

15. Detection and Quantification of *Plasmodium* spp. by 18S rRNA Gene Subunit-Based and Species-Specific Real-Time PCR Assays. Status: Study protocol is waiting for approval.

16. *Leptospira* (LPS assay) RAPID PCR Validation Using JBAIDS Molecular Assay Transition Package. Status: Study is ongoing in trying condition.

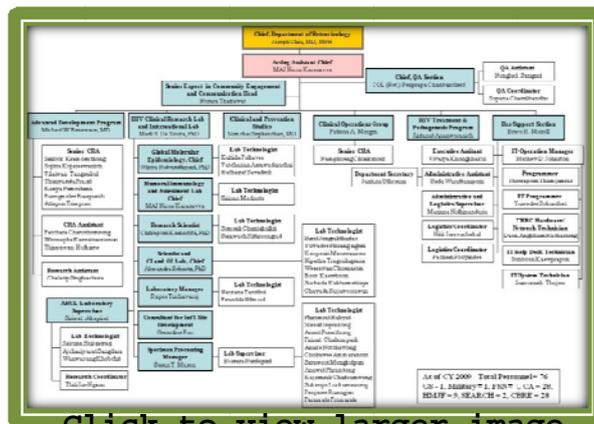
DEPARTMENT OF RETROVIROLOGY

DEPARTMENT MISSION

The mission of the Department of Retrovirology is to prepare for and conduct advanced development of preventive HIV vaccines for soldiers. This mission is achieved collaboratively and supported through i) the performance of preclinical and clinical (phase I-III) trials of candidate vaccines and their evaluations for safety, immunogenicity and efficacy, ii) the identification and characterization of potential cohorts for phase III vaccine trials, iii) the establishment of diagnostic assays which differentiate infection from vaccine-induced immune responses, iv) the characterization of HIV viruses circulating in the region, v) the determination of the natural history of HIV infection and disease.

PERSONNEL

The Department of Retrovirology consists of 76 staff that includes 1 GS and 2 Active-Duty Army Officers (1 Medical Corps, 1 Medical Service Corps). Within the department, there are 21 clinical research (4 MDs) and quality assurance staff, 42 laboratory personnel (5 PhD staff scientists), and 13 logistics/IT support staff. An overview of the organization chart is provided below as well as listing of each departmental staff member. The Department of Retrovirology has added personnel due to initiation of new MIDRP-funded activities and increased laboratory work load resulting from the recently completed Phase III trial that showed modest efficacy in preventing HIV infection.



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2009 OUTSIDE TRAINING OF PERSONNEL

- APTIMA HIV-1 RNA Qualitative Assay training, GEN-PROBE INC., San Diego, USA, 04-11 January 2009.
- 12th Bangkok International Symposium on HIV Medicine by HIV-NAT, Queen Sirikit National Convention Centre, 14-16 January 2009.
- Genomic Epidemiology of Malaria, Advanced Courses Genome Campus, WellcomeTrust, Cambridge, England, 16-22 June 2009.
- HIV/SIV-Pathogenesis from Lab Bench to Clinics, Siriraj Hospital, Royal River Hotel, 10 July 2009.
- New Employee Facility Safety Orientation, Ms. Julia Oussava, US Military HIV Research Program, Rockville MD, USA, 18 July 2009.
- Full-length Sequencing of HIV from Plasma Training, Dr. Sodsai Tovanabutra, US Military HIV Research Program, Rockville MD. USA, 18 July - 20 September 2009.
- Annual Study Coordinators and CRAs Training 2009, Thailand Center of Excellence for Life Sciences and Pharmaceutical Research & Manufacturers Association, Thailand, 21 August 2009.
- New Employee Facility Safety Orientation, Ms. Julia Oussava, US Military HIV Research Program, Rockville MD USA, 21 September 2009.
- CA-VIMC: Scientific Advisory Board/Site Visit Full Group Meeting, Durham, North Carolina, USA (BMGF/CAVD/CA-VIMC), 8-9 October 2009.
- Powered Air Purifying Respirator Use Training, Mr. Pramote, 3M Thailand, 30 October 2009.
- Chemical Spill Control, Mr. Thanyakiat, NPC Safety, Thailand, 30 October 2009.
- Dangerous Goods Awareness with Concentration on Preparing, Handling and Transporting Infectious Substances by Air, World Courier, Thailand, 03 November 2009.
- MHRP Site Investigator Meeting, Chiangmai, Thailand, 8-13 November 2009.
- Full-length Sequencing of HIV from Plasma Training, Dr. Sodsai Tovanabutra, US Military HIV Research Program, Rockville MD USA, 12 September - 15 November 2009.
- International Conference on Information Systems, The Association for Information Systems, JW Marriott, Phoenix, AZ, 15-18 December 2009.

