Final Report

Analysis of Factors Contributing to Immune Reconstitution in HIV-Infected Patients during Combination Antiretroviral Therapy

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Analysis of Factors Contributing to Immune Reconstitution in HIV-Infected Patients during Combination Antiretroviral Therapy

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Abstract

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Project Title : Analysis of factors contributing to immune reconstitution in HIV-infected patients during combination of antiretroviral therapy

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Abstract: Combination antiretroviral therapy (cART) can lead to viral suppression and increase of $CD4^+$ T-cells in HIV-infected patients; however, not all patients fully respond to cART. Identifying factors contributing to different immune responses among HIV-infected patients receiving cART will help to define the most effective treatment strategy. We analyzed, among HIV-infected patients with full reponse (FR, CD4 cell count ≥500 cells/μL and confirmed HIV RNA load undetectable) or partial immune response (PR, CD4 cell count < 500 cells/μL and confirmed HIV RNA load undetectable) after at least 5 years cART, the relationship between interleukin-7 (IL-7), a cytokine playing a major role in T-cell generation, and other molecules related to its function. Plasma and peripheral blood mononuclear cells collected from HIV-infected patients on cART were measured for levels of IL-7, soluble IL-7Rα (s IL-7Rα or sCD127), using ELISA technique and thymic output so-called T cell receptor excision circles (TRECs) by real-time quantitative PCR. Additionally, levels of CD127 expression on PBMCs, lymphocytes, T-helper and cytotoxic T cells isolated from EDTA blood were analyzed by flow cytometry. Older Age and percentage of PBMC expressing CD127 were associated with partial response to ART. Levels of IL-7, sIL-7Rα and TRECs were not statistically significant different among patients with full response (FR) and those with partial response (PR) to cART.

Keywords : IL-7, TRECs, HIV-1

Final report content:

1. Abstract

Combination antiretroviral therapy (cART) can lead to viral suppression and increase of $CD4^+$ T-cells in HIV-infected patients; however, not all patients fully respond to cART. Identifying factors contributing to different immune responses among HIV-infected patients receiving cART will help to define the most effective treatment strategy. We analyzed, among HIV-infected patients with full reponse (FR, $CD4^+$ cell count ≥500 cells/μL and confirmed HIV RNA load undetectable) or partial immune response (PR, $CD4^+$ cell count < 500 cells/μL and confirmed HIV RNA load undetectable) after at least 5 years cART, the relationship between interleukin-7 (IL-7), a cytokine playing a major role in T-cell generation, and other molecules related to its function. Plasma and peripheral blood mononuclear cells collected from HIV-infected patients on cART were measured for levels of IL-7, soluble IL-7Rα (s IL-7Rα or sCD127), using ELISA technique and thymic output so-called T cell receptor excision circles (TRECs) by real-time quantitative PCR. Additionally, levels of CD127 expression on PBMCs, lymphocytes, T-helper and cytotoxic T cells isolated from EDTA blood were analyzed by flow cytometry. Older Age and percentage of PBMC expressing CD127 were associated with partial response to ART. Levels of IL-7, sIL-7Rα and TRECs were not statistically significant different among patients with full response (FR) and those with partial response (PR) to cART.

2. Executive summary

Combination antiretroviral therapy (cART) can lead to viral suppression and increase of $CD4^+$ T-cells in HIV-infected patients; however, not all patients fully respond to cART It is now admitted that the recovery of immune cells on cART occur over 3 phases. During the first phase, there is a rapid increase of $CD4^+$ T lymphocytes during the first 2-3 months due to the redistribution of memory CD4 T cells from the lymphoid tissues to peripheral blood. In the second phase, CD4 T-cell increase slowly, about 5 to 10 naïve $CD4^+$ T cells /L monthly, until the end of the second year of therapy. This is followed by a third phase during which there is a much lower production of CD4 T-cell, about 2 to 5 cells /L monthly. Tthymus plays a major role in supplying new naïve T cells. To date, factors contributing to immune restoration are still unclear. Identifying these factors and their roles in contributing to different responses among HIV-infected patients on cART will help to define the most effective treatment strategy. Cells recovered on cART are those redistributed from lymph nodes or generated from the thymus. Roles of cytokines regulating T-cell development and homeostasis during cART treatment are still unclear. IL-7 is a major cytokine involving in these processes.

