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Complete List of Authors:	Lee, Shui Shan; The Chinese University of Hong Kong, Stanley Ho Centre for Emerging Infectious Diseases Wong, Ngai Sze; The Chinese University of Hong Kong, Stanley Ho Centre for Emerging Infectious Diseases Wong, Bonnie; Department of Health, Hong Kong Special Administrative Region Government, Special Preventive Programme Wong, Ka Hing; Department of Health, Hong Kong Special Administrative Region Government, Special Preventive Programme Chan, Kenny; Department of Health, Hong Kong Special Administrative Region Government, Special Preventive Programme
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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

Running title: Immune outcome following antiretroviral therapy

Shui Shan Lee[1], Ngai Sze Wong[1,2*], Bonnie Chun Kwan Wong[3], Ka Hing Wong[3], Kenny Chi Wai Chan[3]

[1]Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, People's Republic of China;

[2]Institute for Global Health & Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, United States;

[3]Special Preventive Programme, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region Government, Hong Kong, People's Republic of China;

***Corresponding author**

Dr. Ngai Sze Wong

Stanley Ho Centre for Infectious Diseases

Postgraduate Education Centre

Prince of Wales Hospital

Shatin

Hong Kong

Tel: (852) 22528813

Fax: (852) 26354977

Email: candy_wong@cuhk.edu.hk

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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 ≥500/μL 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≥500/μL, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥0.8. Patients with a pre-HAART CD8 ≤800/μL (428 out of 704) were more likely to be optimal immune outcome achievers with CD4≥500/μL 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 ≤800/μL. 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.

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

been replaced by early initiation of HAART irrespective of baseline CD4 level.[8] Achievement of a high CD4 count of, say, over 500/μL remains a commonly used marker of immune restoration. Knowingly, prompt treatment and full viral suppression do not imply freedom from co-morbidities, as HIV disease is also characterised by a state of immune activation, with the emergence of non-AIDS morbidity and mortality.[9] This morbid state of immune activation cannot be inferred from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following HAART has however been linked to this scenario of immune activation.[10 11] A high CD8 count following HAART was shown to be associated with inflammatory non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction has been reported.[4 12] Apparently, a target CD4 count is inadequate for reflecting effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly recognized as an important marker of immune reconstitution.[10 13]

To better monitor immunological recovery following HAART, new biomarkers are needed, which should preferably be derived from routinely collected laboratory data. Optimal outcome could be founded on CD4/CD8 normalization on top of the regularly monitored CD4 count. In this study, we define HAART associated immune recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to examine its predictors by analysing regularly collected viral load and immunological data, the latter including CD8 count, in a cohort of HIV patients following HAART.

METHODS

Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical service in Hong Kong were accessed for this observational study. 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). HIV patients of age ≥18 diagnosed in 1985-2012, on HAART continuously for ≥4 years without treatment interruption, with at least 1 CD4 measurement during treatment and with viral load fully suppressed (without consecutive viral load >500copies/mL in the first 4 years on treatment) were selected. We included patients who were treatment naïve or have been on non-standard treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b) viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as other regimens were rarely used for treatment naïve patients

Estimated cumulative viral load was expressed as years*$\log_{10}$ copies/mL, in accordance with the method reported by Zoufaly et al.[14] with modifications. Patients with available negative HIV testing result within 3 years before HIV diagnosis were included, so that one's seroconversion date could be estimated with confidence.[15] In brief, the products of the $\log_{10}$ viral load were summed from estimated seroconversion to subsequent specified time-point(s), with the computation of the highest viral load for the undiagnosed interval and an upward adjustment by 1 $\log_{10}$ for the presumed

primary infection period. The time of seroconversion was determined as the mid-point between last negative and first positive HIV antibody testing dates. On the other hand, optimal immune outcome was defined as the achievement of a CD4 count of ≥500/μL and a CD4/CD8 ratio ≥0.8 while conventional outcome was defined as achieving CD4 count of ≥500/μL but not CD4/CD8 ratio ≥0.8 within specific time. Late HIV diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis. The latest CD4 and CD8 measurements ≤30 days before HAART initiation were used as the baseline.

Comparisons between pre-HAART CD8>800/μL vs ≤800/μL were made by odds ratio (OR) and multivariable logistic regression with pre-HAART CD4 as confounder, while correlation coefficients were calculated to test the associations between CD4 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking reference from the criteria of high CD8 count (i.e. over 800/μL) during primary infection reported in another study.[16] CD4 (maximum value), CD8 (minimum value) and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome and conventional outcome by Year 4 on HAART over time (≤12 months, 12.1-24 months, … , >96 months) were compared in generalized estimating equations (GEE). Multivariable logistic regression model (stepwise) was applied to examine the predictors of optimal immune outcome and conventional outcome. Complete case analysis was performed. Loss to follow-up and death were data end points. Statistical tests were performed in SPSS.

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 18857clinical 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 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 first regimen, but 3 patients had changed to raltegravir-based regimen afterwards.(Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients

(44%) had reached 500/μL or above, of which 105 (33%) gave a CD4/CD8 ratio of ≥0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio. On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients could not reach the CD4 target. (Table 3) The temporal changes of CD4 count, CD8 count and CD4/CD8 ratio over time is shown in figure 1. Whereas both CD4 count (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a steady rise from the first time-point following HAART, the temporal pattern of CD8 counts was inconspicuous (figure 1(c)). Patients with optimal immune outcome had significantly higher median CD4 and CD4/CD8 ratio but lower CD8 count than those only with satisfactory CD4 recovery (conventional outcome) in all time points (GEE model results in Supplementary Table 1). The CD4 count at Year 4 was positively correlated with pre-HAART CD4 ($r= 0.38$, $p<0.001$)(See web-only Supplementary Figure 1(a)) Categorised by one's pre-HAART CD8 count, about half (n=428, 61%) had a lower count of ≤800/μL. The 2 groups had similar demographic, cumulative viral load levels and had received similar treatment regimens. The CD4 count at Year 4 was positively correlated with pre-HAART CD8 count ($r=0.18$, $p<0.001$) (See web-only Supplementary Figure 1(b)) whereas the latter was also positively correlated with CD8 at Year 4.($r=0.35$, $p<0.001$) (See web-only Supplementary Figure 1(c)). After adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had higher chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR (aOR)=64.63, 95%C.I.=23.47 to 177.98) (Table 2). Likewise, a low pre-HAART CD8 count of ≤800/μL was associated with the optimal immune outcome at Year 4, with an increased odds (aOR=5.07, 95%C.I.=2.74-9.41) after adjusting for pre-HAART CD4. There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8

at Year 4 (r=0.33, p<0.001) could be demonstrated (See web-only Supplementary Figure 1(f)).

The following independent variables were then tested for their prediction of optimal immune outcome and conventional outcome achieved since treatment initiation throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART age, treatment duration and male gender. In the final model, both high pre-HAART CD4 and low pre-HAART CD8 were significant predictors of optimal immune outcome, while only the former was a significant predictor of conventional outcome (Table 4). Patients who were male and started HAART at younger age were more likely to achieve both outcomes. Patients on treatment for longer time (≥97months) had higher odds to achieve optimal immune outcome (aOR=3.34, 95%C.I.=2.17 to 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78, 95%C.I.=1.89 to 4.09, 49-72 months as reference). As a substudy (results not shown), cumulative viral load was measured but it did not show any correlation with the pattern of immune response.

DISCUSSION

Pre-HAART CD4 count has long been shown to be a predictor of immunological outcome 3-5 years following antiretroviral therapy.[1] Our previous longitudinal studies in a cohort of Chinese HIV patients have demonstrated positive associations between nidus and maximum CD4 count over 5 years irrespective of the causative virus subtype or the regimens prescribed.[17 18] In assessing antiretroviral treatment response,

however, CD4 count alone appeared to add little to viral load monitoring.[19] To account for the potential risk of non-AIDS related comorbidities including metabolic complications,[9] parallel CD4/CD8 ratio testing is gaining popularity as it reflects also the intensity of chronic inflammation implicated.[9 10] In this study, a CD4 count ≥500/μL in conjunction with a ratio of ≥0.8 was examined as a target outcome indicator for chronically infected patients on continued antiretroviral therapy. This target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year treatment period. Both pre-HAART CD4 and CD8 count, as well as the treatment interval were independent predictors of this new outcome target. While CD4 remained a useful prognostic marker, using it as the sole marker might overestimate treatment performance by including patients with high CD4 count but high CD8 count and low CD4/CD8 ratio as achiever (205 out of 715 achieved CD4 target only).

In this study, we have shown that 44% of patients on HAART achieved a CD4 count ≥500/μL at the end of 4 years, an outcome slightly poorer than that of 59% reported by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our shorter observation period (4 instead of 5 years) and the lower median pre-HAART CD4 count (158/μL compared to 180/μL).[20] As concluded in the recently published "START" study examining the benefits of the initiation of antiretroviral therapy in HIV-positive adults with a CD4 count >500/μL, CD4 count *per se* could not capture all outcome effects arising from immediate HAART in chronic HIV infection.[21] Our study confirmed that CD4/CD8 ratio could be a readily available supplementary marker to monitor immune recovery. Evidently, the ratio may vary with lengths of observations, demographics, and/or even HAART regimens.[22-24] As the CD4/CD8 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio

of 0.8 [22] to assess the state of immune recovery at 4 years after HAART initiation. Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7 years, the median observation interval of our cohort.

