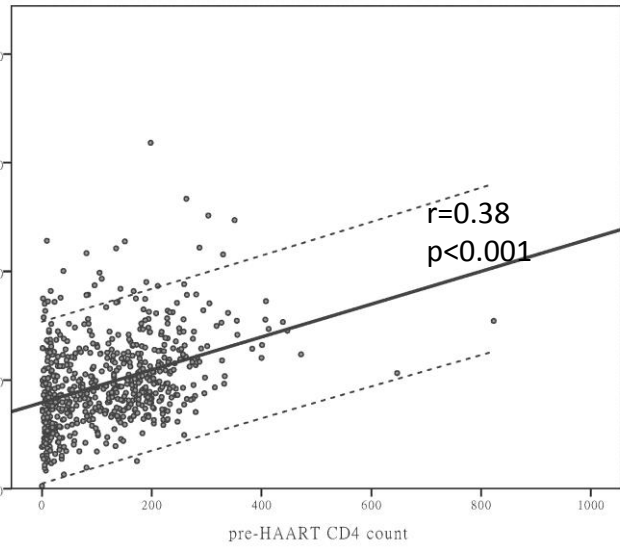
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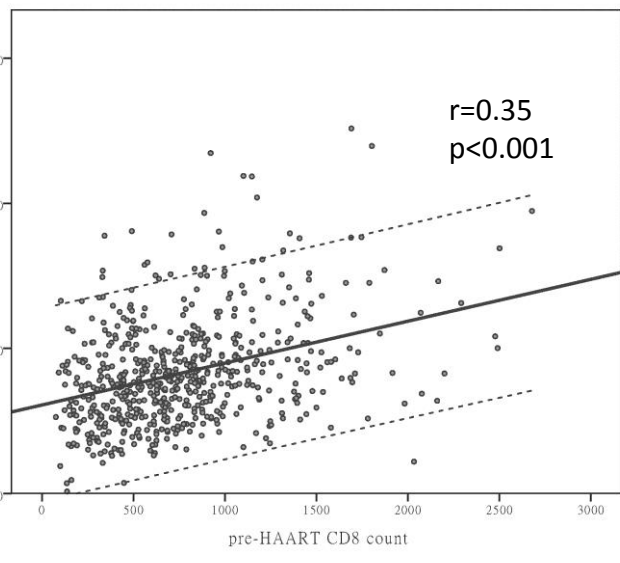
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

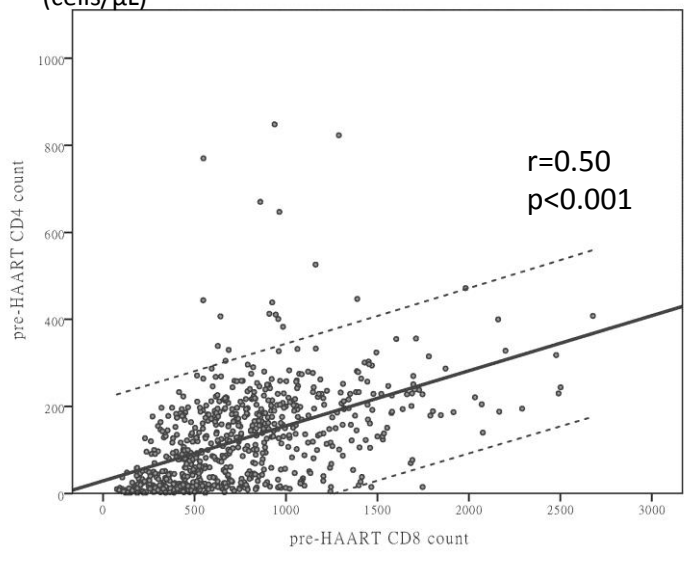
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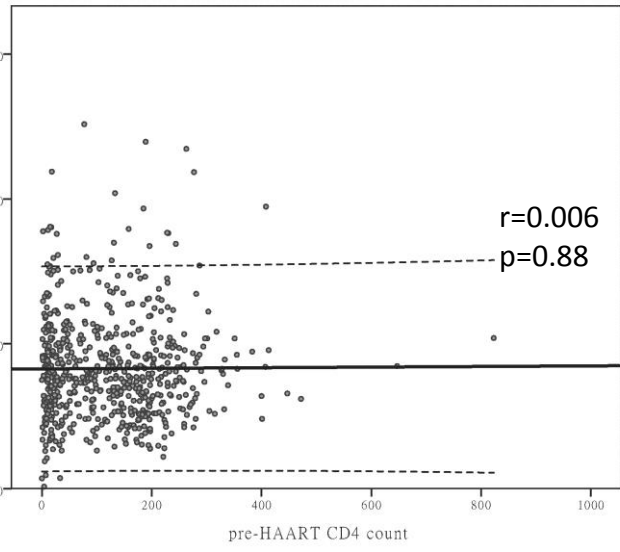
(c) pre-HAART CD8 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



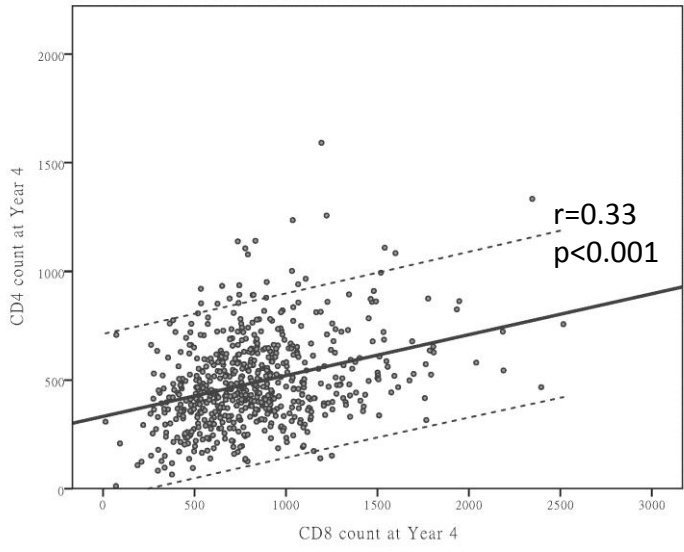
(d) pre-HAART CD8 (cells/ μ L) vs pre-HAART CD4 (cells/ μ L)



(e) pre-HAART CD4 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



(f) year 4 CD8 (cells/ μ L) vs year 4 CD4 (cells/ μ L)



Supplementary Table 1. Relationship between CD4 count and corresponding CD4/CD8 ratio at (a) baseline before initiation of highly active antiretroviral therapy (pre-HAART) and (b) outcome at the end of Year 4 following HAART

(a) Baseline (pre-HAART)

		CD4/CD8 ratio			
		<0.4	0.4-0.79	>=0.8	<u>Total</u>
CD4	<50	240 (34%)	0 (0%)	0 (0%)	<u>240 (34%)</u>
	50-199	293 (42%)	23 (3%)	0 (0%)	<u>316 (45%)</u>
	200-499	124 (18%)	17 (2%)	1 (0.1%)	<u>142 (20%)</u>
	>=500	0 (0%)	4 (1%)	2 (0.3%)	<u>6 (1%)</u>
	Total	<u>657 (93%)</u>	<u>44 (6%)</u>	<u>3 (0.4%)</u>	<u>704 (100%)</u>

(b) Outcome at the end of Year 4

		CD4/CD8 ratio			
		<0.4	0.4-0.79	>=0.8	<u>Total</u>
CD4	<50	1 (0.2%)	0 (0%)	0 (0%)	<u>1 (0.2%)</u>
	50-199	33 (5%)	6 (1%)	0 (0%)	<u>39 (6%)</u>
	200-499	130 (21%)	193 (31%)	36 (6%)	<u>359 (58%)</u>
	>=500	24 (4%)	134 (22%)	65 (10%)	<u>223 (36%)</u>
	Total	<u>188 (30%)</u>	<u>333 (54%)</u>	<u>101 (16%)</u>	<u>622 (100%)</u>

Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

Model:	a. CD4 (cells/ μ L)		b. CD8 (cells/ μ L)		c. CD4/CD8 ratio	
	B	95%C.I.	B	95%C.I.	B	95%C.I.
(Intercept)	282.86	273.99 to	1142.76	1105.97 to	0.24	0.22 to 0.25*
		291.74*		1179.55*		
Months from HAART initiation						
>96 months	421.75	400.9 to 442.59*	-54.91	-95.36 to -14.47*	0.49	0.46 to 0.52*
84-96	388.40	366.41 to 410.39*	-18.28	-61.83 to 25.27	0.44	0.41 to 0.47*
72-84	375.13	358.62 to 391.64*	-18.79	-58.14 to 20.57	0.41	0.39 to 0.43*
60-72	328.63	314.74 to 342.52*	-50.54	-85.11 to -15.98*	0.37	0.35 to 0.39*
48-60	297.14	283.97 to 310.3*	-19.01	-48.33 to 10.3	0.34	0.31 to 0.37*
36-48	267.56	256.63 to 278.49*	-14.72	-42.71 to 13.28	0.28	0.26 to 0.3*
24-36	207.20	196.89 to 217.52*	19.10	-7.03 to 45.24	0.20	0.19 to 0.22*
12-24	119.10	111.79 to 126.42*	61.80	37.11 to 86.48*	0.10	0.08 to 0.12*
<=12 months	0 ^a		0 ^a		0 ^a	
Achievement by year 4						
optimal	97.55	80.71 to 114.39*	-401.33	-437.93 to	0.41	0.37 to 0.45*
immune				-364.74*		

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16 *p<0.05

17 optimal immune outcome – achievement of CD4≥500/μL and CD4/CD8 ratio ≥0.8 by
18 Year 4;

19 conventional outcome – achievement of only CD4≥500/μL by Year 4;

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	14-15
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7-8
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	Table 1,2
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 3
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(b) Report category boundaries when continuous variables were categorized	Table 1,2
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Combining CD4 recovery and CD4/CD8 ratio restoration as an indicator for evaluating the outcome of continued antiretroviral therapy: an observational cohort study

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Immunology (including allergy), Infectious diseases
Keywords:	antiretroviral therapy, CD4, CD8, CD4/CD8 ratio, immune outcome

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3 **Combining CD4 recovery and CD4/CD8 ratio restoration as an indicator for**
4 **evaluating the outcome of continued antiretroviral therapy: an observational**
5 **cohort study**
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9 **Running title:** Immune outcome following antiretroviral therapy
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14 Shui Shan Lee¹, Ngai Sze Wong^{1,2*}, Bonnie Chun Kwan Wong³, Ka Hing Wong³,
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16 Kenny Chi Wai Chan³
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5 **Keywords:** antiretroviral therapy; CD4; CD8; CD4/CD8 ratio; immune outcome
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9 **Word Count:**
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11 **Abstract:** 239 words; **Main text:** 3278 words
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For peer review only

ABSTRACT

Objectives. Immune recovery following highly active antiretroviral therapy (HAART) is commonly assessed by the degree of CD4 reconstitution alone. In this study, we aimed to assess immune recovery by incorporating both CD4 count and CD4/CD8 ratio.

Design: Observational cohort study

Setting and participants. Clinical data from Chinese HIV+ patients attending the largest HIV service in Hong Kong and who had been on HAART for ≥ 4 years were accessed.

Main outcome measures. Optimal immune outcome was defined as a combination of a CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 .

Results. A total of 718 patients were included for analysis (6353 person-years). At the end of Year 4, 318 out of 715 patients achieved CD4 $\geq 500/\mu\text{L}$, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥ 0.8 . Patients with a pre-HAART CD8 $\leq 800/\mu\text{L}$ (428 out of 704) were more likely to be optimal immune outcome achievers with CD4 $\geq 500/\mu\text{L}$ and CD4/CD8 ratio ≥ 0.8 , the association of which was stronger after adjusting for pre-HAART CD4 counts. In a multivariable logistic model, optimal immune outcome was positively associated with male gender, younger pre-HAART age and higher pre-HAART CD4 count, longer duration of HAART and pre-HAART CD8 $\leq 800/\mu\text{L}$. Treatment regimen and cumulative viral loads played no significant role in the pattern of immune recovery.

Conclusions. A combination of CD4 count and CD4/CD8 ratio could be a useful approach for the characterisation of treatment outcome over time, on top of monitoring CD4 count alone.

Strengths and limitations of this study

- The combined use of CD4 and CD4/CD8 ratio as an outcome measure offers a new perspective for measuring immune recovery following antiretroviral therapy.
- The combined marker could avoid overestimation of treatment performance in patients with CD4 count but low CD4/CD8 ratio.
- The study was limited by not having included clinical events in the analysis, a gap which should be filled in larger scale cohort studies.

INTRODUCTION

Highly active antiretroviral therapy (HAART) forms the cornerstone of modern day treatment of human immunodeficiency virus (HIV) infection. In monitoring treatment outcome, peripheral blood CD4+ lymphocyte (hereafter referred as CD4) count measurement is widely used, the results of which feature a rapid rise in the first 3-6 months followed by a second phase of gradual increase, plateauing 4 to 6 years afterwards.¹ Nadir CD4 counts and advanced age are associated with poorer CD4 recovery, a well-reported phenomenon that has been reviewed in the literature.^{1,2}

While high and persistently elevated CD8+ lymphocytes (hereafter referred as CD8) is commonly observed in chronically infected HIV patients, relatively little attention has been paid to its impact on immunological recovery.³ A large cohort study suggested that markedly elevated CD8 count at HAART initiation was associated with a poor increase in CD4 count.⁴ Host factors aside, virus burden exerted by HIV could also impact immunological recovery. In the absence of timely and effective HAART, HIV cumulate over time leading to a state of cumulative viraemia, a predictor of suboptimal immunological outcome in primary HIV infection.⁵ Separate studies have shown that high cumulative viral load was a potential marker for progression to acquired immune deficiency syndrome (AIDS) in chronic HIV infection.⁶ Despite effective therapy, some 20-30% of patients were unable to achieve optimal immunological recovery,^{1,7} an outcome resulting from the interaction of a good range of host and viral factors, as well as co-infection with other pathogens, notably hepatitis C virus.³

Over the last decade, a CD4-guided approach to treatment initiation has gradually

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3 been replaced by early initiation of HAART irrespective of baseline CD4 level.⁸
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5 Achievement of a high CD4 count of, say, over 500/ μ L remains a commonly used
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7 marker of immune restoration. Knowingly, prompt treatment and full viral
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9 suppression do not imply freedom from co-morbidities, as HIV disease is also
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11 characterised by a state of immune activation, with the emergence of non-AIDS
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13 morbidity and mortality.⁹ This morbid state of immune activation cannot be inferred
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15 from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following
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17 HAART has however been linked to this scenario of immune activation.^{10 11} Low
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19 CD4/CD8 ratio was observed in patients despite high CD4 level (>500/ μ L).¹² A high
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21 CD8 count following HAART was shown to be associated with inflammatory
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23 non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction
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25 has been reported.^{4 13} Apparently, a target CD4 count is inadequate for reflecting
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27 effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly
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29 recognized as an important marker of immune reconstitution.^{10 14}
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36 To better monitor immunological recovery following HAART, new biomarkers are
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38 needed, which should preferably be derived from routinely collected laboratory data.
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40 Optimal outcome could be founded on CD4/CD8 normalization on top of the
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42 regularly monitored CD4 count. In this study, we define HAART associated immune
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44 recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to
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46 examine its predictors by analysing regularly collected viral load and immunological
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48 data, the latter including CD8 count, in a cohort of HIV patients following HAART.
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56 **METHODS**