We analyzed the relationship between interleukin-7 (IL-7), a cytokine playing a major role in T-cell generation, and other molecules related to its function among 2 groups of patients; 1) partial responders (PR) to c-ART with $CD4^+$ cell count < 500 cells/μL and confirmed HIV RNA load undetectable after at least 5 years of treatment and 2) Full response (FR) to cART are those whose $CD4^+$ cell count ≥ 500 cells/ μL and confirmed HIV RNA load undetectable after at least 5 years of treatment. Demographic and clinical characteristics at baseline and after at least 5 years cART as well are presented. Older age and percentage of PBMC expressing CD127 are important factors associated with partial response to ART, there were no statistically different in the percentages of

PBMC expressing CD127 or in lymphocytes, T-helper and CTL cells FR and PR. In addition, the levels of newly produced lymphocytes as measured by the production of TRECs did not correlate with levels of IL-7, sIL-7R, percentages of cells expressing CD127 or levels of CD127 expression on cell surface.

3. Objective

3.1. To measure levels of IL-7, sIL-7Rα and IL-7Rα and TRECs.

3.2. To determine factors contributing to partial response (PR) to cART

3.3. To analyze their relationships with immune reconstitution among full responders (FR) and those with partial response (PR) to cART.

4. Research methodology

4.1. Patient Recruitment

A total of 46 HIV-infected patients enrolled within the Program for HIV prevention and treatment (PHPT-GFATM) and on cART for at least 5 years followed were recruited from 2 sites: Nakornping and Sanpatong hospitals (Chiang Mai, Thailand) between September 27, 2005 and March 7, 2007. All participants signed the consent form approved by the Ethics Committee at the Faculty of Associated Medical Sciences, Chiang Mai University.

4.2. Sample Collection and Processing

Forty mL of blood was collected in EDTA tubes from each patient. Six milliliters of blood was sent to the Faculty of Associated Medical Sciences Clinic for CBC and lymphocyte subpopulation counts. Plasma was separated from 10 mL of blood and kept at -20°C until used.

4.3. Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation method using Isoprep with the density of 1.077 g/ml (Robbins Scientific Corporation, Sunnyvale, CA). Approximately 1x10^{6} PBMCs were then used for CD127 analysis, whereas 2x10^{6} cells were aliquoted and kept at -20°C for TREC determination. The remaining PBMCs were divided in half and immediately sorted into CD4- and CD8-T cells.

4.4. CD4- and CD8-T Cell Sorting

CD4- and CD8-T cells were sorted using the EasySep® positive selection kits (StemCell Technologies, British Columbia, Canada) according to the manufacturer's protocol. Briefly, PBMCs at the concentration between 10^{7}-10^{8} cells was incubated with an EasySep® positive selection cocktail, at room temperature (RT) for 15 minutes followed by the addition of dextran magnetic nanoparticles and incubated for an additional of 10 mins. The tube of mixture was placed into the magnet for 5 minutes before the unbound cells were poured off the supernatant fraction. The tube was washed and positively selected cells were collected. The sorted cells were counted, their purity was checked and kept at -20°C until used.

4.5. Detection of CD127 (Surface IL-7Rα)

The percentage of cells expressing CD127 and level of CD127 expression on cell surface was measured by flow cytometry. PBMCs at 1x10^{6} cells/tube/100µL were stained with anti-

CD127:PerCPCy5.5 (BioLegend Inc., CA, USA) alone or in combinations with the following antibodies; anti-CD3:PE (BioLegend Inc., CA, USA) and anti-CD4:FITC (Immunotech Laboratories, Inc., CA, USA), or anti-CD3:PE and anti-CD8:FITC (Immunotech Laboratories, Inc., CA, USA) at the concentrations recommended by the manufacturers. Cells were incubated on ice in the dark for 30 minutes and washed twice with PBS. Then cells were resuspended in 1% paraformaldehyde and analyzed by the flow cytometer (Cytomics™ FC500, Beckman Coulter Inc., Florida, USA).

