Update on influenza vaccination using microneedle delivery

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Mark Prausnitz serves as a consultant and is an inventor on patents licensed to companies developing products related to this presentation. This potential conflict of interest is being managed by Georgia Tech and Emory University.
Selected immunological advantages of skin immunization

- Enhanced humoral immune responses
- Increased duration of immunity
- Increased breadth of immunity
Advantages of microneedle patch vaccine delivery

- Solid Metal
- Polymer

**Advantages**

- Avoid hypodermic needles
- Painless
- Enhanced stability
- Rapid distribution
- Dose sparing
- Low cost
- Potential self-administration
How does microneedle-based immunization initiate immune responses?

MN immunization results in an increase of cytokines produced by the skin resident populations important for neutrophils, monocytes and dendritic cells recruitment, which are key players in the innate immune response.
• **Objective 1**
  - Determine the effectiveness of seasonal influenza subunit vaccine strains encapsulated in dissolving microneedle patches in murine model.

• **Objective 2**
  - Determine contribution of Langerin+ cells in adaptive responses elicited by skin immunization with dissolving microneedle patches.

• **Objective 3**
  - Confirm vaccine stability in dissolving microneedle patches (*in vitro* and *in vivo* studies)

• **Objective 4**
  - Define efficacy of subunit vaccine in non-human primates after microneedle delivery
Objective 1: Efficacy of dissolving microneedles

Vaccine and routes of delivery

- A/Brisbane/59/07 (H1N1)
- A/Victoria/210/09 (H3N2)
- B/Brisbane/60/08

- Cohorts of BALB/c mice were immunized once with 3 µg HA of monovalent vaccine intramuscularly (unprocessed vaccine, IM; vaccine mixed with excipients, IM exc. ) or cutaneously (mMN, pMN)

Gelatin microneedles
Immunization with H1N1 vaccine conferred complete survival in all vaccinated cohorts

Challenge with 5xLD50 mouse-adapted A/Brisbane/59/07
H3N2 vaccine conferred complete survival in pMN cohorts, whereas mMN and IM cohorts showed partial or no survival.

Challenge with 5xLD50 mouse-adapted A/Victoria/210/09
Influenza B vaccine induced robust, long lasting immune responses by skin immunization

**Graph 1:**
- **X-axis:** wk 2, wk 4, wk 10
- **Y-axis:** anti-B/Brisbane/60/08 HAI titers Gmean ± 95%CI
- **Legend:**
  - Naive
  - IM
  - IM exc
  - mMNN
  - pMN

**Graph 2:**
- **X-axis:** wk4, wk10
- **Y-axis:** Anti-B/Brisbane/60/08 NT titers (Gmean±95%CI)
- **Legend:**
  - Naive
  - IM
  - IM exc
  - mMNN
  - pMN

**Notes:**
- a, b indicate statistical significance.
Conclusion 2: Langerin+ cells contribute to adaptive responses

Langerin-DTR skin

Western blot of HA in skin lysate

V: A/Brisbane/59/07
1-3: skin alone
4-6: skin at 0h
7-9: skin at 24h w/ DT
10-12: skin at 24h w/o DT

Antigen removal ± DT

Protective immunity ± DT

Rates of survival (%)

Days post infection

Conclusion 2: Langerin+ cells contribute to adaptive responses

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Antigen removal ± DT

Protective immunity ± DT

Rates of survival (%)

Days post infection
Conclusion 3: Vaccine in pMN is stable for at least 90 days at 25°C

Stability of HA in gelatin patches

Protective immunity after 3 months at 25°C
Objective 4: Vaccine efficacy in non-human primates

Three groups of monkeys (4 animals each) received 45 μg of the trivalent vaccine by:
- Hypodermic needle injection (IM)
- Metal microneedle arrays
- Polymer microneedle patches

<table>
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<th>Prime</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
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<td>Blood and nasopharyngeal swabs</td>
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<table>
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<tr>
<th>Boost</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
<th>Week 52</th>
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<tbody>
<tr>
<td>Blood</td>
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Samples collected
- Whole blood for CBC and WBC phenotype
- Serum for humoral responses (binding Abs, HAI, Neutralizing antibodies and plaque reduction assay)
- Plasma for chemokines/cytokines (Luminex)
- Whole blood for T cell responses (FACS, ELISPOT assays) and B cell responses (ASC and memory B cells)
- Nasopharyngeal swabs for mucosal responses (IgA)
All groups showed similar HAI titers against influenza A viruses after boost.
Mucosal IgA responses were higher in the dissolving microneedle group, with a peak between 14 and 21 days.
Animals immunized with pMN showed higher influenza-specific ASC numbers three weeks after prime and flu specific memory B cells 2 weeks after boost.
Skin vaccination induced similar levels of neutralizing antibody titers against all influenza viruses as the IM group.

**H1N1:** max titers 512-1024

**H3N2:** max titers 128

**B:** max titers 2048
• T cell immune responses were similar among all vaccinated groups:

• Comparable frequencies of activated CD4+ and CD8+ T\textsubscript{CM} and T\textsubscript{EM} cells among all vaccinated cohorts
• Comparable frequencies of proliferating CD8+ T\textsubscript{CM} and T\textsubscript{EM} cells among all vaccinated cohorts
• The dissolving microneedle group demonstrated more consistent proliferative capacity of CD4+ T\textsubscript{EM} cells

In the dissolving microneedle group we observed higher numbers of IL-4 secreting T cells with a peak 3 weeks after prime.

Cellular immune responses
• Although the sample size of this study is too small to draw conclusions the result gives us a hint of what we should expect in clinical trials: diversity of responses and at least non-inferiority between technologies

• More correlates of immunity should be included in vaccine technology development:
  ➢ Systemic humoral responses are not different between intramuscular or skin vaccination but mucosal responses may benefit from skin delivery

  ▪ Skin vaccination may exert quantitative but not qualitative advantages in T cell responses but in vaccines robustness of immune response is critical.
Future plans

- Phase I clinical trials of dissolving microneedles in 2015
- Improving immune responses in high risk populations
- Vaccine stability studies at various temperatures and extended times
- Novel adjuvants for skin immunization
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