NeuroAIDS Research with SEARCH Consortium in Thailand

South East Asia Research Collaboration with Hawaii (SEARCH)

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Professor of Medicine
Hawaii AIDS Clinical Research Program
John A. Burns School of Medicine
University of Hawaii – Manoa
SEARCH is a partnership in Bangkok, Thailand between

- John A. Burns School of Medicine, University of Hawaii (UH) at Manoa in Honolulu

- The Thai Red Cross AIDS Research Centre (TRCARC) in Bangkok

- The Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok
NeuroAIDS Research and SEARCH

- SEARCH NeuroAIDS Research done in collaboration with the Division of Neurology, Phramongkutklao Medical Center/ Royal Thai Army Medical Department

Phramongkutklao Medical Center
SEARCH

- Services as a Coordinating Center
  - Clinical and translational research in HIV/AIDS and other infectious diseases
  - Regional Training Center for HIV/AIDS patient care/management as well as in research-related clinical and laboratory techniques
    - Vietnam military PEPFAR program
    - Advanced Physician Training in HIV Management
    - “Technology transfer”
SEARCH

Operations directed by Jintanat Ananworanich M.D.

Personnel
- 2 Clinical Research Nurses
- A Biostatistician
- Training Coordinator
- Administrative/Fiscal Officer

New office located on the 2nd floor of one of the Water Tower Buildings at the TRCARC

Currently 7 active SEARCH research protocols
Changing phenotype of Dementia

New factors in the HAART era
- Potent/toxic antiretrovirals
- Aging
- Insulin resistance
- Lipodystrophy
- Chronic immune activation
- Mitochondrial toxicity

HIV-infected host

Changing neuroepidemiology of HAD
- Decreased HAD incidence with unchanged prevalence
- Unchanged degree of encephalitis
- Attenuated severity
- Altered phenotype

Emerging markers
- Metabolic disturbance
- Altered redox pathways
- Mitochondrial toxicity
- Amyloid beta/ApoE

Classic Markers
- Inflammation
- Macrophage activation
- Chemotaxis
- Virus/viral particles

Classically described mechanistic pathways
Thai Red Cross AIDS Research Centre
- Substantial HIV clinical trials expertise particularly within HIV-NAT
- Direct in-country knowledge of patient population bases, resources, regulatory requirements

Phramongkutlao Medical Center
- Local Neurology and Infectious Disease expertise
**U.S. AFRIMS**

- CAP certified lab for HIV serology, hematology and VL assays
- Stringent protocols for processing, storing and shipment of specimens
- HIV immunovirologic capabilities:
  - ELISPOT
  - Intracellular cytokine assays (LSRII flow machine with 6 colors)
  - Cromium release CTL and LPA assays
  - ADCC and NK assays
  - Full length viral sequencing
  - HIV neutralization assays
MR Spectroscopy capabilities

- Collaboration with Chulalongkorn University
- Expertise from U.S. side provided by Dr. Napapon Salisuta

Representative spectra from Frontal White Matter (upper image) and parietal grey matter (lower image)
SEARCH 002 Thai Normative Data for the NIMH/WHO Neuropsychological Battery

- Normative neuropsychological data using the International HIV neuropsychological battery
- Goal 300+ enrollees at PMK
- Continued enrollment in the future as more studies enroll
- Data available upon request; SEARCH website (www.searchthailand.org)
### SEARCH 002 Thai Normative Data for the NIMH/WHO Neuropsychological Battery

<table>
<thead>
<tr>
<th>Highest earned certificate</th>
<th>Age 20 - 29 years</th>
<th>Age 30 - 39 years</th>
<th>Age 40 - 49 years</th>
<th>Age 50 - 59 years</th>
<th>Age 60 - 69 years</th>
<th>Total</th>
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<tbody>
<tr>
<td>1: No certificate or primary certificate</td>
<td>11</td>
<td>17</td>
<td>17</td>
<td>7</td>
<td>1</td>
<td>53</td>
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<tr>
<td>2: Secondary certificate</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td>1</td>
<td>42</td>
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<tr>
<td>3: High school or Associates/Technical/Vocational certificate</td>
<td>25</td>
<td>27</td>
<td>16</td>
<td>5</td>
<td>1</td>
<td>74</td>
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<tr>
<td>4: Bachelor of Science or Arts or more than BS/BA</td>
<td>18</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td></td>
<td>46</td>
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<tr>
<td>Total</td>
<td>66</td>
<td>68</td>
<td>57</td>
<td>21</td>
<td>3</td>
<td>215</td>
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</table>
Neuropsychological Assessment of Non HIV-Infected Subjects  Age 20 - 29 years and Education: No certificate or primary certificate

