

## NEW APPROACHES FOR THE DESIGN OF MULTI-REGION HYBRIDIZATION ASSAYS (MHAS) FOR HIV-1 GENOTYPING AND A SECOND-GENERATION ASSAY FOR SOUTHEAST ASIA

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**Background:** The Thai HIV-1 epidemic is rapidly evolving, with the circulation of subtype B, CRF01\_AE and their recombinants. Subtype C and C-containing strains, found in neighboring countries, are expected to impact the HIV-1 genetic diversity in Thailand. A Multi-region Hybridization Assay (MHAbce) (Watanaveeradej et al., XIV Intl AIDS Conf 2002) developed earlier, was revised to accommodate the changing molecular picture. Here we present a new approach for MHA development and a second-generation MHAbce for Southeast Asia.

**Methods:** Nucleic acids were extracted from plasma or PBMCs. First-round amplicons were generated by RT-PCR or PCR. 384-well plate real-time PCR with subtype-specific fluorescent TaqMan probes was used to assess the genotype at 8 genomic regions; the specific amplicon production was monitored by using Sybr-Green (SG) and melting curves (MC). The samples' genotype was deduced from the patterns of probe reactivity.

**Results:** A novel approach was used for the development of probes and primers for real-time PCR. Candidate probes were first synthesized as unlabeled oligonucleotides (ONTs) and were evaluated by MC with SG in their hybridization properties towards synthetic ONTs representing Southeast Asian strains. Those showing the highest specificity and sensitivity were synthesized as labeled probes. Different primer-pair combinations were tested by SG-based real-time PCR with MC, and those showing optimum sensitivity, efficiency and minimum primer-dimer generation were selected. The real-time PCRs were then assessed using the selected primers and labeled probes on a panel of 12 prototypic strains and the assay performed with high specificity and sensitivity. A field test on a larger sample set is underway.

**Conclusions:** New design approaches for real-time PCR HIV-1 genotyping permitted rapid re-design of the MHAbce to accommodate the increasing diversity of the Thai epidemic. Large-scale screening of strains is now possible to support vaccine trials.

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## PHASE III TRIAL OF HIV PRIME-BOOST VACCINE COMBINATION IN THAILAND

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**Background:** The world's first community-based, phase III HIV vaccine trial began in Thailand in late 2003. This is being carried out through the infrastructure of the Ministry of Public Health, augmented by Mahidol University and supported by the Armed Forces Research Institute of Medical Sciences.

**Objectives:** determine if this prime-boost vaccine strategy 1) prevents infection, 2) alters disease course in vaccinees who become infected, and 3) is safe. Vaccines were designed specifically for the predominant circulating HIVs in Thailand (subtypes E and B). Prime: a recombinant canarypox ALVAC-HIV (vCP1521) with a subtype B gag/pro and gp41, and subtype E gp120 (R5) gene insertions (Aventis Pasteur). Boost: AIDSVAX.gp120 B/E, monomers of gp120 B (X4) + gp120 E (R5) with alum (VaxGen).

**Methods:** 16,000 HIV-negative adult Thais, screened and enrolled through the health care system of the Ministry of Public Health.

**Study design:** randomized, placebo-controlled, double-blind phase III trial. Immunization is intramuscular over 6 months with a 3-year follow-up period.

**Results:** Clinical, laboratory & data system infrastructures have been built, qualified and validated; more than 400 staff trained, counseling and treatment networks strengthened, and communities engaged. HIV prevalence in volunteers assessed in a screening protocol was ~3%. During the initial 3-month phase-in period of the trial, more than 500 volunteers were enrolled.

**Conclusions:** With focus on a low-incidence, community-based population, the trial size is large, logistics very demanding and community engagement crucial.

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## **PROSPECTIVE ANALYSES OF HIV-1-SPECIFIC PROLIFERATIVE RESPONSES, RECALL ANTIGEN PROLIFERATIVE RESPONSES, AND CLINICAL OUTCOMES IN AN HIV-1-SEROPOSITIVE COHORT**

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**Background:** In cross-sectional studies of chronically infected individuals, lymphoproliferative responses to human immunodeficiency virus (HIV) type 1 p24 Gag antigen have previously been associated with lower virus load. It was not known whether this association would be predictive of better clinical outcome in longitudinal studies.

**Methods:** In blood samples from 608 HIV-seropositive individuals enrolled in a trial of glycoprotein 160 vaccine therapy over the course of 5-3 years, lymphoproliferative responses to HIV-1 antigens, tetanus toxoid (TT), and mitogens were measured and correlated with clinical outcome and other parameters of progression. Baseline lymphoproliferative responses to antigens and mitogens were used to categorize the cohort into responders or nonresponders.

**Results:** Although response to recall antigens did not correlate with clinical indices of disease progression, positive baseline lymphoproliferative responses to p24 and TT were associated with lower plasma levels of HIV-1 RNA. Persistently positive lymphoproliferative responses to the antigens also inversely correlated with repeated measurements of virus load, although the significance was lost once the measurements were adjusted for virus load and CD4<sup>+</sup> cell count at baseline, by use of generalized estimating equation analysis.