Pre-HAART CD8 count and its normalisation following antiretroviral treatment is relatively under-investigated.[25 26] In our study, pre-HAART CD4 and pre-HAART CD8 counts were positively correlated. Over time, CD4 rise went in parallel with slowly falling CD8 until reaching an optimal CD4 level of ≥500/μL with a near-normalised CD4/CD8 ratio≥0.8 at Year 4. Pre-HAART CD8 was a significant predictor of optimal immune outcome but not conventional outcome. The median CD8 count of former group was lower than latter group of patients in all time points since HAART initiation. Significant expansion of CD8 is known to occur soon after infection and the phenomenon might persist throughout the course of HIV infection. Recent studies suggested that CD8 normalisation was associated with early initiation of HAART during acute infection.[16] HIV-specific CD8 has been proposed to play an important role in effecting functional cure of HIV infection.[27] Its relationship with the absolute count of CD8 before and after HAART has not been established. With the growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS morbidity and chronic inflammation,[9 10 28 29] it is possible that HIV+ patients' clinical outcome could be better explained from both the ratio and CD4 count rather than from the latter alone. From a virological perspective, the estimated cumulative viral load can be viewed as a surrogate of prolonged non-suppression of virus load. It does not however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its immunological impacts could be overtaken by a long interval of HAART, if the pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to

early initiation of HAART in chronic HIV infection to avoid temporal accumulation of virus, a conclusion similar to that for primary HIV infection.[5]

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

CONCLUSIONS

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

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

	frequency median	% (IQR)
(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)	37	(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 median	% (IQR)
estimated cumulative viral load[#] from seroconversion to diagnosis (n=199)	8	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	39	(33 to 46)
Months from diagnosis to treatment initiation	8.67	(2.75 to 33.13)
CD4 count (cells/μL)	109	(29 to 190)
CD4/CD8 ratio [a]	0.14	(0.06 to 0.23)
CD8 count (cells/μL) [a]	673	(441 to 966)
Viral load (log_{10} copies/mL) [b]	5.15	(4.62 to 5.58)
Estimated cumulative viral load[#] from seroconversion to treatment initiation (n=199)	18	(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)	85.38	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years [c]	476	(354 to 630)
Highest CD4/CD8 ratio within 4 years [d]	0.55	(0.39 to 0.76)

	frequency median	% (IQR)
CD4 count ≥500/μL 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*$\log_{10}$ 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/µL) and low (≤800/µL) 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 ≤800 (n=428)**		**Univariate analysis**		**adjusted by pre-HAART CD4**	
	median/ no.	**IQR/ %**	**median/ no.**	**IQR/ %**	**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%	*ref*		*ref*	
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%	*ref*		*ref*	
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/ no.	IQR/ %	median/ no.	IQR/ %	OR	95% CI	aOR	95% CI
C	4	1.9%	4	1.2%	0.56	0.14 to 2.27	1.13	0.25 to 5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to 1.64	1.37	0.6 to 3.17
Age at diagnosis (yrs)	36.80	31.74 to 43.54	37.46	30.27 to 45.17	1.00	0.98 to 1.01	0.99	0.98 to 1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to 3.28*	0.98	0.64 to 1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to 2.88*	0.94	0.63 to 1.41
(b) Pre-HAART virological status								
viral load (log_{10} copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to 5.52	5.20	4.69 to 5.58	1.23	1.03-1.47*	0.80	0.64-0.99*
Viral load log_{10} > 5	145	52.9%	259	61.7%	1.43	1.05 to 1.95*	0.71	0.49 to 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/ no.	IQR/ %	median/ no.	IQR/ %	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 35.52	5.60	2.44 to 30.58	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/ no.	IQR/ %	median/ no.	IQR/ %	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 2.87*	5.07	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 1.004	0.999	0.99 to 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*$\log_{10}$ 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 ≥500, CD4/CD8 ratio ≥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 ≥500/μL and CD4:CD8 ratio ≥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 ≥500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio ≥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 ≥500/μL and a CD4/CD8 ratio ≥0.8, and conventional outcome was defined as only achieved CD4 count ≥500/μL 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	*ref*		*ref*	
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	*ref*		*ref*	
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≤800/μL	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

*p<0.05

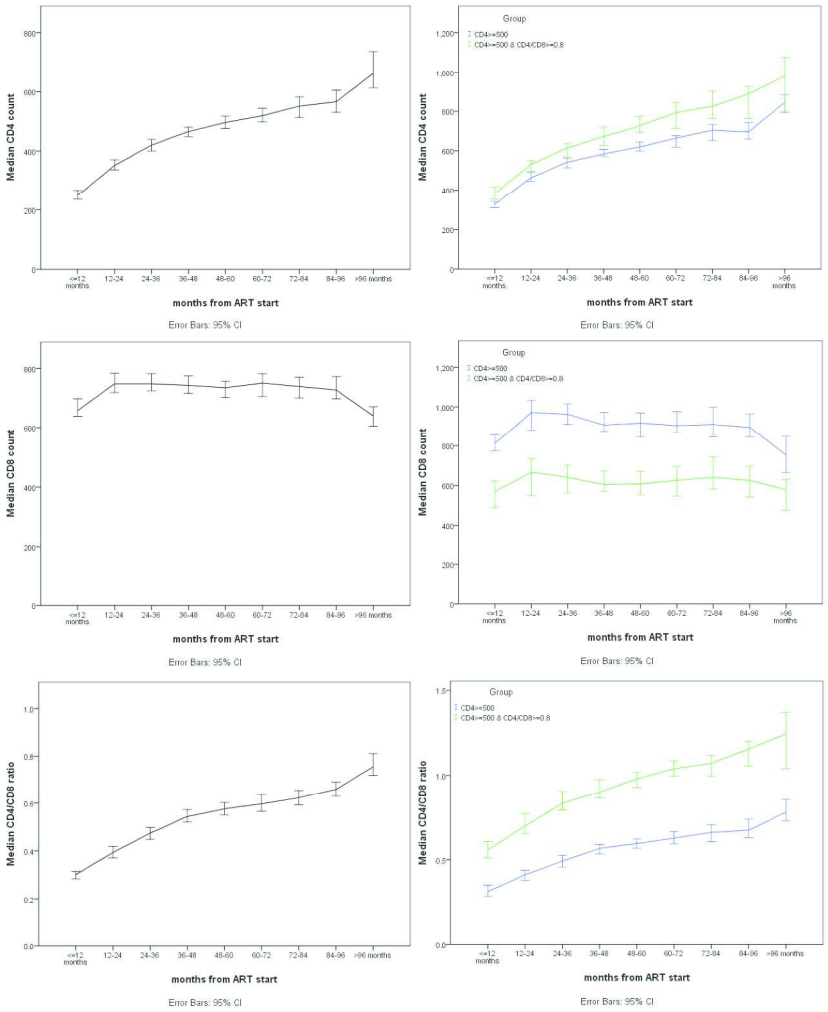
FIGURE LEGENDS

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.

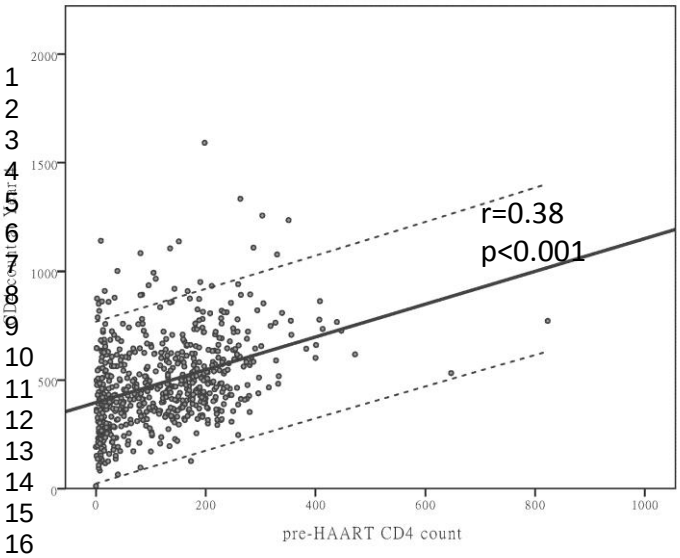
Supplementary Table 1. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations



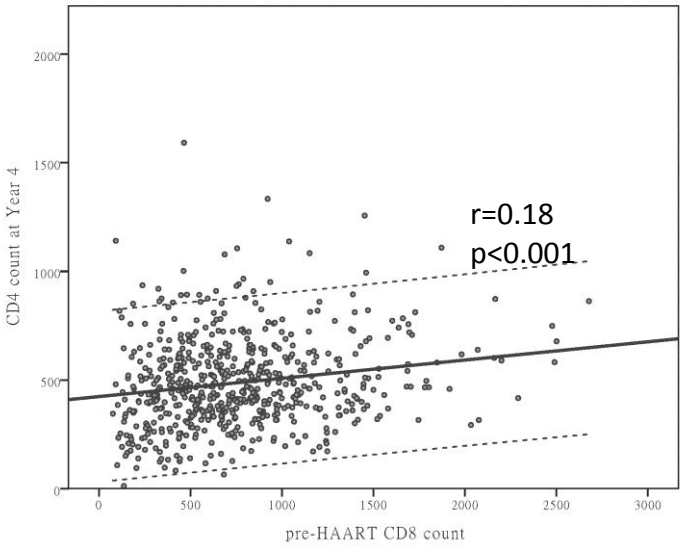
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

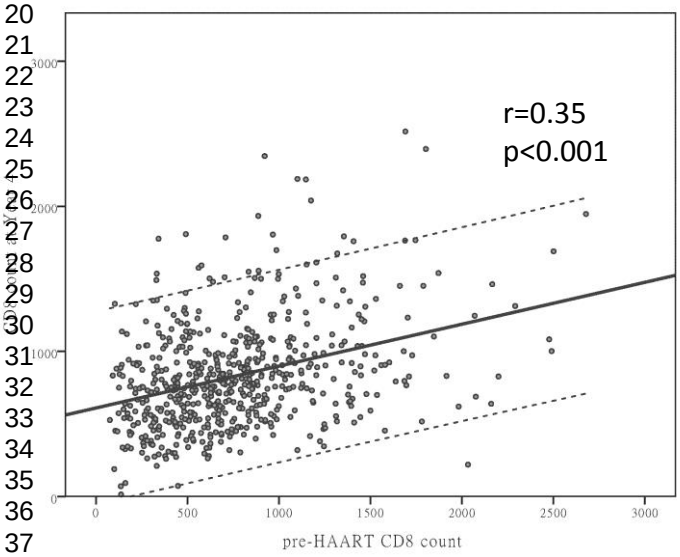
(a) pre-HAART CD4 (cells/μL) vs year 4 CD4 (cells/μL)