ACCOMPLISHMENTS

The vast majority of the Department of Retrovirology's efforts in 2009 were directed to the completion of the world's largest phase III HIV vaccine trial (RV144) conducted in Eastern seaboard of Thailand (Chon Buri and Rayong Provinces) that began in September 2003. The study enrolled 16,402 volunteers with half receiving placebo and half vaccinated with ALVAC canarypox prime (produced by Aventis Pasteur) followed by gp120 boost (produced by VaxGen). The final vaccinations were given on 31 July 2006; 85.2% of volunteers completed vaccination. Follow up of volunteers was completed 30 June 2009. The retention rate at the last surveillance visit was 90%, with a total of 52,985 years of follow-up (92% of potential f/u years for the trial). Final results were announced 24 Sept 2009. The observed vaccine efficacy in the modified intent to treat analysis was 31.2% [$p = 0.04$, 95% CI (OBF) 1.1, 52.1]. The vaccine regimen had no effect in post infection viral load or CD4 in volunteers who acquired HIV infection. There were no differences in adverse events between the two groups. The overall local reactions were higher in the vaccine recipients (88.0%) than the placebo (61.0%), as were the overall systemic reactions



(77.2% vaccine recipients and 59.8% placebo recipients). There was no difference in self-reported risk behavior between the two groups. This collaborative study included more than 500 staff and multiple academic and government partners: the Thai Ministry of Public Health, Mahidol University, Royal Thai Army Medical Department, DAIDS/NIAID, and the USA Medical Research and Materiel Command. While the Phase III Trial is now complete, additional laboratory studies are underway and planned on archive specimens to identify correlates of vaccine efficacy and better understand the immune response. Two advisory groups (Scientific and Product Development) made up of international experts and Trial Collaborators have been established to advise the sponsor on ways to improve the vaccine and future clinical development of the product.

RV152, a study of breakthrough infections in the Phase III trial (RV144) which began in May 2006 closed to enrollment in June 2009 with the completion of RV144. As of January 2010, 53 of 120 enrolled volunteers have reached study endpoint of CD4 less than 350 cells/ μ L. Enrolled volunteers are continued to be followed for clinical outcome. Final analysis will be conducted when approximately 80 primary composite endpoints are accumulated. It is estimated that the final analysis will occur in late 2010. In anticipation of revision of National Treatment Guideline to begin treatment of HIV infected individuals when CD4<350 cells / μ L, volunteers who have already reached this parameters have been contacted starting in January 2010 for counseling and preparation for initiating treatment.

RV158, the Phase I study of a CRF01_AEMVA vaccine which began on 27th November 2007 in Thailand enrolled twelve volunteers and was completed in October 2008. There were no significant safety issues associated with the test product. Immunogenicity analyses conducted in 2009 of chromium release assay showed anti-insert CD8 cytotoxic T lymphocyte (CTL) activity, predominantly ENV directed (60% intramuscular / intra-dermal) and GAG/POL directed (40% in intramuscular/hypodermal injection). The assay also showed strong anti-MVA CD8 CTL with up to 100% in IM/HD and route dependent with IM route responding higher than ID. The IFN- γ Elispot Assay showed low detectable ENV responses in all routes and predominantly ENV, 60% (6/10) ENV response in IM/HD and 23% (9/40) in all routes. Anti MVA IFN- γ response was up to 90% by IM route. The response was dose and route dependent with $10^8 > 10^7 > 10^6$ and IM route greater than ID. Lymphocyte proliferation (LP) assay showed strong ENV directed response that is greater by IM compared to ID. The p24 directed LP response was lower in frequency and magnitude compared to ENV and was route dependence (IM > ID). The multi-function flow cytometry vector specific CD4 responses were predominantly 1 or 2 functions (IL-2 OR IL-2 plus TNF α or IFN γ).

RV212, a cross-sectional study to screen for and generate broadly neutralizing monoclonal antibodies from HIV infected individuals seeks to generate broadly neutralizing monoclonal antibodies (mAbs) from volunteers who are HIV infected and have broadly cross-reactive serum neutralizing activity. The protocol began enrollment in August 2007 and completed in July 2008. Only a few Thai volunteers were identified with broadly, cross-reactive antibodies. Isolation and production of monoclonal antibodies is ongoing. AFRIMS will conduct genotyping, antibody isolation and purification, and construct a CRF01_AE antibody pool for reagents.

