

# Delayed progression to AIDS in volunteers treated with long-term HIV-1 Immunogen (REMUNE<sup>®</sup>) therapy in Thailand

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## SUMMARY

**Objective:** To observe the long-term effects of HIV-1 Immunogen (REMUNE<sup>®</sup>, Vista California) as a first course of treatment to sustain the immune system and thus delay the initiation of therapy with antiretroviral drugs and/or delay disease progression.

**Methods:** For this open-label, multi-institute extended Phase II P2101B study, disease progression, CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts, HIV-1 RNA levels, and genotypic antiretroviral drug resistance were examined in 223 asymptomatic HIV-1 infected Thai volunteers receiving REMUNE<sup>®</sup> every 12 weeks over 132 weeks. A subset of subjects was randomly selected by the physicians to receive antiretroviral drugs for 10 months.

**Results:** Patients treated with REMUNE<sup>®</sup> demonstrated a low clinical disease progression rate (0.72/100 person-year), higher CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts, higher body weight, and stable viral load with no serious adverse events. We were unable to observe any genotypic evidence of drug-resistance in subgroups of patients on REMUNE<sup>®</sup> monotherapy or REMUNE<sup>®</sup> plus ARTs.

**Conclusions:** This Thai study, like previous U.S. and European studies, confirms that therapeutic immunization of HIV- infected volunteers modifies disease progression as evidenced by stabilization of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts, body weight, and viral load. As a majority of asymptomatic patients demonstrated an objective response to immunization, this study suggests that REMUNE<sup>®</sup> may be utilized prior to initiation of antiviral drug therapy when CD4<sup>+</sup> cell counts are still above the current ART guidelines. In addition, further studies should be carried out to examine its potential use in combination with ART in order to reduce the increasingly common occurrence of drug resistance. **Key words:** Immune-based therapy, progression to AIDS rate, delaying antiretroviral drug initiation, drug resistance

## Introduction

Massive government HIV awareness and prevention programs have made significant progress, but there continues to be reports of high incidences of HIV disease in parts of Africa and Asia [1, 2]. While increasingly potent and more effective drugs have also become available in the market, the most recent treatment guidelines in the US and Europe (BHIVA) [3,4] recommend delaying antiretroviral drug therapy for HIV-infected adults until the CD4<sup>+</sup> count falls below 350 cells/ $\mu$ l. Resistance and toxicity are two recognized limitations of long-term antiretroviral therapy (ART), and recent clinical studies suggest that delaying ART has little deleterious effect on disease progression [5]. Therefore, for HIV-infected individuals there is an increasing need for additional treatment alternatives that could impact on HIV disease progression or sustain the level of CD4<sup>+</sup> T-cells, especially in asymptomatic patients with CD4<sup>+</sup> T-cell counts above the cut-off level recommended in treatment guidelines.

Recent treatment approaches include immune-based therapy (IBT) to induce HIV-specific antiviral immune responses or non-specific immunity in order to delay the onset of AIDS before the initiation of antiviral drug therapy. In Thailand, the Ministry of Public Health set up criteria to treat HIV infection with antiretroviral drugs named ‘GPO-VIR’, produced by the Thai Government Pharmaceutical Organization (GPO), which state that treatment should be initiated when the patient’s CD4<sup>+</sup> T-cells decline below 200 cells/ $\mu$ l. At present, there are no registered immune base therapies for the induction of HIV-1 specific immunity.

The intervention approach using REMUNE<sup>®</sup> (Immune Response Corp., Carlsbad, California) for HIV infection involves the induction of HIV-specific antiviral immune responses by immunization with a gp120-depleted, inactivated whole virus in Incomplete Freund’s Adjuvant (HIV-1 Immunogen, REMUNE<sup>®</sup>). REMUNE<sup>®</sup> was given to asymptomatic Thai subjects with CD4<sup>+</sup> T-cell counts over 300 cells/ $\mu$ l and antiretroviral drug-naïve. We initially examined the safety and immunogenicity of the HIV-1 Immunogen (REMUNE<sup>®</sup>) in

an open label study of 30 subjects [6,7], followed by a pivotal, double-blind, randomized, placebo-controlled, multi-center, Phase II trial (P2101B) in asymptomatic, HIV-1-infected antiretroviral drug-naïve subjects [8]. Volunteers, totaling 297 from five university hospitals, received four doses of either REMUNE<sup>®</sup> or IFA at 2:1 ratio. Our analysis utilized the area-under-the-curve-minus-baseline (AUCMB) method which was one of the two specified primary analyses in the proposed analysis plan. Significant increases in CD4<sup>+</sup> T-cell counts analyzed by the (AUCMB) method ( $p < 0.05$ ) were associated with enhanced HIV-specific immunity. Moreover, overall CD4<sup>+</sup> T-cell counts remained stable ( $p < 0.05$ ) as reported over 132 weeks [9].

In this further study, a retrospective examination of viral burden was carried out, as well as a review of volunteers' clinical outcome in order to obtain the rate of progression to AIDS in this cohort. HIV-1 genotypic drug-resistance testing was also performed in a randomly-selected cohort of patients treated with REMUNE<sup>®</sup> alone and REMUNE<sup>®</sup> plus ART. The incidence of genotypic drug resistance in these groups was compared with the incidence of drug resistance in a non-REMUNE<sup>®</sup>-treated cohort on antiretroviral drugs.

## **Methods**

This 132 week P2101B Extended Phase II open-label study followed the 40-week double-blind placebo-controlled trial [8]. It was approved by the Technical Subcommittee on HIV/AIDS Vaccine Development under the Thai National AIDS Committee and by the Ethical Review Committee for Research in Human Subjects of the Ministry of Public Health, Thailand. Approvals were likewise secured from all institutes and university hospitals involved in this study.