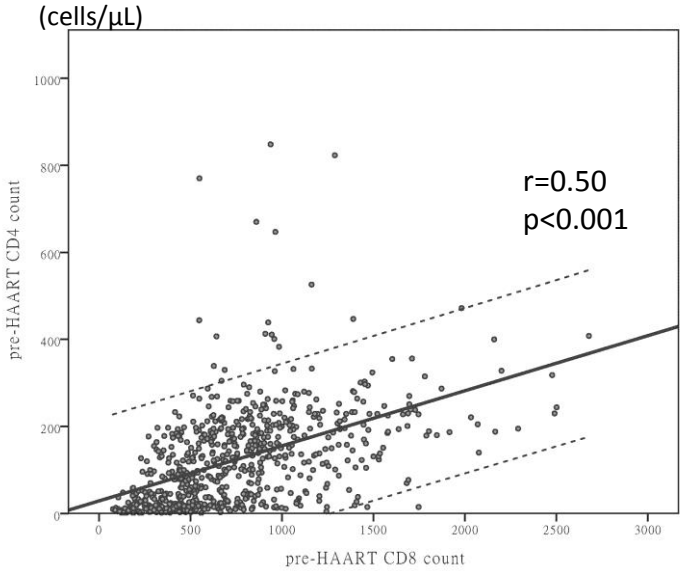
(b) pre-HAART CD8 (cells/μL) vs year 4 CD4 (cells/μL)



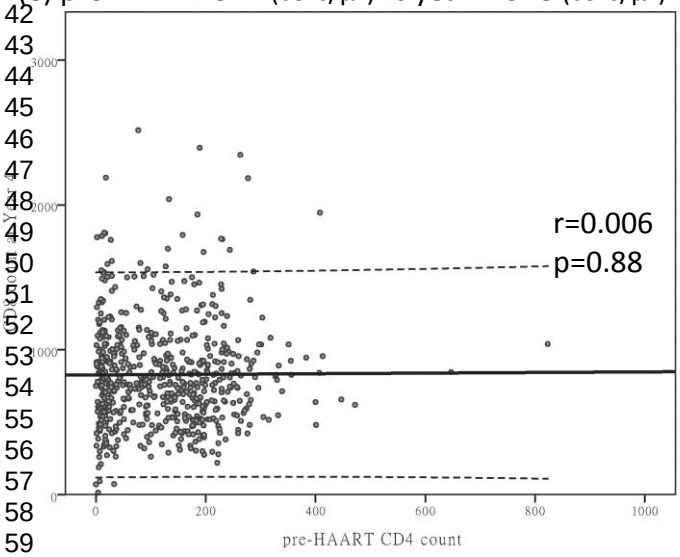
(c) pre-HAART CD8 (cells/μL) vs year 4 CD8 (cells/μL)



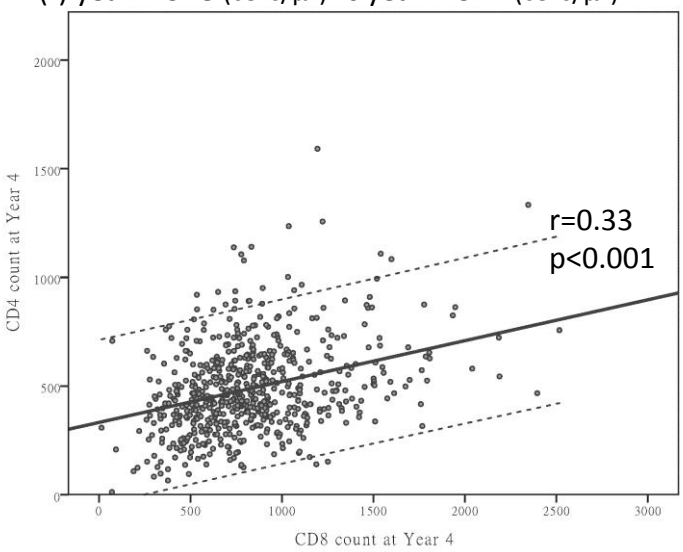
(d) pre-HAART CD8 (cells/μL) vs pre-HAART CD4 (cells/μL)



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



(f) year 4 CD8 (cells/μL) vs year 4 CD4 (cells/μL)



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/μL)		b. CD8 (cells/μL)		c. CD4/CD8 ratio	
	B	**95%C.I.**	**B**	**95%C.I.**	**B**	**95%C.I.**
(Intercept)	282.86	273.99 to 291.74*	1142.76	1105.97 to 1179.55*	0.24	0.22 to 0.25*
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 immune	97.55	80.71 to 114.39*	-401.33	-437.93 to -364.74*	0.41	0.37 to 0.45*

outcome			
conventional	0[a]	0[a]	0[a]
outcome			
(Scale)	31443	151917	0.06

*p<0.05

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

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			

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

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

Running title: Immune outcome following antiretroviral therapy

Shui Shan Lee[1], Ngai Sze Wong[1,2*], Bonnie Chun Kwan Wong[3], Ka Hing Wong[3], Kenny Chi Wai Chan[3]

[1]Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, People's Republic of China;

[2]Institute for Global Health & Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, United States;

[3]Special Preventive Programme, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region Government, Hong Kong, People's Republic of China;

***Corresponding author**

Dr. Ngai Sze Wong

Stanley Ho Centre for Infectious Diseases

Postgraduate Education Centre

Prince of Wales Hospital

Shatin

Hong Kong

Tel: (852) 22528813

Fax: (852) 26354977

Email: candy_wong@cuhk.edu.hk

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Abstract: 239 words; **Main text**: 3242 words

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 ≥500/μL 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≥500/μL, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥0.8. Patients with a pre-HAART CD8 ≤800/μL (428 out of 704) were more likely to be optimal immune outcome achievers with CD4≥500/μL 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 ≤800/μL. 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

been replaced by early initiation of HAART irrespective of baseline CD4 level.[8] Achievement of a high CD4 count of, say, over 500/μL remains a commonly used marker of immune restoration. Knowingly, prompt treatment and full viral suppression do not imply freedom from co-morbidities, as HIV disease is also characterised by a state of immune activation, with the emergence of non-AIDS morbidity and mortality.[9] This morbid state of immune activation cannot be inferred from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following HAART has however been linked to this scenario of immune activation.[10 11] Low CD4/CD8 ratio was observed in patients despite high CD4 level (>500/μL).[12] A high CD8 count following HAART was shown to be associated with inflammatory non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction has been reported.[4 13] Apparently, a target CD4 count is inadequate for reflecting effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly recognized as an important marker of immune reconstitution.[10 14]

To better monitor immunological recovery following HAART, new biomarkers are needed, which should preferably be derived from routinely collected laboratory data. Optimal outcome could be founded on CD4/CD8 normalization on top of the regularly monitored CD4 count. In this study, we define HAART associated immune recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to examine its predictors by analysing regularly collected viral load and immunological data, the latter including CD8 count, in a cohort of HIV patients following HAART.

METHODS

Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical service in Hong Kong were accessed for this observational study. 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). HIV patients of age ≥18 diagnosed in 1985-2012, on HAART continuously for ≥4 years without treatment interruption, with at least 1 CD4 measurement during treatment and with viral load fully suppressed (without consecutive viral load >500copies/mL in the first 4 years on treatment) were selected. We included patients who were treatment naïve or have been on non-standard treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b) viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as other regimens were rarely used for treatment naïve patients

Estimated cumulative viral load was expressed as years*$\log_{10}$ copies/mL, in accordance with the method reported by Zoufaly et al.[15] with modifications. Patients with available negative HIV testing result within 3 years before HIV diagnosis were included, so that one's seroconversion date could be estimated with confidence.[16] In brief, the products of the $\log_{10}$ viral load were summed from estimated seroconversion to subsequent specified time-point(s), with the computation of the highest viral load

for the undiagnosed interval and an upward adjustment by 1 $\log_{10}$ for the presumed primary infection period. The time of seroconversion was determined as the mid-point between last negative and first positive HIV antibody testing dates. On the other hand, optimal immune outcome was defined as the achievement of a CD4 count of ≥500/μL and a CD4/CD8 ratio ≥0.8[17] while conventional outcome was defined as achieving CD4 count of ≥500/μL but not CD4/CD8 ratio ≥0.8 within specific time. Late HIV diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis. The latest CD4 and CD8 measurements ≤30 days before HAART initiation were used as the baseline.

Comparisons between pre-HAART CD8>800/μL vs ≤800/μL were made by odds ratio (OR) and multivariable logistic regression with pre-HAART CD4 as confounder, while correlation coefficients were calculated to test the associations between CD4 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking reference from the criteria of high CD8 count (i.e. over 800/μL) during primary infection reported in another study.[18] CD4 (maximum value), CD8 (minimum value) and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome and conventional outcome by Year 4 on HAART over time (≤12 months, 12.1-24 months, … , >96 months) were compared in generalized estimating equations (GEE). Multivariable logistic regression model (stepwise) was applied to examine the predictors of optimal immune outcome and conventional outcome. Complete case analysis was performed. Loss to follow-up and death were data end points. Statistical tests were performed in SPSS.

