Disclosure: Harold S. Nelson MD

Below I have disclosed relevant commercial associations that might pose a conflict of interest

- **Consultant Arrangements:**
  - Merck
  - Circassia

- **Stock/Other Equity Ownership:**
  - None

- **Patent Licensing Arrangements:**
  - None

- **Grants/Research Support:**
  - Circassia

- **Speakers' Bureau:**
  - None
New Approaches to Immunotherapy

Learning Objectives

Upon completion of this session the attendee should recognize other approaches that enhance the safety and/or efficacy of immunotherapy by:

1) Employing currently available allergen extracts by routes other than SCIT or SLIT

2) Employing modified extracts, adjuvants or stimulants of innate or acquired immunity.
Strategies to Improve the Safety and/or Efficacy of AIT

- Change to a more efficient route of administration.
- Increase the dose of allergen by reducing its allergenicity by chemical modification (allergoid), recombinant modification, or non-IgE-binding peptides.
- Use a Th₁- or Treg-promoting TLR ligand: CpG oligonucleotides, monophosphoryl lipid A or a CRL-ligand (mannan)
- Adjuvants: Probiotics, vitamin D, cytokine antagonists
Which of the following in NOT True

A. Clinical improvement with epicutaneous AIT can persist for 2 years without boosting.
B. Systemic reactions have been reported with epicutaneous AIT.
C. The clinical improvement with intralymphatic AIT can persist 3 years without boosting.
D. There is an increased risk of systemic reactions with intralymphatic AIT compared with standard SCIT.
E. Pain with intralymphatic AIT is similar to that with a venapuncture.
Epicutaneous Immunotherapy (EPIT)

- With EPIT the keratinocytes can be activated by physical irritation or adjuvants (in these studies tape-stripping X 10 was used). This also enhances penetration of allergen through the skin.
- Keratinocyte activation increases secretion of IL-1, IL-6, IL-8, TNF-\(\alpha\) and IFN-\(\gamma\)
- These cytokines favor maturation and emigration of dendritic cells to draining lymph nodes.

G Senti, TM Kundig. Imm Allergy Clin N.A. 2016;36:25-37
### G Senti et al. Allergy 2015;70:707-101
(Symptom reduction vs. baseline year)

<table>
<thead>
<tr>
<th># Active</th>
<th># Patches</th>
<th>Dose</th>
<th>Duration</th>
<th>↓ Active</th>
<th>↓ Placebo</th>
<th>Eczema/Systemic Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>12</td>
<td>21 µg Phl p 5</td>
<td>48 hours</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 50%, 2&lt;sup&gt;nd&lt;/sup&gt; 72%</td>
<td>A &gt; P .02 &amp; .005</td>
<td>64%, 0%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3 µg Phl p 5 15 µg Phl p 5 30 µg Phl p 5</td>
<td>8 hours</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;,44%, 51%,63% 2&lt;sup&gt;nd&lt;/sup&gt;,31% 53%.70%</td>
<td>31%</td>
<td>16-30%, 11%</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>21 µg Phl p 5</td>
<td>8 hours</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 48% 2&lt;sup&gt;nd&lt;/sup&gt; 40%</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; 10% 2&lt;sup&gt;nd&lt;/sup&gt; 15%</td>
<td>18%, 4%</td>
</tr>
</tbody>
</table>

**Note:**
- A > P indicates a statistically significant difference between the active and placebo groups.
- The table shows the number of active and placebo patches used, the dose of Phl p 5, the duration of the study, and the symptoms reduced at 1<sup>st</sup> and 2<sup>nd</sup> assessments, along with the percentage reduction compared to baseline for eczema/systemic reactions.
Intralymphatic Allergen Administration Renders Specific Immunotherapy Faster and Safer: A Randomized Controlled Trial


- Randomized, open label comparison of 3 years of subcutaneous injections (cumulative dose $4 \times 10^6$ SQ-U) or 3 intralymphatic injections at 4-week intervals (cumulative dose 3,000 SQ-U)
- Nasal tolerance was faster with IL injections (4 months versus 1 year) and persisted at 3 years.
- Systemic reactions were fewer with IL injections (6 mild vs. 18 mild and 2 severe).
- Other outcomes at three years were similar for the 2 approaches.
Symptom Scores: IL versus SC

