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Title:

A Phase 1 Randomized, Placebo-Controlled, Observer-Blinded Trial to Evaluate the Safety and Immunogenicity of Inactivated *Streptococcus pneumoniae* Whole-Cell Vaccine in Adults

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Abstract

Background: Broadly protective pneumococcal vaccines that are affordable for low-resource countries are needed. *Streptococcus pneumoniae* whole cell vaccine (PATH-wSP) is an investigational vaccine that contains killed cells from a non-encapsulated strain of *S. pneumoniae* (SPn) formulated with aluminum hydroxide adjuvant. Preclinical studies demonstrated protection against both nasopharyngeal carriage (T-cell mediated) and invasive pneumococcal disease (antibody-mediated). The aim of this randomized, double-blind, placebo-controlled Phase 1 study was to assess the safety, tolerability, and immunogenicity of PATH-wSP in healthy adults and to determine whether this vaccine candidate should progress into a Phase 2 trial.

Methods: Forty-two (42) participants were randomized into three dose cohorts to receive 0.1, 0.3, or 0.6 mg of PATH-wSP or placebo (saline). Participants received a three-dose vaccination schedule spaced by four-week intervals. Post-vaccination assessments included solicited reactogenicity events through day 7, blood chemistry and hematology assessments at day 7, and all adverse events (AEs) through day 84. Participants were monitored for serum antibody and peripheral blood mononuclear cell cytokine responses to pneumococcal antigens. A six-month telephone follow-up was completed to assess for any additional AEs related to vaccination.

Results: PATH-wSP was safe and well tolerated. Reactogenicity was acceptable and no untoward safety signals were observed. PATH-wSP elicited significant immunoglobulin G responses to multiple pneumococcal antigens, including pneumococcal surface protein A and pneumolysin, as measured by enzyme-linked immunosorbent assay. Functional antibody responses were observed with the highest dose of PATH-wSP (0.6 mg) using passive antibody transfer followed by SPn challenge in mice and with a pneumolysin toxin-neutralizing antibody

assay. Increases in T-cell cytokine responses, including interleukin 17A, were also seen among PATH-wSP vaccinees.

Conclusions: PATH-wSP was safe and well tolerated in healthy US adults, eliciting pneumococcal antigen-specific antibody and T-cell cytokine responses.

Key words: vaccine, pneumococcal, Phase 1, immunogenicity, whole-cell, dosing

Clinical Trial Registry: NCT01537185

Introduction

Currently licensed pneumococcal conjugate vaccines (PCVs) were designed to target the 10 to 13 serotypes that are the most prevalent cause of invasive pneumococcal disease (IPD), but no licensed vaccine exists that protects against all pneumococcal serotypes. Following the introduction of PCVs, pneumococcal associated disease in young children has been significantly reduced [1]. A number of studies, however, have documented replacement carriage in the nasopharynx with serotypes not included in the vaccines [2]. Recently, an increase in pneumococcal disease caused by these non-vaccine serotypes has been recorded [3, 4]. Furthermore, currently licensed PCVs are relatively expensive to produce and require substantial donor assistance for low-resource countries to be able to afford them. Additional pneumococcal vaccines are needed that are more affordable to manufacture, provide sufficient global supply, and can offer the broadest protection possible to prevent pneumococcal pneumonia and IPD, including reducing the chance for non-PCV serotype disease emergence.

Vaccines that contain proteins common to essentially all pneumococcal serotypes could potentially offer broad protection to children worldwide. A number of Phase 1 and 2 studies have been conducted or are underway to assess specific *Streptococcus pneumoniae* (SPn) proteins that might be included in a vaccine in the future [5-9]. An alternative strategy is to use whole pneumococcal cells that contain numerous proteins and also have inherent adjuvant properties. Given the manufacturing process for a whole cell vaccine (e.g., high yields and low costs), if such a vaccine induced a strong immune response it would have the potential to provide broad protection at an affordable price. Here we describe a first-in-human Phase 1 clinical study to evaluate the safety, tolerability, and immunogenicity of an experimental SPn whole cell vaccine candidate adsorbed to aluminum hydroxide (Alum) adjuvant (PATH-wSP) in healthy US adults.

Materials and Methods

Study design and participants: This was a Phase 1 study conducted between February 13, 2012 and May 22, 2013 [10]. The study was reviewed and approved by the Western Institutional Review Board and conducted in compliance with the study protocol, international standards of Good Clinical Practice and the Declaration of Helsinki. The study was conducted at a single center, Comprehensive Clinical Development Northwest, in Tacoma, Washington, United States.