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 18857clinical 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

first regimen, but 3 patients had changed to raltegravir-based regimen afterwards.(Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients (44%) had reached 500/μL or above, of which 105 (33%) gave a CD4/CD8 ratio of ≥0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio. On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients could not reach the CD4 target. (Table 2) The temporal changes of CD4 count, CD8 count and CD4/CD8 ratio over time is shown in figure 1, while distribution of CD4 and CD4/CD8 ratio at the end of Year 4 is shown in Supplementary Table 1(b). Whereas both CD4 count (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a steady rise from the first time-point following HAART, the temporal pattern of CD8 counts was inconspicuous (figure 1(c)). Patients with optimal immune outcome had significantly higher median CD4 and CD4/CD8 ratio but lower CD8 count than those only with satisfactory CD4 recovery (conventional outcome) in all time points (GEE model results in Supplementary Table 2). The CD4 count at Year 4 was positively correlated with pre-HAART CD4 ($r= 0.38$, $p<0.001$)(See web-only Supplementary Figure 1(a)) Categorised by one's pre-HAART CD8 count, about half (n=428, 61%) had a lower count of ≤800/μL. The 2 groups had similar demographic, cumulative viral load levels and had received similar treatment regimens. The CD4 count at Year 4 was positively correlated with pre-HAART CD8 count ($r=0.18$, $p<0.001$) (See web-only Supplementary Figure 1(b)) whereas the latter was also positively correlated with CD8 at Year 4.($r=0.35$, $p<0.001$) (See web-only Supplementary Figure 1(c)). After adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had higher chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR (aOR)=64.63, 95%C.I.=23.47 to 177.98) (Table 3). Likewise, a low pre-HAART CD8

count of ≤800/μL was associated with the optimal immune outcome at Year 4, with an increased odds (aOR=5.07, 95%C.I.=2.74-9.41) after adjusting for pre-HAART CD4. There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8 at Year 4 ($r=0.33$, $p<0.001$) could be demonstrated (See web-only Supplementary Figure 1(f)).

The following independent variables were then tested for their prediction of optimal immune outcome and conventional outcome achieved since treatment initiation throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART age, treatment duration and male gender. In the final model, both high pre-HAART CD4 and low pre-HAART CD8 were significant predictors of optimal immune outcome, while only the former was a significant predictor of conventional outcome (Table 4). Patients who were male and started HAART at younger age were more likely to achieve both outcomes. Patients on treatment for longer time (≥97months) had higher odds to achieve optimal immune outcome (aOR=3.34, 95%C.I.=2.17 to 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78, 95%C.I.=1.89 to 4.09, 49-72 months as reference). As a sub-study (results not shown), we have performed another set of GEE models with cumulative viral load as an independent variable (results not shown). The results did not support it as a significant predictor of an optimal immune outcome both in CD4 count and CD4/CD8 ratio, though the number of patients eligible for the analyses was only 187.

DISCUSSION

Pre-HAART CD4 count has long been shown to be a predictor of immunological outcome 3-5 years following antiretroviral therapy.[1] Our previous longitudinal studies in a cohort of Chinese HIV patients have demonstrated positive associations between nidus and maximum CD4 count over 5 years irrespective of the causative virus subtype or the regimens prescribed.[19 20] In assessing antiretroviral treatment response, however, CD4 count alone appeared to add little to viral load monitoring.[21] To account for the potential risk of non-AIDS related comorbidities including metabolic complications,[9] parallel CD4/CD8 ratio testing is gaining popularity as it reflects also the intensity of chronic inflammation implicated.[9 10] In this study, a CD4 count ≥500/μL in conjunction with a ratio of ≥0.8 was examined as a target outcome indicator for chronically infected patients on continued antiretroviral therapy. This target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year treatment period. Both pre-HAART CD4 and CD8 count, as well as the treatment interval were independent predictors of this new outcome target. While CD4 remained a useful prognostic marker, using it as the sole marker might overestimate treatment performance by including patients with high CD4 count but high CD8 count and low CD4/CD8 ratio as achiever (205 out of 715 achieved CD4 target only).

In this study, we have shown that 44% of patients on HAART achieved a CD4 count ≥500/μL at the end of 4 years, an outcome slightly poorer than that of 59% reported by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our shorter observation period (4 instead of 5 years) and the lower median pre-HAART CD4 count (158/μL compared to 180/μL).[22] As concluded in the recently published "START" study examining the benefits of the initiation of antiretroviral therapy in

HIV-positive adults with a CD4 count >500/μL, CD4 count *per se* could not capture all outcome effects arising from immediate HAART in chronic HIV infection.[23] Our study confirmed that CD4/CD8 ratio could be a readily available supplementary marker to monitor immune recovery. Evidently, the ratio may vary with lengths of observations, demographics, and/or even HAART regimens.[17 24 25] As the CD4/CD8 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio of 0.8 [17] to assess the state of immune recovery at 4 years after HAART initiation. Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7 years, the median observation interval of our cohort.

Pre-HAART CD8 count and its normalisation following antiretroviral treatment is relatively under-investigated.[26 27] In our study, pre-HAART CD4 and pre-HAART CD8 counts were positively correlated. It was noted that heterosexuals gave a lower pre-HAART CD8 (Table 3) compared to MSM but the difference became insignificant after the adjustment of pre-HAART CD4. Over time, CD4 rise went in parallel with slowly falling CD8 until reaching an optimal CD4 level of ≥500/μL with a near-normalised CD4/CD8 ratio≥0.8 at Year 4. Pre-HAART CD8 was a significant predictor of optimal immune outcome but not conventional outcome. The median CD8 count of former group was lower than latter group of patients in all time points since HAART initiation. Significant expansion of CD8 is known to occur soon after infection and the phenomenon might persist throughout the course of HIV infection. Recent studies suggested that CD8 normalisation was associated with early initiation of HAART during acute infection.[18] HIV-specific CD8 has been proposed to play an important role in effecting functional cure of HIV infection.[28] Its relationship with the absolute count of CD8 before and after HAART has not been established. With the

growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS morbidity and chronic inflammation,[9 10 29 30] it is possible that HIV+ patients' clinical outcome could be better explained from both the ratio and CD4 count rather than from the latter alone. From a virological perspective, the estimated cumulative viral load can be viewed as a surrogate of prolonged non-suppression of virus load. It does not however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its immunological impacts could be overtaken by a long interval of HAART, if the pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to early initiation of HAART in chronic HIV infection to avoid temporal accumulation of virus, a conclusion similar to that for primary HIV infection.[5]

We acknowledge that our study carries a number of limitations. Foremost, all patients had been on HAART during the time when a CD4-guided approach to treatment initiation was enforced. As the patients had been started on either a PI-based or NNRTI-based regimen, the possible impacts of newer generations of antiretroviral like INSTI could not be ascertained. The results should therefore be interpreted with caution, especially that strong association between INSTI-based regimen and CD4/CD8 normalisation has recently been reported.[31] These were selection bias which might have limited the extrapolation of results to the entire HIV population. In addition, our dataset did not include other inflammatory or infectious outcomes (e.g. HCV and/or cytomegalovirus co-infections [32-35]) and therefore these could not be analysed in perspective. As the main comparative period was 4 years, the minimum treatment duration of study population, the immunological recovery achieved by patients in this study may not necessarily be reflecting the ultimate response to HAART. We have nevertheless evaluated the outcome (comprising both CD4 count

and CD4/CD8 ratio) of all enrolled patients with a median duration of treatment of over 6 years in the final analysis. Theoretically, cohorts with patients observed throughout their lifetime would be invaluable to determine the health benefits of HAART. Analyses from such life-long cohorts should become a reality in the coming years or decades.

CONCLUSIONS

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

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

	frequency median	% (IQR)
(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)	37	(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 median	% (IQR)
estimated cumulative viral load[#] from seroconversion to diagnosis (n=199)	8	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	39	(33 to 46)
Months from diagnosis to treatment initiation	8.67	(2.75 to 33.13)
CD4 count (cells/μL)	109	(29 to 190)
CD4/CD8 ratio [a]	0.14	(0.06 to 0.23)
CD8 count (cells/μL) [a]	673	(441 to 966)
Viral load (log_{10} copies/mL) [b]	5.15	(4.62 to 5.58)
Estimated cumulative viral load[#] from seroconversion to treatment initiation (n=199)	18	(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)	85.38	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years [c]	476	(354 to 630)
Highest CD4/CD8 ratio within 4 years [d]	0.55	(0.39 to 0.76)

	frequency median	% (IQR)
CD4 count ≥500/μL 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*$\log_{10}$ 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 ≥500, CD4/CD8 ratio ≥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 ≥500/μL and CD4:CD8 ratio ≥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 ≥500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio ≥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/µL) and low (≤800/µL) 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 ≤800 (n=428)		Univariate analysis		adjusted by pre-HAART CD4	
	median/ no.	IQR/ %	median/ no.	IQR/ %	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%	*ref*		*ref*	
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%	*ref*		*ref*	
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/ no.	IQR/ %	median/ no.	IQR/ %	OR	95% CI	aOR	95% CI
C	4	1.9%	4	1.2%	0.56	0.14 to 2.27	1.13	0.25 to 5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to 1.64	1.37	0.6 to 3.17
Age at diagnosis (yrs)	36.80	31.74 to 43.54	37.46	30.27 to 45.17	1.00	0.98 to 1.01	0.99	0.98 to 1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to 3.28*	0.98	0.64 to 1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to 2.88*	0.94	0.63 to 1.41
(b) Pre-HAART virological status								
viral load (log_{10} copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to 5.52	5.20	4.69 to 5.58	1.23	1.03-1.47*	0.80	0.64-0.99*
Viral load log_{10} > 5	145	52.9%	259	61.7%	1.43	1.05 to 1.95*	0.71	0.49 to 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/ no.	IQR/ %	median/ no.	IQR/ %	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 35.52	5.60	2.44 to 30.58	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/ no.	IQR/ %	median/ no.	IQR/ %	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 2.87*	5.07	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 1.004	0.999	0.99 to 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*$\log_{10}$ 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 ≥500/μL and a CD4/CD8 ratio ≥0.8, and conventional outcome was defined as only achieved CD4 count ≥500/μL 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	*ref*		*ref*	
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	*ref*		*ref*	
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≤800/μL	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