Symptom Scores: IL versus SC

Years

hayfever

nasal congestion

nasal itching

sneezing

red eyes

ocular itching

asthma

dry cough

PNAS 2008;105:17908-12
Subsequent Studies with Intralymphatic Immunotherapy

- A study with Fel d 1 fused to an HIV-derived translocation peptide & an Ig invariant chain administered every 4 weeks increased nasal allergen tolerance 74-fold vs. < 3-fold for placebo (p < .001) (JACI 2012;129:1290-6)

- A double-blind study with 3 or 6 injections of grass pollen extract at 2-week intervals had no effect on symptoms in the following grass pollen season (JACI 2013;132:1248).
Strategies to Improve the Safety and/or Efficacy of AIT

- Change to a more efficient route of administration.
- Increase the dose of allergen by reducing its allergenicity by chemical modification (allergoid), recombinant modification, or non-IgE-binding peptides.
- Use a Th$_1$- or Treg-promoting TLR ligand: CpG oligonucleotides, monophosphoryl lipid A or a CRL-ligand (mannan)
- Adjuvants: Probiotics, vitamin D, cytokine antagonists
Which of the Following is True of All Recombinant Allergens?

A. They do not react with specific IgE
B. They do not induce an IgE response
C. They react with T-cell epitopes
D. All of the above
E. None of the above
A Highly Polymerized Grass Pollen Extract is Efficacious and Safe, etc.


- 121 subjects with ARC treated with a grass allergoid in A D-B, P-C 1-year study.
- Monthly maintenance contained 12 μg group 1 plus group 5 grass allergens
- Up-dosing with 2 injections 30 minutes apart on 2 days one week apart.
- Systemic reactions all grade 1.
- Compared to placebo symptoms reduced 34% and medication use 40%.
Cluster SCIT with High Polymerized Extract

Double-blind, Placebo-controlled, Dose-ranging Study of New Recombinant Hypoallergenic Bet v 1 in an Environmental Exposure Chamber


- 37 adults with allergic rhinitis due to birch pollen underwent 10 weekly injections (7 up-dosing and 3 maintenance) followed by 8 hours exposure to birch pollen in an EEC.

- Received placebo or a rBet v 1 with a stable folding variation that had reduced reactivity with IgE but retained all T cell epitopes.
Percent Change in Symptoms EEC (8 hour AUC)

- Systemic reactions: grade 2 systemic reactions (one each) with 160 mcg & 320 mcg
- Specific IgG1: Similar increase with 80-320 μg

Development and Preliminary Clinical Evaluation of a Peptide Immunotherapy Vaccine for Cat Allergy

Determined binding affinities of Fel d 1 peptides for 10 commonly expressed HLA-DR molecules.

Functional immunodominant peptides were identified by means of peptide induced proliferation and cytokine secretion from PBMC of allergic donors.

Histamine releasing activity was assessed to rule out reactivity with IgE.

Peptides of ragweed, grass, birch and house dust mite also developed.
Chamber Study with Fel d 1-derived Peptides

167 cat-allergic subjects received either placebo or two dosing regimens: 4 weekly intradermal injections of 6 nmol or 8 weekly intradermal injections of 3 nmol of Fel d 1-derived peptides.

Challenged in exposure chamber for 4 consecutive days at baseline & after 18-22 weeks.

94 returned after one year and 50 after 2 years for repeat chamber exposures.

