PCVs: the First Vaccines Against Normal Nasopharyngeal Microbiota Constituents

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“What you’re seeing and what you’re reading is not what’s happening”
“There are no facts, only interpretations.”

Friedrich Nietzsche
1844-1900

“The world is always interpretable otherwise, it has no meaning behind it, but countless meanings”

The value of provocation...

Banksy is an anonymous England-based street artist, vandal, political activist, and film director
The value of provocation...
The value of provocation...

EVE...
The “orthodox” PCV endpoint targets description

Colonization of vaccine serotypes

- Invasion
- Aspiration
- Local spread

IPD
- Bacteremia
- Meningitis
- Pneumonia

Otitis media

Cleary & Clarke, *Emerging Topics in Life Sciences*, 1:297–312, 2017
The Microbiology of the Nasopharynx and Upper Respiratory Tract

Streptococcus pneumoniae is a constituent of the normal nasopharyngeal flora!
Below are Vaccine-preventable Pathogens: Which is the exception?

- Smallpox
- Tuberculosis
- Diphtheria
- Tetanus
- B pertussis
- Poliovirus
- Hepatitis B
- Hepatitis A
- H. influenzae b
- S. pneumoniae
- Measles
- Rubella
- Varicella zoster virus
- Influenza virus
- Yellow fever
- Malaria
- Dengue
- Ebola
- Japanese encephalitis virus
- Rotavirus

Japanese encephalitis virus is the exception.
Inter-bacterial Associations Within the Nasopharyngeal Microbiome

- Pneumococcal serotypes compete with other pneumococcal serotypes for colonization

Cleary & Clarke, *Emerging Topics in Life Sciences*, 1:297–312, 2017
Inter-bacterial Associations Within the Nasopharyngeal Microbiome

- Pneumococcal serotypes compete with other pneumococcal serotypes for colonization
- Pneumococci interact with other flora components (i.e. NTHi, *M. catarrhalis*)
- Not all pneumococcal serotypes are similar in their characteristics (i.e. invasiveness, interaction with other flora components)

Cleary & Clarke, *Emerging Topics in Life Sciences*, 1:297–312, 2017
The “orthodox” PCV endpoint targets

Colonization of vaccine serotypes

- Invasion
- Aspiration
- Local spread

IPD
- Bacteremia
- Meningitis
- Pneumonia

Vaccine


Cleary & Clarke, Emerging Topics in Life Sciences, 1:297–312, 2017

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The “orthodox” PCV endpoint targets

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IPD

Bacteremia

Meningitis

Pneumonia

Otitis media

Non-vaccines serotypes

Interaction

antibiotics

Other constituents of the microbiota

virus
The “orthodox” PCV endpoint targets

Insights about a potential paradox
Vaccine against a normal flora constituent: The paradox
Licensed and Near Future PCVs

CRM_{197} conjugate (Pfizer)

PCV7

<table>
<thead>
<tr>
<th>Serum Type</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
</tr>
</thead>
</table>

>95 serotypes are currently recognized

CRM_{197} conjugate (Pfizer)

PCV13

<table>
<thead>
<tr>
<th>Serum Type</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
<th>1</th>
<th>5</th>
<th>7F</th>
<th>3</th>
<th>6A</th>
<th>19A</th>
</tr>
</thead>
</table>

*H. Influenzae* Protein D (4, 6B, 9V, 14, 23F, 1, 5, 7F); Tetanus toxoid (18C); Diphtheria toxoid (19F) (GSK)

CRM_{197} conjugate (Merck)

PCV15

<table>
<thead>
<tr>
<th>Serum Type</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
<th>1</th>
<th>5</th>
<th>7F</th>
<th>3</th>
<th>6A</th>
<th>19A</th>
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</thead>
</table>

22F 33F

CRM_{197} conjugate (Pfizer)

PCV20

<table>
<thead>
<tr>
<th>Serum Type</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
<th>1</th>
<th>5</th>
<th>7F</th>
<th>3</th>
<th>6A</th>
<th>19A</th>
</tr>
</thead>
</table>

22F 33F 8 10A 11A 12F 15B/C
Serotypes Compete with Other Serotypes on the “Privilege” of Being the Predominant Serotype

- A model of serotype prevalence suggested that serotypes with polysaccharides that are less metabolically costly (i.e. fewer carbons per repeat unit) will be more heavily encapsulated, will be able to avoid neutrophil-mediated killing, and will be more likely to be carried.
Serotypes Compete with Other Serotypes on the “Privilege” of Being the Predominant Serotype

- A model of serotype prevalence suggested that serotypes with polysaccharides that are less metabolically costly (i.e. fewer carbons per repeat unit) will be more heavily encapsulated, will be able to avoid neutrophil-mediated killing, and will be more likely to be carried.