*p<0.05

FIGURE LEGENDS

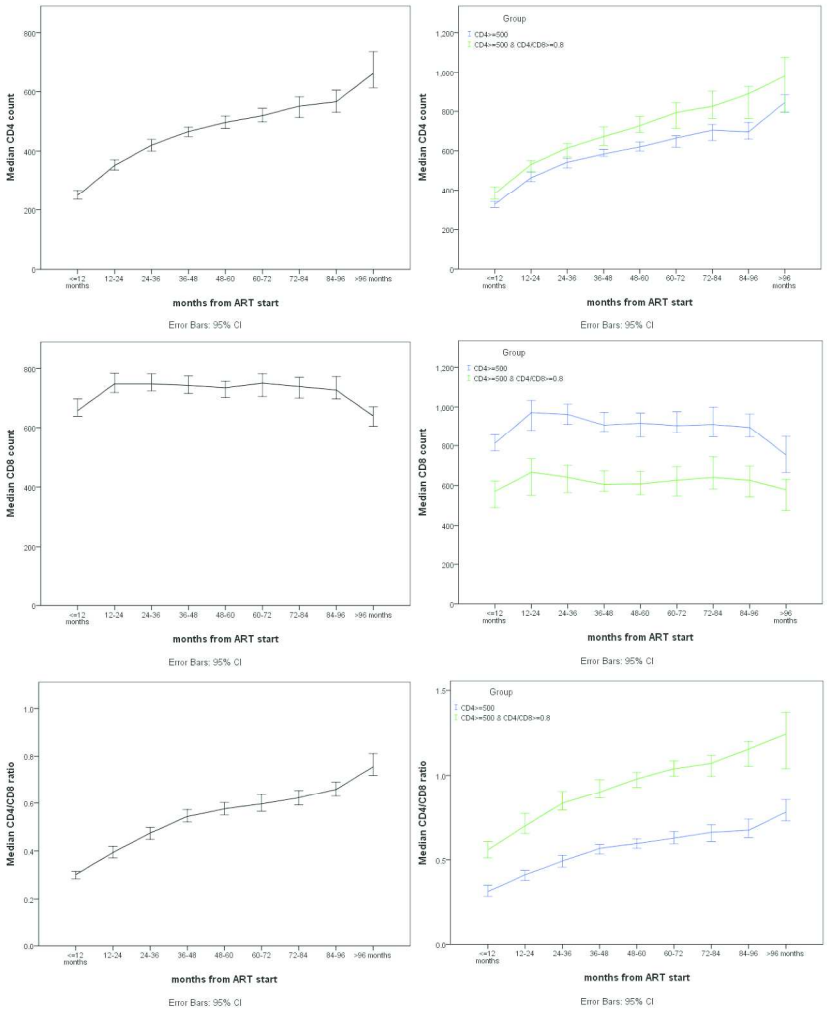
Figure 1. Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards.

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

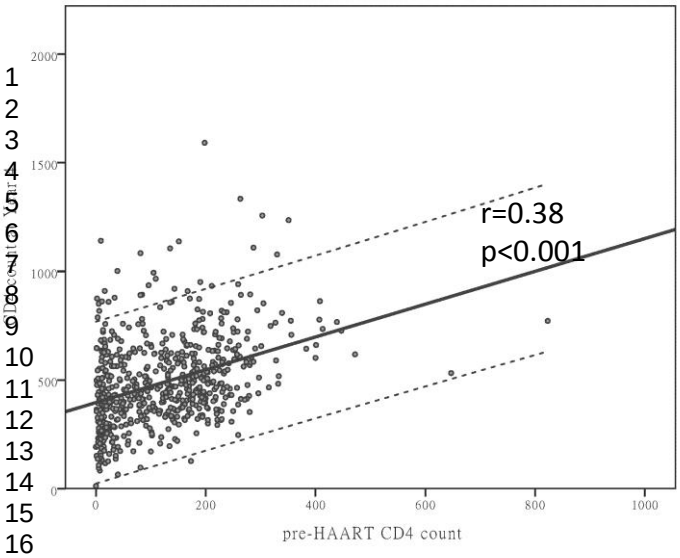
Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations



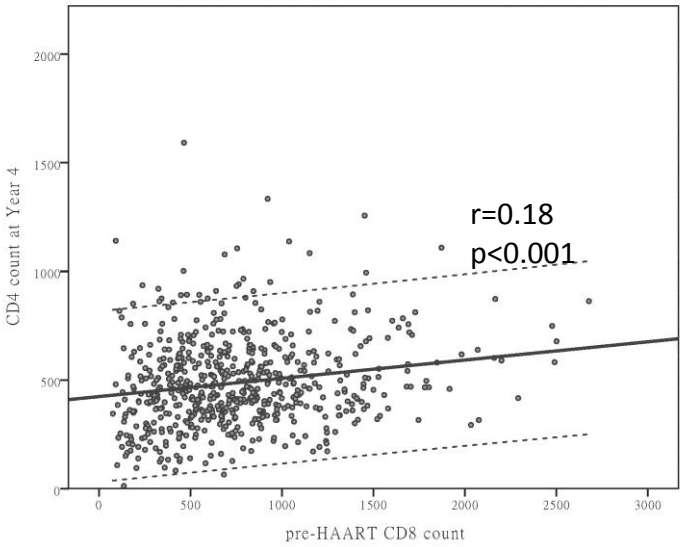
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

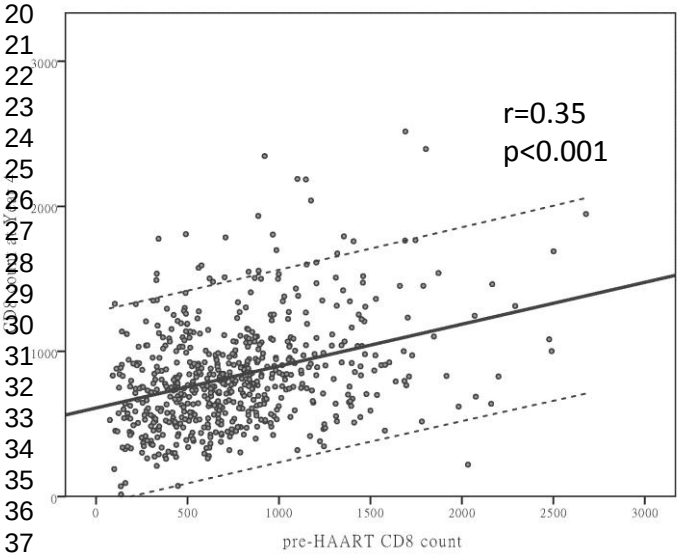
(a) pre-HAART CD4 (cells/μL) vs year 4 CD4 (cells/μL)



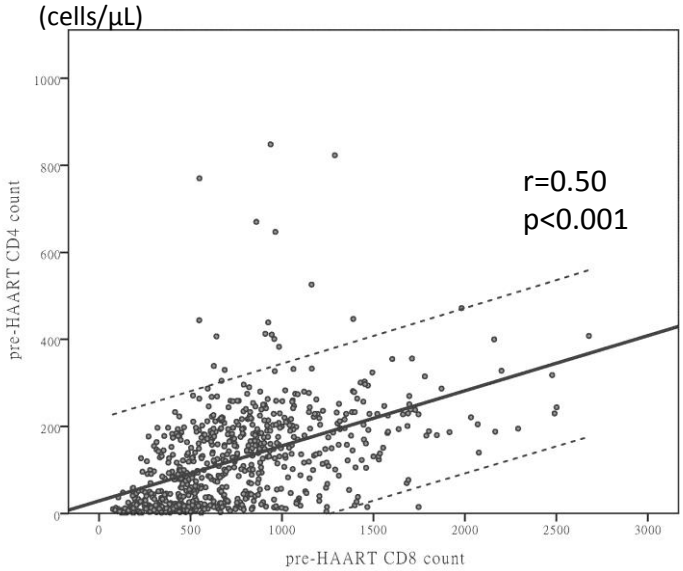
(b) pre-HAART CD8 (cells/μL) vs year 4 CD4 (cells/μL)



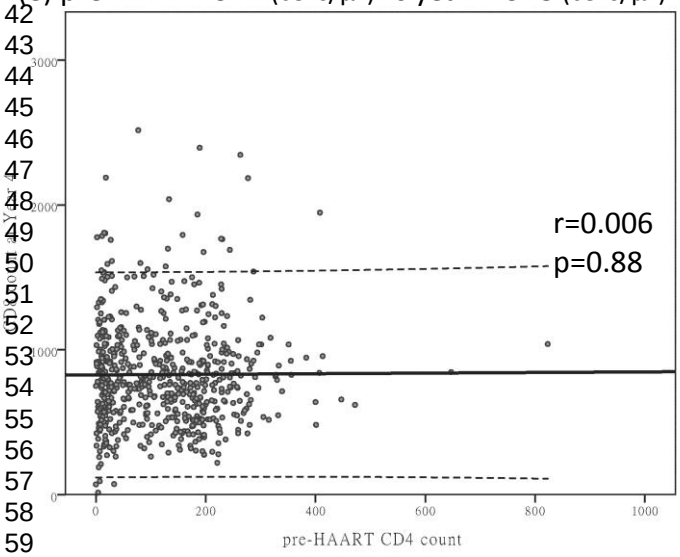
(c) pre-HAART CD8 (cells/μL) vs year 4 CD8 (cells/μL)



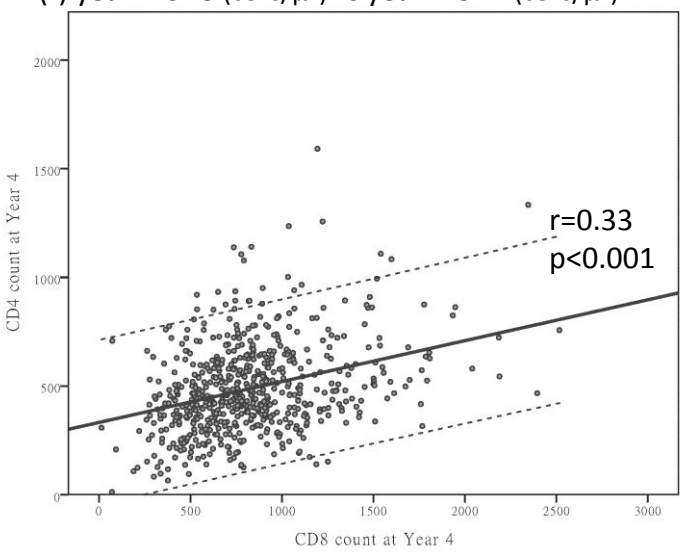
(d) pre-HAART CD8 (cells/μL) vs pre-HAART CD4 (cells/μL)



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



(f) year 4 CD8 (cells/μL) vs year 4 CD4 (cells/μL)



