The findings in this presentation have not been formally disseminated by the FDA and should not be construed to represent any Agency determination or policy.
Vaccine Adjuvants

A paradigm shift in Vaccine Production

- Whole viral particles, live or inactivated: Very potent, Low safety
- Subunit antigens (peptides or proteins): Better safety, Lower potency

Why adjuvants are included in vaccines

- Improve immunogenicity of recombinant vaccines
- Increase breadth of protection
- Overcome reduced immune responses in targeted populations
- Reduce dose of antigen
Vaccine Adjuvants

• **Adjuvant** - an agent that is added to, or used in conjunction with a vaccine antigen to augment or potentiate (and possibly target) the specific immune response to the antigen

• Use in licensed vaccines:
  – **Adjuvants alone are not licensed; each specific antigen/adjuvant formulation is licensed**
**Peripheral organ**

**Immature dendritic cell**

1- Antigen uptake and Activation

2- Migration

**Antigen**

**Danger signals** (microbial products, RNA, CpG DNA...)

**Toll-Like Receptors, NOD, RLR, Inflammasome**

**Peripheral organ**

**Mature dendritic cell**

3- Antigen Presentation

Pathogen-specific response

**Lymph node**

**Lymph**

**T cell, B cells**

Cytokine & Antibody production

**Adjuvant activity**
Some Adjuvants can activate dendritic cells by mimicking components of microbes that bind to *Pattern Recognition Receptors (PRR)* on APCs. DNA genetic material from viruses and bacteria can activate dendritic cells. RNA genetic material from viruses can also activate dendritic cells.
## Adjuvants Categories and Status

<table>
<thead>
<tr>
<th>Adjuvant category</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral salts</td>
<td>Aluminum hydroxide</td>
<td>In many licensed vaccines</td>
</tr>
</tbody>
</table>
| Micro-fluidized detergents; emulsions; saponins | MF59, AS03, AF03 QS21, ISCOMS,W₈₀5EC | In clinical trials  
AS03/H5N1 influenza licensed in 2013 |
| TLR agonists                             | CpG(TLR9), MPL (TLR4), GLA-SE (TLR4), R848 (TLR7/8), Flagellin (TLR5), poly I:C, poly:C₁₂U (TLR3) | In clinical trials |
| Particulate delivery                     | nanoparticles, PLGA, VLP, Liposomes, Virosomes, CAF01, CAF-04 | In clinical trials |
| Carbohydrate-based                      | δ-inulin, chitin/chitosan,        | In clinical trials |
| Human cytokines, chemokines, activating ligands, DC targeting | IL-12, IL-15, GM-CSF, MCP-1, CD40L, DEC-205 | In clinical trials |
| Bacterial exotoxins                      | CT, LT (modified)                 | In clinical trials |
| **Combination Adjuvants**                | AS01, AS02, GLA-SE, ISCOMS, AS04 (MPL+Alum) | In clinical trials.  
AS04 in licensed HPV vaccine (Cervarix) |
Regulatory considerations of adjuvanted vaccines

- Regulatory pathways supporting development and approval of vaccines formulated with novel adjuvant are the same as for unadjuvanted vaccines

- Adjuvants are considered “constituent materials” (21 CFR 610.15)

- Efficient planning of the development pathway for any adjuvanted vaccine requires careful attention to preclinical testing, study design, dosing decisions, and safety monitoring

- Although manufacturers are not required to demonstrate the “added benefit” of adjuvanted vs. unadjuvanted vaccines in clinical comparative phase 3 studies, manufacturers should provide a justification for including an adjuvant in the vaccine

- Evaluation of safety of an adjuvanted vaccine needs to include special safety considerations
Licensure Pathways for adjuvanted vaccines

The same for adjuvanted and unadjuvanted vaccines!

• “Traditional” Approval
  – 21 CFR 601 Subpart A and C
    • Approval “…based on data… which demonstrate that the manufactured product meets prescribed requirements of safety, purity and potency…”

• Accelerated Approval
  – 21 CFR 601 Subpart E
    • Approval “…on the basis of adequate and well-controlled clinical trials establishing that the product has an effect on a surrogate endpoint …reasonably likely…to predict clinical benefit…”
    • Approval “..subject to the requirement that the applicant study the biological product further to verify and describe its clinical benefit…[such] studies would usually be already underway…such studies must also be adequate and well controlled. The applicant shall carry out such studies with due diligence”
When & how should the “added benefit” of the adjuvant be demonstrated?

