PNAG: broadening infection protection

by Michael J. Haas, Senior Writer

A multi-institutional team has added more than 20 pathogens to the list of known bacteria that express poly-*N*-acetylglucosamine, a bacterial capsule polysaccharide that provides a potentially broad-spectrum target for

vaccination.¹ The findings open up new indications for **Alopetx Vaccine LLC** and **Alopetx Pharmaceuticals LLC**, which are already developing a vaccine and an antibody for passive immunization, respectively.

Over the past two decades, studies by multiple groups have identified poly-*N*-acetylglucosamine (PNAG), a polysaccharide encoded by a conserved four-gene locus, as a component of the surface capsule in at least seven species of bacteria.²⁻⁷

PNAG is one of many polysaccharides and other carbohydrate polymers found on the outer surface of bacteria that help prevent host macrophages and other cells from engulfing the pathogens.

Anti-PNAG antibodies have been found in circulation in humans and other animals, but these naturally occurring antibodies do not effectively kill PNAG-expressing bacteria or provide adequate immune protection against them, casting doubts on their prophylactic potential.⁸⁻¹⁰

Between 2005 and 2007, teams led by Gerald Pier showed that a

Table 1. Adding pathogens to the PNAG list. A study in *Proceedings of the National Academy of Sciences*¹ has added 24 more species of Gram-positive bacteria, Gram-negative bacteria, fungi, yeast and parasites to the existing list of pathogens that express the surface antigen poly-*N*-acetylglucosamine (PNAG). There now are about 40 known species of pathogens that express the molecule.

Pathogen species ^A	Pathogen type	Type of infection or disease ^B	Effect of anti-PNAG antibody ^C	
			<i>in vitro</i>	<i>in vivo</i>
<i>Bacillus subtilis</i>	Gram-positive bacteria	Commensal; food poisoning	Pathogen death	Unknown
<i>Campylobacter jejuni</i>	Gram-negative bacteria	Gastroenteritis	Unknown	Unknown
<i>Citrobacter rodentium</i>	Gram-negative bacteria	Veterinary (only in mice)	Unknown	Unknown
<i>Clostridium difficile</i>	Gram-positive bacteria	Nosocomial infections	Unknown	Unknown
<i>Enterococcus faecalis</i>	Gram-positive bacteria	Nosocomial infections	Pathogen death	Unknown
<i>Haemophilus ducreyi</i>	Gram-negative bacteria	Chancroid (genital ulcers)	Unknown	Unknown
<i>H. influenzae</i>	Gram-negative bacteria	Bacteremia, pneumonia, other infections	Unknown	Unknown
<i>Helicobacter pylori</i>	Gram-negative bacteria	Gastritis; gastric ulcers	Unknown	Unknown
<i>Listeria monocytogenes</i>	Gram-positive bacteria	Listeriosis	Unknown	Pathogen death
<i>Mycobacterium smegmatis</i>	Gram-positive bacteria	None known	Unknown	Unknown
<i>M. tuberculosis</i>	Gram-positive bacteria	Tuberculosis	Unknown	Unknown
<i>Neisseria meningitidis</i>	Gram-negative bacteria	Meningitis	Pathogen death	Pathogen death
<i>N. gonorrhoeae</i>	Gram-negative bacteria	Gonorrhea	Pathogen death	Unknown
<i>Salmonella enterica</i> serovars Typhi and Typhimurium	Gram-negative bacteria	Gastrointestinal infections	Unknown	Unknown
<i>Streptococcus dysgalactiae</i>	Gram-positive bacteria	Veterinary	Unknown	Unknown
<i>S. pneumoniae</i>	Gram-positive bacteria	Pneumonia, otitis media and other infections	Pathogen death	Pathogen death
<i>S. pyogenes</i>	Gram-positive bacteria	Group A streptococcal infections	Pathogen death	Pathogen death
<i>Candida albicans</i>	Yeast	Nosocomial, eye, urinary tract and other infections	Pathogen death	Pathogen death
<i>Aspergillus flavus</i>	Fungus	Aspergillosis	Unknown	Unknown
<i>Fusarium solani</i>	Fungus	Corneal infection	Unknown	Unknown
<i>Cryptococcus neoformans</i>	Fungus	Respiratory	Unknown	Unknown
<i>Plasmodium berghei</i>	Parasite	Malaria (in animal models)	Unknown	Pathogen death
<i>P. falciparum</i>	Parasite	Malaria	Unknown	Unknown
<i>Trichomonas vaginalis</i>	Parasite	Vaginitis and urogenital infections	Unknown	Unknown