Fel d 1-derived Synthetic Peptide Immuno-regulatory Epitopes Show a Long-term Treatment Effect in Cat Allergy Subjects.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>Baseline</th>
<th>18-22 Weeks</th>
<th>1 Year</th>
<th>2 Years</th>
<th>P-value 2nd year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>22</td>
<td>15.02</td>
<td>-3.81</td>
<td>-2.99</td>
<td>-2.02</td>
<td></td>
</tr>
<tr>
<td>8 X 3 nmol</td>
<td>17</td>
<td>16.18</td>
<td>-5.15</td>
<td>6.5%</td>
<td>7.9%</td>
<td>0.64</td>
</tr>
<tr>
<td>4 X 6 nmol</td>
<td>11</td>
<td>15.30</td>
<td>-6.38</td>
<td>34.6%</td>
<td>29.6%</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Fel d 1-derived Peptides: Results of a Phase III Study

- 1,245 subjects, 12-65 years of age who were sensitized to cats, had regular cat dander exposure, and a prescribed baseline level of symptoms were randomized to:
  - 4 or 8 intradermal injections of 6nmol Fel d 1-derived peptides, or
  - Placebo injections.

- Primary outcome combined rhinitis and medication scores one year after initiating treatment.

Circassia press release 20 June 2016
## Fel d 1-derived Peptides:
### Results of a Phase III Study

<table>
<thead>
<tr>
<th></th>
<th>Baseline TRSS</th>
<th>1-Year TRSS</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 X 6 nmol</td>
<td>14.5</td>
<td>5.7</td>
<td>-61.0%</td>
</tr>
<tr>
<td>8 X 6 nmol</td>
<td>14.2</td>
<td>5.5</td>
<td>-61.1%</td>
</tr>
<tr>
<td>Placebo</td>
<td>14.5</td>
<td>5.9</td>
<td>-59.5%</td>
</tr>
</tbody>
</table>

Company will cancel grass registration study and ragweed dose-ranging study. Continuation of phase IIb HDM study and birch phase II study, nearing completion, will continue.

Circassia press release 20 June 2016
Efficacy of 2 Months of Allergen-specific Immunotherapy with Bet v 1-derived Contiguous Overlapping Peptides in Patients with Allergic Rhinoconjunctivitis: Results of a Phase IIb Study..


- A randomized, double-blind, placebo controlled trial in 239 adults allergic to birch pollen.
Efficacy of 2 Months of Allergen-specific Immunotherapy with Bet v 1-derived Contiguous Overlapping Peptides

- 3 peptides of 49-71 amino acid length which encompass the entire Bet v 1 molecule with overlapping.
- Administered subcutaneously a half dose, followed by 4 full doses over 8 weeks completing 1 month before the birch pollen season.
- Treatments were: 50 μg, 100 μg, or placebo.
- Primary outcome symptom/medication scores in 2013.

Symptom/medication Scores during the Birch Pollen Season

Pollen Exposure (grains/m³)

Green = Placebo
Yellow = 100 μg
Red = 50 μg

50 μg reduced RSMS 26%
p = 0.015

Immunotherapy with Bet v 1-derived Contiguous Overlapping Peptides: Safety

- Late respiratory symptoms were reported by 13% on placebo, 35% of 50 μg and 50% on 100 μg; 6.5% of subjects reported drops in FEVs of ≥ 30% but none resulted in emergency treatment.
- These reactions were most severe with the first two injections and suggested T-cell activation.
- Two grade 3 systemic reactions requiring treatment occurred more than 60 minutes after injections.
- Dropouts due to TRAEs were 2.8% in the 50 μg and 9.8% in the 100 μg groups.

Allergen-specific Immunotherapy with Bet v 1-derived Contiguous Overlapping Peptides: Conclusions

- 5 injections of 50μg of overlapping peptides derived from Bet v 1 produced a 26% reduction in rhinitis symptom/medication scores during the ensuing birch pollen season.
- Systemic symptoms suggesting T-cell activation were common with early injections.
- A previous study reported 6/15 developed positive SPTs to the peptides by the end of treatment.

Development and Characterization of a Recombinant, Hypoallergenic, Peptide-based Vaccine for Grass Pollen Allergy


- Derived peptides from the major IgE-binding sites of the 4 major allergens of timothy (1,2,5,6)
- Selected those with: absent IgE binding, induction of IgG that blocked IgE-binding, reduced T-cell proliferation and release of inflammatory cytokines.
- Fused selected peptides with hepatitis B-derived PreS domain to serve as carrier and provide T-cell help
None of the fusion proteins showed detectable IgE reactivity. Antibodies were induced in rabbits with the fusion proteins and tested for blocking of IgE-binding. Fusion proteins produced less T-cell stimulation. A single protein with 4 recombinant PreS-fused grass pollen allergen peptides was selected for further studies.