• Manufacturers should provide a rationale for the use of adjuvant in their vaccine formulation, supportive data may be derived from:
  – Preclinical studies (e.g., in *vitro* assays and/or proof-of-concept studies in animal models)
  – Early clinical immunogenicity trials comparing adjuvanted vs. unadjuvanted vaccines to include
    • evidence of enhanced immune response,
    • antigen sparing effects, or
    • other advantages
  – Data from use of adjuvant with related vaccine antigens

• If available, information about the presumed mechanism of action of the adjuvant
Adjuvant Mode of Action (MOA)

- May help to predict desired effectiveness and unwanted safety signals

- What are the most informative studies?
  - in animals
  - In tissue culture (which cells? which species?)
  - Genomics / transcriptome / System Biology

_Do we have all the needed scientific tools to evaluate the safety and effectiveness of adjuvants?_
HOW TO IMPROVE PANDEMIC INFLUENZA VACCINES: Can adjuvants do the job?

Higher virus-neutralizing antibody titers

Increase breadth of protection

Improved antibody “quality”

Affinity maturation:
Stronger antibody binding to protective targets

Additional desired attributes:
• Reduced antigen dose
• Reduced number of vaccinations
• Long-term immunological memory
UNDERSTANDING ANTIBODY RESPONSES FOLLOWING INFLUENZA VACCINATION & INFECTION

- TRADITIONAL ASSAYS USED FOR VACCINE RESPONSES:
  - Hemagglutination Inhibition assay: HI
  - Virus Neutralization in MDCK cells: VN

NOVEL APPROACHES

WHOLE GENOME PHAGE DISPLAY LIBRARIES (GFPDL)
- ANTIBODY EPI TOPE REPERTOIRE (Hemagglutinin & RBD)

SURFACE PLASMON RESONANCE (SPR)
- ANTIBODY KINETICS (AFFINITY MATURATION)
  - On-Rate ($K_{on}$) / Disassociation Rate ($K_{off}$)
  - ANTIBODY ISOTYPE
CONSTRUCTION OF GENE-FRAGMENT PHAGE DISPLAY LIBRARY (GFPDL) OF INFLUENZA GENOME

Library of phages, each displaying a unique peptide sequence fused to gIIIp on the surface

AVIAN:
- H5N1 - A/Vietnam/1203/2004
- A/Indonesia/5/05
- H7N7 - A/Netherlands/2003
- H7N9 - A/Shanghai/1/2013

SWINE:
- H1N1 - A/California/04/2009
ADJUVANTS STUDIED

ALUMINUM ADJUVANT COMPOUNDS

- "ALUMINUM HYDROXIDE" (ALUMINUM OXYHYDROXIDE ALO(OH), BOEHMITE)
- "ALUMINUM PHOSPHATE" (AMORPHOUS ALUMINUM HYDROXYPHOSPHATE)


Oil-in-Water adjuvants

MF59 adjuvant

AS03 adjuvant

Other adjuvants in clinical development
IMMUNE RESPONSE GENERATED FOLLOWING SUBUNIT H5N1-Vietnam VACCINATION

NIAID-VANDERBILT TRIAL
No Adjuvant vs Alum vs MF59-Adjuvant

Bernstein et al, JID; 2008:1977; 1-9
MF59-ADJUVANTED H5N1-VIETNAM VACCINE GENERATED BROADER ANTIBODY PROFILE COMPARED TO UNADJUVANTED VACCINE (HAI-80)

ANTIGENIC DOMAINS IDENTIFIED FROM GFPDL ANALYSIS FOR FURTHER EVALUATION OF INDIVIDUAL ANTIBODY RESPONSES FOLLOWING H5N1 VACCINATION

H5N1 NEUTRALIZING MAbs BIND ONLY TO HA1 PURIFIED AT pH 7.2

ANTIBODY AVIDITY MEASUREMENTS IN POLYCLONAL SERUM BY OFF-RATE CONSTANTS USING SPR REAL TIME KINETICS ASSAY

HETEROGENEOUS SAMPLE MODEL

\[ L_1 + A \xleftrightarrow[k_{a1}]{k_{d1}} L_1 A \quad \text{and} \quad L_2 + A \xleftrightarrow[k_{a2}]{k_{d2}} L_2 A \]

\[ L + A_1 \xleftrightarrow[k_{a1}]{k_{d1}} LA_1 \quad L + A_2 \xleftrightarrow[k_{a2}]{k_{d2}} LA_2 \quad LA_1 + A_2 \xleftrightarrow[k_x]{k} LA_2 + A_1 \]
MF59 ENHANCES AFFINITY MATURATION FOR ANTI-H5N1-HA1 ANTIBODIES THAT CORRELATES WITH VIRUS NEUTRALIZATION

Khurana S, et al. (2011) Science Transl Med: 3 (85)
SUMMARY: Adjuvants improve the quality of Humoral responses against H5N1

• GFPDL & SPR analytical tools provided important new information on the potential of Adjuvants to reshape vaccine immune responses

• *Oil-in-water adjuvants and several prime-boost approaches generated broader antibody Diversity & stronger Antibody binding avidity against the HA1 globular head (blocking virus entry) compared with unadjuvanted H5N1 vaccines.*

  • Stronger HA1 antibody avidity correlated with improved neutralization antibody titers against multiple H5N1 viruses.