RV225, the molecular epidemiology study of HIV-1 among HIV blood testing clients attending the Thai Red Cross Anonymous Clinic in Bangkok, Thailand, was completed in 2009 with the transfer of the Principle Investigator (CPT Miguel A. Arroyo). Publications are in preparation; a summary of the final results of the study follows.

1. The frequency of responses to all or some of the questions of the self-administered questionnaire was highly variable: 29.3-99.7%.



2. The estimated overall HIV prevalence was 14%. This HIV-1 prevalence and the demographic predictors were in accordance with previous results for the TRCAC published by Khongphatthanayothine et al 2006. Among the risk factors found to be associated with HIV prevalence were age (25-29 years), risk behavior men who have sex with men (MSM), marital status (not single), education (< high school) among others.

3. Condom use was found to be protective from HIV infection.

4. Overall, non-CRF01AE strains accounted for 18.9% of the infections; 5.9%, B; 0.3%, C; B/C, 1.6% and 11.2% CRF01/B. Cross-sectional analysis between the high risk TRCAC and low risk MTCT cohorts revealed a direct association between HIV-1 2

5. None of the samples (n=50) analyzed showed an anti-retroviral resistance profile, which suggest that the majority of the population attending the TRCAC during the study period were drug naive.

The following conclusions were made based on the study results.

1. There is a concentrated and genetically complex HIV epidemic among MSM individuals in Thailand.

2. These findings advocate for targeted intervention and prevention measures and continued surveillance of circulating HIV-1 strains among high-risk populations in Thailand.

3. Although there is a benefit to target high-risk populations for their participation in HIV vaccine trials, the implications of the HIV molecular diversity on vaccine effectiveness in this population is unknown and requires further study.

In collaboration with the University of Hawaii and the Thai Red Cross AIDS Research Centre (SEARCH – South East Asia Research Collaboration with Hawaii), the Department is currently working on several protocols. RV233 is a HIV incidence cohort study in clients receiving anonymous HIV counseling and testing service. In addition the study investigated HIV subtype and genotypic resistance among HIV seroconverters. A total of 992 subjects, from 1075 subjects screened, were enrolled between 1 August 2008 - 5 August 2009. Among 992 subjects enrolled, 36.5% were heterosexual men, 31.2% were men who have sex with men (MSM) and 32.3% were women. Prevalent HIV infection was identified among 105 subjects (10.6%) at baseline visit, 4.7% of heterosexual men, 20.6% of MSM and 7.2% of women. There were 2 HIV seroconversion, both MSM, during the study period. This gave the calculated HIV incidence of 2.3 infections/100 person-years (95% confidence interval, CI, 0.6, 9.1) among MSM and the overall calculated HIV incidence of 0.56 infections/100 person-years (95%CI 0.14, 2.55). Due to the very low HIV seroconversion rate among the overall study participants along with the lower than expected follow-up rate, the early study closure plan was developed in August 2009. The last study visit occurred in December 2009.

RV254, another collaboration with SEARCH and the Thai Red Cross investigated the incidence, demographics, HIV subtype and genotypic resistance in acute HIV infection within a high-risk Thai cohort at the Thai Red Cross Anonymous Clinic (TRCAC), which has an HIV prevalence of about 17%. TRCAC uses 4th generation enzyme-linked immunoassay (AxSYM) for HIV diagnosis. AxSYM-negative samples are pooled to detect acute HIV infection by nucleic acid testing (NAT) using Roche Amplicor v 1.5 ultrasensitive assay. Acute HIV infection samples were AxSYM-negative, NAT positive. Additional acute HIV infections were identified if the sample is AxSYM and NAT positive but first generation sensitive EIA (HIV-1 Microelisa System, Organon Teknika, Durham, NC). Demographic and risk behavior data from the TRCAC questionnaires were collected. Between April to December 2009, 15 acute HIV infected subjects were identified, 9 from pooled NAT and 6 from sequential EIA. The predicted incidence of acute HIV infection for 28 days was 2.33/100 person-years (95% CI, 1.96-2.71). Ten enrolled