141 grass-allergic subjects received 3 injections of BM32 over 2 months, a fall booster, and 3 injections prior to the second season (2014).

- Dose second year 20 μg each of 4 components
- Rhinoconjunctivitis symptom score reduced 25% vs. placebo (p=0.042)
- VAS reduced (p=0.014), RQLQ reduced (p<0.005), Symptom/medication score reduced (p=0.085).

“Planning to move into Phase III trials.”
Strategies to Improve the Safety and/or Efficacy of AIT

- Change to a more efficient route of administration.
- Increase the dose of allergen by reducing its allergenicity by chemical modification (allergoid), recombinant modification, or non-IgE-binding peptides.
- Use a Th$_1$- or Treg-promoting TLR ligand: CpG oligonucleotides, monophosphoryl lipid A (MPL), or a CRL-ligand (mannan).
- Adjuvants: Probiotics, vitamin D, cytokine antagonists
CpG DNA bound to Amb a 1: Results for the First Ragweed Season

Results for the Second Ragweed Season

A

B

CpG-Amb a 1
Study Design

- First preseason 6 weekly injections:
  - CpG-Amb a 1 - 1.2 mg to 30 mg n = 310
  - Placebo n = 152

- Second preseason 2 injections:
  - CpG-Amb a 1/CpG-Amb a 1 (booster) n = 135
  - CpG-Amb a 1/Placebo n = 130
  - Placebo/Placebo n = 133
The Effect of CpG-amb a 1 on Peak Season TNSS: Change From Baseline

<table>
<thead>
<tr>
<th>Year</th>
<th>CpG-Amb a 1 / PLACEBO</th>
<th>PLACEBO</th>
<th>CpG-Amb a 1 / CpG-Amb a 1 /PLACEBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>21.2% P=0.04 1.88</td>
<td>2.35</td>
<td>28.5% P=0.02 1.83</td>
</tr>
<tr>
<td>2005</td>
<td>21.2% P=0.04 1.88</td>
<td>2.35</td>
<td>21.2% P=0.04 1.88</td>
</tr>
</tbody>
</table>
January 8, 2007:

“One-year data from the two-year DARTT ragweed trial indicated that no meaningful ragweed-specific allergic disease was observed in the study population.” The trial was stopped.

May 16, 2008:

In an Environmental Exposure Chamber study ISS-Amb a 1-treatment reduced total nasal symptom score by 41% vs. placebo in 253 subjects in the ITT population (p=0.09). “Due to the wide variation in reported response, we have decided to discontinue clinical development”.

The Novel TLR-9 Agonist QbG10 shows Clinical Efficacy in Persistent Allergic Asthma


- QbG10 consists of: A protein shell derived from the bacteriophage Qbeta & DNA oligomer G10 rich in non-methylated CpG motifs.
- G10 is an A-type CpG that induces IFN-α production by pDCs and suppresses Th2 & stimulates Tregs.
- 63 patients with allergic asthma treated with ICS received 7 injections over 10 weeks of QbG10 or placebo.
The Novel TLR-9 Agonist QbG10 shows Clinical Efficacy in Persistent Allergic Asthma

- Subjects did not receive injections of allergen extracts since preliminary studies demonstrated equal effectiveness with or without allergen administration.
- After 4 weeks ICS dose decreased by 50% and after 8 weeks ICS discontinued.
- Evaluated at end of 12 weeks.

Immunotherapy with QβG10 (yellow) versus Placebo (red)

Weeks after Randomization

Delta FEV1 (mL) Delta ACQ Score

P-value (repeated measures): 0.002

* * * * * * * * *

Inhaled CS

↑50% reduction  ↑100% reduction

Cytos Biotechnology Announces Negative Topline Results of Clinical Trial.