<table>
<thead>
<tr>
<th>Assessment</th>
<th>- 2SD</th>
<th>- 1SD</th>
<th>MEAN</th>
<th>+ 1SD</th>
<th>+ 2SD</th>
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</thead>
<tbody>
<tr>
<td>Finger tapping average dominant</td>
<td>37.44</td>
<td>45.63</td>
<td>53.82</td>
<td>62.01</td>
<td>70.20</td>
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<tr>
<td>Finger tapping average non-dominant</td>
<td>34.86</td>
<td>43.12</td>
<td>51.38</td>
<td>59.64</td>
<td>67.90</td>
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<tr>
<td>WHO/UCLA auditory verbal learning test- total 1-5 trial</td>
<td>43.11</td>
<td>50.56</td>
<td>58.00</td>
<td>65.44</td>
<td>72.89</td>
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<td>WHO/UCLA auditory verbal learning test- trial 5</td>
<td>11.58</td>
<td>12.61</td>
<td>13.64</td>
<td>14.66</td>
<td>15.69</td>
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<tr>
<td>WHO/UCLA auditory verbal learning test- trial 8</td>
<td>10.03</td>
<td>11.52</td>
<td>13.00</td>
<td>14.48</td>
<td>15.97</td>
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<tr>
<td>BVMT-R learning - total 1-3 trial</td>
<td>7.43</td>
<td>15.72</td>
<td>24.00</td>
<td>32.28</td>
<td>40.57</td>
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<td>BVMT-R delay trial</td>
<td>2.94</td>
<td>6.11</td>
<td>9.27</td>
<td>12.44</td>
<td>15.60</td>
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<tr>
<td>Color trials 1 (sec.)</td>
<td>58.66</td>
<td>49.34</td>
<td>40.02</td>
<td>30.70</td>
<td>21.37</td>
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<tr>
<td>Color trials 2 (sec.)</td>
<td>146.02</td>
<td>117.08</td>
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<td>EWIA digital symbol</td>
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<td>Grooved Pegboard, dominant time (sec.)</td>
<td>72.36</td>
<td>65.28</td>
<td>58.21</td>
<td>51.14</td>
<td>44.06</td>
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<tr>
<td>Grooved Pegboard, non-dominant time (sec.)</td>
<td>82.81</td>
<td>73.30</td>
<td>63.79</td>
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<td>44.77</td>
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<td>Timed gate average (sec.)</td>
<td>91.49</td>
<td>56.45</td>
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<td>-48.67</td>
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<tr>
<td>Verbal fluency first names</td>
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<td>15.41</td>
<td>20.27</td>
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<tr>
<td>Verbal fluency animals</td>
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<td>19.37</td>
<td>22.27</td>
<td>25.17</td>
<td>28.08</td>
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<tr>
<td>Trial-making A time (sec.)</td>
<td>46.94</td>
<td>38.12</td>
<td>29.30</td>
<td>20.48</td>
<td>11.66</td>
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<td>EIWA block design total trial 1-10</td>
<td>23.23</td>
<td>29.70</td>
<td>36.18</td>
<td>42.66</td>
<td>49.14</td>
</tr>
</tbody>
</table>
Neuropsychological Assessment of Non HIV-Infected Subjects  Age 20 - 29 years and Education: No certificate or primary certificate