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5 Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical
6 service in Hong Kong were accessed for this observational study. Data access
7 approval was granted by Department of Health, Hong Kong Special Administrative
8 Region Government in compliance with the Personal Data (Privacy) Ordinance.
9
10 Individual consent for the study was waived following approval of the Joint Chinese
11 University of Hong Kong – New Territories East Cluster Clinical Research Ethics
12 Committee (CREC). HIV patients of age ≥ 18 diagnosed in 1985-2012, on HAART
13 continuously for ≥ 4 years without treatment interruption, with at least 1 CD4
14 measurement during treatment and with viral load fully suppressed (without
15 consecutive viral load > 500 copies/mL in the first 4 years on treatment) were selected.
16
17 We included patients who were treatment naïve or have been on non-standard
18 treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and
19 CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b)
20 viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as
21 appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease
22 inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as
23 other regimens were rarely used for treatment naïve patients
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45 Estimated cumulative viral load was expressed as $\text{years} * \log_{10} \text{copies/mL}$, in
46 accordance with the method reported by Zoufaly et al.¹⁵ with modifications. Patients
47 with available negative HIV testing result within 3 years before HIV diagnosis were
48 included, so that one's seroconversion date could be estimated with confidence.¹⁶ In
49 brief, the products of the \log_{10} viral load were summed from estimated seroconversion
50 to subsequent specified time-point(s), with the computation of the highest viral load
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3 for the undiagnosed interval and an upward adjustment by $1 \log_{10}$ for the presumed
4 primary infection period. The time of seroconversion was determined as the mid-point
5 between last negative and first positive HIV antibody testing dates. On the other hand,
6 optimal immune outcome was defined as the achievement of a CD4 count of $\geq 500/\mu\text{L}$
7 and a CD4/CD8 ratio ≥ 0.8 ¹⁷ while conventional outcome was defined as achieving
8 CD4 count of $\geq 500/\mu\text{L}$ but not CD4/CD8 ratio ≥ 0.8 within specific time. Late HIV
9 diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis.
10 The latest CD4 and CD8 measurements ≤ 30 days before HAART initiation were used
11 as the baseline.
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25 Comparisons between pre-HAART CD8 $> 800/\mu\text{L}$ vs $\leq 800/\mu\text{L}$ were made by odds ratio
26 (OR) and multivariable logistic regression with pre-HAART CD4 as confounder,
27 while correlation coefficients were calculated to test the associations between CD4
28 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking
29 reference from the criteria of high CD8 count (i.e. over $800/\mu\text{L}$) during primary
30 infection reported in another study.¹⁸ CD4 (maximum value), CD8 (minimum value)
31 and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome
32 and conventional outcome by Year 4 on HAART over time (≤ 12 months, 12.1-24
33 months, ... , > 96 months) were compared in generalized estimating equations (GEE).
34 Multivariable logistic regression model (stepwise) was applied to examine the
35 predictors of optimal immune outcome and conventional outcome. Complete case
36 analysis was performed. Loss to follow-up and death were data end points. Statistical
37 tests were performed in SPSS.
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RESULTS

As of the end of 2012, data of 2974 diagnosed adults were accessed. Of these, 718 eligible treatment-naïve diagnosed cases who had been on HAART continuously for ≥ 4 years were included in the study. Their case records contained 18857 clinical measurements (18693 CD4, 18521 CD8 and/or 17776 viral load measurements) at multiple time points spanning over 6353 person-years' follow-up. General characteristics of the study population are displayed in Table 1. Overall, a majority (84%) were male with a median age at diagnosis of 37 years (interquartile range (IQR): 31 – 45 years). The median interval from diagnosis to the latest assessment was 100 months (IQR= 74-141 months). Most were infected by either HIV-1 subtype B (31%) or CRF01_AE (38%), with men who have sex with men (MSM) accounting for 39% of the study population. The pre-HAART median CD4 and CD8 counts were 109/ μL and 673/ μL respectively, which were positively correlated (Pearson correlation coefficient $r = 0.50$, $p < 0.001$) (See web-only Supplementary Figure 1(d)). The distribution of CD4 and CD4/CD8 ratio at baseline before initiation of HAART is shown in Supplementary Table 1(a). The life-time estimated cumulative viral load at the last assessment increased with the interval between seroconversion and HAART initiation ($r = 0.94$, $p < 0.001$).

During the study period, a CD4-guided approach was in place, implying that HAART was recommended when one's CD4 count fell below 350/ μL . A majority of the patients (74%) had been started on a PI-based with 25% on NNRTI-based regimen, and 1 % had been started on non-standard regimen subsequently changed to HAART within 1 year. Integrase Inhibitors (INSTI) had not been used as a component of one's

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3 first regimen, but 3 patients had changed to raltegravir-based regimen
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5 afterwards.(Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to
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7 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients
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9 (44%) had reached 500/ μ L or above, of which 105 (33%) gave a CD4/CD8 ratio of
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11 ≥ 0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio.
12
13 On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients
14
15 could not reach the CD4 target. (Table 2) The temporal changes of CD4 count, CD8
16
17 count and CD4/CD8 ratio over time is shown in figure 1, while distribution of CD4
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19 and CD4/CD8 ratio at the end of Year 4 is shown in Supplementary Table 1(b).
20
21 Whereas both CD4 count (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a
22
23 steady rise from the first time-point following HAART, the temporal pattern of CD8
24
25 counts was inconspicuous (figure 1(c)). Patients with optimal immune outcome had
26
27 significantly higher median CD4 and CD4/CD8 ratio but lower CD8 count than those
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29 only with satisfactory CD4 recovery (conventional outcome) in all time points (GEE
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31 model results in Supplementary Table 2). The CD4 count at Year 4 was positively
32
33 correlated with pre-HAART CD4 ($r = 0.38$, $p < 0.001$)(See web-only Supplementary
34
35 Figure 1(a)) Categorised by one's pre-HAART CD8 count, about half ($n = 428$, 61%)
36
37 had a lower count of $\leq 800/\mu$ L. The 2 groups had similar demographic, cumulative
38
39 viral load levels and had received similar treatment regimens. The CD4 count at Year
40
41 4 was positively correlated with pre-HAART CD8 count ($r = 0.18$, $p < 0.001$) (See
42
43 web-only Supplementary Figure 1(b)) whereas the latter was also positively correlated
44
45 with CD8 at Year 4. ($r = 0.35$, $p < 0.001$) (See web-only Supplementary Figure 1(c)).
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47 After adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had
48
49 higher chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR
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51 (aOR)=64.63, 95% C.I.=23.47 to 177.98) (Table 3). Likewise, a low pre-HAART CD8
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3 count of $\leq 800/\mu\text{L}$ was associated with the optimal immune outcome at Year 4, with an
4 increased odds (aOR=5.07, 95%C.I.=2.74-9.41) after adjusting for pre-HAART CD4.
5
6
7 There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See
8 web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8
9
10 web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8
11 at Year 4 ($r=0.33$, $p<0.001$) could be demonstrated (See web-only Supplementary
12 Figure 1(f)).
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20 The following independent variables were then tested for their prediction of optimal
21 immune outcome and conventional outcome achieved since treatment initiation
22 throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART
23 age, treatment duration and male gender. In the final model, both high pre-HAART
24 CD4 and low pre-HAART CD8 were significant predictors of optimal immune
25 outcome, while only the former was a significant predictor of conventional outcome
26 (Table 4). Patients who were male and started HAART at younger age were more
27 likely to achieve both outcomes. Patients on treatment for longer time (≥ 97 months)
28 had higher odds to achieve optimal immune outcome (aOR=3.34, 95%C.I.=2.17 to
29 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78,
30 95%C.I.=1.89 to 4.09, 49-72 months as reference). As a sub-study (results not shown),
31 we have performed another set of GEE models with cumulative viral load as an
32 independent variable (results not shown). The results did not support it as a significant
33 predictor of an optimal immune outcome both in CD4 count and CD4/CD8 ratio,
34 though the number of patients eligible for the analyses was only 187.
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56 DISCUSSION