- The Phase 2b study in patients with moderate to severe allergic asthma did not achieve a statistically significant reduction of the ACQ score or other endpoints compared to placebo.

- Cytos Management is evaluating the options of liquidation of the company or possible bankruptcy.

Media Release 14 April 2014
Ultrashort-specific Immunotherapy Successfully Treats Seasonal Allergic Rhinoconjunctivitis to Grass Pollen


- 1028 subjects were randomized to 4 injections of grass allergoid, tyrosine adsorbed with monophosphoryl lipid A (Grass MATA MPL) or placebo.
- Grass MATA MPL provided 13.4% benefit over placebo over all, but 38.3% at sites with a higher burden of disease.
- 2.5% active withdrew due to AEs, mostly local.
“The G204 trial was designed to explore higher dose regimens using the mobile environmental exposure chamber”
“The results did not determine a recommended dose for the Phase III trial”.
“Elsewhere in the pipeline we successfully completed a Phase IIb dose finding study for birch and have set up protocols for the HDM program in Spain and peanut in Switzerland”
Novel Vaccines Targeting Dendritic Cells by Coupling Allergoids to Nonoxidized Mannan Enhance Allergen Uptake and Induce Functional Regulatory T Cells through Programmed Death Ligand 1.


- C-type lectin receptors (CLR) are pattern recognition receptors expressed by DC that bind glycans, mediate endocytosis and cell signaling.
- The main CLRs expressed by DCs recognize mannose-terminated sugars.
- Mannan is a polysaccharide found in plants, fungi and yeast.
In this study it was shown that glutaraldehyde-polymerized allergens coupled to nonoxidized mannan were hypoallergenic vaccines, targeting DCs, enhancing allergen uptake and promoting FOXp3 Tregs cells through programmed death – ligand 1 (PD-L1).

Skin Prick Test with Grass Extracts

N = Unmodified extract
P = Polymerized extract
PM = Polymerized extracts coupled to mannan.
C+ = positive control

Allergen Uptake by Monocyte-derived Human Dendritic Cells

Cytokine Release by Grass Extracts from PBMCs from Allergic Donors

Strategies to Improve the Safety and/or Efficacy of AIT

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- Use a Th$_1$- or Treg-promoting TLR ligand: CpG oligonucleotides, monophosphoryl lipid A or a CRL-ligand (mannan).
- Adjuvants: Vitamin D, Probiotics, cytokine antagonists
The Clinical Effect of Vitamin D Supplementation Combined with Grass-specific Immunotherapy in children with Allergic Rhinitis


- 50 children, ages 5-12 years, enrolled in a randomized, double-blind, placebo-controlled trial of grass SLIT (300 IR tablet) with or with vitamin D 1000 IU daily for 5 months.
- Mean serum 25(OH)D rose from 48.8 to 94.8 ng/mL.
- SLIT plus vitamin D was more effective in reducing nasal symptoms (17.3 vs. 22.5, p = 0.04) and asthma symptoms (7.7 vs. 12.8, p = 0.001) than SLIT alone.
Combination of Specific Allergen and Probiotics Induces Specific Regulatory B Cells and Enhances Specific Immunotherapy Effect on Allergic Rhinitis


- 158 Chinese patients with perennial allergic rhinitis, monosensitized to HDMs were randomized to: placebo, SCIT with HDM extract, twice daily oral clostridium butyricum, or SCIT plus probiotic.
- Treated 6 months, with 6 month follow-up.
Scores for Nasal Symptom (left) and Medication (right)

Humoral Responses: IgG4 (left) and IgE (right)

Serum IL-4 (left) and Skin Prick Tests (right)

New Approaches to Immunotherapy

Conclusions

- A number of promising new approaches to AIT have failed, in follow-up studies.
- Never the less there remain promising approaches to improve the safety and/or efficacy of AIT by:
  1) Employing currently available allergen extracts by routes other than SCIT or SLIT
  2) Employing modified extracts, adjuvants or stimulants of innate or acquired immunity.