The study volunteers were those previously enrolled in P2101B who opted voluntarily to continue in this 132-week open label extension study (P2101B Extended Phase II).

Informed consents were newly obtained from these volunteers. Similar inclusion and exclusion criteria of the previously completed P2101B study were used. These volunteers were confirmed HIV-1 positive by Western blot and enzyme-linked immunosorbent assay (ELISA) prior to enrollment and 95% were found to be infected with subtype E [8].

After week 40, all subjects were administered open label REMUNE<sup>®</sup> beginning at week 48 and the immunization continued onwards at 12-week intervals up to 132 weeks (8 injections). Out of the originally enrolled 297 subjects from the previous 40-week study, 48 volunteers (16%) discontinued, 17 volunteers (6%) were placed randomly on antiviral drug combinations as a subset study before unblinding, but after completion of 40 week study, and 9 subjects (3%) were taking other antiretroviral drug treatments of their choice. The remaining 223 subjects (75%), with their mean CD4<sup>+</sup> cell of 532.78 cells/ $\mu$ L at baseline, were treated solely with REMUNE<sup>®</sup> up to 132 weeks, with a maximum of 11 doses, and minimum of 7 doses from the placebo-control group. The mean CD4<sup>+</sup> cell count in the REMUNE<sup>®</sup>-treated group (11 doses) changed from 535.52 cells/ $\mu$ L (day 1) to 587.84 cells/ $\mu$ L (week 132). The mean CD4<sup>+</sup> count in the group that originally was randomized to the placebo-control group, but then received REMUNE<sup>®</sup> (7 doses), changed from 527.46 cells/ $\mu$ L (day 1) to 531.95 cells/ $\mu$ L (week 132).

REMUNE<sup>®</sup> consists of gp120-depleted, inactivated HIV-1 at a dose of 10 units of p24 antigen in Incomplete Freund's Adjuvant (IFA). Gp120-depleted HIV-1 is highly purified by ultrafiltration and ion exchange chromatography [10,11] from filtered (0.45  $\mu$ m) extracellular supernatant fluid of HIV-1 HZ321 infected HuT-78 cells [12]. We monitored CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts, humoral antibodies, viral load and drug resistance and also carried out complete physical examination that included assessment of clinical disease progression.

Lymphocyte phenotyping analysis to count CD4<sup>+</sup> T-cells on fresh cells using the same validated methodology [13] was done at the BioService Unit, National Science and

Technology Development Agency, Ministry of Science and Technology, Bangkok, Thailand. Enumeration of the CD4<sup>+</sup> and CD8<sup>+</sup> cells in blood samples from HIV-infected patients was determined by three-color immunofluorescence, (e.g. CD3/CD4/CD45) and the use of TRUECOUNT Absolute Count Tube (Immune Response Corp., Carlsbad, California) with a known amount of fluorescent dye beads to allow absolute counts of the leukocyte subset to be determined [8]. Calculation of the absolute cells (no. of cells/ $\mu$ l) was done using the following equation as recommended by the laboratory investigators and agreed upon by the reagent supplier:

$$\frac{\text{No. of events in quadrant containing cell population (Q2)}}{\text{No. of events in absolute count bead region (R2)}} \times \frac{\text{Total No. of Beads}}{\text{Blood volume } (\mu\text{l})}$$

The absolute cells were calculated using the average values from both reactions. The percentage of CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes were obtained directly from the percentage of the lymphocyte population that was gated. Monitoring of CD4<sup>+</sup> T-cells from blood samples drawn from HIV-1-infected patients was performed on day 1, and at weeks 12, 24, 36, 40, 48, 88, 96, and 132.

Plasma HIV-1 RNA was assayed from blinded samples using the Nucleic Acid Sequence-Based Amplification (NASBA) method at the Division of Virology, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand from day 1 up to week 40 [14]. At weeks 88 and 132, plasma HIV-1 RNA was assayed at the Virology and Molecular Microbiology Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand. Plasma viral RNA and EDTA-plasma were isolated using the NucliSens<sup>TM</sup> Extractor for automated nucleic acid isolation (bioMérieux l'Etoile, France) [15, 16]. Before extraction, three RNA internal control standards (QA, QB and QC) from the NASBA HIV-1 Quantitative (QT) were added to lysis mixture (100  $\mu$ l of plasma + 900  $\mu$ l Guanidine Isothiocyanate (GuSCN) lysis buffer). Subsequently 50  $\mu$ l of activated silica

particle solution was added, to bind all nucleic acids (DNA and RNA) in lysate. After drying of the silica particles, the nucleic acids were eluted in 50 µl of elution buffer (1mm Tris at pH 8.5). Five micro liters of the extracted nucleic acids were used as the input for isothermal nucleic acid amplification with the addition of 5 µL of primer mix. The reaction was incubated at 65°C and 41°C for 5 minutes each, followed by the addition of three enzymes, reverse transcriptase, Rnase H and T7 RNA polymerase and further incubation for 90 minutes at 41°C. The RNA concentration of HIV-1, QA, QB and QC amplicates was measured in four separated aliquots. Calculation of the relative amount of the four amplicates revealed the amount of HIV-1 RNA in the sample [17]. Manual extraction for plasma samples was used in day 1 and week 40 and from week 88 up to week 132. Samples were extracted using the NucliSens™ Extractor.