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

(b) Outcome at the end of Year 4

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

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/μL)**		**b. CD8 (cells/μL)**		**c. CD4/CD8 ratio**	
	B	**95%C.I.**	**B**	**95%C.I.**	**B**	**95%C.I.**
(Intercept)	282.86	273.99 to 291.74*	1142.76	1105.97 to 1179.55*	0.24	0.22 to 0.25*
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 immune	97.55	80.71 to 114.39*	-401.33	-437.93 to -364.74*	0.41	0.37 to 0.45*

outcome			
conventional	0[a]	0[a]	0[a]
outcome			
(Scale)	31443	151917	0.06

*p<0.05

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

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			

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

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

Running title: Immune outcome following antiretroviral therapy

Shui Shan Lee[1], Ngai Sze Wong[1,2*], Bonnie Chun Kwan Wong[3], Ka Hing Wong[3], Kenny Chi Wai Chan[3]

[1]Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong, People's Republic of China;

[2]Institute for Global Health & Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, United States;

[3]Special Preventive Programme, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region Government, Hong Kong, People's Republic of China;

***Corresponding author**

Dr. Ngai Sze Wong

Stanley Ho Centre for Infectious Diseases

Postgraduate Education Centre

Prince of Wales Hospital

Shatin

Hong Kong

Tel: (852) 22528813

Fax: (852) 26354977

Email: candy_wong@cuhk.edu.hk

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

Word Count:

Abstract: 239 words; **Main text**: 3278 words

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 ≥500/μL 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≥500/μL, of which only 33% (105 out of 318) concurrently achieved CD4/CD8 ratio ≥0.8. Patients with a pre-HAART CD8 ≤800/μL (428 out of 704) were more likely to be optimal immune outcome achievers with CD4≥500/μL 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 ≤800/μL. 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

been replaced by early initiation of HAART irrespective of baseline CD4 level.[8] Achievement of a high CD4 count of, say, over 500/μL remains a commonly used marker of immune restoration. Knowingly, prompt treatment and full viral suppression do not imply freedom from co-morbidities, as HIV disease is also characterised by a state of immune activation, with the emergence of non-AIDS morbidity and mortality.[9] This morbid state of immune activation cannot be inferred from the pattern of CD4 recovery alone. Failure of CD4/CD8 normalisation following HAART has however been linked to this scenario of immune activation.[10 11] Low CD4/CD8 ratio was observed in patients despite high CD4 level (>500/μL).[12] A high CD8 count following HAART was shown to be associated with inflammatory non-AIDS-related clinical events, and in fact a higher risk of myocardial infarction has been reported.[4 13] Apparently, a target CD4 count is inadequate for reflecting effective immune recovery. Concurrent rise of the CD4/CD8 ratio is increasingly recognized as an important marker of immune reconstitution.[10 14]

To better monitor immunological recovery following HAART, new biomarkers are needed, which should preferably be derived from routinely collected laboratory data. Optimal outcome could be founded on CD4/CD8 normalization on top of the regularly monitored CD4 count. In this study, we define HAART associated immune recovery by a combination of CD4 outcome and CD4/CD8 restoration. We set out to examine its predictors by analysing regularly collected viral load and immunological data, the latter including CD8 count, in a cohort of HIV patients following HAART.

METHODS

Anonymous clinical data from Integrated Treatment Centre, the largest HIV clinical service in Hong Kong were accessed for this observational study. 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). HIV patients of age ≥18 diagnosed in 1985-2012, on HAART continuously for ≥4 years without treatment interruption, with at least 1 CD4 measurement during treatment and with viral load fully suppressed (without consecutive viral load >500copies/mL in the first 4 years on treatment) were selected. We included patients who were treatment naïve or have been on non-standard treatment for < 1 year before HAART initiation. Data retrieved were: (a) CD4 and CD8 counts at diagnosis, before HAART initiation and 3-4 monthly subsequently, (b) viral load levels at the respective time-points, (c) AIDS diagnosis and the timing, as appropriate, (d) antiretroviral treatment date and regimens, differentiated as protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) based as other regimens were rarely used for treatment naïve patients

Estimated cumulative viral load was expressed as years*$\log_{10}$ copies/mL, in accordance with the method reported by Zoufaly et al.[15] with modifications. Patients with available negative HIV testing result within 3 years before HIV diagnosis were included, so that one's seroconversion date could be estimated with confidence.[16] In brief, the products of the $\log_{10}$ viral load were summed from estimated seroconversion to subsequent specified time-point(s), with the computation of the highest viral load

for the undiagnosed interval and an upward adjustment by 1 $\log_{10}$ for the presumed primary infection period. The time of seroconversion was determined as the mid-point between last negative and first positive HIV antibody testing dates. On the other hand, optimal immune outcome was defined as the achievement of a CD4 count of ≥500/μL and a CD4/CD8 ratio ≥0.8[17] while conventional outcome was defined as achieving CD4 count of ≥500/μL but not CD4/CD8 ratio ≥0.8 within specific time. Late HIV diagnosis was defined as the diagnosis of AIDS within 3 months of HIV diagnosis. The latest CD4 and CD8 measurements ≤30 days before HAART initiation were used as the baseline.

Comparisons between pre-HAART CD8>800/μL vs ≤800/μL were made by odds ratio (OR) and multivariable logistic regression with pre-HAART CD4 as confounder, while correlation coefficients were calculated to test the associations between CD4 and CD8 before and 4 years after HAART. The CD8 threshold was adopted by taking reference from the criteria of high CD8 count (i.e. over 800/μL) during primary infection reported in another study.[18] CD4 (maximum value), CD8 (minimum value) and CD4/CD8 ratio (maximum value) of patients achieving optimal immune outcome and conventional outcome by Year 4 on HAART over time (≤12 months, 12.1-24 months, … , >96 months) were compared in generalized estimating equations (GEE). Multivariable logistic regression model (stepwise) was applied to examine the predictors of optimal immune outcome and conventional outcome. Complete case analysis was performed. Loss to follow-up and death were data end points. Statistical tests were performed in SPSS.

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 18857clinical 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

first regimen, but 3 patients had changed to raltegravir-based regimen afterwards.(Table 1). The median treatment duration was 85.38 months (IQR: 63.39 to 117.32). As of the end of a 4-year observation period, the CD4 count of 318 patients (44%) had reached 500/μL or above, of which 105 (33%) gave a CD4/CD8 ratio of ≥0.8 concurrently, while 205 (64%) patients reached the CD4 target but not the ratio. On the other hand, 145 patients reached the optimal ratio, of which 32 (22%) patients could not reach the CD4 target. (Table 2) The temporal changes of CD4 count, CD8 count and CD4/CD8 ratio over time is shown in figure 1, while distribution of CD4 and CD4/CD8 ratio at the end of Year 4 is shown in Supplementary Table 1(b). Whereas both CD4 count (figure 1(a)) and CD4/CD8 ratio (figure 1(e)) showed a steady rise from the first time-point following HAART, the temporal pattern of CD8 counts was inconspicuous (figure 1(c)). Patients with optimal immune outcome had significantly higher median CD4 and CD4/CD8 ratio but lower CD8 count than those only with satisfactory CD4 recovery (conventional outcome) in all time points (GEE model results in Supplementary Table 2). The CD4 count at Year 4 was positively correlated with pre-HAART CD4 ($r= 0.38$, $p<0.001$)(See web-only Supplementary Figure 1(a)) Categorised by one's pre-HAART CD8 count, about half (n=428, 61%) had a lower count of ≤800/μL. The 2 groups had similar demographic, cumulative viral load levels and had received similar treatment regimens. The CD4 count at Year 4 was positively correlated with pre-HAART CD8 count ($r=0.18$, $p<0.001$) (See web-only Supplementary Figure 1(b)) whereas the latter was also positively correlated with CD8 at Year 4.($r=0.35$, $p<0.001$) (See web-only Supplementary Figure 1(c)). After adjusting for pre-HAART CD4, patients with lower pre-HAART CD8 had higher chance of achieving a higher CD4/CD8 ratio at Year 4 (adjusted OR (aOR)=64.63, 95%C.I.=23.47 to 177.98) (Table 3). Likewise, a low pre-HAART CD8

count of ≤800/μL was associated with the optimal immune outcome at Year 4, with an increased odds (aOR=5.07, 95%C.I.=2.74-9.41) after adjusting for pre-HAART CD4. There was no significant correlation between Year 4 CD8 and pre-HAART CD4 (See web-only Supplementary Figure 1(e)), but positive association between CD4 and CD8 at Year 4 ($r=0.33$, $p<0.001$) could be demonstrated (See web-only Supplementary Figure 1(f)).

The following independent variables were then tested for their prediction of optimal immune outcome and conventional outcome achieved since treatment initiation throughout the observation period: pre-HAART CD4, pre-HAART CD8, pre-HAART age, treatment duration and male gender. In the final model, both high pre-HAART CD4 and low pre-HAART CD8 were significant predictors of optimal immune outcome, while only the former was a significant predictor of conventional outcome (Table 4). Patients who were male and started HAART at younger age were more likely to achieve both outcomes. Patients on treatment for longer time (≥97months) had higher odds to achieve optimal immune outcome (aOR=3.34, 95%C.I.=2.17 to 5.15, 49-72 months as reference) than conventional outcome (aOR=2.78, 95%C.I.=1.89 to 4.09, 49-72 months as reference). As a sub-study (results not shown), we have performed another set of GEE models with cumulative viral load as an independent variable (results not shown). The results did not support it as a significant predictor of an optimal immune outcome both in CD4 count and CD4/CD8 ratio, though the number of patients eligible for the analyses was only 187.