![Graph showing the relationship between serotype prevalence and number of carbons per repeat unit.]

<table>
<thead>
<tr>
<th>PCV7</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV13</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Non-vaccine types</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

$r = -0.80; p < 0.001$ (excluding type 3)
A model of serotype prevalence suggested that serotypes with polysaccharides that are less metabolically costly (i.e. fewer carbons per repeat unit) will be more heavily encapsulated, will be able to avoid neutrophil-mediated killing, and will be more likely to be carried.
Pneumococcal Carriage in Children <5 Yrs Attending Pediatric Emergency Room, Southern Israel

8 children <5 yrs old seen each working day at the medical Pediatric ER since Nov 09

PCV7 + catch-up
PCV13 gradual

PCV13 serotype carriage 2016-2017 vs 2009-2010: -85%
Non-vaccine serotype carriage 2016-2017 vs 2009-2010: +45%

For the period of 07-12/09, data for Jewish children are from Nov-Dec only and for Bedouin children for Dec only

Ben Shimol et al, Human Vaccine and Immunotherapeutic 12:2, 268-276, 2016, updated
Presented at Paraguayan Conference of Infectious Diseases 16–17 April 2017; Asuncion, Paraguay
S. pneumoniae Serotype Distribution by Age Group among 641 Children Hospitalized in Utah with Invasive Pneumococcal Disease in Relation to Vaccination period

Square size denotes the number of cases

- 30
- 25
- 20
- 16
- 12
- 8
- 4
- 2
- 1

The Red Queen Hypothesis (in Pneumococcal Infections)

Stockmann et al. *conclusion*: “This vaccine-driven example of human/bacterial coevolution appears to confirm the Red Queen hypothesis, which reveals a limitation of serotype-specific vaccines and offers insights that may facilitate alternative strategies for the elimination of IPD”

Corrected trends in age-group specific IPD incidence in England and Wales 2000/01 - 2016/17

Ladhani et al, Lancet ID, 18: 441–51, 2018
Corrected trends in age-group specific IPD incidence in England and Wales 2000/01 - 2016/17

Other constituents of the microbiota:

- Antibiotics
- Viruses

Non-vaccine serotypes

Interaction

Colonization of vaccine serotypes

- Invasion
- Aspiration
- Local spread

Spread

- Indirect effects

Vaccine

IPD

Bacteremia

Meningitis

Pneumonia

Otitis media

Cleary & Clarke, *Emerging Topics in Life Sciences*, 1:297–312, 2017

Positive pneumococcus-NTHi association is serotype-specific

Selective – only few serotypes can do it

Permissive – any serotype can do it

Simpson's Diversity Index (%)

88 90 92 94 96

Colonization

$\text{p} < 10^{-4}$

$\text{p} = 0.022$

$\text{p} = 0.012$

$\text{p} = 0.11$

Otitis media

$\text{(Spn only)}$

$\text{(Spn+NTHi)}$

$\text{(Spn only)}$

$\text{(Spn+NTHi)}$

Pneumococcal Serotype Diversity in Carriage and MEF Isolates, With and Without Co-occurring NTHI


Permissive

Selective
Prevalence of Specific Pneumococcal Serotypes in S-OM and M-OM in 5,035 Pneumococcal OM Episodes* in Children <3 Years, Southern Israel, 1999-2008

Serotypes significantly more frequent in S-OM

Serotypes significantly more frequent in M-OM

S. pneumoniae S-OM (n=3039)  S. pneumoniae M-OM (n=1996)

* Submitted to Tympanocentesis or Presenting with Acute Otorrhea, Dagan, Leibovitz, Greenberg, Bakaletz, Givon Lavi, J Infect Dis, 208:1152–60, 2013
The nasopharyngeal microbial profiles of 97 PCV-7–vaccinated infants and 103 control infants participating in a randomized controlled trial in the Netherlands were analyzed by sequencing of the 16S rDNA gene, 12 and 24m after vaccination.

