Staphylococcal protein A vaccines
Therapeutics against recurrent S. aureus infection

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Conflicts of interest: research support NOVARTIS AG, GSK, JANSSEN; founder IMMUNARTIS, LLC; board member AVACYN, LLC; consulting JANSSEN, CRUCELL, CONTRAFFECT, GSK, MEDIMMUNE, NOVARTIS
**S. aureus** and MRSA infections in the United States of America

- **S. aureus** is a commensal of the human nares, skin and GI tract as well as an invasive pathogen
- US Department of Defense 2005-2010: **S. aureus skin and soft tissue infection (SSTI)** 122-168/100,000; **bacteremia** 3.6-6/100,000/year
- US DoD 2005-2010 annual incidence: community onset **MRSA bacteremia** 1.2-1.7/100,000; hospital onset 0.4-0.7/100,000
- 2010-2012 prospective study of 30,209 military trainees: 4.15% SSTI; 1.1% MRSA SSTI
- **Very-low-birth-weight infants** (VLBW) in the US 60,000/yr: 3.6% late onset (>72 h post delivery) **bacteremia/meningitis** (26% mortality)
- **End-stage renal disease patients** undergoing hemodialysis annual incidence: invasive MRSA infection 4.2/100 patients
- MRSA infection in **surgical patients** occurs in spite of antibiotic prophylaxis (0.8-1%); **recurrence** is frequent (8-21% for bacteremia patients)
- Are there non-antibiotic means of preventing **Staphylococcus aureus** infection in high risk patients? Immunotherapy, vaccination?

M. Landrum et al. 2012, JAMA 308:50  
M.W. Ellis et al. 2014, CID 58:1540  
A. Shane et al. 2012, Pediatrics 129:914  
D.B. Nguyen et al. 2013, CID 57:1393
Previous and current attempts to develop *Staphylococcus aureus* vaccines *misled by mouse models for preclinical efficacy?*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Mechanism</th>
<th>Target</th>
<th>Status</th>
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<tr>
<td>StaphVAX</td>
<td>NABI</td>
<td>Vaccine</td>
<td>CP5/CP8</td>
<td>failed phase 3</td>
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<tr>
<td>Altastaph</td>
<td>NABI</td>
<td>Antibody</td>
<td>CP5/CP8</td>
<td>ended</td>
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<tr>
<td>Pentastaph</td>
<td>NABI/GSK</td>
<td>Vaccine</td>
<td>CP5/CP8</td>
<td>failed phase 3</td>
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<td>Aurograb</td>
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<td>Antibody</td>
<td>lipoprotein</td>
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<tr>
<td>Veronate</td>
<td>INHIBITEX</td>
<td>Antibody</td>
<td>ClfA</td>
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<td>Tefibazumab</td>
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<td>ClfA</td>
<td>ended</td>
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<tr>
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<td>BIOSYNEXUS</td>
<td>Antibody</td>
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<tr>
<td>V710</td>
<td>MERCK</td>
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<td>IsdB</td>
<td>failed phase 3</td>
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<tr>
<td>SAR279356</td>
<td>SANOFI</td>
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<td>PNAG</td>
<td>ended</td>
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<tr>
<td>NVD3</td>
<td>NOVADIGM</td>
<td>Vaccine</td>
<td>Als3</td>
<td>phase 1/2</td>
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<tr>
<td>STEBVax</td>
<td>IBT</td>
<td>Vaccine</td>
<td>Seb</td>
<td>phase 1</td>
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<tr>
<td>SA3Ag</td>
<td>PFIZER</td>
<td>Vaccine</td>
<td>CP5+8/ClfA</td>
<td>phase 2a</td>
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<td>PF-06290510</td>
<td>PFIZER</td>
<td>Vaccine</td>
<td>CP5+8/ClfA/MntC</td>
<td>phase 2b</td>
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<tr>
<td>MEDI4893</td>
<td>MEDIMMUNE</td>
<td>Antibody</td>
<td>Hla</td>
<td>phase 2b</td>
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</tbody>
</table>
Staphylococcal protein A (SpA)

- Staphylococcal protein A, a surface protein, binds vertebrate immunoglobulin on the bacterial surface.
- Protein A is comprised of five immunoglobulin binding domains with high sequence conservation.
- Region X spans the cell wall; the sorting signal promotes SpA anchoring to peptidoglycan.
- Protein A blocks antibody-induced opsonophagocytosis of staphylococci.
- All clinical *S. aureus* isolates express protein A.