DISCUSSION

Pre-HAART CD4 count has long been shown to be a predictor of immunological outcome 3-5 years following antiretroviral therapy.[1] Our previous longitudinal studies in a cohort of Chinese HIV patients have demonstrated positive associations between nidus and maximum CD4 count over 5 years irrespective of the causative virus subtype or the regimens prescribed.[19 20] In assessing antiretroviral treatment response, however, CD4 count alone appeared to add little to viral load monitoring.[21] To account for the potential risk of non-AIDS related comorbidities including metabolic complications,[9] parallel CD4/CD8 ratio testing is gaining popularity as it reflects also the intensity of chronic inflammation implicated.[9 10] In this study, a CD4 count ≥500/μL in conjunction with a ratio of ≥0.8 was examined as a target outcome indicator for chronically infected patients on continued antiretroviral therapy. This target was achieved in 15% (105 out of 715) of our patients at the end of a 4-year treatment period. The association of pre-HAART CD8 with optimal immune outcome was stronger with a cut-off ratio of ≥1 but the proportion of patients achieving the target outcome would be very low at 6% (46 out of 718). Both pre-HAART CD4 and CD8 count, as well as the treatment interval were independent predictors of this new outcome target. While CD4 remained a useful prognostic marker, using it as the sole marker might overestimate treatment performance by including patients with high CD4 count but high CD8 count and low CD4/CD8 ratio as achiever (205 out of 715 achieved CD4 target only).

In this study, we have shown that 44% of patients on HAART achieved a CD4 count ≥500/μL at the end of 4 years, an outcome slightly poorer than that of 59% reported by the Swiss HIV Cohort Study, a discrepancy which could be attributed to our

shorter observation period (4 instead of 5 years) and the lower median pre-HAART CD4 count (158/μL compared to 180/μL).[22] As concluded in the recently published "START" study examining the benefits of the initiation of antiretroviral therapy in HIV-positive adults with a CD4 count >500/μL, CD4 count *per se* could not capture all outcome effects arising from immediate HAART in chronic HIV infection.[23] Our study confirmed that CD4/CD8 ratio could be a readily available supplementary marker to monitor immune recovery. Evidently, the ratio may vary with lengths of observations, demographics, and/or even HAART regimens.[17 24 25] As the CD4/CD8 ratio tended to rise more slowly than CD4 recovery, we have chosen an interim ratio of 0.8 [17] to assess the state of immune recovery at 4 years after HAART initiation. Normalisation to a ratio of 1 could in fact be demonstrated in 13% of patients within 7 years, the median observation interval of our cohort.

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

of HAART during acute infection.[18] HIV-specific CD8 has been proposed to play an important role in effecting functional cure of HIV infection.[28] Its relationship with the absolute count of CD8 before and after HAART has not been established. With the growing evidence of the role of CD4/CD8 ratio as a new biomarker for non-AIDS morbidity and chronic inflammation,[9 10 29 30] it is possible that HIV+ patients' clinical outcome could be better explained from both the ratio and CD4 count rather than from the latter alone. From a virological perspective, the estimated cumulative viral load can be viewed as a surrogate of prolonged non-suppression of virus load. It does not however independently predict CD4 or CD4/CD8 ratio outcomes. Apparently, its immunological impacts could be overtaken by a long interval of HAART, if the pre-HAART CD4 and CD8 status were optimal. Overall, our results lent support to early initiation of HAART in chronic HIV infection to avoid temporal accumulation of virus, a conclusion similar to that for primary HIV infection.[5]

We acknowledge that our study carries a number of limitations. Foremost, all patients had been on HAART during the time when a CD4-guided approach to treatment initiation was enforced. As the patients had been started on either a PI-based or NNRTI-based regimen, the possible impacts of newer generations of antiretroviral like INSTI could not be ascertained. The results should therefore be interpreted with caution, especially that strong association between INSTI-based regimen and CD4/CD8 normalisation has recently been reported.[31] These were selection bias which might have limited the extrapolation of results to the entire HIV population. In addition, our dataset did not include other inflammatory or infectious outcomes (e.g. HCV and/or cytomegalovirus co-infections [32-35]) and therefore these could not be analysed in perspective. As the main comparative period was 4 years, the minimum

treatment duration of study population, the immunological recovery achieved by patients in this study may not necessarily be reflecting the ultimate response to HAART. We have nevertheless evaluated the outcome (comprising both CD4 count and CD4/CD8 ratio) of all enrolled patients with a median duration of treatment of over 6 years in the final analysis. Theoretically, cohorts with patients observed throughout their lifetime would be invaluable to determine the health benefits of HAART. Analyses from such life-long cohorts should become a reality in the coming years or decades.

CONCLUSIONS

Conventionally, CD4 count has been commonly used as the main outcome marker following HAART. In light of the increasing incidence of co-morbidities associated with HIV related chronic inflammations, CD4 count per se appears to carry little prognostic value in predicting HAART-associated immune recovery. Our results suggested that a combination of CD4 count and CD4/CD8 ratio offers another potentially useful approach to assessing immune outcome, compared to the use of CD4 alone. In evaluating immune recovery following long-term HIV viral suppression, pre-HAART CD8 count could be as important as nidus CD4 count as the independent predictors of the ultimate immune outcome. As both CD4 and CD8 are often routinely collected in the course of HIV management, an assessment of the temporal trends of CD4, CD8 and CD4/CD8 ratio could conveniently predict the immunological outcome without the need for sophisticated immune markers. Virological impact, as inferred from the estimated cumulative viral load after infection,

does not however add to the outcome reflected from viral load suppression. The monitoring of the host immunological responses remains the most important approach 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.

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

	frequency median	% (IQR)
(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)	37	(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 median	% (IQR)
estimated cumulative viral load[#] from seroconversion to diagnosis (n=199)	8	(3 to 18)
(c) Pre-HAART status		
Age (yrs)	39	(33 to 46)
Months from diagnosis to treatment initiation	8.67	(2.75 to 33.13)
CD4 count (cells/μL)	109	(29 to 190)
CD4/CD8 ratio [a]	0.14	(0.06 to 0.23)
CD8 count (cells/μL) [a]	673	(441 to 966)
Viral load (log_{10} copies/mL) [b]	5.15	(4.62 to 5.58)
Estimated cumulative viral load[#] from seroconversion to treatment initiation (n=199)	18	(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)	85.38	(63.39 to 117.32)
AIDS Free during treatment (n=479)	456	95%
Highest CD4 count within 4 years [c]	476	(354 to 630)
Highest CD4/CD8 ratio within 4 years [d]	0.55	(0.39 to 0.76)

	frequency median	% (IQR)
CD4 count ≥500/μL 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*$\log_{10}$ 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 ≥500, CD4/CD8 ratio ≥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 ≥500/μL and CD4:CD8 ratio ≥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 ≥500/μL only	205	622 (552 to 723)	29.10 (17.43 to 38.37)	0.59 (0.49 to 0.69)	/
Ratio ≥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/µL) and low (≤800/µL) 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 ≤800 (n=428)		Univariate analysis		adjusted by pre-HAART CD4	
	median/ no.	IQR/ %	median/ no.	IQR/ %	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%	*ref*		*ref*	
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%	*ref*		*ref*	
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/ no.	IQR/ %	median/ no.	IQR/ %	OR	95% CI	aOR	95% CI
C	4	1.9%	4	1.2%	0.56	0.14 to 2.27	1.13	0.25 to 5.07
Others	13	6.3%	18	5.6%	0.77	0.36 to 1.64	1.37	0.6 to 3.17
Age at diagnosis (yrs)	36.80	31.74 to 43.54	37.46	30.27 to 45.17	1.00	0.98 to 1.01	0.99	0.98 to 1.01
Late HIV diagnosis	48	17.4%	138	32.2%	2.26	1.56 to 3.28*	0.98	0.64 to 1.51
AIDS before treatment	66	23.9%	168	39.3%	2.06	1.47 to 2.88*	0.94	0.63 to 1.41
(b) Pre-HAART virological status								
viral load (log_{10} copies/mL)	(n=274)		(n=420)					
	5.04	4.55 to 5.52	5.20	4.69 to 5.58	1.23	1.03-1.47*	0.80	0.64-0.99*
Viral load log_{10} > 5	145	52.9%	259	61.7%	1.43	1.05 to 1.95*	0.71	0.49 to 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/ no.	IQR/ %	median/ no.	IQR/ %	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 35.52	5.60	2.44 to 30.58	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/ no.	IQR/ %	median/ no.	IQR/ %	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 2.87*	5.07	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 1.004	0.999	0.99 to 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*$\log_{10}$ 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 ≥500/μL and a CD4/CD8 ratio ≥0.8, and conventional outcome was defined as only achieved CD4 count ≥500/μL 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	*ref*		*ref*	
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	*ref*		*ref*	
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≤800/μL	0.998	0.998 to 0.999*		
Constant	0.48		3.30	

aOR – adjusted odds ratio;

*p<0.05

FIGURE LEGENDS

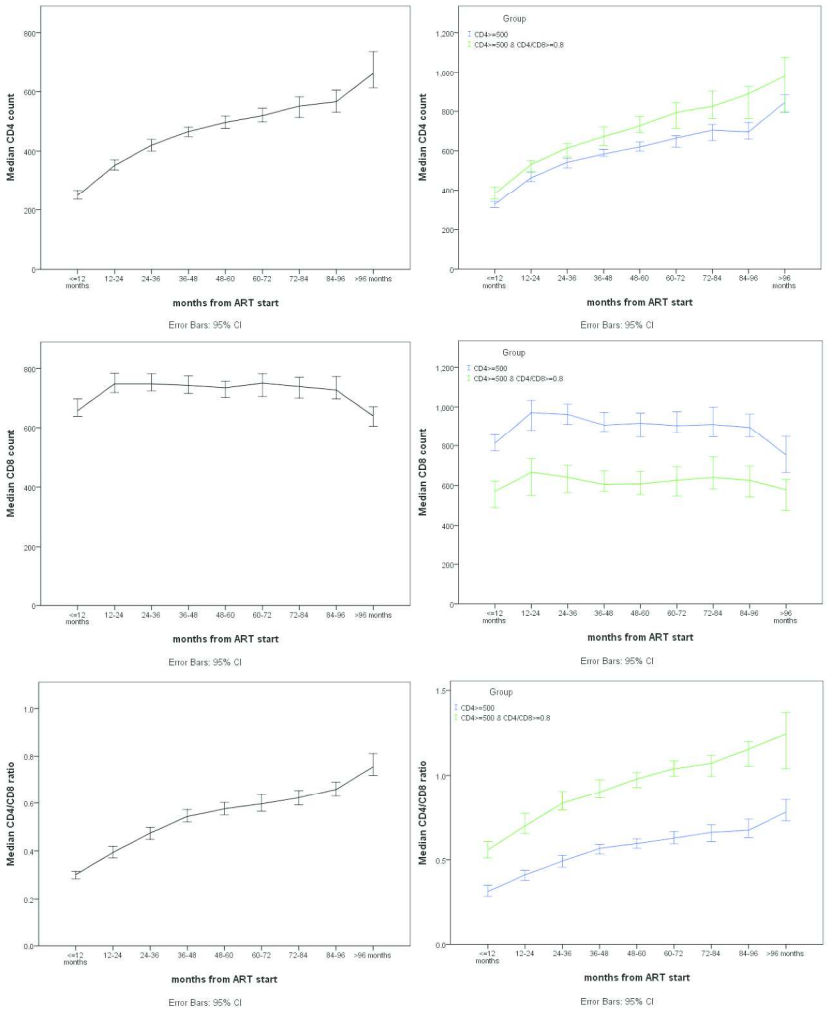
Figure 1. Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards.