4.6. Measurement of IL-7 Level in Plasma

The levels of IL-7 in plasma were measured using Human IL-7 Duoset ELISA kit (R&D Systems, Inc., Minnesota, USA). Briefly, a polystyrene flat-bottom 96-well microplate (Nunc, Fisher Scientific GmbH, Schwerte, Germany) was coated with the Capture Antibody (mouse anti-human IL-7) at a concentration of 2 μg/mL at RT overnight. Plate was then washed with wash buffer (0.05% Tween-20 in PBS) using microtitration plate washer (SLT Lab Instrument, Strasbourg-Schiltigheim, France) and blocked with 1%BSA in PBS for 1 hour at RT. After washing to remove an excess blocking solution, plasma samples or standards (recombinant human IL-7) was added to each well, covered plate with an adhesive strip and incubated for 2 hours at RT. Washed to remove any unbound or non-specific proteins followed by the addition of the Detection Antibody (biotinylated goat anti-human IL-7) and incubated at RT for 2 more hours. After washing, the streptavidin-HRP was added to each well and incubated at RT protected from the light for 20 minutes. Plate was then washed and the TMB Substrate Solution (Substrate Reagent Pack, R&D Systems, MN, USA) was added, incubated for 20 minutes at RT protecting from the light before stopping the reaction with 2N H_2SO_4. The optical density was measured at 450nm with the wavelength correction set at 570nm. The concentration of IL-7 of each sample was calculated using a four parameter logistic (4-PL) curve-fit.

4.7. Soluble IL-7Rα (IL-7Rα) Measurement

The levels of soluble IL-7Rα in plasma were measured using Human sIL-7R ELISA kit (Cusabio Biotech Co., Ltd, Hubei, China), based on a quantitative sandwich enzyme immunoassay. According to the manufacturer's protocol, an assay plate precoated with antibody specific for sIL-7R was added with standards, samples or sample diluent in duplicate/sample, covered with the adhesive strip and incubated for 2 hours at 37°C. Then the liquid of each well was removed followed by the addition of biotin-antibody to each well and incubate for 1 hour at 37°C. Plate was then washed with wash buffer using an aspirate/wash cycle of the ELISA plate autowasher for 4 cycles prior to adding of HRP-avidin and incubated for another hour at 37°C. Then washed the plate 5 times and added the TMB Substrate to each well, incubated the plate for 30 minutes at 37°C protecting from the light followed by the addition of the stop solution and reading the OD at 450nm and correct the wavelength at 570nm.

4.8. DNA Extraction

DNA was extracted using Nucleospin Blood® Prep/Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. About 2-3 x 10^6 PBMCs, sorted CD4- or CD8-T cells were first lysed by incubating with Buffer AL at 56°C for 10 minutes. Then the ethanol was added, mixed and carefully

applied to the column to spin at 6000 x g for 1 minute before transferring the column to a new clean collection tube. To wash the unbound materials from the column, Buffer AW1 was added, centrifuged at 6000 x g for 1 minute, discarded the filtrate and transfered into a new collection tube. Then Buffer AW2 was added, centrifuged at 20,000 x g for 3 minutes and filtrate was again discarded. To ensure that Buffer AW2 will not be carried over, the column was placed into a new collection tube and spun at full speed for 1 minute before discarding the collection tube. The column was placed into a new clean 1.5 mL microcentrifuge tube to elute the DNA by adding Buffer AE, incubate at RT for 1 minute followed by the centrifugation at 6000 x g for 1 minute. The extracted DNA was then kept at -20°C until used.