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5 Pre-HAART CD4 count has long been shown to be a predictor of immunological
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7 outcome 3-5 years following antiretroviral therapy.¹ Our previous longitudinal studies
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9 in a cohort of Chinese HIV patients have demonstrated positive associations between
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11 nidus and maximum CD4 count over 5 years irrespective of the causative virus
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13 subtype or the regimens prescribed.^{19 20} In assessing antiretroviral treatment response,
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15 however, CD4 count alone appeared to add little to viral load monitoring.²¹ To
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17 account for the potential risk of non-AIDS related comorbidities including metabolic
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19 complications,⁹ parallel CD4/CD8 ratio testing is gaining popularity as it reflects also
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21 the intensity of chronic inflammation implicated.^{9 10} In this study, a CD4 count
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23 $\geq 500/\mu\text{L}$ in conjunction with a ratio of ≥ 0.8 was examined as a target outcome
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25 indicator for chronically infected patients on continued antiretroviral therapy. This
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27 target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year
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29 treatment period. The association of pre-HAART CD8 with optimal immune outcome
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31 was stronger with a cut-off ratio of ≥ 1 but the proportion of patients achieving the
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33 target outcome would be very low at 6% (46 out of 718). Both pre-HAART CD4 and
34
35 CD8 count, as well as the treatment interval were independent predictors of this new
36
37 outcome target. While CD4 remained a useful prognostic marker, using it as the sole
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39 marker might overestimate treatment performance by including patients with high
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41 CD4 count but high CD8 count and low CD4/CD8 ratio as achiever (205 out of 715
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43 achieved CD4 target only).
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52 In this study, we have shown that 44% of patients on HAART achieved a CD4 count
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54 $\geq 500/\mu\text{L}$ at the end of 4 years, an outcome slightly poorer than that of 59% reported
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56 by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our
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3 shorter observation period (4 instead of 5 years) and the lower median pre-HAART
4 CD4 count (158/ μ L compared to 180/ μ L).²² As concluded in the recently published
5 “START” study examining the benefits of the initiation of antiretroviral therapy in
6 HIV-positive adults with a CD4 count $>500/\mu$ L, CD4 count *per se* could not capture
7 all outcome effects arising from immediate HAART in chronic HIV infection.²³ Our
8 study confirmed that CD4/CD8 ratio could be a readily available supplementary
9 marker to monitor immune recovery. Evidently, the ratio may vary with lengths of
10 observations, demographics, and/or even HAART regimens.^{17 24 25} As the CD4/CD8
11 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio
12 of 0.8¹⁷ to assess the state of immune recovery at 4 years after HAART initiation.
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14 Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7
15 years, the median observation interval of our cohort.

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32 Pre-HAART CD8 count and its normalisation following antiretroviral treatment is
33 relatively under-investigated.^{26 27} In our study, pre-HAART CD4 and pre-HAART
34 CD8 counts were positively correlated. It was noted that heterosexuals gave a lower
35 pre-HAART CD8 (Table 3) compared to MSM but the difference became insignificant
36 after the adjustment of pre-HAART CD4. Over time, CD4 rise went in parallel with
37 slowly falling CD8 until reaching an optimal CD4 level of $\geq 500/\mu$ L with a
38 near-normalised CD4/CD8 ratio ≥ 0.8 at Year 4. Pre-HAART CD8 was a significant
39 predictor of optimal immune outcome but not conventional outcome. The median
40 CD8 count of former group was lower than latter group of patients in all time points
41 since HAART initiation. Significant expansion of CD8 is known to occur soon after
42 infection and the phenomenon might persist throughout the course of HIV infection.
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44 Recent studies suggested that CD8 normalisation was associated with early initiation

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3 of HAART during acute infection.¹⁸ HIV-specific CD8 has been proposed to play an
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5 important role in effecting functional cure of HIV infection.²⁸ Its relationship with the
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7 absolute count of CD8 before and after HAART has not been established. With the
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9 growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS
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11 morbidity and chronic inflammation,^{9 10 29 30} it is possible that HIV+ patients' clinical
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13 outcome could be better explained from both the ratio and CD4 count rather than from
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15 the latter alone. From a virological perspective, the estimated cumulative viral load
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17 can be viewed as a surrogate of prolonged non-suppression of virus load. It does not
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19 however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its
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21 immunological impacts could be overtaken by a long interval of HAART, if the
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23 pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to
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25 early initiation of HAART in chronic HIV infection to avoid temporal accumulation
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27 of virus, a conclusion similar to that for primary HIV infection.⁵
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34 We acknowledge that our study carries a number of limitations. Foremost, all patients
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36 had been on HAART during the time when a CD4-guided approach to treatment
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38 initiation was enforced. As the patients had been started on either a PI-based or
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40 NNRTI-based regimen, the possible impacts of newer generations of antiretroviral
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42 like INSTI could not be ascertained. The results should therefore be interpreted with
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44 caution, especially that strong association between INSTI-based regimen and
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46 CD4/CD8 normalisation has recently been reported.³¹ These were selection bias
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48 which might have limited the extrapolation of results to the entire HIV population. In
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50 addition, our dataset did not include other inflammatory or infectious outcomes (e.g.
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52 HCV and/or cytomegalovirus co-infections³²⁻³⁵) and therefore these could not be
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54 analysed in perspective. As the main comparative period was 4 years, the minimum
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3 treatment duration of study population, the immunological recovery achieved by
4 patients in this study may not necessarily be reflecting the ultimate response to
5 HAART. We have nevertheless evaluated the outcome (comprising both CD4 count
6 and CD4/CD8 ratio) of all enrolled patients with a median duration of treatment of
7 over 6 years in the final analysis. Theoretically, cohorts with patients observed
8 throughout their lifetime would be invaluable to determine the health benefits of
9 HAART. Analyses from such life-long cohorts should become a reality in the coming
10 years or decades.
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25 CONCLUSIONS

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29 Conventionally, CD4 count has been commonly used as the main outcome marker
30 following HAART. In light of the increasing incidence of co-morbidities associated
31 with HIV related chronic inflammations, CD4 count per se appears to carry little
32 prognostic value in predicting HAART-associated immune recovery. Our results
33 suggested that a combination of CD4 count and CD4/CD8 ratio offers another
34 potentially useful approach to assessing immune outcome, compared to the use of
35 CD4 alone. In evaluating immune recovery following long-term HIV viral
36 suppression, pre-HAART CD8 count could be as important as CD4 count as the
37 independent predictors of the ultimate immune outcome. As both CD4 and CD8 are
38 often routinely collected in the course of HIV management, an assessment of the
39 temporal trends of CD4, CD8 and CD4/CD8 ratio could conveniently predict the
40 immunological outcome without the need for sophisticated immune markers.
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56 Virological impact, as inferred from the estimated cumulative viral load after infection,
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3 does not however add to the outcome reflected from viral load suppression. The
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5 monitoring of the host immunological responses remains the most important approach
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7 in assessing treatment outcome following HAART.
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15
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34 **Contributors**

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36 SSL motivated and designed the study. KHW, BCKW, KCWC contributed the data
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38 and their interpretation. NSW analysed the data. SSL wrote the article. All authors
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40 contributed to interpretation of results and critically reviewed and edited the article.
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53 interpretation of data and drafting the manuscript.
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Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Data access approval was granted by Department of Health, Hong Kong Special Administrative Region Government in compliance with the Personal Data (Privacy) Ordinance. Individual consent for the study was waived following approval of the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC).

Data sharing statement

No additional data are available

Disclaimer

The opinions and assertions contained herein are private views of the authors and do not necessarily reflect those of the Centre for Health Protection, Hong Kong Special Administrative Region Government Department of Health, or the other affiliating institutions.

REFERENCES

1. Battegay M, Nüesch R, Hirschel B, et al. Immunological recovery and antiretroviral therapy in HIV-1 infection. *The Lancet Infectious Diseases* 2006;6:280-87. doi: 10.1016/S1473-3099(06)70463-7
2. Gazzola L, Tincati C, Bellistri GM, et al. The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009;48(3):328-37. doi: 10.1086/595851
3. Tsiara CG, Nikolopoulos GK, Dimou NL, et al. Effect of hepatitis C virus on immunological and virological responses in HIV-infected patients initiating highly active antiretroviral therapy: a meta-analysis. *J Viral Hepat* 2013;20(10):715-24. doi: 10.1111/jvh.12101
4. Helleberg M, Kronborg G, Ullum H, et al. Course and Clinical Significance of CD8+ T-Cell Counts in a Large Cohort of HIV-Infected Individuals. *J Infect Dis* 2015;211(11):1726-34. doi: 10.1093/infdis/jiu669
5. Seng R, Goujard C, Krastinova E, et al. Influence of lifelong cumulative HIV viremia on long-term recovery of CD4+ cell count and CD4+/CD8+ ratio among patients on combination antiretroviral therapy. *AIDS (London, England)* 2015;29:595-607. doi: 10.1097/QAD.0000000000000571
6. Marconi VC, Grandits G, Okulicz JF, et al. Cumulative viral load and virologic decay patterns after antiretroviral therapy in HIV-infected subjects influence CD4 recovery and AIDS. *PLoS One* 2011;6(5):e17956. doi: 10.1371/journal.pone.0017956
7. Gaardbo JC, Hartling HJ, Gerstoft J, et al. Incomplete immune recovery in HIV infection: mechanisms, relevance for clinical care, and possible solutions. *Clin Dev Immunol* 2012;2012:670957. doi: 10.1155/2012/670957
8. World Health Organization. WHO | Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV 2015 [updated 2015-10-09 06:58:51].
9. Saracino A, Bruno G, Scudeller L, et al. Chronic inflammation in a long-term cohort of HIV-infected patients according to the normalization of the CD4:CD8 ratio. *AIDS research and human retroviruses* 2014;30:1178-84. doi: 10.1089/aid.2014.0080
10. Mussini C, Lorenzini P, Cozzi-Lepri A, et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. *Lancet HIV* 2015;2(3):e98-106. doi: 10.1016/S2352-3018(15)00006-5
11. Lu W, Mehraj V, Vyboh K, et al. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc* 2015;18:20052. doi: 10.7448/IAS.18.1.20052
12. Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog* 2014;10(5):e1004078. doi:

- 10.1371/journal.ppat.1004078
13. Badejo OA, Chang CC, So-Armah KA, et al. CD8+ T-cells count in acute myocardial infarction in HIV disease in a predominantly male cohort. *Biomed Res Int* 2015;2015:246870. doi: 10.1155/2015/246870
 14. Serrano-Villar S, Deeks SG. CD4/CD8 ratio: an emerging biomarker for HIV. *Lancet HIV* 2015;2(3):e76-7. doi: 10.1016/S2352-3018(15)00018-1
 15. Zoufaly A, Stellbrink H-J, Heiden MAd, et al. Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. *The Journal of Infectious Diseases* 2009;200:79-87. doi: 10.1086/599313
 16. Pantazis N, Porter K, Costagliola D, et al. Temporal trends in prognostic markers of HIV-1 virulence and transmissibility: an observational cohort study. *Lancet HIV* 2014;1(3):e119-26. doi: 10.1016/S2352-3018(14)00002-2
 17. Menozzi M, Zona S, Santoro A, et al. CD4/CD8 ratio is not predictive of multi-morbidity prevalence in HIV-infected patients but identify patients with higher CVD risk. *J Int AIDS Soc* 2014;17(4 Suppl 3):19709. doi: 10.7448/IAS.17.4.19709
 18. Cao W, Mehraj V, Trottier B, et al. Early Initiation Rather Than Prolonged Duration of Antiretroviral Therapy in HIV Infection Contributes to the Normalization of CD8 T-Cell Counts. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016;62(2):250-7. doi: 10.1093/cid/civ809
 19. Naftalin CM, Wong NS, Chan DP, et al. Three different patterns of CD4 recovery in a cohort of Chinese HIV patients following antiretroviral therapy - a five-year observational study. *International journal of STD & AIDS* 2015;26:803-09. doi: 10.1177/0956462414553826
 20. Wong NS, Reidpath DD, Wong KH, et al. A multilevel approach to assessing temporal change of CD4 recovery following HAART initiation in a cohort of Chinese HIV positive patients. *J Infect* 2015;70(6):676-8. doi: 10.1016/j.jinf.2014.10.012
 21. Ford N, Meintjes G, Pozniak A, et al. The future role of CD4 cell count for monitoring antiretroviral therapy. *Lancet Infect Dis* 2015;15(2):241-7. doi: 10.1016/S1473-3099(14)70896-5
 22. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2005;41(3):361-72. doi: 10.1086/431484
 23. INSIGHT START Study Group, Lundgren JD, Babiker AG, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N Engl J Med* 2015;373(9):795-807. doi: 10.1056/NEJMoa1506816
 24. Leung V, Gillis J, Raboud J, et al. Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. *PLoS One* 2013;8(10):e77665. doi: 10.1371/journal.pone.0077665
 25. Lichtenstein KA, Armon C, Nagabhushanam V, et al. A pilot study to assess inflammatory biomarker changes when raltegravir is added to a virologically suppressive HAART regimen in HIV-1-infected patients with limited immunological responses. *Antiviral Therapy* 2012;17:1301-09. doi:

- 10.3851/IMP2350
26. Cao W, Mehraj V, Kaufmann DE, et al. Elevation and persistence of CD8 T-cells in HIV infection: the Achilles heel in the ART era. *J Int AIDS Soc* 2016;19(1):20697. doi: 10.7448/IAS.19.1.20697
 27. Mudd JC, Lederman MM. CD8 T cell persistence in treated HIV infection. *Curr Opin HIV AIDS* 2014;9(5):500-5. doi: 10.1097/COH.0000000000000086
 28. Jones RB, Walker BD. HIV-specific CD8(+) T cells and HIV eradication. *J Clin Invest* 2016;126(2):455-63. doi: 10.1172/JCI80566
 29. Serrano-Villar S, Perez-Elias MJ, Dronza F, et al. Increased risk of serious non-AIDS-related events in HIV-infected subjects on antiretroviral therapy associated with a low CD4/CD8 ratio. *PLoS One* 2014;9(1):e85798. doi: 10.1371/journal.pone.0085798
 30. Sainz T, Serrano-Villar S, Diaz L, et al. The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. *AIDS* 2013;27(9):1513-6. doi: 10.1097/QAD.0b013e32835faa72
 31. De Salvador-Guillouet F, Sakarovitch C, Durant J, et al. Antiretroviral Regimens and CD4/CD8 Ratio Normalization in HIV-Infected Patients during the Initial Year of Treatment: A Cohort Study. *PLoS One* 2015;10(10):e0140519. doi: 10.1371/journal.pone.0140519
 32. Saracino A, Bruno G, Scudeller L, et al. CD4 and CD4/CD8 ratio progression in HIV-HCV infected patients after achievement of SVR. *J Clin Virol* 2016;81:94-9. doi: 10.1016/j.jcv.2016.05.019
 33. Brites-Alves C, Netto EM, Brites C. Coinfection by Hepatitis C Is Strongly Associated with Abnormal CD4/CD8 Ratio in HIV Patients under Stable ART in Salvador, Brazil. *J Immunol Res* 2015;2015:174215. doi: 10.1155/2015/174215
 34. Freeman ML, Mudd JC, Shive CL, et al. CD8 T-Cell Expansion and Inflammation Linked to CMV Coinfection in ART-treated HIV Infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016;62(3):392-6. doi: 10.1093/cid/civ840
 35. Smith DM, Nakazawa M, Freeman ML, et al. Asymptomatic CMV Replication During Early Human Immunodeficiency Virus (HIV) Infection Is Associated With Lower CD4/CD8 Ratio During HIV Treatment. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2016;63(11):1517-24. doi: 10.1093/cid/ciw612

Table 1. General characteristics of study population (n=718)

	frequency	%
	<u>median</u>	<u>(IQR)</u>
(a) Demographics		
Male gender	605	84%
Ethnicity		
Chinese	581	81%
Asian (Asian other than Chinese)	87	12%
White	47	7%
African	3	0.4%
Age (yrs, at HIV diagnosis)	<u>37</u>	(31 to 45)
(b) HIV infection and diagnosis		
Mode of transmission		
heterosexual	394	55%
man-to-man sex	280	39%
injection drug use	34	5%
contaminated blood transfusion	6	1%
undetermined	4	1%
HIV-1 Subtype		
CRF01_AE	270	38%
B	224	31%
C	8	1%
Others	31	4%
unavailable	185	26%
AIDS diagnosis before treatment	239	33%
Late HIV diagnosis*	192	27%

	frequency	%
	<u>median</u>	<u>(IQR)</u>
estimated cumulative viral load [#] from seroconversion to diagnosis (n=199)	<u>8</u>	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	<u>39</u>	(33 to 46)
Months from diagnosis to treatment initiation	<u>8.67</u>	(2.75 to 33.13)
CD4 count (cells/ μ L)	<u>109</u>	(29 to 190)
CD4/CD8 ratio ^a	<u>0.14</u>	(0.06 to 0.23)
CD8 count (cells/ μ L) ^a	<u>673</u>	(441 to 966)
Viral load (log ₁₀ copies/mL) ^b	<u>5.15</u>	(4.62 to 5.58)
Estimated cumulative viral load [#] from seroconversion to treatment initiation (n=199)	<u>18</u>	(11 to 29)
(d) Antiretroviral treatment and clinical outcomes		
First HAART regimen		
NNRTI-based	182	25%
PI-based	131	18%
PI-based with booster	397	55%
non-standard	8	1%
Total treatment duration (months)	<u>85.38</u>	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years ^c	<u>476</u>	(354 to 630)
Highest CD4/CD8 ratio within 4 years ^d	<u>0.55</u>	(0.39 to 0.76)

	frequency	%
	<u>median</u>	<u>(IQR)</u>
CD4 count $\geq 500/\mu\text{L}$ within 4 years ^c	318	44%
CD4/CD8 ratio ≥ 0.8 within 4 years ^e	145	20%
Deceased	39	5%