^APathogen species listed are the PNAG-expressing pathogens identified in ref. 1. ^BKey illnesses associated with the pathogen. ^CPathogens that F598 (SAR279356), a human mAb against PNAG and deacetylated PNAG made by **Alopetx Pharmaceuticals LLC** and **Sanofi**, killed *in vitro* and/or in mice, as reported in ref. 1. Previous studies also reported that F598 killed three additional pathogens *in vitro* and in mice: the *Burkholderia cepacia* complex, including *B. dolosa*, which causes pneumonia in patients with cystic fibrosis (CF);¹⁰ *Escherichia coli*¹¹ and *S. aureus*,¹³ including methicillin-resistant strains (MRSA).

deacetylated form of PNAG (dPNAG) elicited bacteria-killing antibodies in mice.^{6,11} His teams also developed a human mAb—dubbed F598—that bound to both dPNAG and PNAG and killed bacteria.¹²

Pier is professor of medicine in microbiology and immunology at

Harvard Medical School and a microbiologist in the department of medicine at **Brigham and Women's Hospital**.

In the current study, Pier's team investigated whether PNAG was expressed on bacteria and other pathogens in which the four-gene PNAG-producing locus has not yet been identified.

The group used F598 conjugated to a fluorescent agent to detect PNAG on the surfaces of pathogens and found the molecule in 24 pathogens not previously known to express it. Among the new pathogens were disease-causing bacteria such as *Streptococcus pneumoniae*, *Clostridium difficile* and *Mycobacterium tuberculosis*, as well as the yeast *Candida albicans* and the parasite *Plasmodium falciparum* (see Table 1, "Adding pathogens to the PNAG list").

In human cell-based bactericidal assays, F598 increased killing of *C. albicans*, *Neisseria meningitidis*, *S. pneumoniae* and other pathogens compared with an inactive control antibody.

Lastly, the team tested the protective effect of the mAb in mice challenged with selected pathogens. In mice exposed to *C. albicans*, *N. meningitidis*, *S. pneumoniae*, *S. pyogenes* or *P. burghei*, pretreatment with the mAb led to decreased symptoms of eye infection, meningitis, lung infection, skin infection or cerebral malaria, respectively, compared with pretreatment with a control antibody or control serum.

"Our new study extends the list of PNAG-expressing organisms to most major human pathogens, including 9 of the top 10 causes of nosocomial infections" as reported by the **Centers for Disease Control and Prevention (CDC)**, Pier told *SciBX* (see Table 2, "Nine out of ten for PNAG"). "This implies that a PNAG vaccine or passive immunotherapy with the anti-PNAG antibody could target a huge range of organisms with just one inoculation or treatment."

Pier noted that *Pseudomonas aeruginosa* is the only bacterium on the CDC's top 10 list that does not express PNAG.

Pier's team included researchers from **Boston University School of Medicine**, **Dana-Farber Cancer Institute**, **Harvard School of Public Health**, **University of Massachusetts Medical School**, **The Ohio State University College of Medicine**, **The Research Institute at Nationwide Children's Hospital**, **Walter Reed Army Institute of Research**, **N.D. Zelinsky Institute of Organic Chemistry** and **Sanofi**, which contributed some of the bacteria studied and tested F598 in the animals challenged with *S. pneumoniae*.

Data were reported in *Proceedings of the National Academy of Sciences*.