4.9. Measurement of Thymic Output

To measure thymic output, DNA extracts were used for the quantification of TREC levels by real-time quantitative PCR as described by Pornprasert *et al.* (4). The real-time PCR amplification was performed in a 25μL reaction mixture containing 5μL DNA extract or sterile distilled water as a no template control, 1x real-time PCR Master Mix (Thermo Scientific ABsolute™ qPCR ROX Mix, Surrey, UK), 400nM of each primer (forward, 5'-CACATCCCTTTCAACCATGCT-3'; reverse, 5'-GCCAGCTGCAGGGTTTAGG-3': GenBank sequence accession number DQ858179.1) and 200nM of the fluorogenic probe (5'-ACACCTCTGGTTTTTGTAAAGGTGCCCACT-3') conjugated with FAM (6-carboxyfluorescein) at the 5'-end, and TAMRA (6-carboxytetramethilrhodamine) at the 3'-end. To normalize for cell equivalents, the β-actin primer and probe concentrations were as follows: 300nM of forward primer (5'-TCACCCACACTGTGCCCATCTACGA-3'), 600nM of reverse primer (5'-CAGCGGAACCGCTCATTGCCAATGG-3') and 200nM of the fluorogenic probe (5'-ATGCCCTCCCCCATGCCATCCTGCGT-3') conjugated with the hexachlorofluorescein (HEX), (Human β-actin gene GenBank sequence accession number DQ858179.1). The real-time PCR reaction was performed using Mastercycler realplex4, Eppendorf (Mondotech Co., Ltd., Germany). The mixture was preheated at 95°C for 15 minutes, followed by 50 cycles of 15 seconds at 95°C and 1 minute at 60°C. Each sample was run in duplicate.

4.10. Statistical analysis

Summary statistics for continuous variables are presented as medians and inter-quartile ranges (IQR) and for categorical variables as frequencies and percentage. A composite criterion will be used to define: Partial response (PR) to cART; $CD4^{+}$ cell count <500 cells/μL and confirmed HIV RNA load undetectable, Full response (FR) to c-ART; $CD4^{+}$ cell count $\geq$ 500 cells/ μL and confirmed HIV RNA load undetectable after at least 5 years of treatment. For the comparison between FR and PR groups: continuous variables will be analyzed using Wilcoxon rank sum tests; categorical variables and or frequency will be evaluated by the chi-square test. Multiple regression analysis will be used to predict TRECs from independent variables. Logistic regression analysis will be used to identify risk factors contributing to partial response to cART. Using backward stepwise elimination of variables with p-values <0.25 in the univariate logistic regression, a multivariate logistic regression model will be fitted to assess adjusted associations between the variables and the outcome. Also, confounder and interaction will be considered to the model. All reported p-values are two-sided, and a p-value $\leq$0.05 was considered to be significant. All analyses were performed using STATA, version 10.1.

5. Result

5.1. Demographic and baseline characteristics of patients

Of all 46 participants, 58.70% were males and 41.30% were female. The median [interquartile (IQR)] age of participants was 43.6 (39.8-49.5) years and cART-naïve with median (IQR) $CD4^+$ cell count 161 (89-217) cells/μL and viral load 5.02 (4.44-5.27) log_{10} copies/mL before starting first line NNRTI-based cART under PHPT-GFATM program at the age over 18 years old. At baseline, there were 13 (28.26%) participants whose $CD4^+$ cell count were less than 100 cells/μL, 32 (69.57%) were less than 200 cells/μL, 45 (97.83%) were less than 350 cells/μL and all (100%) were less than 500 cells/μL. At the censoring date for collecting blood samples, participants had reached at least 5 years of cART with the median (IQR) cART duration of 6 (5.6-6.4) years. Participants had median (IQR) of other hematological parameters at baseline within the normal ranges including hemoglobin, hematocrit, platelet, WBC and neutrophil of 12.6 (11.2-13.9) gm/dL, 38.8 (34.9-42.0)%, 249,500 (201,000-291,000) cells/μL, 4,850 (4,300-5,940) cells/ μL and 47.2 (41.8-54.5)%, respectively.