<table>
<thead>
<tr>
<th>Composite Variables</th>
<th>- 2SD</th>
<th>- 1SD</th>
<th>MEAN</th>
<th>+ 1SD</th>
<th>+ 2SD</th>
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<tbody>
<tr>
<td>Motor NPZ</td>
<td>47.14</td>
<td>51.97</td>
<td>56.80</td>
<td>61.63</td>
<td>66.46</td>
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<td>Psychomotor NPZ</td>
<td>36.97</td>
<td>45.72</td>
<td>54.46</td>
<td>63.20</td>
<td>71.94</td>
</tr>
<tr>
<td>Verbal Fluency NPZ</td>
<td>14.53</td>
<td>17.90</td>
<td>21.27</td>
<td>24.64</td>
<td>28.02</td>
</tr>
<tr>
<td>Verbal Learning NPZ</td>
<td>26.94</td>
<td>31.22</td>
<td>35.50</td>
<td>39.78</td>
<td>44.06</td>
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<tr>
<td>Visual Learning NPZ</td>
<td>5.32</td>
<td>10.98</td>
<td>16.64</td>
<td>22.29</td>
<td>27.95</td>
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<tr>
<td>Motor Speed/Fine Motor NPZ</td>
<td>42.57</td>
<td>46.42</td>
<td>50.26</td>
<td>54.11</td>
<td>57.95</td>
</tr>
<tr>
<td>Sustained Attn NPZ</td>
<td>23.70</td>
<td>38.10</td>
<td>52.49</td>
<td>66.88</td>
<td>81.27</td>
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<tr>
<td>Selective Attn NPZ</td>
<td>52.77</td>
<td>63.51</td>
<td>74.25</td>
<td>85.00</td>
<td>95.74</td>
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</tbody>
</table>
SEARCH 003 Mitochondrial toxicity potential of various NRTI regimens

- Open label, 3 arm, head-to-head evaluation of the 18 month mitochondrial consequences of a [6-month lead in with d4T followed by AZT] vs continuous AZT or TDF-based first line HAART

- Anticipated Start Date of October 2007

- Funding Commitment from Thai Government Pharmaceutical Organization (GPO) and MitoScience; Tenofovir supply from Gilead

- R01 grant funding request to support extended laboratory evaluation for lipoatrophy under review at NIH (PI: M. Gerschenson)

- Similar grant funding request planned soon to support neuropathy extended laboratory evaluations
Background and Rationale

- GPOvir, a generic co-formulation of d4T+3TC+NVP, is widely used in Thailand.
- Lipoatrophy and peripheral neuropathy are common side-effects of d4T.
- A co-formulation utilizing ZDV [GPOvir-Z (ZDV+3TC+NVP)] has been developed.
- However, there is concern regarding the potential rates of anemia with use of ZDV.
  - The risk of severe anemia particularly high early in Rx course in severely ill patients with low CD4 counts.
Background and Rationale

- One suggested alternative to balance the risks and benefits of these two medications:
  - 6 months lead-in with d4T-containing GPOvir followed by a switch to ZDV-containing GPOvir-Z
- However, Tenofovir is now more affordable in Thailand and is increasingly a viable antiretroviral option for Thais.
### SEARCH 003 SCHENA

<table>
<thead>
<tr>
<th></th>
<th>0 – 6 months</th>
<th>6 – 18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm I</td>
<td>GPOvir [(d4T)+3TC+NVP]</td>
<td>GPOvir-Z [AZT+3TC+NVP]</td>
</tr>
<tr>
<td>Arm II</td>
<td>GPOvir-Z [AZT+3TC+NVP]</td>
<td>GPOvir-Z [AZT+3TC+NVP]</td>
</tr>
<tr>
<td>Arm III</td>
<td>TDF+3TC+NVP</td>
<td>TDF+3TC+NVP</td>
</tr>
</tbody>
</table>
SEARCH 003

- 150 patients at the Thai Red Cross AIDS Research Centre and Queen Savang Vadhana Hospital
- Neuropathy objectives: To compare among the 3 arms at baseline, 6 mo. and 18 mo. of therapy
  - Relative frequency of peripheral neuropathy (by clinical neuropathy exams and pain scores)
  - Changes in epidermal nerve fiber density (ankle and thigh skin punch biopsies)

Initial regimen 6 mo. | Subsequent regimen 12 mo
Intensive Laboratory Analyses planned

- **Lipoatrophy – Related Assays**
  - mtDNA levels in fat, PBMCs, and buccal cells
  - Mitochondrial OXPHOS protein and enzyme activity levels (CI and CIV) in fat, PBMCs and buccal cells
  - Immunohistochemical assessments in fat tissue of cytokines [TNF-a, IL-1, IL6], degree of apoptosis by TUNEL and CD68 macrophage levels
  - Mitochondrial specific 8-oxo-deoxyguanine in PBMCs
  - Intracellular NRTI- triphosphate concentrations within PBMCs
  - HIV DNA assays in CD14+ (monocytes) PBMCs

