Novel Immunogens with Intrinsic Adjuvant

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Vision of NHRI Vaccine Center

NHRI Vaccine Center (VC) strives to be the leader in developing novel vaccines and immunotherapeutic candidates to fulfill Asian healthcare needs.
Mission of NHRI Vaccine Center

- Optimize utilization of resources to complete Government assignments
- Continue recruiting PI with different platform technologies to enhance vaccine research and development to meet local needs
- Form Strategic Alliance to maximize the functions of cGMP Pilot Plants and BCG manufacturing facility
- Assist local Universities or Biotech to produce GMP-grade vaccine candidates to initiate phase 1 and 2 clinical trials in Taiwan and Asia
- Establish constant forum for training and educating local young scientists in vaccine-related biotechnology, and
- Build up capability to respond to Taiwan government emergency request for vaccines against pandemic diseases and bioterrorism.
Vaccine Research and Development Center

Vaccine Center & cGMP Facility

NHRI R1-7F (R&D)
Vaccine Center Floor Plan (1st floor)

- BCG
- Bacterial Pilot Plant
- Core Facility
- Viral Pilot Plant
- cGMP Warehouse
- Central Filling
- Biological Manufacturing Plant
- QC Animal Facility
Platform Technologies

Genetic Engineering
- E. coli, baculovirus, yeast, CHO and Vero cell expression systems
- Bacterial and Viral vectors for mucosal vaccine development
- Host modification for high virus titer production
- Plasmid-based Chimeric Reassortant for live vaccine and vectors

Molecular Immunology
- HLA reagents for Asian populations (A02, A11 and A24 tetramers and transgenic mice)
- Molecular monitoring systems for human immune responses to Vaccines
- Human dendritic cell technology for vaccinology

Formulation and Vaccine Delivery Systems
- Controlled-release microencapsulation technology for multivalent vaccine development
- Develop novel lipoprotein-based technology
- Synthetic Adjuvant development

Bioanalytical and Product Characterization
- “State of the art” Bioanalytical and Serological core facility
- GLP/GMP QC testing systems
- Product characterization according to ICH guidelines

Bioprocess Development
- Scale up and optimization cell-culture based vaccine development
- Single-use technology development
- Downstream purification process improvement and validation
- Leading roles to assist local vaccine industry
<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Stages of Development</th>
<th>Licensed</th>
<th>Strategic Partners</th>
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<tbody>
<tr>
<td>BCG</td>
<td>Research</td>
<td>Clinical</td>
<td>Licensed</td>
</tr>
<tr>
<td>Horse anti-Snake venom IgG</td>
<td>Pre-clinical completed</td>
<td></td>
<td>Taiwan</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
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<tr>
<td>MDCK Cell-based H5N1 flu vaccine</td>
<td>Pre-clinical completed</td>
<td>MVSS, MCB phase 1 completed</td>
<td>CDC, Medigen</td>
</tr>
<tr>
<td>Vero Cell based EV-71</td>
<td>Pre-Clinical Completed</td>
<td>MVS, MCB phase 1 completed</td>
<td>CDC, Adimmune, Medigen</td>
</tr>
<tr>
<td>Meningococcal group B Subunit vaccine</td>
<td>Pre-clinical completed</td>
<td>MCB Phase 1 allowed</td>
<td>CDC</td>
</tr>
<tr>
<td>Vero Cell based JEV</td>
<td>Pre-Clinical</td>
<td>MVS ready</td>
<td>CDC</td>
</tr>
<tr>
<td>Mucosal RSV</td>
<td>Pre-clinical</td>
<td>Q4/2015</td>
<td>TWi</td>
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<tr>
<td>CAV16/EV71 Vaccine</td>
<td>Pre-clinical</td>
<td>2016</td>
<td></td>
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<tr>
<td>CTL-based Cervical cancer vaccine</td>
<td>Pre-clinical</td>
<td>2016</td>
<td></td>
</tr>
<tr>
<td>Dengue Vaccine (D3 of E protein)</td>
<td>Pre-clinical</td>
<td>2016</td>
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</table>
Recombinant antigen processes very good safety profile but these vaccine-candidates are usually poor immunogens.