Genotypic drug-resistance testing was conducted on 60 plasma samples randomly taken from 37 volunteers (37/297 or 12.4%) at Weeks 12, 96, 132, 144 and 232. This was done at the Virology and Molecular Microbiology Unit at Ramathibodi Hospital to identify point mutations in HIV-1 *polymerase gene* region that might confer phenotypic drug resistance. HIV-1 RNAs from 0.5 mL samples of plasma were isolated, sequenced, and analyzed using the US Food and Drug Administration (FDA) approved TRUGENE HIV-1 Genotyping system (Bayer Health Care LLC, Diagnostics Division; Berkeley, CA, USA) [18]. From the 37 volunteers, 20 cases were from the REMUNE® monotherapy cohort and 17 cases were treated with REMUNE® in combination with antiretroviral drug therapy for 40-week period (Wk 48-Wk 88 or Wk 52-Wk 92). These 17 subjects were randomly selected to receive antiretroviral drugs in two different clinical sites. Mean CD4<sup>+</sup> T-cell count before ART initiation of these 17 subjects is 443.88 cells/µL (range from 221 cells to 724 cells).

The AUCMB metric of analysis was used to examine the effect of REMUNE® on the CD4<sup>+</sup> T-cells, CD8<sup>+</sup> T-cells and viral load (log<sub>10</sub> HIV-1 RNA copies/mL) levels of the

volunteers by measuring these levels at different time points from day 1 up to week 132. Subjects were stratified based on change of CD4<sup>+</sup> cell count over time. Subjects with positive CD4<sup>+</sup> T-cell changes at week 132 from baseline (positive AUCMB) were classified as responders and patients with negative AUCMB were otherwise classified as non-responders. Then, we compared the means of the CD4<sup>+</sup> T-cells, CD8<sup>+</sup> T-cells, viral load and body weight between the responder and non-responder cohorts using the independent samples t-test. All the tests were calculated with a 95% confidence level.

The incidence of clinical AIDS defining events, Category C of the Center for Diseases Control (CDC) 1993 AIDS surveillance case definition [19], was determined in this study by comparison with historical controls as reported in other Thai studies (similar patient populations were compared)[20,21,22].

The conduct of this clinical trial adhered to the guidelines of the Good Clinical Practice-International Conference on Harmonization (ICH) and Good Laboratory Practices in certified laboratories.

## **Results**

Seventy five percent of volunteers from the original study remained in the extension study up to 132 weeks. The average CD4<sup>+</sup> T-cell count at the beginning of the study of these asymptomatic HIV+ volunteers was 532.78 cell/ $\mu$ L. The statistical method, AUCMB, was the same metric used in the previously completed and published Phase II, double-blinded, randomized, placebo-controlled, 40-week study (P2101B) [8]. This method was also recommended by the British HIV Association (BHIVA) guidelines released in July 2003 [4].

As shown in Figure 1, the overall mean CD4<sup>+</sup> T-cell count of 223 subjects who received REMUNE<sup>®</sup> was sustained above the baseline value up to week 132 with an average increase of 36.01 cells/ $\mu$ L [9]. Responders (135) with positive AUCMB of CD4<sup>+</sup> cell count

changes and non-responders (88) were identified at week 132 using the approach described above. Overall, 60.5% (135/223) of subjects were identified as responders with an average increase from the baseline in CD4<sup>+</sup> (176.55 cells) [Fig. 1], CD8<sup>+</sup> T-cells (603.28 cells) [Fig. 2], and body weight (1.76 kgs) [Fig.3]. The mean CD4<sup>+</sup> T-cell count of responders is significantly higher than that of non-responders at week 132 ( $p < 0.001$ ) [Table 3]. The mean CD8<sup>+</sup> T-cell count of the responder cohort is also significantly higher than the non-responder cohort at week 132 ( $p < 0.001$ ) [Table 3] and the mean body weight of the responders is also significantly higher as compared to the non-responders ( $p = 0.002$ ) [Table 3].

Figure 4 shows changes of viral load ( $\log_{10}$  HIV-RNA) over time. At 132 weeks, the increase from baseline (Day 1) of 0.13  $\log_{10}$  is observed in the responder group as compared to 0.35  $\log_{10}$  in the non-responder group. The responder cohort's viral load is significantly lower than the non-responder cohort at week 132 ( $p < 0.001$ ) [Table 3]. Overall, an increase of 0.22  $\log_{10}$  viral load at week 132 from baseline was observed. The increase in mean viral load however, was within  $\pm 0.5 \log_{10}$  variability of the assay indicating some immune control of viral replication by REMUNE<sup>®</sup> up to 132 weeks.

From 297 volunteers initially recruited for the 40-week P2101B protocol, thirteen subjects (2.35/100 person-year) progressed to AIDS by immunological definition (CD4<sup>+</sup> < 200 cells/ $\mu$ l) and four subjects (0.72/100 person-year) progressed to AIDS by clinical definition, as defined by clinical documentation of tuberculosis. The opportunistic infections (tuberculosis) of the four subjects were recorded to have separately occurred at study weeks 24, 60, 72 and 48. At week 132, 93.4% survived AIDS-free by immunological definition while 98.5% survived AIDS-free by clinical definitions [Figure 5].

Antiviral drug resistance was also examined in two subsets of patients in this cohort. First patients who received REMUNE<sup>®</sup> alone were examined. Twenty cases from the REMUNE<sup>®</sup> monotherapy cohort showed mutation unrelated to antiretroviral drug resistant

strains. These specimens all had viral loads higher than  $3.0 \log_{10}$ . The protease resistance mutation and Reverse Transcriptase mutation profiles were identical in each of the pairs of samples from the same patients collected at week 12 and week 232 indicating no evidence of a rapid and significant overgrowth in HIV-1 drug resistant strains. The mutations found were the following: M36I, L63P, L10I, K20R, I63L, A71V, L63H, and I93L, which are usually evident in all HIV infected individuals treated with antiretroviral drugs but do not lead to drug resistant strains. The most frequent is M36I in 20 cases, while the others were found in a few cases at certain time points.