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

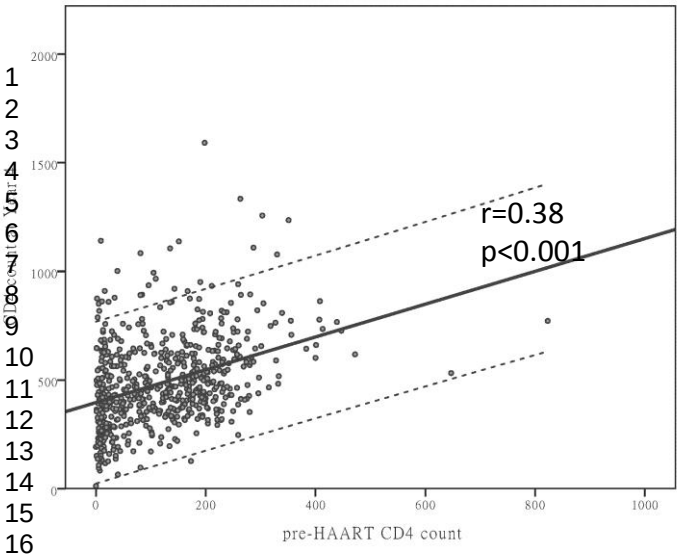
Supplementary Table 2. Comparison of CD4 count, CD8 count and CD4/CD8 ratio between patients achieved optimal immune outcome and conventional outcome by Year 4 in generalized estimating equations



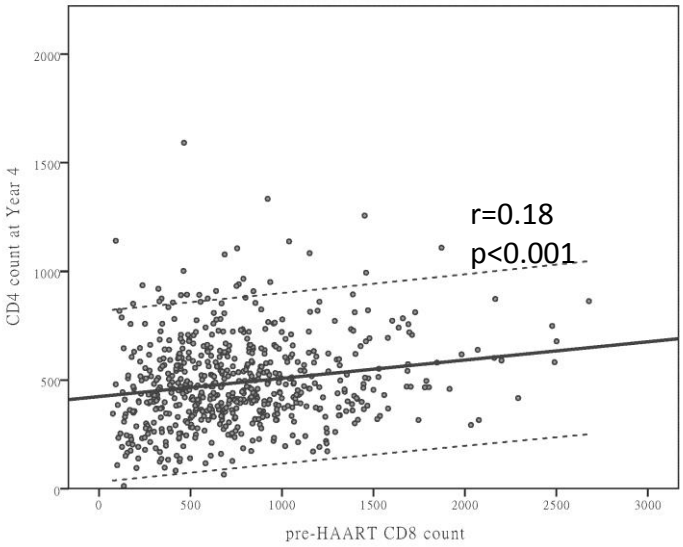
Yearly changes of (a) CD4 count, (b) CD8 count, and (c) CD4/CD8 ratio from HAART initiation to 6 years afterwards

275x397mm (300 x 300 DPI)

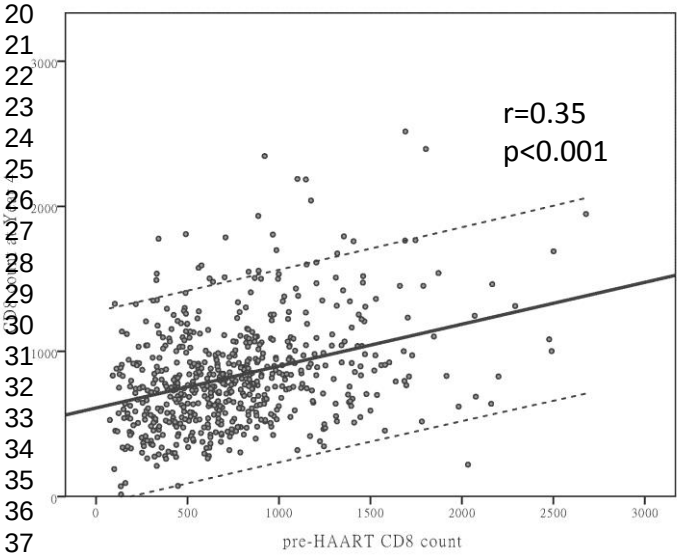
(a) pre-HAART CD4 (cells/μL) vs year 4 CD4 (cells/μL)



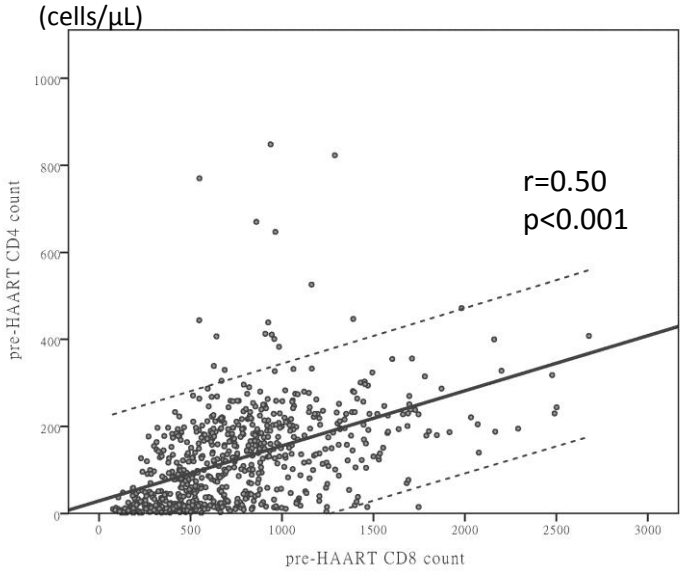
(b) pre-HAART CD8 (cells/μL) vs year 4 CD4 (cells/μL)



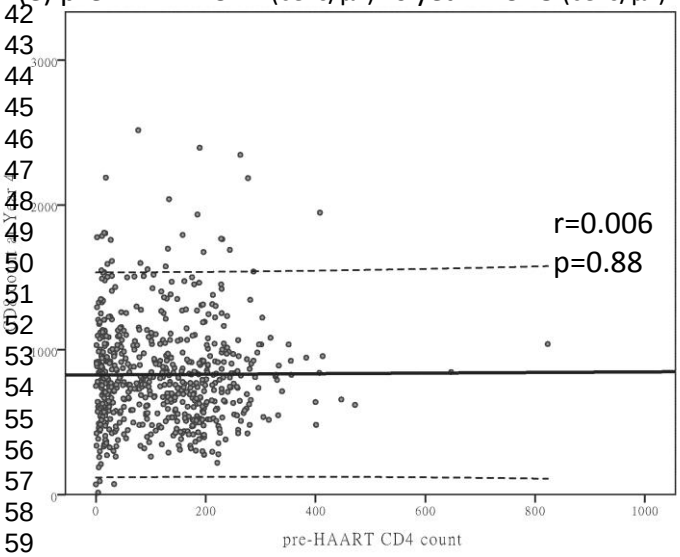
(c) pre-HAART CD8 (cells/μL) vs year 4 CD8 (cells/μL)



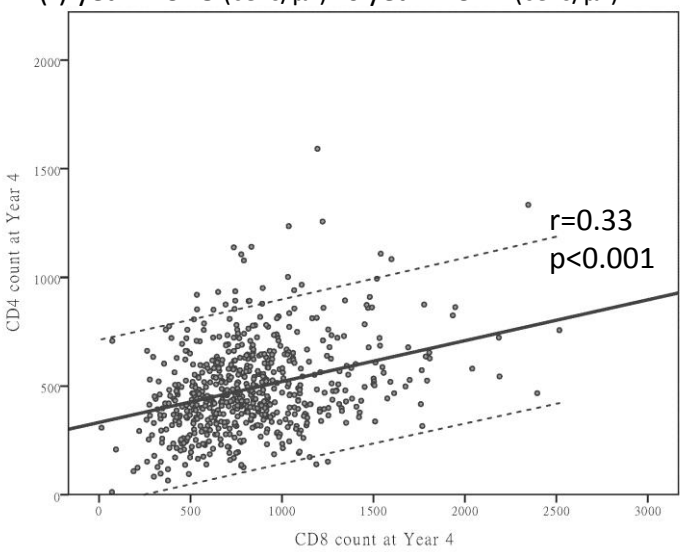
(d) pre-HAART CD8 (cells/μL) vs pre-HAART CD4 (cells/μL)



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



(f) year 4 CD8 (cells/μL) vs year 4 CD4 (cells/μL)



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

(b) Outcome at the end of Year 4

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

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/μL)**		**b. CD8 (cells/μL)**		**c. CD4/CD8 ratio**	
	B	**95%C.I.**	**B**	**95%C.I.**	**B**	**95%C.I.**
(Intercept)	282.86	273.99 to 291.74*	1142.76	1105.97 to 1179.55*	0.24	0.22 to 0.25*
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 immune	97.55	80.71 to 114.39*	-401.33	-437.93 to -364.74*	0.41	0.37 to 0.45*

outcome			
conventional	0[a]	0[a]	0[a]
outcome			
(Scale)	31443	151917	0.06

*p<0.05

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

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			

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.