They require appropriate adjuvant to be efficacious.

Is it possible to have solution for this dilemma?
Ideal Immunogens

✓ Activate T-cell immune responses by Antigen Presenting cells (DC)
  ➢ Pro-inflammatory cytokines release
  ➢ Up-regulate T-cell Biomarkers (MHC II, CD86, etc)
  ➢ induce the right effective T-cells (CTL)

✓ Enhance Antibody responses
  ➢ Fast and strong
  ➢ functional and long memory

Novel immunogen with
Intrinsic adjuvant
Studies of Outer surface protein A (OspA)

OspA of Borrelia Burgdorferi is a bacterial lipoprotein which is efficacious when it is lipidated as the key component of lyme disease vaccine.
Expression of lipoproteins in E. coli often results in low expression, incomplete modification or entirely absence of lipid moiety (Hansson et al. 1995; Shang et al. 1996; Madurawe et al. 2000).
Schematic structure of Lipoprotein

Hydrophobic region (h-region) → Mature sequence

- Positively charged region (n-region)
- Lipobox with invariant Cysteine (c-region)

Diacylglycerol \[\text{C-O-C} \quad \text{C-O-C} \quad \text{C-O-C} \quad \text{C-O-C}\] → Amide linked fatty acid (R)

Thioether Linkage
Expression of lipoproteins in E. coli system

Native apyrase → (Lipid modified form)
The structure of C43(DE3) strain after expression of membrane protein

37°C (3 hours after induction)  
25°C (18 hours after induction)

C43(DE3)

Ignacio et al. (2000) FEBS Letters 482; 215-219
Expression and purification of the lipidated immunogen at high level

Ag473(lipoprotein), an antigen from *N. meningitidis*, Hsu et al. *Proteomics*, 2008
Mass spectra analysis of recombinant Ag473

Mass difference by 14 amu
$MS^n$ structural analysis of Lipoprotein

**Figure 2**

(A) Triacyl-CSQEA$k$ m/z 1452.3
(B) acyl-dAla-SQEAK m/z 867.0
(C) dAla-SQEAK m/z 612.2
(D) Triacyl-Cys m/z 890.8
(E) diacyl Cys m/z 636.6
(F) acyl-Cys m/z 370.2
(G) Palmitic acid m/z 256.1
Cost-effectiveness of MGBvac downstream processing

Clarified *E. coli* supernatant (TX100-soluble membrane fraction/0.22uM filtrated)

MPE Process (Mixed phase extraction)

Membrane S Chromatography

Membrane E Chromatography

Concentrated by Ultrafiltration (TFF)

Reversed Phase Chromatography (Needed due to false positive LAL value from Triton X-100)
# Mouse Challenge Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunization</td>
<td>r-Ag473 (10ug/dose)</td>
<td>r-Ag473 (10ug/dose)</td>
<td>PBS</td>
<td>PBS</td>
</tr>
<tr>
<td>Challenge strain</td>
<td>NmB-R3 10^2CFU</td>
<td>NmB-R3 10^1CFU</td>
<td>NmB-R3 10^2CFU</td>
<td>NmB-R3 10^1CFU</td>
</tr>
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</table>

**Mortality**

![Mortality Chart]

**Group A**

![Group A IgG Graph]

**Group B**

![Group B IgG Graph]
Establishment of lipid-based intrinsic adjuvant for subunit vaccine development

US patent No. 7,833,776 “Novel Lipidation Sequence for Lipoprotein Expression in E. coli system “
Rationale of using envelope protein domain III (ED III)

- A large proportion of the antibody responses following natural infection target prM. The anti-prM responses were highly cross-reactive and unable to effectively neutralize virus.
- ED II and NS1 contains epitopes which can induce autoantibodies bind to plasminogen, platelets and endothelial cells.
- ED III is the critical site associated with receptor binding & contains several neutralizing epitopes.
- Most identified CTL epitopes locate NS3 and NS5.
Rationale of using lipidated vaccine candidates
Conversion of non-lipoprotein to lipoprotein