We also examined a subset of patients who were randomly assigned to receive REMUNE<sup>®</sup> plus antiretroviral drugs for 40 weeks. When specimens from all these 17 patients were analyzed, two of the 10 HAART (AZT, 3TC, and Viracept) cases, Subjects KK118 and KK125, showed drug resistant strains 4 weeks after drug cessation at weeks 96 and 92 respectively. Only one from the 7 COMBID (AZT and 3TC) cases, Subject PM201, showed a mutation which lead to drug resistant strains at week 96 (Table 1).

We then compared the two patients groups (REMUNE<sup>®</sup> monotherapy and REMUNE<sup>®</sup> plus ART) with a larger cohort of patients receiving ART alone for evidence of drug resistance. In comparison, the routine testing of 500 patients on ART during the year 2003 (Table 2), done at the same period and at the same laboratory, Ramathibodi Hospital, showed increased mutation frequencies compared to the years 2000-2003 (AZT 46-50.2%; ddI 11-46.8%; 3TC 46-43.8; d4T4 -45.1; Nevirapine 6-46.3).

## **Discussion**

HIV infection is characterized by progressive immune dysfunction progressively. CD4<sup>+</sup> T-cell count is an immunological marker that monitors antiretroviral drug effectiveness

or clinical progression. In various natural history cohorts (including Thailand), there is typically an average loss of approximately 50-100 CD4<sup>+</sup> T-cells/ $\mu$ L per year [20, 21, 22].

Immunization of HIV-1 Immunogen (REMUNE<sup>®</sup>) in antiviral drug-naïve volunteers was found to enhance HIV1 specific immunity as measured by Western blot [6, 7, 8], *in vitro* HIV-specific T-cell proliferation and increase cytotoxic T-cells activity [8, 23]. In a large 3-year Phase III REMUNE<sup>®</sup> clinical trial, where an antiviral drug switching was permitted, low clinical progression rates were observed. Instead of the usual 6% progression rate per year, at the end of 3 years the progression rate was less than 1% per year. The introduction of protease inhibitors, and constant drug switching, made it impossible to determine whether REMUNE<sup>®</sup> could truly inhibit clinical progression [24, 25]. However, in a predefined subset of this trial (252 subjects), REMUNE<sup>®</sup> treated patients showed a significant decrease in viral load at multiple time points over a 3-year period [25]. This decrease in virus correlated with HIV-1 specific immunity. The other large double-blinded placebo-controlled study, Spain's STIR 2102, revealed that patients treated with REMUNE<sup>®</sup> showed a significant delay in time to virological failure [23].

Previously, we reported a sustained level of CD4<sup>+</sup> T-cells up to week 132 [9]. In the present study we evaluated the response to treatment using the metric AUCMB. By this analysis, 60.5% of these subjects were classified as responders by virtue of having CD4<sup>+</sup> T-cell counts above baseline. Besides CD4<sup>+</sup> T-cells, we observed CD8<sup>+</sup> T-cell counts to have increased. No accelerated weight loss was observed and mean body weight improved by 1.08 kg over 132 weeks. Viral load, another important surrogate marker, was also measured in this follow-up study. Overall, subjects showed a mean increase of 0.22 log<sub>10</sub> copies/mL in their viral load over time (within variability of  $\pm 0.5$  log<sub>10</sub> copies/mL). This suggests that no clinically significant changes occurred in viral load in this cohort over the observation period.

The incidence of clinical AIDS defining events is examined in this study. The rate of clinical progression to clinical AIDS in Thailand has been reported in previous similar cohort studies to be from 6.8/100 person-year [21] to 12.2/100 person-year in untreated cohorts [26]. In comparison, the rate of clinical progression to AIDS in this study is only 0.72 /100 person-year. Thus, the event rate observed in this trial appears to be discordant with the events rates observed in natural history studies of HIV-infected people in Thailand. Interestingly, all of the clinical cases of clinical AIDS were observed to be tuberculosis, the most common opportunistic infection in HIV-1 infected individuals in Thailand [26,27]. Thus, the clinical spectrum of opportunistic infections in Thailand may be quite different from that described in developed countries where *Pneumocystis carinii Pneumonia* (PCP) or lymphoma may be more common opportunistic diseases.

This long-term treatment of HIV-1 infection with REMUNE<sup>®</sup> confirms our previous reports. REMUNE<sup>®</sup> is safe and confers a stabilization of CD4<sup>+</sup> T-cell counts, CD8<sup>+</sup> T-cell counts, viral load and body weight and slows progression to AIDS-defining diseases. These results are discordant with the expected decline in CD4<sup>+</sup> T-cell counts, increase in viral load and rates of clinical progression observed in untreated natural history cohorts. It has been reported in previous studies that the number of CD4<sup>+</sup> T-cells would typically decline approximately 50-100 cells/ $\mu$ L per year [20, 21, 22]. This is in contrast to the findings in this study where treatment with REMUNE<sup>®</sup> resulted in the majority of patients showing an increase in their CD4<sup>+</sup> T-cell counts. It is tempting to speculate that immunization with REMUNE<sup>®</sup> resulted in stabilization of CD4<sup>+</sup> T-cell counts compared to other HIV-infected natural history cohorts. For example, in the European Delta study, treatment with a two drug combination resulted in over-all CD4<sup>+</sup> T-cell declines below baseline level at week 96. This time to declining CD4 counts is significantly shorter than that observed in the current study of REMUNE<sup>®</sup> monotherapy [28].

An interesting observation of this study is that a subset of subjects who received concomitant antiviral drug therapy with REMUNE<sup>®</sup> had a very low rate of antiviral drug resistance compared to natural cohorts. Whether this was due to better care or the result of immune pressure resulting from immunization with REMUNE<sup>®</sup> needs to be further explored.