"The new study underscores that we have something unusual and even unique in our PNAG vaccine," said Daniel Vlock. "First, we have a target that is expressed incredibly broadly in pathogens. Second, we can produce the vaccine via chemical synthesis, which is certainly cleaner than recombinant methods, and we hope it will be simpler and cheaper as well."

In 2006, Pier and Vlock cofounded Alopexx Pharmaceuticals to develop F598 as a passive immunotherapy to protect against bacterial infections. Four years later, the duo cofounded Alopexx Vaccine, which has the dPNAG-based vaccine AV0318 in preclinical development to prevent bacterial infections in humans and production animals.

Vlock is CEO of both companies, which are part of the group **Alopexx Enterprises LLC**, which provides investment, managerial and development capabilities to its multiple subsidiaries.

In 4Q13, Alopexx Vaccine plans to begin testing AV0318 in cows to prevent mastitis caused by *S. aureus* and *Escherichia coli* and in pigs to prevent a severe form of pneumonia caused by *Actinobacillus pleuropneumoniae*, Vlock said.

"The immunogenicity and any toxicity we see in these animals will have an impact on what we can do in humans," he added.

Alopexx Vaccine expects to begin a Phase I trial of AV0318 in humans next year. Vlock declined to disclose the target populations or indications.

Meanwhile, Pier's team is testing F598 *in vitro* and in animals challenged with *E. coli* K1, Group B *Streptococcus* and other pathogens that cause neonatal infections in humans.

Table 2. Nine out of ten for PNAG. Multiple studies have determined that the surface antigen poly-*N*-acetylglucosamine (PNAG) is expressed on all but one of the pathogens responsible for the 10 most frequent nosocomial infections as reported to the **Centers for Disease Control and Prevention (CDC)** for 2006–2007.¹⁴ These 10 pathogens accounted for 87% of reported nosocomial infections in the U.S., including central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia and surgical-site infections. Source: Refs. 1,10,13,15; **Alopexx Vaccine LLC**

Pathogen	Type	PNAG expression ^A	% of all nosocomial infections
<i>Staphylococcus epidermidis</i> and other coagulase-negative staphylococci	Bacteria	Yes	15
<i>S. aureus</i>	Bacteria	Yes ¹³	15
<i>Enterococcus</i> species, including <i>E. faecalis</i>	Bacteria	Yes (<i>E. faecalis</i>) ¹	12
<i>Candida albicans</i>	Yeast	Yes ¹	11
<i>Escherichia coli</i>	Bacteria	Yes	10
<i>Pseudomonas aeruginosa</i>	Bacteria	No ¹	8
<i>Klebsiella pneumoniae</i>	Bacteria	Yes	6
<i>Enterobacter</i> species, including <i>E. cloacae</i>	Bacteria	Yes (<i>E. cloacae</i>) ¹⁰	5
<i>Acinetobacter baumannii</i>	Bacteria	Yes ¹⁵	3
<i>K. oxytoca</i>	Bacteria	Yes	2

^AIndicates whether the pathogen expresses PNAG on its surface, as reported by the source cited.

Pier said Brigham and Women's Hospital and **Beth Israel Deaconess Medical Center** hold a portfolio of patents covering the PNAG technology.

The antibody-related IP from that portfolio is licensed to Alopexx Pharmaceuticals, while the vaccine-related IP is licensed to Alopexx Vaccine, Vlock said.

In 2010, Sanofi exercised an option from Alopexx Pharmaceuticals to in-license F598 (SAR279356), which the pharma now has in Phase II testing to prevent bacterial infections.

Last December, Sanofi terminated a Phase II trial of SAR279356 for patients on mechanical ventilation in intensive care units because of difficulties with patient recruitment.

Sanofi declined to discuss the antibody's status or how Pier's new study might affect its development plans.

Haas, M.J. *SciBX* 6(23); doi:10.1038/scibx.2013.566

Published online June 13, 2013

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COMPANIES AND INSTITUTIONS MENTIONED

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