M. Uhlén et al. 1984, JBC 259:1695
O. Schneewind et al. 1992, Cell 70:267
O. Schneewind et al. 1995, Science 268:103
Anchoring surface proteins to the envelope of *Staphylococcus aureus*
Contribution of surface proteins & sortase to *S. aureus* abscess formation in mice

<table>
<thead>
<tr>
<th><em>S. aureus</em></th>
<th>P-value</th>
<th># Abscess (5d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>------</td>
<td>4.4</td>
</tr>
<tr>
<td>sasA</td>
<td>0.2568</td>
<td>2.3</td>
</tr>
<tr>
<td>sdrE</td>
<td>0.5023</td>
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<tr>
<td>fnbpA</td>
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<tr>
<td>fnbpB</td>
<td>0.2074</td>
<td>2.0</td>
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<tr>
<td>clfB</td>
<td>0.1298</td>
<td>1.9</td>
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<tr>
<td>sasB</td>
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<td>1.7</td>
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<tr>
<td>sasD</td>
<td>0.1272</td>
<td>1.5</td>
</tr>
<tr>
<td>sasC</td>
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<td>1.4</td>
</tr>
<tr>
<td>sasF</td>
<td>0.3187</td>
<td>1.3</td>
</tr>
<tr>
<td>sasG</td>
<td>0.0770</td>
<td>1.2</td>
</tr>
<tr>
<td>clfA</td>
<td>0.0848</td>
<td>1.1</td>
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<tr>
<td>isdH</td>
<td>0.0859</td>
<td>1.1</td>
</tr>
<tr>
<td>isdC</td>
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<tr>
<td>sdrD</td>
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<tr>
<td>isdB</td>
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<tr>
<td>isdA</td>
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<td>0.4</td>
</tr>
<tr>
<td>spa</td>
<td>0.0356</td>
<td>0.4</td>
</tr>
<tr>
<td>srtA</td>
<td>0.0216</td>
<td>0.0</td>
</tr>
</tbody>
</table>

M. McAdow et al. 2011, PLoS Pathog. 7:e1002307
Staphylococcal protein A (SpA)

**S. aureus Infection**

- **S. aureus**
- Protein A (SpA)
- IgG

**Inhibition of opsonophagocytosis**

**Inhibition of antibody responses**

*References:*


C. Goodyear & G. Silverman 2003, JEM 197:1125
S. aureus spa mutants that cannot bind immunoglobulin

F. Falugi et al. 2013, mBio 4:e00575
Host immunoglobulin is required for \textit{S. aureus} pathogenesis

F. Falugi \textit{et al.} 2013, mBio 4:e00575
Virulence defects of *S. aureus spa* mutants

<table>
<thead>
<tr>
<th>S. aureus</th>
<th>Load / $\log_{10}$CFU g$^{-1}$</th>
<th>Significance P</th>
<th># Abscesses</th>
<th>Significance P</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>6.20 ± 0.43</td>
<td>--</td>
<td>8.50 ± 1.75</td>
<td>--</td>
</tr>
<tr>
<td>$spa_{KK}$</td>
<td>5.29 ± 0.41</td>
<td>0.0924</td>
<td>2.50 ± 0.74</td>
<td>0.0023</td>
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<tr>
<td>$spa_{AA}$</td>
<td>4.70 ± 0.53</td>
<td>0.0528</td>
<td>5.11 ± 1.41</td>
<td>0.1383</td>
</tr>
<tr>
<td>$spa_{KKAA}$</td>
<td>4.24 ± 0.47</td>
<td>0.0069</td>
<td>2.85 ± 0.98</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

F. Falugi *et al.* 2013, mBio 4:e00575
Prior infection with the $spa_{KKAA}$ mutant elicits protective immunity against S. aureus re-infection.