*Late HIV diagnosis refers to the diagnosis of AIDS within 3 months of HIV diagnosis

Estimated cumulative viral load expressed as years*log₁₀ viral load copies/mL

^a 14 missing values; ^b 18 missing values; ^c 2 missing values; ^d 8 missing values; ^e 3 missing values

Table 2 The profiles of immunological outcomes of patients by achievement of none, one or both of the 2 target immunological markers (CD4 \geq 500, CD4/CD8 ratio \geq 0.8) before the end of a 4-year observation period#

	no.	median peak or highest CD4 count (/μL) (IQR)	median months to CD4 target (IQR)	median peak or highest CD4:CD8 ratio (IQR)	median months to target CD4:CD8 ratio (IQR)
CD4 \geq 500/μL and CD4:CD8 ratio \geq 0.8	105	741 (618 to 876)	20.63 (12.6 to 30.53)	0.98 (0.86 to 1.2)	28.90 (14.43 to 42.95)
Concurrent achievement of both targets	15	694 (569 to 1182)	20.27 (13.07 to 28.17)	1.05 (0.9 to 1.49)	20.27 (13.07 to 28.17)
CD4 target before ratio target	57	788 (660 to 921)	15.13 (8.7 to 22.88)	0.89 (0.83 to 0.99)	39.23 (30.78 to 45.98)
Ratio target before CD4 target	33	650 (547 to 764)	31.13 (22.3 to 39.4)	1.17 (1.02 to 1.56)	14.40 (8.68 to 24.08)
CD4 \geq 500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio \geq 0.8 only	32	431 (369 to 475)	/	1.05 (0.89 to 1.17)	29.32 (18.48 to 40.33)
CD4 target then changed to ratio target	4	588 (519 to 660)	20.02 (12.23 to 35.36)	0.86 (0.81 to 0.95)	36.83 (20.68 to 49.72)
Ratio target then changed to CD4 target	4	583 (521 to 636)	29.68 (20.52 to 40.38)	0.87 (0.86 to 1.01)	13.87 (5.48 to 25.45)
Failure to achieve both targets	365	362 (253 to 432)	/	0.43 (0.31 to 0.55)	/

#Equivalent to a maximum of <52 months with the inclusion of a 3-month buffer period;

Table 3. Comparison between patients with high (>800/ μ L) and low (\leq 800/ μ L) pre-HAART CD8 counts

Variables included in the analyses were (a) general baseline characteristics, (b) pre-HAART virological status, (c) antiretroviral therapy, and (d) outcome at year 4.

	pre-HAART CD8 >800 (n=276)		pre-HAART CD8 \leq 800 (n=428)		Univariate analysis	adjusted by pre-HAART CD4		
	median/ <u>no.</u>	IQR/ <u>%</u>	median/ <u>no.</u>	IQR/ <u>%</u>	OR	95% CI	aOR	95% CI
(a) Baseline characteristics								
Male gender	242	87.7%	352	82.2%	0.65	0.42 to 1.01	1.82	1.12 to 2.96*
Chinese ethnicity	222	80.4%	351	82.0%	1.11	0.75 to 1.63	1.10	0.71 to 1.71
Mode of transmission	(n=372)		(n=427)					
MSM	120	44.0%	153	35.8%	<i>ref</i>		<i>ref</i>	
Heterosexual	140	51.3%	249	58.3%	1.39	1.02 to 1.91*	0.93	0.65 to 1.33
injection drug user	13	4.8%	19	4.4%	1.15	0.54 to 2.41	0.47	0.21 to 1.08
contaminated blood transfusion	0	0.0%	6	1.4%	/		/	
Subtype	(n=206)		(n=322)					
CRF01_AE	95	46.1%	171	53.1%	<i>ref</i>		<i>ref</i>	
B	94	45.6%	129	40.1%	0.76	0.53 to 1.1	1.35	0.88 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4				
	median/IQR/ <u>no.</u>	% <u>no.</u>	median/IQR/ <u>no.</u>	%	OR	95% CI	aOR	95% CI
								2.06
C	4	1.9%	4	1.2%	0.56	0.14 to	1.13	0.25 to
						2.27		5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to	1.37	0.6 to 3.17
						1.64		
Age at diagnosis (yrs)	36.80	31.74 to	37.46	30.27 to	1.00	0.98 to	0.99	0.98 to
		43.54		45.17		1.01		1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to	0.98	0.64 to
						3.28*		1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to	0.94	0.63 to
						2.88*		1.41
(b) Pre-HAART virological status								
viral load (log ₁₀ copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to	5.20	4.69 to	1.23	1.03-1.47*	0.80	0.64-0.99*
		5.52		5.58				
Viral load log ₁₀ > 145	52.9%		259	61.7%	1.43	1.05 to	0.71	0.49 to
5						1.95*		1.02
Estimated cumulative viral load #	(n=96)		(n=101)					
	17.74	10.00 to	18.53	10.88 to	1.004	0.98 to	1.004	0.98 to

	pre-HAART CD8 >800 (n=276)	pre-HAART CD8 ≤800 (n=428)	Univariate analysis	adjusted by pre-HAART CD4
	median/IQR/ <u>no.</u> %	median/IQR/ <u>no.</u> %	OR	95% CI
			aOR	95% CI
	29.61	27.73	1.03	1.03
(c) Antiretroviral treatment				
Months from diagnosis to HAART initiation	12.80	3.87 to 5.60	2.44 to 1.00	0.99 to 1
	35.52	30.58		1.01
NNRTI-based initial regimen	70	25.4%	105	24.5%
			0.96	0.67 to 1.84
			1.36	1.22 to 2.78*
(d) Outcome at Year 4				
CD4 count /μL	(n=246)	(n=370)		
	488	386 to 437	332 to 589	0.999
	625			0.998 to 1
				1.001
				0.9997 to 1.002
CD4>500/μL	117	47.6%	141	38.1%
			0.68	0.49 to 1.29
			0.94*	0.88 to 1.91
CD4/CD8 ratio	(n=246)	(n=370)		
	0.49	0.36 to 0.57	0.41 to 3.61	1.93 to 64.63
	0.68	0.79	6.75*	23.47 to 177.98*
Viral load (log ₁₀ copies/mL)	(n=245)	(n=366)		
	1.88	1.88 to 1.88	1.88 to 1.18	0.73 to 0.83
	2.6	2.6	1.91	0.48 to 1.44
Suppressed viral	245	100.0%	364	99.5%
			/	/

	pre-HAART CD8 >800 (n=276)		pre-HAART CD8 ≤800 (n=428)		Univariate analysis	adjusted by pre-HAART CD4		
	median/IQR/ <u>no.</u>	%	median/IQR/ <u>no.</u>	%	OR	95% CI	aOR	95% CI
load (≤500 copies/mL)								
CD4>500/μL & CD4/CD8 ratio>0.8	(n=243)		(n=370)					
	24	9.9%	59	15.9%	1.73	1.04 to	5.07	2.74 to
						2.87*		9.41*
Treatment (months)	83.83	62.13 to	85.05	64.17 to	1.000	0.997 to	0.999	0.99 to
		117.42		116.75		1.004		1.003

Note: all analyses were performed in logistic regression: simple logistic regression for univariate analyses, and multivariable logistic regression with selected confounders for multivariable analyses.