• In ferrets, higher antibodies avidity (HA1) correlated with better protection against lethal challenge with highly pathogenic H5N1 strains and reduced viral loads
Potential Benefits/Risks of Vaccine Adjuvants

**Benefits**

- Enhance / accelerate the immune response
- Prolong the immune response
- Focus the immune response (CMI vs. Ab, Th1/Th2)
- Diversify the immune response
- Increase antibody affinity
- Improve long term memory
- Special Patient Populations
- Dose sparing

**Risks**

- Increase reactogenicity
  - Local
  - Systemic effects, e.g., inflammation, fever, myalgia
- Nonspecific immune activation
  - Immune mediated diseases
  - Organ specific
  - Inflammatory diseases
  - Autoimmunity
Species specific adjuvant effects:
- Source and targets of proinflammatory cytokines, immune-modulators, pyrogenic (absence of rabbit reagents)
- Different organ and cell-type distribution of innate immune receptors: TLR 11/12 (mouse only); TLR8 (human only) different TLR9 cellular expression
- Differences in primary sequences and specificity of PRR between mice and humans (TLR4)
- Differences in skin composition and mucosal sites (lungs, URT, GI)
Human monocytoid cell line Mono Mac 6 (MM6):
Selection of Endotoxin as a comparator

- Studies in rabbits showed that 0.5 EU of endotoxin IM induced a 0.5°C increase in body temperature: 
  \textit{in vivo pyrogenicity threshold}

- Reference Endotoxin Standard was used to induce proinflammatory cytokines in MM6 cells in vitro. The response to 0.5 EU of endotoxin was considered as \textit{in vitro “safety” threshold}
Use of endotoxin standard to establish a threshold between "safe" and "non-safe" levels of pro-inflammatory cytokines in MM6 cell culture.

Zaitseva et al., Vaccine, 2012
Proinflammatory cytokines above the safe threshold were secreted by MM6 cells in response to TLR agonists Pam3CSK4, FSL-1, and flagellin.

IL-1β

TNF-α

IL-6

IL-8

Zaitseva et al., Vaccine, 2012
R848 but not MF59, Alum, or MPL, induced IL-1β above safety threshold

Zaitseva et al., Vaccine, 2012
Rabbits Study: IM administration of flagellin and FSL-1 induced rapid febrile response that was followed by up-regulation of CRP at 24 hrs

Zaitseva et al., Vaccine, 2012
Increase in PGE$_2$ in the plasma preceded febrile response in rabbits following i. m. FSL-1 administration

Zaitseva et al., Vaccine, 2012
Search for earlier marker of toxicity:

**PGE₂ contributes to initiation of febrile responses in vivo**

**In vitro:** TLR agonists but not adjuvants induced PGE₂ in human monocytes and in MM6 cells in vitro

(Zaitseva et. al. Vaccine 2012)
Summary

• The Risk/Benefit considerations are key to the evaluation of novel adjuvant-antigen products.

• Research on mechanisms of action in animal models and human cells to assess the activity of new adjuvants alone and in conjunction with antigen(s) is critical for early screening and safety assessments of novel adjuvants (down selection).
Assessing Safety of Vaccines containing Novel Adjuvants: clinical trials

• Specific inquiries regarding symptoms consistent with autoimmune and neuroinflammatory diseases
• Consider targeted laboratory screening assessment (e.g., CRP, fibrinogen, ANA, ANCA, Rheumatoid factor)
• Maintain banked serum and cell specimens when possible
• One-year clinic safety follow-up suggested
• Suggested comparisons (early in clinical development):
  – Adjuvanted vaccine vs. saline placebo
  – Adjuvanted vaccine vs. unadjuvanted antigen
• Favorable risk/benefit assessments will support continued investigation of novel adjuvants
Acknowledgements

CBER, FDA
Surender Khurana
Marina Zaitseva
Tatiana Romantseva
Yukinori Endo
Jody Manischewitz
Lisa King

Collaborators
Kathy Edwards (Vanderbilt)
Kanta Subbarao (NIAID)
Ruth Karron (Johns Hopkins)
Novartis
VRC/NIH

Marion Gruber
(OVRR Director)