**Plausible speculation:** Replacement form PCV7 serotypes (often single-colonizers) to non-PCV7 serotypes (often mixed-colonizers with NTHi) → facilitating interaction with NTHi?
**Structural Equation Modelling (SEM) Analysis of Risk Factors for S. pneumoniae and H. influenzae Colonization**

Children <6 years old, 2012, Italy

<table>
<thead>
<tr>
<th>SEM analysis</th>
<th>S. pneumoniae</th>
<th>H. influenzae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff (95%CI)</td>
<td>p</td>
</tr>
<tr>
<td>Age standardized</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Young siblings</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Day-care centres attendance</td>
<td>0.54 (0.14; 0.94)</td>
<td>0.009</td>
</tr>
<tr>
<td>Parent’s smoking</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous respiratory infections</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vaccination status</td>
<td>PCV7 0.09 (-0.27; 0.45) 0.629</td>
<td>PCV7/PCV13 + PCV13* 0.43 (0.07; 0.79) 0.021</td>
</tr>
<tr>
<td></td>
<td>PCV7 -0.02 (-0.40; 0.35) 0.909</td>
<td>PCV7/PCV13 + PCV13* 0.45 (0.08; 0.82) 0.018</td>
</tr>
</tbody>
</table>

*Children who received the first doses of PCV7 followed by a dose of PCV13 or children under 24 months of age who had already received the full PCV7 vaccination series followed by one PCV13 dose.

*No pneumococcal change (no replacement)*

*NTHi Increase*

The Impact of PCV13 Era on the Nasal Microbiota Based on 16S rRNA Sequencing

Selected were the reads of the 5 most abundant bacterial families (*Moraxellaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Pasteurellaceae*, and *Corynebacteriaceae*), which were defined as the core microbiota, and oligotyping was done.

<table>
<thead>
<tr>
<th>Bacterial Family</th>
<th>OT</th>
<th>SNV</th>
<th>Taxonomic assignment</th>
<th>Abundance-based NBR</th>
<th>Binary-based LME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCV7</td>
<td>PCV13</td>
</tr>
<tr>
<td>Pasteurellaceae</td>
<td>P1</td>
<td>CA</td>
<td><em>H. influenzae</em> 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>TCC</td>
<td><em>H. influenzae</em> 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>CCC</td>
<td><em>H. influenzae</em> 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>CCT</td>
<td><em>H. influenzae</em> 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P5</td>
<td>TA</td>
<td><em>H. influenzae</em> 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P6</td>
<td>TCTTA</td>
<td><em>H. influenzae</em> 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacteriaceae</td>
<td>C1</td>
<td>G</td>
<td><em>C. pseudophrophiticum/propinquum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>T-</td>
<td><em>C. accolens</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcaceae</td>
<td>Sta1</td>
<td>TC</td>
<td><em>S. aureus</em> 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sta2</td>
<td>AA</td>
<td><em>S. capitis/caprae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sta3</td>
<td>TT</td>
<td><em>S. aureus</em> 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sta4</td>
<td>AGA</td>
<td><em>S. pasteurii/warneri</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxellaceae</td>
<td>M1</td>
<td>CA</td>
<td><em>M. nonliquefaciens/catarrhalis</em> 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>TA</td>
<td><em>M. lincolnii</em> 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>CG</td>
<td><em>M. nonliquefaciens/catarrhalis</em> 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M4</td>
<td>TGT</td>
<td><em>M. lincolnii</em> 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M5</td>
<td>AC</td>
<td><em>M. osloensis</em></td>
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<tr>
<td>Streptococcaceae</td>
<td>Stre1</td>
<td>G</td>
<td><em>S. mitis group</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stre2</td>
<td>CT</td>
<td><em>S. dentisani/oralis/tigurinus/oligofermentans/infantis</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Impact of PCVs on Otitis Media

* Complex = Recurrent/non responsive/spontaneously draining/often bilateral

PCV7

Acute OM

Complex* OM

Chronic OM

SP
NTHi
Mixed NTHi+SP
NTHi in biofilms
Culture negative

SP
NTHi
Mixed NTHi+SP
NTHi in biofilms
Culture negative

SP
NTHi
Mixed NTHi+SP
NTHi in biofilms
Culture negative

Palmu et al, PIDJ, 23:732-8, 2004
Impact of the Sequential PCV7/PCV13 Introduction to the NIP on Pneumococcal OM, Children <24m with MEF Culture*

Rate Reduction

* Many of whom had complex OM

88% (85 – 90%)

80% (77 – 82%)

61% (56 – 65%)

Each study year is July 1st through June 30th

Ben-Shimol, Givon-Lavi, Leibovitz, Raiz Greenberg, Dagan, Clin Infect Dis, 63:611-8, 2016, Updated