in the study, two women and 8 men. The staging of acute HIV infection was Fiebig I/II in 2, Fiebig III in 7 and Fiebig IV in 1 subject. Risk factors were heterosexual transmission in 3 and homosexual transmission (men) in 7 subjects. Eight of 10 subjects had acute retroviral syndrome with the most common symptoms being fever, lymphadenopathy, oral ulcers and diarrhea. The mean HIV RNA and CD4 at enrollment were 119, 325 copies/ml (min 1202 - max 337,105) and 426 cells/mm³ (min 218 - max 740). HIV subtypes were CRF_01AE in 4, B in 1, non typable in 3 and pending results in 2. Two subjects who were partners had K103N (resistance to non nucleoside reverse transcriptase inhibitors) and the rest had no drug resistance. Most subjects agreed to undergo the optional procedures except 2 who did not undergo gut biopsy and 1 who could not undergo MRI/MRS and lumbar puncture due to the presence of metal rods in his leg. All subjects elected to begin antiretroviral therapy as part of a local protocol. Two-thirds have reached at least week 12 and all have undetectable HIV RNA below 50 copies/ml. In 2010, the investigators will perform immunologic and virologic analysis using the stored samples in order to understand what occurs in the different compartments very early in HIV infection. These tests will include immunophenotyping (blood, gut), immunohistochemistry (gut), viral load (blood, gut, CSF, genital secretion), HIV sequence (blood, gut, genital secretion) and cytokines and chemokines (blood, CSF). Several manuscripts are in preparation to include 6 month follow-up data from the first 10 patients. Protocol version 1.2 is being reviewed by the institutional review boards that will include leukopheresis, neuropsychological testing and comprehensive neurological and psychological assessments.

The RV 243 protocol entitled “Assessment of neutralizing antibody (NAb) in participants from phase I/II Trials of ALVAC-HIV (vCP1521) priming with Chiron gp120 B/E, Sanofi-Pasteur oligomeric gp160, or AIDSVAX™ B/E gp120 B/E boosting against a newly developed, standardized panel of HIV-1 isolates”. This study aims to use the TZM-bl cell line Luciferase Reporter Pseudovirus NAb assay method. It will also aim to use archived plasma samples from previous phase I/II prime-boost HIV vaccine studies (RVs 132 and 135). The objectives of this protocol are (1) to compare cross-clade NAb among samples from HIV uninfected volunteers who have received prime-boost regimens of ALVAC vCP1521 and three different Env subunit protein boosts (2) to compare the frequency and titers of NAb induced among the three protein boost regimens and (3) to evaluate the evolution of NAb, i.e. the change in immunogenic responses whether NAb is detectable and/or shows variation in its expression, among volunteers during the prime-boost regimen. Luciferase Reporter Pseudovirus Nab assay method has successfully transferred to AFRIMS Retrovirology laboratory to assay RVs 132 and 135 clinical samples from Dr. Montefiori’s Laboratory for AIDS Vaccine Research and Development, Duke University Medical Center, Durham, NC, U.S.A. The laboratory facility underwent Good Clinical Laboratory Practice (GCLP) audit in January 2009. GCLP certification was granted in June and the external neutralization assay proficiency testing panel from the CA-VIMC was successfully completed in September 2009. Equivalency testing between AFRIMS and the CA-VIMC, was undertaken for further quality assurance. Initial equivalency assays involved testing plasma samples from RV243. There were some discrepancies between the two laboratories, and the assays were extended to serum, with enhanced concordance of results. A substantial number of samples used in this protocol will be sent to Dr. Montefiori’s laboratory and Humoral Immunology Core Laboratory, Division of Retrovirology, WRAIR, Rockville, MD, U.S.A. for NAb assay using TZM-bl platform. This is for quality assurance purpose i.e. equivalency and proficiency, to validate the assays performed at AFRIMS Laboratories, Bangkok, Thailand, for clinical specimen analysis under Comprehensive Antibody - Vaccine Immune Monitoring Consortium. It is also to assess the level of quantitative agreement in the results among laboratory locations. In preparation



for an assessment of the neutralizing antibody (Nab) response in RV144, the investigators will evaluate NAb responses in an earlier phase I/II trial RV135. The primary goals are to: (1) determine the longevity of binding and NAb response post final boosting, (2) define optimal sample conditions for the detection of neutralizing antibodies in RV135 and (3) determine whether results in the TZM-bl and PBMC neutralization assays are concordant and correlate with binding and other types of antibodies as measured by other groups. NAbs will be assessed using the TZM-bl luciferase neutralizing antibody assay. The TZM-bl assay has achieved a high level of optimization and formal validation. The immediate goal of RV243 will be to perform further equivalency tests with CA-VIMC, but will expand to include testing of all RV135 ALVAC-vCP1521 prime with VaxGen AIDSVax B/E (600mcg dose) boost vaccine recipients. Placebo recipients will also be included. The study will later be extended to include subjects who received ALVAC and one of two HIV Env protein boosts: Chiron B/E gp120 or Aventis Pasteur (TH023) on the same immunization schedule to compare the frequency and magnitude of Nab responses.