Overall, this Thai study as well as other U.S. and European studies, has confirmed that therapeutic immunization of Thai infected volunteers modifies disease as evidenced by stabilization of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell counts, body weight, and viral load. As a majority of asymptomatic patients demonstrated an objective response to immunization, this study suggests that REMUNE<sup>®</sup> may be used for intervention purposes prior to initiation of antiviral drug therapy when CD4<sup>+</sup> cell counts are still above the treatment guideline level. In addition, further studies should examine its potential use in combination with ART in order to decrease the increasingly high occurrence of drug resistance.

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**Table 1****Genotypic Drug Resistance in Patients Treated with REMUNE+HAART (KK118 & KK125) and with REMUNE+COMBID (PM201)**

<b>Patients</b>	<b>Period Sample tested</b>	<b>Protease resistance mutation</b>	<b>Reverse Transcriptase resistance mutation</b>	<b>Viral Load (Log<sub>10</sub>)</b>
<b>KK 118</b>	<b>WK96</b>	<b>M361</b>	<b>M184I</b>	<b>4.20</b>
			<b>Didanosine (Possible Resistance)</b>	
			<b>Zalcitabine (Possible Resistance)</b>	
			<b>Lamivudine (Resistance)</b>	
<b>KK 125</b>	<b>WK92</b>	<b>M361, L10V, L63P</b>	<b>M184V</b>	<b>2.7</b>
			<b>Didanosine (Possible Resistance)</b>	
			<b>Zalcitabine (Possible Resistance)</b>	
			<b>Lamivudine (Resistance)</b>	
<b>PM 201</b>	<b>WK96</b>	<b>M361</b>	<b>M184V</b>	<b>3.26</b>
			<b>Didanosine (Possible Resistance)</b>	
			<b>Zalcitabine (Possible Resistance)</b>	
			<b>Lamivudine (Resistance)</b>	

**Table 2 Genotypic Drug-Resistance Routine testing of non-REMUNE treated HIV-1-infected patients**

Region	Number of Patients			
	2000	2001	2002	2003
RT	83	88	387	<b>406</b>
PR	83	88	314	<b>298</b>

Distribution of Drug Resistance				
Mutations	Patients (%)			
	2000	2001	2002	2003
<b>Mutations to NRTIs</b>				
M41L	28	38	31.3	<b>26.6</b>
E44D	0	9	10.3	<b>6.2</b>
A62V	2	3	2.8	<b>3.7</b>
K65R	2	3	5.2	<b>4.2</b>
D67N	38	40	46	<b>45.6</b>
T69D/G/E/A	5	6	5.4	<b>6.4</b>
K70R	25	23	27.6	<b>28.8</b>
L74V	2	6	4.9	<b>5.4</b>
V75M/T/A/S	0	15	9.8	<b>10.6</b>
F77L	0	4	1.8	<b>2.0</b>
W88S	-	-	0.5	<b>0.7</b>
Y115F	-	-	1	<b>2.0</b>
F116Y	0	4	4.7	<b>5.2</b>
V118I	0	22	20.9	<b>15.5</b>
Q151M	3	3	5.9	<b>4.9</b>
V179D	2	3	3.9	<b>3.9</b>
M184V/I	46	27	32.3	<b>42.6</b>
Y188L	-	-	5.7	<b>6.7</b>
H208Y	0	6	0	<b>0.0</b>
L210W	19	24	22.7	<b>17.7</b>
T215Y/F/S/C/D	36	45	43.9	<b>41.6</b>
K219Q/F/E	23	16	27.9	<b>26.4</b>
F227L	-	-	1.8	<b>1.0</b>
M230L	-	-	1.3	<b>1.0</b>

Distribution of Drug Resistance				
Mutations	Patients (%)			
	2000	2001	2002	2003
<b>Mutations to NNRTIs</b>				
A98G	2	5	7.5	<b>6.4</b>
L100I	0	3	2.1	<b>3.4</b>
K101E/Q	4	5	8.8	<b>13.1</b>
K103N	1	12	18.1	<b>21.7</b>
V106A	-	-	1.6	<b>0.5</b>
V108I	0	6	7	<b>7.9</b>
Y181C	4	8	11.1	<b>15.8</b>
G190A/S/E	7	12	16	<b>18.7</b>
P225H	-	-	3.6	<b>2.5</b>

Distribution of Drug Resistance				
Mutations	Patients (%)			
	2000	2001	2002	2003
<b>Mutations to PIs</b>				
L10I/V/F	64	18	26.1	<b>32.2</b>
K20R	11	12	17.5	<b>20.8</b>
D30N	-	-	1.3	<b>0.7</b>
V32I	-	-	0.3	<b>0.7</b>
<b>L33F</b>	ND	ND	ND	<b>1.7</b>
M36I	88	70	92	<b>91.9</b>
M46I	8	3	5.1	<b>5.4</b>
I47V	-	-	0.3	<b>0.3</b>
G48V	10	3	2.2	<b>2.3</b>
F53L	6	4	2.2	<b>1.0</b>
I54V	11	6	4.1	<b>4.7</b>
L63P	36	20	25.2	<b>25.5</b>
A71T/V	10	4	6.7	<b>4.0</b>
<b>G73S</b>	ND	ND	ND	<b>0.7</b>
V82A/F/T	13	8	4.5	<b>3.4</b>
I84V	-	-	1.6	<b>2.3</b>