5.2. Clinical characteristics and immune responses of Partial and Full Responders

At the cut-off date (at least 5 years of cART treatment), none of the participants had $CD4^+$ cell count <200 cells/μL (critical levels, risk of having opportunistic infection). However, there were 10 (21.74%) participants had $CD4^+$ cell count <350 cells/μL (recommended level of starting cART) and 32 (69.67%) still had $CD4^+$ cell count <500 cells/μL (within normal reference range of $CD4^+$ cell count). Participants were divided into 2 groups based on the number of $CD4^+$ cell count reached at the censoring date; Full Responders, FR) participants who had $CD4^+$ cell count $\geq$ 500 cells/μL with viral load at undetectable levels, and Partial Responders, PR) patients with $CD4^+$ cell count < 500 cells/μL with viral load at undetectable levels after at least 5 years of cART. There were 14 (30.43%) FR and 32 (69.57%) PR. Within FR group, 6 (42.86)% were female and 8 (57.14)% were male, whereas, there were 13 (40.63%) female and 19 (59.38%) male within the PR group. The median (IQR) age of FR and PR groups were 42.8 (38.7-46.9) and 46.0 (40.4-49.7) years, respectively. There were no sex and age different between these 2 groups of patients. In addition, the median (IQR) ARV duration, $CD4^+$ cell count and viral load at baseline were 6.0 (5.5-6.1) years, 206 (152-239) cells/μL, 5.07 (4.49-5.27) log_{10} copies/mL, respectively, in FR group, and 6.1 (5.7-6.5) years, 139 (68-193) cells/μL and 4.94 (4.41-5.31) log_{10} copies/mL, respectively, in PR group. There was a statistically significant different ($P = 0.02$) of $CD4^+$ cell count at baseline between FR and PR. Other hematological parameters of these 2 groups were all within the normal reference ranges at both baseline and censoring date and there were no significant different levels of these parameters between groups at baseline. However, at the censoring date the PR group had the significantly higher ($P= 0.01$) levels of percentage of neutrophil and significantly lower ($P = 0.01$) percentage of lymphocytes than the FR group [median (IQR) of neutrophil and lymphocyte of 66.4 (60.5-73.1)% and 26 (20.8-33.1)%, respectively, in PR *v.s.* 59.5 (46.2-63.8)% and 33.6 (26.5-44.8)%, respectively, in FR].

5.3. IL-7, sIL-7R, CD127 and TREC levels in Partial and Full Responders

The median (IQR) IL-7 plasma levels in FR and PR were 0.75 (0-7.21) and 0 (0-0.26) pg/mL, respectively. This IL-7 level was significantly higher ($P = 0.05$) in FR than in PR. The median plasma

levels of sIL-7R between 2 groups of participants were not significantly different [median (IQR) of 1.15 (0.76-2.26) ng/mL in FR *v.s.* 1.57 (0.61-2.48) ng/mL in PR]. FR participants had the median (IQR) percentages of PBMCs, lymphocytes, $CD4^+$ and $CD8^+$ T cells that express CD127 of 1.52 (0.63-24.28), 31.51 (8.28-93.44), 0.2 (0.05-52.55) and 0.12 (0-35.05)%, respectively. Whereas, the median (IQR) levels of CD127 (Mean Fluorescent Intensity, MFI) expressed on PBMCs, lymphocyte, $CD4^+$ and $CD8^+$ T cells in FR group were 25.80 (12.60-34.40), 2.02 (1.95-3.79), 1.62 (1.00-6.66) and 0.89 (0-4.15), respectively. PR participants had the median (IQR) percentages of PBMCs, lymphocytes, $CD4^+$ and $CD8^+$ T cells that express CD127 of 2.46 (0.98-26.88), 22.48 (17.04-68.17), 2.59 (0.70-43.54) and 4.14 (0.07-34.74)%, respectively, and had the median (IQR) levels of CD127 expressed on PBMCs, lymphocyte, $CD4^+$ and $CD8^+$ T cells of 21.1 (12.95-40.07), 2.16 (1.96-3.23), 1.73 (1.61-4.18) and 1.70 (0.70-3.81), respectively. Neither the percentages of cells expressing CD127 nor the level of CD127 expressed on each cell in all 4 subpopulations of cells analyzed were statistically different between FR and PR participants. Percentages of TRECs (%TRECs) detected in PBMCs, $CD4^+$ and $CD8^+$ T cells of PR participants were slightly higher than those detected in FR group, however not significant, with median (IQR) %TRECs of PBMCs, $CD4^+$ and $CD8^+$ T cells in PR of 4.67 (1.27-13.37), 6.08 (2.95-12.37) and 5.31 (1.70-10.51)%, respectively, *v.s.* 2.63 (1.02-5.72), 4.50 (1.34-7.02) and 2.32 (0.60-9.11)%, respectively, in FR participants.

5.4. Risk factors associating with the generation of partial immune response after cART

When gender, age, ARV duration, viral load, levels of IL-7 and sIL-7R in plasma and percentages of different subpopulations of cells expressing CD127 were determined whether they contribute to the partial immune response after receiving the treatment within this cohort of patients, most factors analyzed above were found not associated with the occurrence of the partial immune response. However, age and the percentages of PBMCs expressing CD127 demonstrated the association.