Estimated cumulative viral load from seroconversion expressed as years*log₁₀ viral load copies/mL

*p<0.05

^a 1 missing value; ^b 2 missing value

Table 4. Multivariable logistic regression for evaluating variables associated with a) an optimal immunological outcome and b) conventional outcome

An optimal immunological outcome was defined as achieved CD4 count $\geq 500/\mu\text{L}$ and a CD4/CD8 ratio ≥ 0.8 , and conventional outcome was defined as only achieved CD4 count $\geq 500/\mu\text{L}$ by study end-point

	a) optimal immune outcome		b) conventional outcome	
	aOR	95%C.I.	aOR	95%C.I.
Male gender	2.23	1.4 to 3.53*	1.81	1.11 to 2.96*
Age at HAART initiation	0.98	0.97 to 0.9996*	0.96	0.94 to 0.97*
Pre-HAART CD4 (μL)				
<=100	<i>ref</i>		<i>ref</i>	
101-200	2.91	1.83 to 4.62*	2.30	1.57 to 3.37*
201-300	4.61	2.53 to 8.39*	3.52	2.1 to 5.9*
>300	20.36	7.51 to 55.17*	12.84	3.6 to 45.75*
Months on treatment				
49-72	<i>ref</i>		<i>ref</i>	
73-96	1.58	0.93 to 2.67	1.67	1.08 to 2.57*
≥ 97	3.34	2.17 to 5.15*	2.78	1.89 to 4.09*
Pre-HAART CD8 $\leq 800/\mu\text{L}$	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

* $p < 0.05$

FIGURE LEGENDS

Figure 1. Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards.

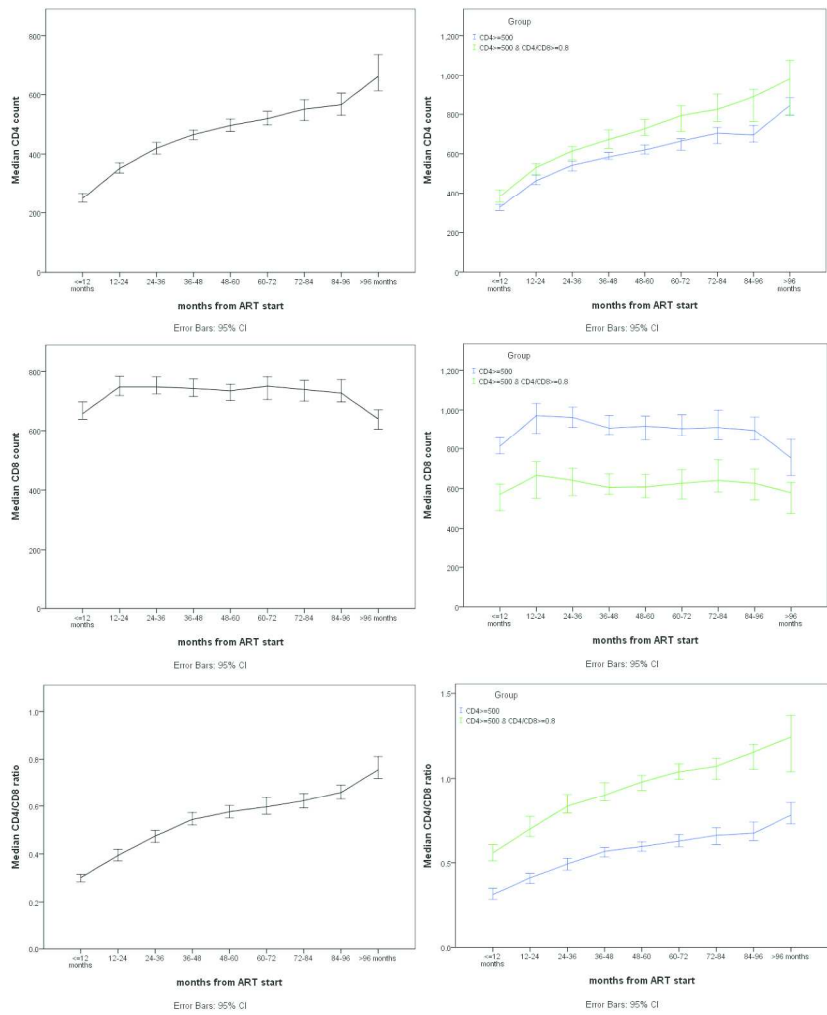
Supplementary material

Supplementary Figure 1. Correlations between immunological markers of the study population in scattered plots with fitting line and 95% confidence interval (dotted lines): (a) pre-HAART CD8 versus pre-HAART CD4; (b) CD4 at year 4 versus pre-HAART CD4; (c) CD8 at year 4 versus pre-HAART CD4; (d) CD8 at year 4 versus CD4 at year 4; (e) CD8 at year 4 versus pre-HAART CD8; (f) CD4 at year 4 versus pre-HAART CD8.

Supplementary Table 1. Relationship between CD4 count and corresponding CD4/CD8 ratio at (a) baseline before initiation of highly active antiretroviral therapy (pre-HAART) and (b) outcome at the end of Year 4 following HAART

Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

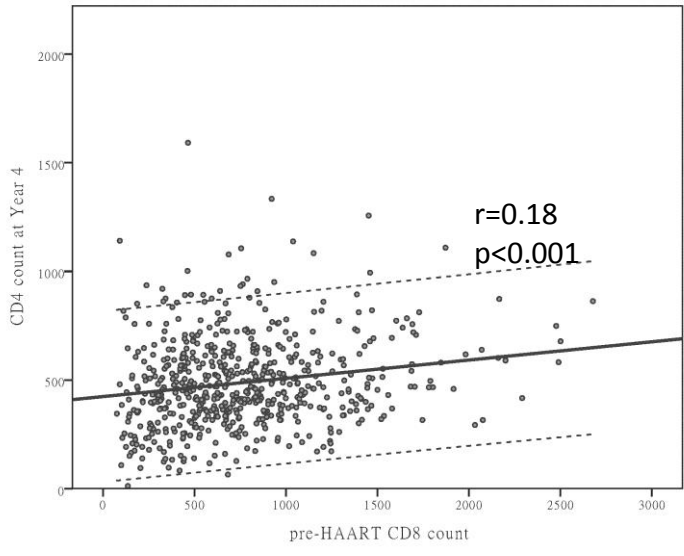
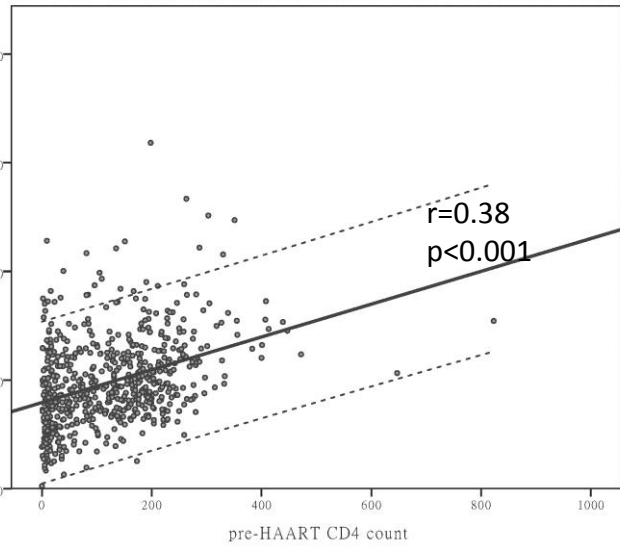
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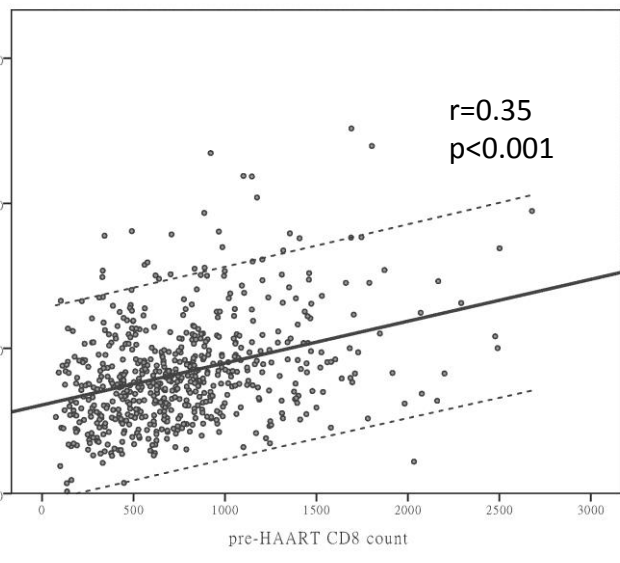
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