ESCMID eLibrary © by author
Impact of PCVs on Otitis Media

* Complex = Recurrent/non responsive/spontaneously draining/often bilateral

PCV7

Acute OM

Complex*

OM

Burden OM

Chronic OM

SP

NTHi

Mixed NTHi+SP

NTHi in biofilms

Culture negative

SP

NTHi

Mixed NTHi+SP

NTHi in biofilms

Culture negative

SP

NTHi

Mixed NTHi+SP

NTHi in biofilms

Culture negative

SP

NTHi

Mixed NTHi+SP

NTHi in biofilms

Culture negative

Palmu et al, PIDJ, 23:732-8, 2004

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Effect of PCV7 Followed by Polysaccharide Pneumococcal Vaccine on Recurrent AOM: a Randomized Study


Nasopharyngeal carriage of pneumococci

ITT analysis: rate ratio 1.25 (95% CI 0.99–1.57)

Vaccines
- Pneumococcal conjugate vaccine
- Hepatitis vaccine

Cumulative hazard

Time after complete vaccination (months)

Soeters, *CID*, 67:881–9, 2018

NTHi: Annual increase of 3%
Notification Rate for Cases of Invasive Haemophilus influenzae Disease, by Serotype and Year of Notification, in 12 Countries in Europe, 2007–2014, All Ages

8,781 cases notified from Belgium, Cyprus, the Czech Republic, Denmark, Finland, Ireland, Italy, the Netherlands, Norway, Slovenia, Spain, and the UK

NTHi: Annual increase of 7.4% (5.3% to 9.6%)

Whittaker, Emerg Infect Dis, 23:396-404, 2017
Cleary & Clarke, Emerging Topics in Life Sciences, 1:297–312, 2017

Colonization of vaccine serotypes

- Invasion
- Aspiration
- Local spread

IPD
- Bacteremia
- Meningitis
- Pneumonia

Otitis media

Indirect effects

Interaction

Non-vaccines serotypes

Viruses

Other constituents of the microbiota

Antibiotics

Spread
A Nonmetric Multidimensional Scaling (NMDS) Plot to Visualize the Associations between Nasopharyngeal Microbiota Clusters and Host Characteristics in Regard to RSV Infections in Children

A Nonmetric Multidimensional Scaling (NMDS) Plot to Visualize the Associations between Nasopharyngeal Microbiota Clusters and Host Characteristics in Regard to RSV Infections in Children

The Use of Nasopharyngeal Microbiota to Discriminate LRTIs from Health – A case Control Study

5 wks -5 yrs old children
- 154 LRI cases
- 307 matched healthy controls

Man et al, Lancet ID, http://dx.doi.org/10.1016/S2213-2600(18)30449-1, 2019
Relative Distribution of Carried Serotypes During RSV(+) CAAP and No-virus CAAP in Children <5 Yrs

Serotypes collaborating with NTHi

Serotypes antagonistic to NTHi

PCV13 serotype
Non-PCV13 serotype

Greenberg et al, J Infect Dis, 215:1111–6, 2017
Colonization of vaccine serotypes

Interaction

Viruses

Other constituents of the microbiota

Non-vaccines serotypes

Antibiotics

Invasion

Aspiration

Local spread

IPD

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Cleary & Clarke, Emerging Topics in Life Sciences, 1:297–312, 2017

Dynamics in Non-susceptibility Within each of the 6 Most Common Non-PCV13 Serotypes Carried in Children in Southern Israel

Upon PCV implementation

6 years post PCV7/13 implementation

15A (n=178)

15B/C (n=367)

16F (n=220)

23B (n=202)

11A (n=160)

Dynamics in Non-susceptibility Within each of the 6 Most Common Non-PCV13 Serotypes Carried in Children in Southern Israel

Danino et al, Clinical Infect Dis, DOI: 10.1093/cid/ciy926, 2019
For in much wisdom is much grief: and he that increaseth knowledge increaseth sorrow

Ecclesiastes Chapter 1(18)

King Salomon

Marc Chagall 1887-1985
“Your assumptions are your windows on the world. Scrub them off every once in a while, or the light won't come in.”

Alan Alda,
Born 1936
Actor, writer and director
"Orthodox" approach
"Orthodox" approach

Paradigm shift

Vaccine
Am I going too far with my speculations?

We do not really fully know where and how far we are going!!!
“Only those who will risk going too far can possibly find out how far one can go.”

T.S. Eliot, poet
1888 - 1965