Table 1. Demographic and clinical characteristics of HIV-infected patients

Variables	Overall (N = 46) n (%) or Median (IQR)	Patients with FR (N=14)	Patients PR (N=32)	p-value
Gender:				1.00
Female	19 (41.30)	6 (42.86)	13 (40.63)	
Male	27 (58.70)	8 (57.14)	19 (59.38)	
Age (years):				
Median age	43.6 (39.8-49.5)	42.8 (38.7-46.9)	46.0 (40.35-49.7)	0.44
Duration of ARV				
Median	6 (5.6-6.4)	5.95 (5.5-6.1)	6.1 (5.65-6.5)	0.35
CD4abs at baseline				
Median	160.5 (89.0-217.0)	205.5 (152-239)	139 (68.0-192.5)	0.02
Viral load at baseline				
Median	5.015 (4.44-5.27)	5.07 (4.49-5.27)	4.94 (4.41-5.305)	0.77
Hgb at baseline				
Median	12.55 (11.2-13.9)	12.75 (12.3-14.6)	12.35 (10.95-13.35)	0.18
Hct at baseline				
Median	38.8 (34.9-42.0)	38.85 (37.5-44.3)	38.65 (34.5-40.75)	0.40
Plt at baseline				
Median	249500 (201000- 291000)	245000 (227000-271000)	252000(200000-293000)	0.97
WBC at baseline				
Median	4850 (4300-5940)	5700 (4400-6000)	4750 (4100-5490)	0.29
Neu at baseline				
Median	47.2 (41.8-54.5)	44.5 (41.2-47.5)	50.3 (43.05-59.7)	0.07
Lym at baseline				
Median	31.95 (25.5-39.2)	37.55 (28.3-41.2)	30.9 (23.95-37.95)	0.06
Hgb at cvd visit date				
Median	13.7 (12.8-14.6)	13.95 (13-14.8)	13.6 (12.3-14.3)	0.39
Hct at cvd visit date				
Median	43.05 (40.1-45.3)	43.45 (40.7-45.9)	42.8 (39.75-45.3)	0.50
Plt at cvd visit date				
Median	272000 (257000-312000)	286500(259000-311000)	271500(256500-319500)	0.78
WBC at cvd visit date				
Median	6500 (5500-7700)	7100 (5900-8100)	6300 (5350-7350)	0.16
Neu at cvd visit date				
Median	63.10 (55.70-72)	59.5 (46.20-63.80)	66.4 (60.50-73.10)	0.01
Lym at cvd visit date				
Median	27.30 (22.4-34.80)	33.55 (26.50-44.80)	26 (20.80-33.05)	0.01

NOTE. IQR = Interquartile range, FR = Full Responder, PR = Partial responder

Wilcoxon rank-sum test were used to compare patients' characteristics.

Table 2. Levels of IL-7 and sIL-7R in plasma, CD127 cell surface expression and TRECs measured in HIV-infected patients

Variables	Overall (N = 46) n (%) or Median (IQR)	Patients with FR (N=14)	Patients PR (N=32)	p-value
CD127_CTL				
Median	2.98 (0-35.05)	0.12 (0-35.05)	4.14 (0.07-34.74)	0.41
CD127MFI_CTL				
Median	1.70 (0-3.81)	0.89 (0-4.15)	1.70 (0.70-3.81)	0.38
CD 127_Th				
Median	2.14 (0.15-49.55)	0.2 (0.05-52.55)	2.59 (0.70-43.54)	0.47
CD127MFI_Th				
Median	1.72 (1.01-5.84)	1.62 (1.00-6.66)	1.73 (1.61-4.18)	0.52
CD127_Lym				
Median	23.57 (14.53-82.07)	31.51 (8.28-93.44)	22.48 (17.04-68.17)	0.70
CD127MFI_Lym				
Median	2.14 (1.95-3.79)	2.02 (1.95-3.79)	2.16 (1.96-3.25)	0.46
CD127_PBMC				
Median	1.99 (0.84-24.28)	1.52 (0.63-24.28)	2.46 (0.98-26.88)	0.22
CD127MFI_PBMC				
Median	21.8 (12.7-40)	25.8 (12.6-34.40)	21.1 (12.95-40.70)	0.77
Il7_pg/ml				
Median	0 (0-1.51)	0.75 (0-7.21)	0 (0-0.26)	0.05
SIl7R_ng/ml				
Median	1.42 (0.65-2.41)	1.15 (0.76-2.26)	1.57 (0.61-2.48)	0.84
%TREC_PBMC				
Median	3.37 (1.20-9.66)	2.63 (1.02-5.72)	4.67 (1.27-13.37)	0.42
%TREC_CD4				
Median	5.09 (1.66-11.65)	4.50 (1.34-7.02)	6.08 (2.95-12.37)	0.38
%TREC_CD8				
Median	4.71 (1.51-9.94)	2.32 (0.60-9.11)	5.31 (1.70-10.51)	0.14