- **Peripheral Neuropathy-Related Assays**
  - Anti-PGP9.5 immunostaining and quantitation of the epidermal nerve fibers in epidermal tissue
PBMC and Fat Correlations

mtDNA correlation PBMC vs Fat
r= .169, p=0.40

CI correlation PBMC vs Fat
r= 0.459, p=0.02

CIV correlation PBMC vs Fat
r= 0.606, p=0.001

Shikuma et al, manuscript under review, Antiretroviral Therapy
SEARCH 003 Mitochondrial toxicity potential of various NRTI regimens

Questions:
- How much neuropathy damage occurs with a short 6 months of d4T use?
  - Does the damage persist at 18 months?
- Does HIV-induced neuropathy improve following AZT or TDF-based HAART?
- Can oxidative phosphorylation protein/enzyme activity levels in blood cells or buccal cells be used to monitor for toxicity?
- What is role of HIV induced inflammation (cytokines) and mitochondrial-specific oxidative stress in the pathogenesis of neuropathy?
SEARCH 005 Neurocognition in Long Term VL suppressed Thais on NNRTI-based Rx

- Collaboration with HIV-NAT
- What is the long-term neurocognitive status of subjects fully suppressed on NNRTI-based Rx?
- Are there neurocognitive differences between subjects who initiated such ARV Rx with CD4 < 200 cells/mm$^3$ vs CD4 > 200 cells/mm$^3$?
- Patients (n=135) in the HIV-NAT 2NN cohort with > 3 years VL suppression on NNRTI-based first line treatment
  - One time neurologic and neuropsychologic assessments
- MRI/MRS substudy in patients with HIV dementia
- Complete in August 2007
SEARCH 007

T cell correlates to HIV dementia

- Newly NIH-funded (NINDS) Study [PI: Silvia Ratto-Kim]
- Does loss of specific HIV-1 T cell response result in activation/dysregulation of monocytes/macrophages leading to their accumulation in the brain and development of HIV associated dementia?
- Define HIV-1 specific CD4+ T helper cells and CD8+ CTL responses in 20 HAD and matched non-HAD patients; correlate responses to
  - Monocyte/macrophage subpopulation cell number, percentage and immune function, and HIV DNA levels
  - Autologous viral sequences (viral escape sequences and HIV quasispecies)
- Evaluate the impact of ARV on dementia related to changes in immunological responses.
Preliminary data on 20 HAD, 20 non-HAD and 10 HIV negative controls showing a trend toward increased %CD8+/CD38+ T cells in HAD.
Other collaboration with HIV-NAT

- **MOST** [Monotherapy in Switzerland and Thailand]
  - NeuroAIDS Sub-study
    - Collaboration with the Swiss Cohort Study
    - Evaluation of Kaletra monotherapy as an approach to second line treatment for NNRTI failure (n=200)
    - Neurocognitive outcomes in association with CSF VL

- **PREDICT** [Pediatric Randomized to Early versus Deferred ART Initiation in Cambodia and Thailand]
  - Neurodevelopment Sub-study
    - NIMH/NICHD-funded 3- year substudy
    - Evaluation of neurodevelopment outcomes in children ages 1- 12 years with mild or moderate HIV symptoms and CD4 15-24% randomized to immediate vs deferred (CD4 below 15%) ARV initiation.
Efforts in Building Expertise for NeuroAIDS Research

- Search Research Forum on NeuroAIDS
- NeuroAIDS workshop at the Bangkok Symposium on HIV Medicine
- Neurologic examination training for physicians
- Neuropsychological test training for nurses
Future Plans

- Establishing a NeuroAIDS inception cohort
  - Evaluate the neuropathogenesis during primary HIV infection and the natural course of disease in Clade A/E
- Neurocognitive studies in other populations
  - HCV-infected patients in Vietnam
- Participate in building NeuroAIDS expertise in the South East Asia region
Thank You for Your Attention

www.SearchThailand.org