Participants considered for eligibility were healthy adults aged 18 to 40 years at the time of consent, without evidence of the following: chronic health issues; abnormal screening clinical labs; history of invasive pneumococcal disease or pneumococcal vaccination; contraindications to vaccination; recent vaccination; or receipt of blood products. Forty-two participants were enrolled into one of three dose cohorts to receive 0.1, 0.3, or 0.6 mg (protein content) of PATH-wSP, or placebo (saline) using an electronic randomization block design with sequential subject assignment by data management. Pharmacy staff were unblinded and responsible for preparing and administering vaccinations. Study participants and all others involved in conducting the trial, including laboratories, remained blinded to treatment assignment.

Each dosing cohort received a series of three vaccinations at 28-day intervals with a dose escalation design. In each cohort, participants were randomized to either PATH-wSP (n=10) or placebo (n=4). Participants were monitored for one hour post-vaccination before release from the clinic and then self-reported local and systemic reactogenicity events (REs) for seven days post-vaccination using a standard diary scoring card. Local REs included injection site pain, tenderness, erythema, induration, and itching. Systemic REs included headache, muscle pain, temperature (oral), nausea, vomiting, fatigue, diarrhea, joint aches, and chills. Safety laboratory testing occurred at seven days following each vaccination. Adverse events (AEs) were assessed

at each visit and categorized by Medical Laboratory for Regulatory Activities System Organ Class (MedDRA SOC) and MedDRA Preferred Term (PT) and analyzed by study cohort, severity, duration, and relationship to vaccine. An internal safety team reviewed blinded reactogenicity and laboratory results weekly. An unblinded independent data safety monitoring committee gave authority to allow dose escalation or to alter the study should a safety signal emerge. Pre-specified pause rules included any serious AE (SAE), a grade 3 clinical or laboratory abnormality or >2 subjects having the same grade 3 injection site reaction or grade 2 laboratory abnormality.

Vaccines. *S. pneumoniae* whole-cell antigen bulk, Lot No. 1676, was manufactured by Walter Reed Army Institute of Research from strain RM200 RX1E PdT ΔlytA (genetically modified to remove the *lytA* gene). The virulence factor pneumolysin gene was replaced with a gene encoding for a pneumolysoid containing three point mutations that abolish cytolytic activity and complement activation [11]. Beta-propriolactone was utilized to inactivate cells during processing, and the final drug product (*S. pneumoniae* whole cell antigen [SPWCA]) was stored at -80°C until the day of use. Dosage was specified by protein content as determined by the Kjeldahl assay, which represents approximately half the dry weight. Alum, Lot No. 1008198, was formulated at Instituto Butantan by diluting commercial Alhydrogel® with normal saline and stored at 2 to 8°C. PATH-wSP doses were formulated on the day of vaccination by adsorbing SPWCA to Alum at room temperature for one hour prior to vaccination. The final formulation contained 0.6 mg of elemental aluminum per dose. Normal saline was used as the placebo. All vaccinations were given intramuscularly in the lateral deltoid muscle.

Study hypothesis and objectives. The primary hypothesis was that PATH-wSP would be safe and well tolerated. This objective was evaluated by solicited reactogenicity through 7 days and

by unsolicited AEs through 84 days post-vaccination. A secondary hypothesis was that an increase in antibody responses over baseline to PATH-wSP vaccination would be measurable. An extensive number of assays were included in this early stage of vaccine development and, therefore, a staggered approach to sample analysis was performed.

Assays were either developed specifically or adapted for use in this vaccine development program. Briefly, the SPWCA, Pneumolysoid (L460D), and pneumococcal surface protein A (PspA) enzyme-linked immunosorbent assays (ELISAs) were developed and validated by Charles River Laboratories, Montreal, and specific antibody responses were measured following PATH-wSP vaccination. The Antibodies in Lymphocyte Supernatant (ALS) assay measured the acute response of B-cells recently stimulated by PATH-wSP vaccination by culturing peripheral blood mononuclear cells (PBMCs) and measuring antibody responses in culture supernatants by ELISA [12]. The Boston Children's Hospital (BCH) ELISA measured antibodies to SPWCA, eight SPn specific proteins, and pneumococcal cell wall polysaccharide. Three assays were utilized for assessing cytokine responses. An Intracellular Cytokine Staining (ICS) assay identified the T-cell phenotype ($CD4^+$ or $CD8^+$) and the cytokines/cell surface markers produced following *in vitro* stimulation of PBMCs with SPWCA [13]; the Multiplex Bead Array (MBA) used a Luminex® platform to measure multiple cytokines after *in vitro* stimulation of PBMCs with SPWCA; and interleukin 22 (IL-22) was measured by standard ELISA.