NOTE. IQR = Interquartile range, FR = Full Responder, PR = Partial responder

Wilcoxon rank-sum test were used to compare patients' characteristics.

6. Conclusion and Discussion

There were slightly more male than female HIV-infected patients enrolled in this study with the age ranged from 28 years old to nearly sixty (median = 43.6 years and IQR= 39.8-49.5 years) and the median duration of ART exposure of 6 years (IQR = 5.6-6.4 years). Of 46 patients, 14 were full responders (FR) and 32 were partial responders (PR) and there were no statistically different in sex, age and duration of ART exposure between 2 groups (P = 1.00, 0.44 and 0.35, respectively). Additionally, the clinical characteristics at baseline including viral load, hemoglobin, hematocrit, platelet, WBC count, neutrophil and lymphocyte also showed no statistically different between 2 groups. However, the median of absolute CD4-cell number at baseline in FR was significantly higher than PR (206 v.s.139 cells/μL, p = 0.018) and this number remained significantly higher in FR than PR by the cut-off date. Most of clinical characteristics at cut-off date between 2 groups were also found similar. Nevertheless, the median of the percentages of neutrophils in FR was found significantly lower than in PR (59.5% v.s. 66.4%, p = 0.01), whereas, the median of percentages of lymphocytes at the censoring date of FR was significantly higher than in PR (33.55% v.s. 26%, p = 0.01). This result might indicate a lower immune activation by microbial flora and a higher immune restoration in FR compared to PR. Though from multivariate logistic regression analysis indicated that age and percentage of PBMC expressing CD127 are important factors causing the partial response to ART in this group of patients, there were no statistically different in the percentages of PBMC expressing CD127 or in lymphocytes, T-helper and CTL cells between FR and PR. This influential might be more prominent in the case of immunological non-responders. By measuring TRECs, we have analyzed the capacity of the thymus to produce new lymphocytes and have estimated that about 3.4% of PBMCs 5.1% of $CD4^+$ T cells and 4.7% of $CD8^+$ T-cells are newly produced lymphocytes. But the levels of these newly produced lymphocytes did not correlate with the production of IL-7, sIL-7R, percentages of cells expressing CD127 or levels of CD127 expression on cell surface in this group of patients.

7. Appendix

8. Output (Acknowledge the Thailand Research Fund)

8.1 International Journal Publication: Under preparation.

8.2 Application

8.3 Others e.g. national journal publication, proceeding, international conference, book chapter, patent

Poster:

1. Levels of IL-7, IL-7Rs and thymic outputs in HIV-infected patients on combination antiretroviral therapy. Singboottra P, Pornprasert S, Dettrairat S, Klungsinsirikul P, Leenasirimakul P, Klinbuayaem V, Ngo-Giang-Huong N. TRF Annual Conference “ประชุมนักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว. ครั้งที่ 13”. October 16th – 18th, 2013.
2. Analysis of Factors Contributing to Immune Reconstitution in HIV-Infected Patients on Combination Antiretroviral Treatment. Singboottra P, Pornprasert S, Dettrairat S, Klungsinsirikul P, Leenasirimakul P, Klinbuayaem V, Ngo-Giang-Huong N. Academic conference “วิถีวิจัย: มช ก้าวไกลรับใช้แผ่นดิน ครั้งที่ 9”. December 2nd -3rd. 2013.