F. Falugi et al. 2013, mBio 4:e00575
Antigen-specificity of antibodies in human blood with or without *S. aureus* infection

N. Pauli et al. 2014, JEM 211:2331
**Staphylococcus aureus** infection expands VH3 plasmablasts (PB) in human blood

N. Pauli *et al.* 2014, JEM 211:2331
Antigen-specificity of PB BCRs (antibodies) in human blood with or without *S. aureus* infection

N. Pauli et al. 2014, JEM 211:2331
S. aureus infection elicits V_H3 clonal immunoglobulin expansion in mice

H. K. Kim et al. 2016, under revision
A model for protein A release from the staphylococcal cell wall

S. Becker et al. 2014, PNAS 111:1574
Peptidoglycan-linked SpA, not recombinant SpA, triggers $V_H^3$ clonal expansion

H. K. Kim et al. 2016, under revision
Non-toxigenic protein A vaccine ($\text{SpA}_{\text{KKAA}}$)

H. K. Kim et al. 2010, JEM 207:1863
Efficacy of the SpA<sub>KKAA</sub> vaccine against *S. aureus* USA300 LAC infection in mice

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Staphylococcal load and abscess formation in renal tissue</th>
<th>log&lt;sub&gt;10&lt;/sub&gt; CFU</th>
<th>P-value</th>
<th>Reduction (log&lt;sub&gt;10&lt;/sub&gt;CFU)</th>
<th>IgG Titer</th>
<th>Number lesions</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
<td></td>
<td>7.20 ± 0.24</td>
<td>–</td>
<td>–</td>
<td>&lt;100</td>
<td>4.0 ± 0.8</td>
<td>–</td>
</tr>
<tr>
<td>SpA</td>
<td></td>
<td>6.81 ± 0.26</td>
<td>0.2819</td>
<td>0.39</td>
<td>476</td>
<td>3.3 ± 1.0</td>
<td>0.5969</td>
</tr>
<tr>
<td>SpA&lt;sub&gt;KKAA&lt;/sub&gt;</td>
<td></td>
<td>3.66 ± 0.76</td>
<td>0.0001</td>
<td>3.54</td>
<td>10,200</td>
<td>1.2 ± 0.5</td>
<td>0.0109</td>
</tr>
</tbody>
</table>

H. K. Kim *et al*. 2010, JEM 207:1863
SpA$_{KKAA}$ as a therapeutic vaccine
Immune responses to S. aureus in vaccinated mice

H. K. Kim et al. 2010, JEM 207:1863
$\text{SpA}_{\text{KKAA}}$-derived monoclonal antibodies (SpA$_{\text{KKAA}}$-mAbs)

SpA<sub>KKAA</sub>-mAbs prevent <i>S. aureus</i> infection in mice

H. K. Kim <i>et al.</i> 2012, Infect. Immun. 80:3460
SpA_{KKAA}-mAb prevents *S. aureus* sepsis in neonatal mice and promotes immunity

V. Thammavongs a *et al.* 2015, *Vaccine* 33:523
Humanized $\text{SpA}_{\text{KKAA}}$-mAb prevents $S.\ aureus$ sepsis in neonatal mice

V. Thammavongsa et al. 2015, Vaccine 33:523
Host adaptation of *S. aureus* interpreted as SpA binding to vertebrate immunoglobulin
Guinea pig bloodstream infection with the *S. aureus* $spa_{KKAA}$ mutant

H. K. Kim *et al.* 2015, *mBio* 6:e002369
Immunization of guinea pigs with the SpA\textsubscript{KKAA} vaccine

H. K. Kim et al. 2015, mBio 6:e002369
Summary

• SpA blocks the effector function of human, guinea pig and mouse antibodies directed against staphylococci

• SpA blocks B cell responses in humans > guinea pigs > mice

• SpA\textsubscript{KKAA} immunization protects mice and guinea pigs against \textit{S. aureus} bloodstream infection

• SpA\textsubscript{KKAA} immunization elicits SpA-neutralizing antibodies that enable broad spectrum immune responses against \textit{S. aureus} antigens.

• SpA\textsubscript{KKAA}–mAb administration also enables broad spectrum immune responses in animals with \textit{S. aureus} infection

• The guinea pig model for \textit{S. aureus} bloodstream infection may be useful to predict clinical trial success
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