In addition, four functional assays were assessed for future utility in the PATH-wSP vaccine development program. Serum antibodies were assessed for their ability to neutralize wildtype pneumolysin-induced lysis of rabbit red blood cells (Ply-nAb). The validated multiplex opsonophagocytic assay (MOPA) was performed according to the methods of Romero-Steiner and assessed the ability of antibodies to facilitate the killing of 14 *S. pneumoniae* serotypes (6C

and those contained in Prevnar13®) by phagocytes [14]. The Surface Killing Assay (SKA) measured opsonized pneumococci after overnight growth on blood agar plates overlaid with polymorphonuclear cells [15]. An intravenous challenge model for pneumococcal sepsis, which has been described previously, was utilized as the Passive Protection Assay (PPA) [5]. Briefly, mice were injected intraperitoneally with 100 μL of various dilutions of pre- and post-immunization serum. After four hours, mice were challenged intravenously with a lethal dose of virulent serotype 3 SPn (A66.1). Mice were monitored for 14 days at 4-hour intervals and scored for moribund status.

Statistical Methods: Safety data were descriptive in nature and summarized by treatment group, vaccination period, and, in the case of AEs, by MedDRA SOC and PT. The intention-to-treat population was analyzed for all safety evaluations. Immunogenicity testing was by treatment group (pre- and post-baseline or change from baseline) and tested using the *t* test and Fisher's exact test or other test as indicated in the results section. Analyses did not include any unmatched (pre/post vaccination) sample pairs. The study was designed to provide preliminary safety and immunogenicity data to support testing the study product in additional larger cohorts of adults and in age-descending studies, and was not statistically powered for pre-specified endpoints.

Results

One hundred forty-seven (147) participants gave informed consent and were evaluated. Eighty-eight (88) failed to meet eligibility requirements, 17 withdrew consent, and 42 were randomized into the trial. The demographics of trial participants can be found in Table 1. Compliance with the vaccination schedule was high, with only three participants having a delay in vaccination (all at the final vaccination).

Safety

Local REs were reported among 60 to 100% of participants given varying doses of PATH-wSP and 16.7 to 25% of those given the placebo. The higher rate of local REs is a common occurrence among participants receiving Alum-adjuvanted vaccines when compared to injection with saline. Maximum local reactogenicity tended to occur with the first dose of PATH-wSP, and was typically reduced with subsequent vaccinations. Nearly all the local REs were graded as mild or moderate, with duration ranging from one to four days. The most common solicited REs were pain and/or tenderness at the site of injection. Two participants who received 0.3 mg of PATH-wSP reported severe pain with the first vaccination but did not seek medical attention, and on repeat vaccinations pain was classified as mild. No events of local necrosis or abscess formation were observed. No volunteer refused further vaccination due to REs.

Solicited systemic REs were mild in nature and did not increase with repeated injections of the vaccine. No participant reported a severe systemic RE. Overall, systemic REs were less frequent than local REs—ranging from 10 to 60% among participants given varying doses of PATH-wSP and 8 to 25% among those given the placebo. No obvious trends were observed across successive injections or reactogenicity type, and no one event appeared dominant when considering dosage or vaccination sequence. There were no safety laboratory changes of clinical significance observed, and fluctuations were consistent with normal day-to-day variations. Forty-five (45) unsolicited AEs were reported by 24 participants in the study (18 of 30 participants receiving PATH-wSP and 6 of 12 participant receiving placebo, respectively). Of these, five participants had mild AEs rated as possibly related to receipt of clinical trial material. These cases included three cases of injection pain, one headache that extended beyond the seven-day

post-vaccination period (all resolved by day 10), and one episode of dysfunctional uterine bleeding three days post vaccination (n=2, n=3 for PATH-wSP 0.3 mg and 0.6 mg, respectively). The other 40 AEs were distributed relatively equally between all four treatment groups, and all resolved by day 84 of the study. A single SAE (ruptured ectopic pregnancy with inadequate contraceptive method) occurred during the trial, resolved without sequelae, and was deemed not related to vaccination. At the six-month follow-up phone call there were no AEs reported related to vaccination and no new SAEs.