Vaccine 2009 27:1400-1409
The residue of LPS is < 3EU/mg. This preparations were used for characterization and immunogenicity study.
APCs are activated by lipidated vaccine candidates

PLoS One 2011 6:e23319
Effect of lipidated vaccine candidates on cytokine production of BM-DC
APCs activation by lipidated vaccine candidates is mediated by TLR-2

![Graphs showing cytokine production](Mol Immunol 2010 47:2015-2021)
Lipoprotein induce neutralizing antibody responses without exogenous adjuvants

**Dengue-1**

- **D1ED III**
- **D1ED III + Alum**
- **LD1ED III**

**Dengue-2**

- **D2ED III**
- **D2ED III + Alum**
- **LD2ED III**

**Dengue-3**

- **D3ED III**
- **D3ED III + Alum**
- **LD3ED III**

**Dengue-4**

- **D4ED III**
- **D4ED III + Alum**
- **LD4ED III**

Neutralization titer
Lipidated vaccine candidates induce higher ED III-specific antibody responses
Lipidated vaccine candidates induce quickly anti-ED III antibody responses with high affinity.
Infection of dengue virus is blocked by non-lipidated and lipidated vaccine candidates

Lipidated vaccine candidates are in proper conformation and destroyed by heating.
Lipidated vaccine candidate induce long-lasting protective immunities

Thirty seven weeks after the first immunization, mice were challenged with dengue-2-infected K562 cells intraperitoneally and bled at 32 hours after challenge. Plasma virus titers were determined by focus-forming assays using BHK-21 cells. The detection limit of the assay is $2.3 \log_{10} \text{ ffu/mL}$. 
Summary

• TLR-2 mediates lipidated vaccine candidates to activate APCs.

• Lipidated vaccine candidates without exogenous adjuvant can elicit memory neutralizing antibody responses.

• Lipidated vaccine candidates without exogenous adjuvant can long-lasting protective immunities.

• Lipidated vaccine candidates reduce the risk of antibody-dependent enhancement.
Rationale of therapeutic HPV vaccines

**Prophylactic HPV vaccine**
- HPV L1
- VLPs
- B cells
- Plasma cells
- Block virus entry

**Therapeutic HPV vaccine**
- HPV E7
- DCs
- CD8
- IFN-γ
- IL-2/IFN-γ
- CD4
- Kill cancer cells
- HPV specific CTLs
- HPV infected cancer cells
Co-delivery of TLR2 ligands and antigens enhance immune responses

The selected target is E7 oncoprotein of human papillomavirus type 16.
Induction of a Th1-biased immune and CTL responses

Induction of higher levels of E7-specific cytotoxic T lymphocyte activity
Immunization with rlipo-E7m induces a strong anti-tumor effect.
Recombinant lipo-E7m combined with TLR9 agonist synergistically enhance T cells responses

16 Days tumor with TLR9 agonist
The rlipo-E7m could induce protective anti-tumor effect in vivo
Conclusion

Our current results have successfully demonstrated the merit of lipo-immunogens for novel subunit vaccine development.

Successfully applied to other antigens:

- TB
- pneumococcal
- C. difficile
- A. baumannii
- TTA
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<tr>
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<tbody>
<tr>
<td>Production of Lipidated Proteins In <em>E. coli</em></td>
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<td>TW I376385/US 8,426,163</td>
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<tr>
<td>Lipidating Sequences and Use thereof for Producing Lipidated Proteins in <em>E. coli</em></td>
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<td>TW I354023/US 7,833,776</td>
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<td>Adjuvants</td>
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<td>US 8466259</td>
<td>W/US/CN/EU</td>
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<tr>
<td>Lipidated Vaccine against Dengue Virus Infection</td>
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<td>US 8,287,880</td>
<td>TW</td>
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Awards

- Platform technology (lipidating signal sequence)
- The 7th National Innovation Award (第九屆國家新創獎)
- The silver invention award 2012 (國家發明獎銀牌)
- Therapeutic HPV vaccine
- The 9th National Innovation Award (第九屆國家新創獎)
Acknowledgement

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Dr. Steve Hsieh (Process development)

Dr. Yan Kwok (Bioanalytical & Product Characterization)
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