HIV-1 mutation conferring high resistance to antiretroviral drugs (ARTs)				
Antiretroviral drugs (ARTs)	Patients (%)			
	2000	2001	2002	2003
<b>NRTIs</b>				
Azidovudine (AZT)	46	54	55.6	<b>50.2</b>
Didanosine (ddI)	11	43	46.8	<b>43.6</b>
Zalcitabine (ddC)	41	43	44.7	<b>42.1</b>
Lamivudine (3TC)	46	27	31.5	<b>43.8</b>
Stavudine (d4T)	4	46	43.4	<b>45.1</b>
Abacavir (ABC)	NA	42	38.8	<b>36.9</b>
Tenofovir	NA	NA	NA	<b>15.3</b>
Foscarnet	NA	NA	NA	<b>0.0</b>
<b>NNRTIs</b>				
Nevirapine (NVP)	6	25	39	<b>46.3</b>
Delavirdine (DLV)	ND	22	32.8	<b>38.9</b>
Efavirenz (EFV)	1	18	31	<b>32.3</b>
<b>PIs</b>				
Nelfinavir (NFV)	5	12	11.8	<b>6.7</b>
Saquinavir (SQV)	30	9	8.3	<b>7.4</b>
Indinavir (IDV)	30	9	7.6	<b>7.4</b>
Ritonavir (RTV)	21	9	7.6	<b>9.1</b>
Amprenavir (APV)	NA	6	4.1	<b>4.4</b>
Lopinavir+Ritonavir (LPV+RTV)	NA	-	0.3	<b>1.7</b>

ND, Not determined  
 NA, Not Available in Thailand  
 -, Undetectable

Table 3. AUCMB of CD4, CD8, and viral copies (log10) at week 132 and Mean Difference of Body Weight at week 132 from baseline

	Responders (n=135)		Non-responders (n=88)		P-value <sup>1</sup>	95 % CI of (Resp-Non-resp.)	
	Mean	S.D.	Mean	S.D.			
CD4	160.31	127.72	-133.68	118.75	< 0.001	260.438	327.543
CD8	367.12	377.80	-74.78	405.60	< 0.001	336.868	546.925
Log RNA	0.03	0.62	0.36	0.54	< 0.001	-0.489	-0.172
Body Weight	1.77	4.11	0.01	3.90	0.002	2.838	0.663

<sup>1</sup> by independent samples t-test

## Figure Legends

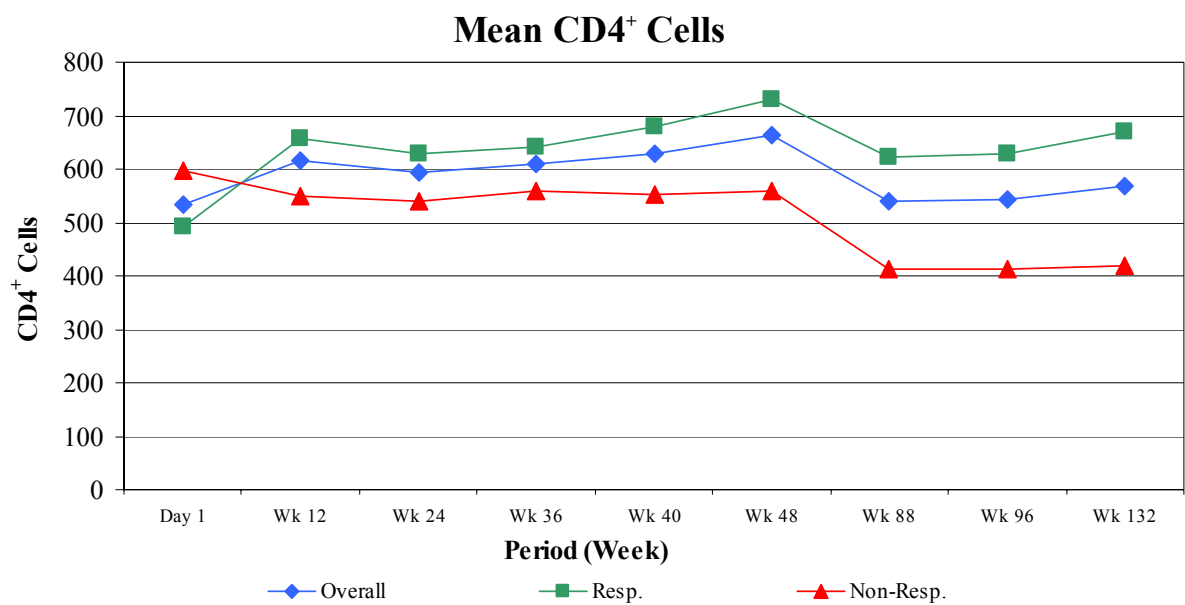
Figure 1: Mean Changes of CD4<sup>+</sup> T-Cell Counts of Responders and Non-responders

Figure 2: Mean Changes of CD8<sup>+</sup> T-cell Counts of Responders and Non-responders