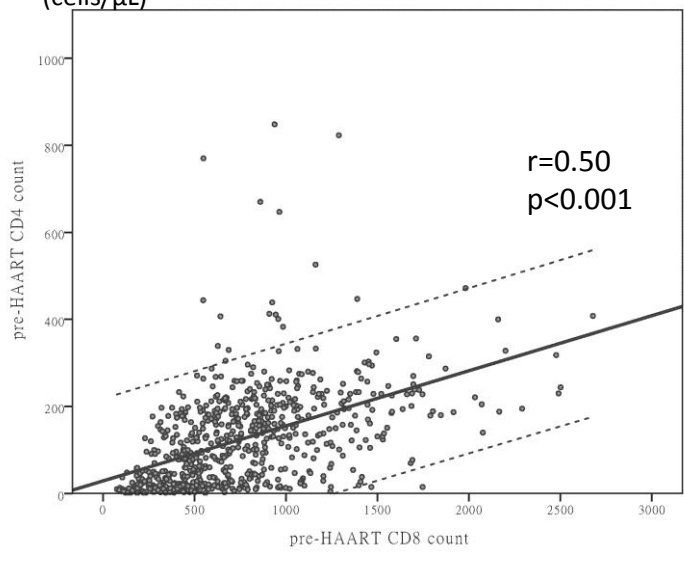
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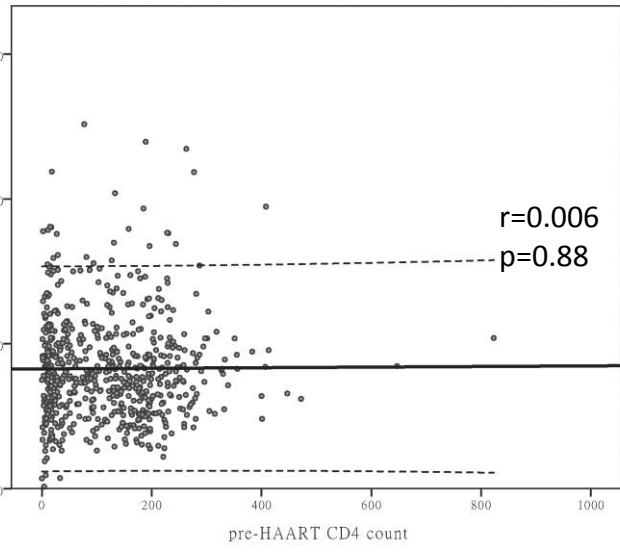
(c) pre-HAART CD8 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



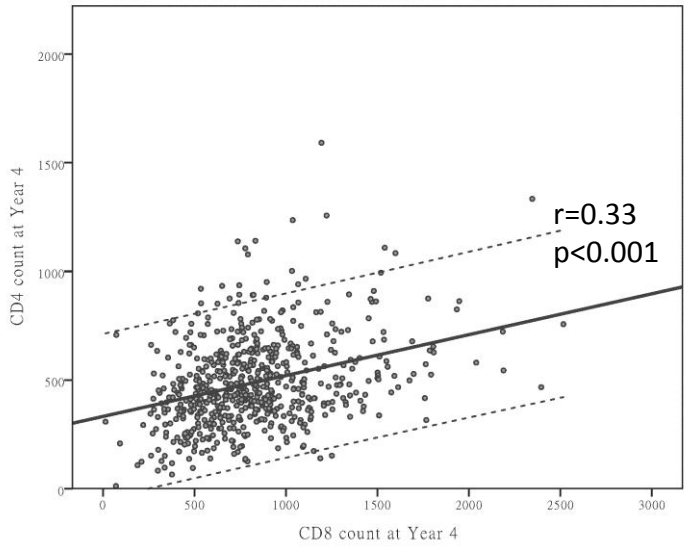
(d) pre-HAART CD8 (cells/ μ L) vs pre-HAART CD4 (cells/ μ L)



(e) pre-HAART CD4 (cells/ μ L) vs year 4 CD8 (cells/ μ L)



(f) year 4 CD8 (cells/ μ L) vs year 4 CD4 (cells/ μ L)



Supplementary Table 1. Relationship between CD4 count and corresponding CD4/CD8 ratio at (a) baseline before initiation of highly active antiretroviral therapy (pre-HAART) and (b) outcome at the end of Year 4 following HAART

(a) Baseline (pre-HAART)

		CD4/CD8 ratio			
		<0.4	0.4-0.79	>=0.8	<u>Total</u>
CD4	<50	240 (34%)	0 (0%)	0 (0%)	<u>240 (34%)</u>
	50-199	293 (42%)	23 (3%)	0 (0%)	<u>316 (45%)</u>
	200-499	124 (18%)	17 (2%)	1 (0.1%)	<u>142 (20%)</u>
	>=500	0 (0%)	4 (1%)	2 (0.3%)	<u>6 (1%)</u>
	<u>Total</u>	<u>657 (93%)</u>	<u>44 (6%)</u>	<u>3 (0.4%)</u>	<u>704 (100%)</u>

(b) Outcome at the end of Year 4

		CD4/CD8 ratio			
		<0.4	0.4-0.79	>=0.8	<u>Total</u>
CD4	<50	1 (0.2%)	0 (0%)	0 (0%)	<u>1 (0.2%)</u>
	50-199	33 (5%)	6 (1%)	0 (0%)	<u>39 (6%)</u>
	200-499	130 (21%)	193 (31%)	36 (6%)	<u>359 (58%)</u>
	>=500	24 (4%)	134 (22%)	65 (10%)	<u>223 (36%)</u>
	<u>Total</u>	<u>188 (30%)</u>	<u>333 (54%)</u>	<u>101 (16%)</u>	<u>622 (100%)</u>

Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations

Model:	a. CD4 (cells/ μ L)		b. CD8 (cells/ μ L)		c. CD4/CD8 ratio	
	B	95%C.I.	B	95%C.I.	B	95%C.I.
(Intercept)	282.86	273.99 to	1142.76	1105.97 to	0.24	0.22 to 0.25*
		291.74*		1179.55*		
Months from HAART initiation						
>96 months	421.75	400.9 to 442.59*	-54.91	-95.36 to -14.47*	0.49	0.46 to 0.52*
84-96	388.40	366.41 to 410.39*	-18.28	-61.83 to 25.27	0.44	0.41 to 0.47*
72-84	375.13	358.62 to 391.64*	-18.79	-58.14 to 20.57	0.41	0.39 to 0.43*
60-72	328.63	314.74 to 342.52*	-50.54	-85.11 to -15.98*	0.37	0.35 to 0.39*
48-60	297.14	283.97 to 310.3*	-19.01	-48.33 to 10.3	0.34	0.31 to 0.37*
36-48	267.56	256.63 to 278.49*	-14.72	-42.71 to 13.28	0.28	0.26 to 0.3*
24-36	207.20	196.89 to 217.52*	19.10	-7.03 to 45.24	0.20	0.19 to 0.22*
12-24	119.10	111.79 to 126.42*	61.80	37.11 to 86.48*	0.10	0.08 to 0.12*
<=12 months	0 ^a		0 ^a		0 ^a	
Achievement by year 4						
optimal	97.55	80.71 to 114.39*	-401.33	-437.93 to	0.41	0.37 to 0.45*
immune				-364.74*		

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16 *p<0.05

17 optimal immune outcome – achievement of CD4≥500/μL and CD4/CD8 ratio ≥0.8 by
18 Year 4;

19 conventional outcome – achievement of only CD4≥500/μL by Year 4;

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(b) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	14-15
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7-8
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	Table 1,2
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 3
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(b) Report category boundaries when continuous variables were categorized	Table 1,2
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.