A new study protocol, RV217d was initiated during 2009 in a field site in Pattaya, Thailand in conjunction with similar studies being under taken in other MHRP sites in Africa. Volunteers considered at higher risk for HIV (MSM, SWs, TGs) are enrolled and followed for two years with blood collection every 6 months after baseline studies. Alternating 6 months the volunteers will get counseling and education. Twice a week the volunteers will provide a capillary blood specimen for sensitive testing of very early HIV infection. Those identified as recently infected will be studied intensively for ten visits and then followed for an additional 5 years.

The objectives of this study include:

1. Define the risk behavior, prevalence and incidence of HIV infection and retention of a high risk cohort of adults in Thailand, Uganda, Kenya and Tanzania.
2. Obtain 150 acute HIV infections (AHI) with at least 30% captured within Fiebig stages I and II to support the full characterization of host responses and viral dynamics.
3. Observe an incidence of 3.7% with at least 30% of the incident cases identified prior to the advent of detectable antibody.

Secondary/Exploratory Objectives

1. Assess and optimize HIV diagnostic strategies in HIV primary infection across multiple subtypes and risk groups.
2. Define the genetic diversity and evolution of HIV-1 in the prevalent and incident HIV cases with particular emphasis on characterization of acute, primary HIV infection.
3. Characterize immune activation, innate and adaptive cellular immunity in the early acute HIV-1 infection.
4. Characterize B cell responses in peripheral and mucosal compartments arising in early acute HIV-1 infection.
5. Characterize genetic polymorphisms in genes controlling host restriction, innate and adaptive Immunity, and their influence on HIV acquisition and early control of HIV infections.
6. Characterize clinical events including endemic infection as risk factors for HIV acquisition.

The study protocol began in July 2009 and more than 393 potential volunteers have been interviewed and 220 volunteers have taken the ACASI questionnaire. 204 volunteers passed the screening visit and 185 were successfully enrolled. Twenty-five potential volunteers were already HIV infected at baseline for an overall prevalence of those tested of 12%. Retention to the large blood draw visits and to finger stick small blood volume (twice weekly collections) has been approximately 90%. Two hundred volunteers will be enrolled (with replacements

for those that are lost until enrollment is complete). The two-hundred volunteers is Part A, if Part A is successful the plan is to proceed to Part B which continues the procedures in Part A but increases the volunteer population by an additional 300 volunteers. The results of Part A will be reviewed and modifications made if necessary and then the enrollment for Part B will continue. Again volunteers will be followed for 2 years; incident HIV cases will be followed for an additional 5 years.

COLLABORATIONS

- Ministry of Public Health, Department of Disease Control (DDC), Nonthaburi
- Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, Bangkok
- Division of AIDS, NIAID, NIH
- Siriraj Hospital, Faculty of Medicine, Mahidol University, Bangkok
- AFRIMS- Division of Research (Thai component)
- Phramongkutklao Army Medical Center, Bangkok
- Data Management Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok
- The Thai Red Cross AIDS Research Centre
- Hawaii AIDS Clinical Research Program, John A. Burns School of Medicine, University of Hawaii
- Laboratory for AIDS Vaccine Research and Development, Duke University Medical Center, Durham, NC, U.S.A.
- Collaboration of AIDS Vaccine Discovery
- Comprehensive Antibody - Vaccine Immune Monitoring Consortium (CA-VIMC)
- Sanofi-Pasteur
- VaxGen, Inc. (now Global Solutions for Infectious Diseases)

SUMMARY OF FUTURE PLANS AND STRATEGIES

The immediate focus of the coming year is additional laboratory studies of archive RV144 specimens to identify correlates of vaccine efficacy and better understand the immune response. Two advisory groups (Scientific and Product Development) made up of international experts and Trial Collaborators have been established to advise the sponsor on ways to improve the ALVAC prime gp120 boost vaccine regimen and future clinical development of the vaccines. Plans are underway to begin two follow-up studies to investigate immunologic response following late boosting and intense evaluation of immunologic profile following each vaccination. A phase IIB trial is planned for FY2012 to evaluate early efficacy of this vaccine regimen in community risk and high risk (MSM) population. In preparation of this phase IIB study, cohort studies to characterized suitable population will begin in Q3 2010. These studies will involve new collaborators at Chiang Mai University and Phramongkutklao Hospital and Medical College of Medicine of the Royal Thai Army.

The Department of Retrovirology will continue to serve as one of the USMHRP's major testing platforms for phase I-II studies of newer vaccine candidates which will involve further testing of the subtype E (CRF01_AE) MVA vaccine candidate currently in phase I testing and newer DNA vaccine candidate. Further development of populations suitable for more advanced testing in phase IIB and III vaccine trials will be pursued through cohort studies of high-risk populations in Bangkok and Pattaya.