Figure 3: Mean Changes of Body Weight (kg) of Responders and Non-responders

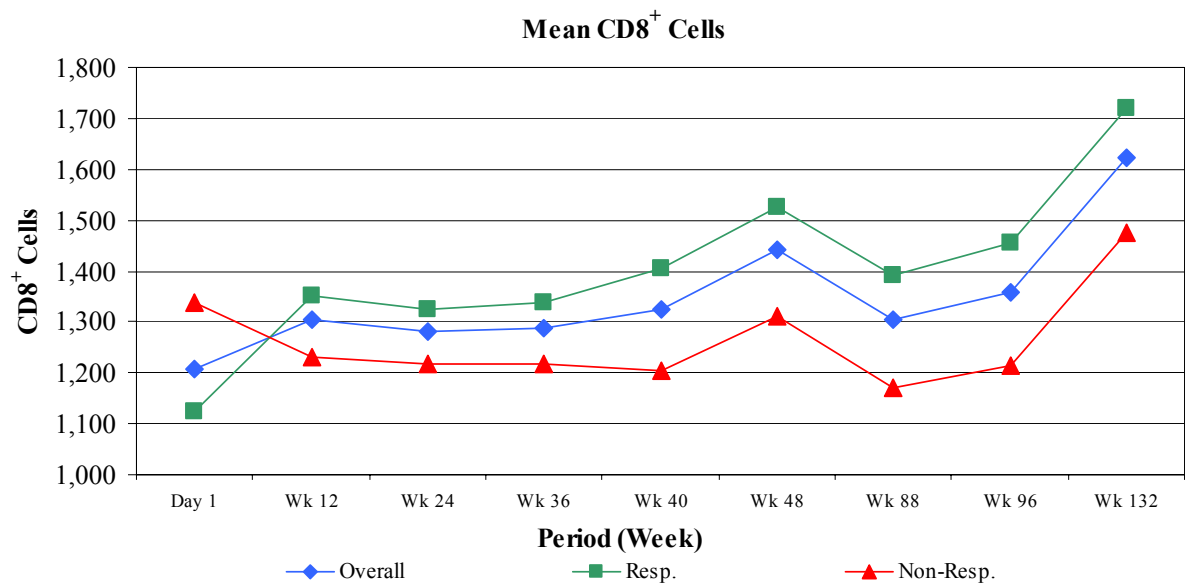
Figure 4: Mean Changes of Log HIV-1 RNA of Responders and Non-responders

Figure 5: Kaplan-Meier product-limit estimates of AIDS-free survival based upon the clinical definition of AIDS

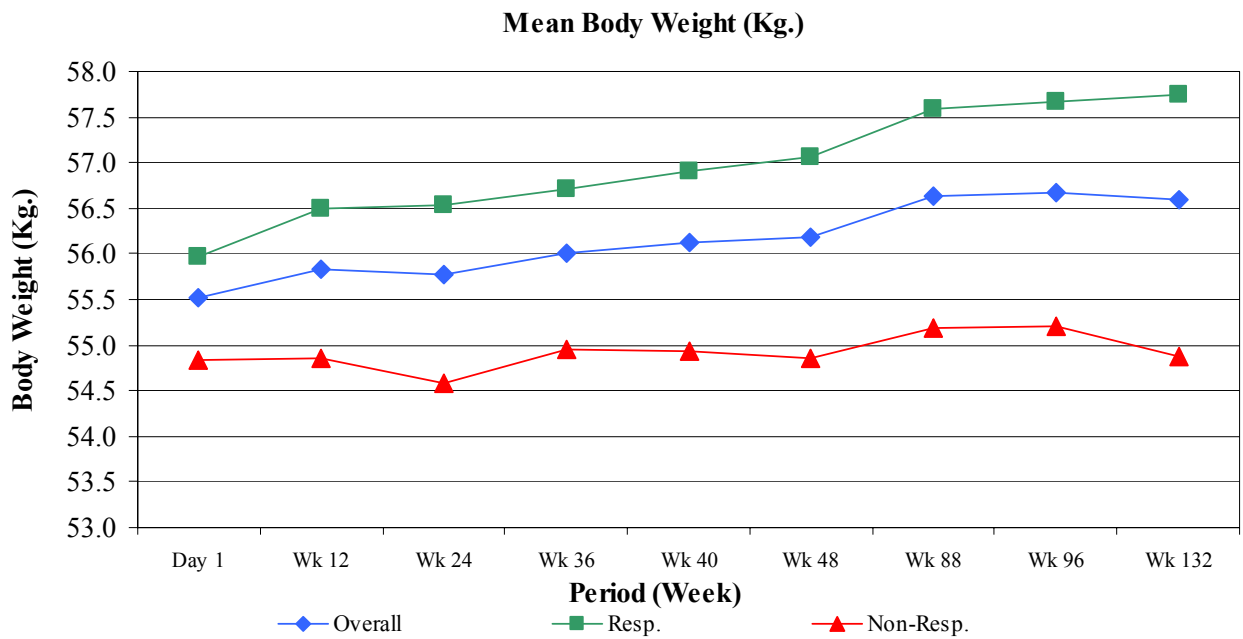


		Day 1	Wk 12	Wk 24	Wk 36	Wk 40	Wk 48	Wk 88	Wk 96	Wk 132
Overall	N	223	223	223	223	222	223	223	222	223
	Mean	532.78	614.46	594.48	608.25	628.55	662.52	538.76	541.89	568.79
Resp.	N	135	135	135	135	135	135	135	134	135
	Mean	492.99	658.16	630.73	641.01	681.22	731.99	623.19	628.19	669.54
Non-resp.	N	88	88	88	88	87	88	88	88	88
	Mean	593.81	547.43	538.86	557.99	546.82	555.94	409.24	410.48	414.24

**Figure 1. Mean Changes of CD4<sup>+</sup> T-cell counts of Responders and Non-responders**

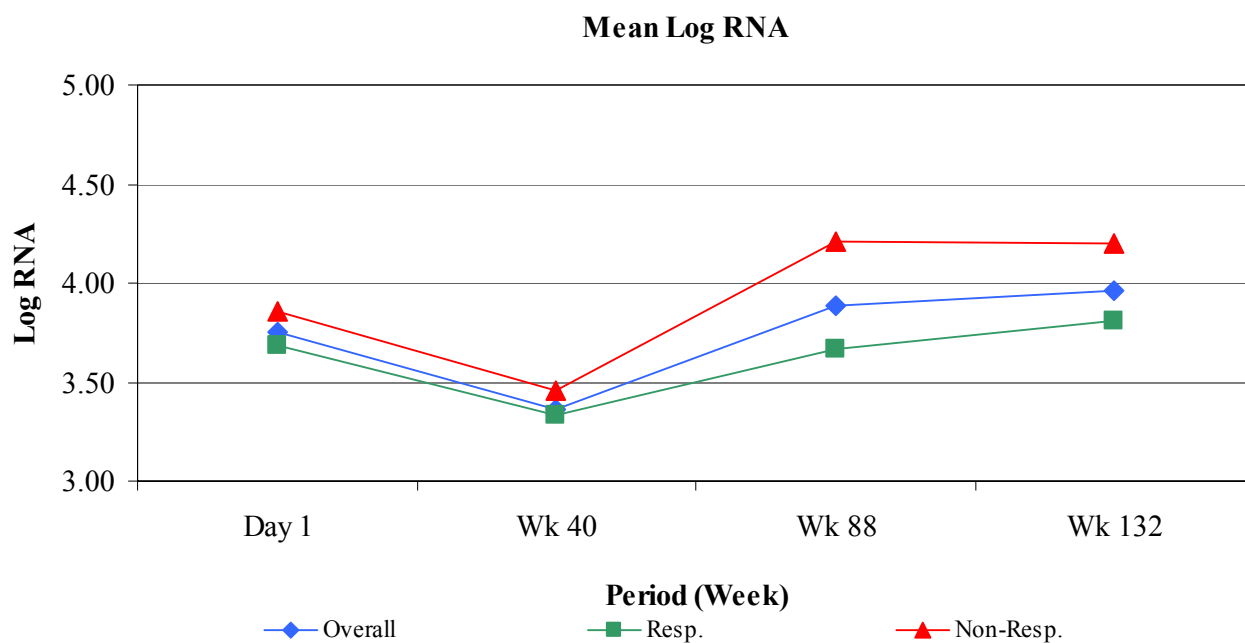


**Figure 2. Mean Changes of CD8<sup>+</sup> T-cell counts of Responders and Non-responders**



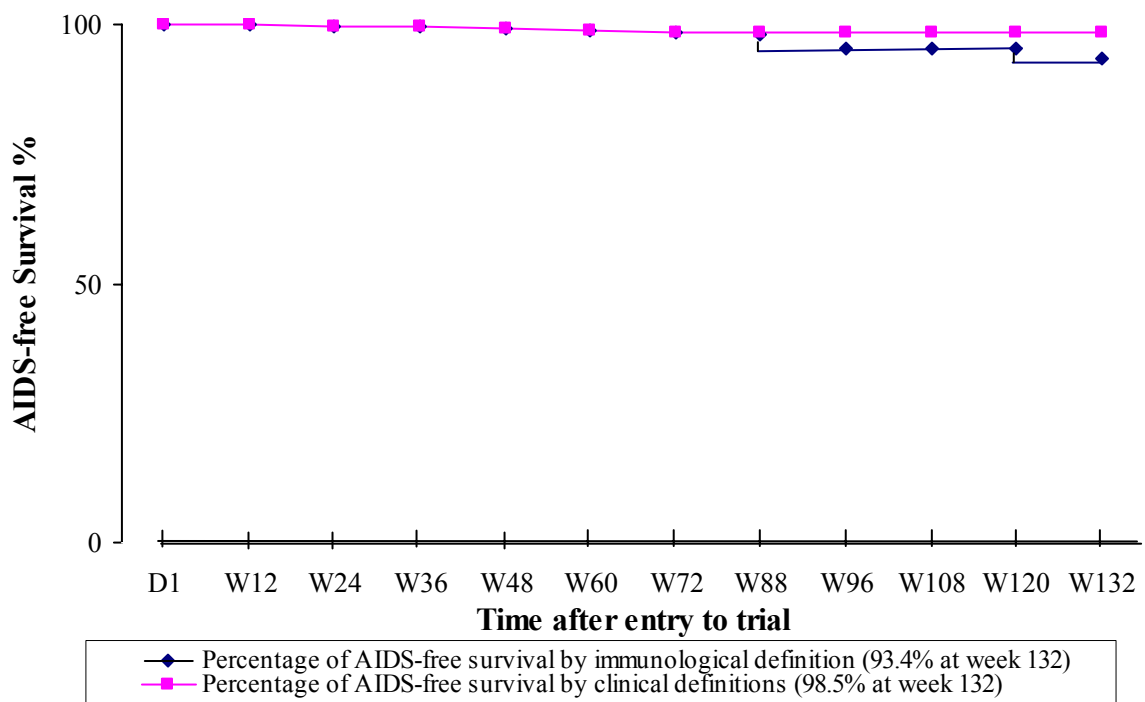
		Day 1	Wk 12	Wk 24	Wk 36	Wk 40	Wk 48	Wk 88	Wk 96	Wk 132
Overall	N	223	223	223	223	222	223	223	222	223
	Mean	55.52	55.84	55.76	56.01	56.13	56.18	56.63	56.68	56.60
Resp.	N	135	135	135	135	135	135	135	134	135
	Mean	55.98	56.50	56.53	56.70	56.91	57.05	57.60	57.69	57.74
Non-Resp.	N	88	88	88	88	87	88	88	88	88
	Mean	54.83	54.83	54.59	54.95	54.91	54.84	55.14	55.14	54.84

**Figure 3 Mean Changes of body weight (kg) of Responders and Non-responders**



		Day 1	Wk 40	Wk 88	Wk 132
Overall	N	223	85	223	223
	Mean	3.75	3.36	3.88	3.97
Resp.	N	135	69	135	135
	Mean	3.69	3.34	3.67	3.82
Non-Resp.	N	88	16	88	88
	Mean	3.85	3.46	4.21	4.18

**Figure 4 Mean Changes of log HIV-RNA of Responders and Non-responders**



**Figure 5** Kaplan-Meier product-limit estimates of AIDS-free survival based upon the clinical definition of AIDS