Immunogenicity

SPWCA ELISA and ALS assays. The anti-SPWCA serum immunoglobulin G (IgG) response, as measured by ELISA, was chosen as the secondary endpoint since it measured responses to a broad array of antigens present in the vaccine. Anti-SPWCA IgG was assessed for each subject comparing day 0 baseline to day 28 post each vaccination (days 28, 56, and 84). No significant change from baseline was detected at any PATH-wSP dose, nor at any post vaccination time point using the SPWCA ELISA (Figure 1A). Recognizing the potential limitations of the SPWCA ELISA with respect to pre-existing antibody responses in adult subjects, the ALS assay was selected as a potential way to reduce background responses and, therefore, increase sensitivity. The ALS assay is designed to “capture” B-cells recently stimulated (e.g., by vaccination) and to, therefore, measure PATH-wSP-specific stimulated antibody responses without being confounded by high pre-existing pneumococcal antibody titers [12]. The ALS assay demonstrated that PBMCs from individuals [previously?] vaccinated with PATH-wSP secreted significantly greater concentrations of pneumococcal antibodies compared to baseline, whereas the placebo subjects showed no response (Figure 1B).

Antibody responses to specific SPn antigens following PATH-wSP vaccination. A variety of individual SPn antigens contained within the whole-cell vaccine may be immunogenic. We selected cohort 3 subset samples (days 0 and 84) to test prospectively using the BCH ELISAs. Median fold-rises in antibody levels were statistically significant ($P<0.05$) in vaccinated participants for antibodies against eight of the ten antigens tested using the two-fold cut off criteria (data not shown). Collectively, all eight of the 0.6-mg-vaccinated subjects made a response to at least one of the pneumococcal-specific proteins (Table 3).

After analysis of the BCH ELISA results, PspA and Pneumolysoid (L460D) ELISAs were developed and validated after data lock and used to further assess immune responses among PATH-wSP vaccinees. Geometric mean titers were significantly increased at 28 days following final vaccination (day 84) with 0.6 mg PATH-wSP for Ply (2.6-fold) and PspA (2.4-fold) ($P<0.05$ and $P<0.001$, respectively), with Ply demonstrating a dose-dependent response (Fig. 2). No changes were observed in the placebo treatment group.

T-cell cytokine responses to PATH-wSP vaccination (day 0 versus day 84). Significant increases in PBMC $CD4^+$ ICS responses were seen for 0.6 mg PATH-wSP recipients (but not other treatment groups) with specific increases in IL-17A ($P<0.01$), CD40L ($P<0.05$) , IL-2 ($p<0.01$), and TNF-α ($P<0.05$) (data not shown). For the MBA assay of PBMC culture supernatants, only IL-17A demonstrated a consistent increased response to SPWCA stimulation *in vitro* when comparing day 84 to baseline, with a significant increase seen following vaccination in participants receiving 0.6 mg of PATH-wSP- ($p <0.01$) and a trend in the 0.3 mg vaccinated group (Figure 3). The IL-22 ELISA did not demonstrate a measurable increase with any treatment group (data not shown).

Functional immune responses to PATH-wSP vaccination. PPA was utilized to assess functional responses for placebo- and 0.6-mg PATH-wSP-vaccinated recipients (n=9 and n=9, respectively) who had paired serum samples from day 0 and day 84. Testing was initially performed to compare pre- versus post-immunization responses using a 1:50 dilution of sera with additional assessment performed at dilutions of 1:10 or 1:100 for recipients noted to have a weak post-response or a strong baseline response, respectively.

A significant increase in median time to moribund state in SPn-challenged mice was seen with the sera from six of the nine participants vaccinated with 0.6 mg PATH-wSP; whereas, serum from only one of nine placebo-treated individuals provided increased protection at day 84 compared to pre-vaccination (Table 4). One 0.6-mg-vaccinated participant had high levels of protective antibody at baseline and a change in response post-vaccination could not be ascertained.

The MOPA and Ply-nAb assays were performed on the cohort-3 subset using baseline and 84 day post-vaccination sera. PATH-wSP did not induce opsonophagocytic activity to any of the serotypes tested (data not shown). Four of nine participants receiving 0.6 mg PATH-wSP demonstrated at least a four-fold rise in Ply-nAb titer versus none of the placebo-treated participants (data not shown).

Discussion

Developed through a partnership between PATH, Instituto Butantan, and BCH, PATH-wSP has been shown in preclinical studies to mediate its protective responses via both T-cell (IL-17A) and B-cell immune pathways, thereby having the potential to reduce both pneumococcal carriage and disease. Pneumococcal vaccines that incorporate common protein antigens also have the potential to overcome some of the major limitations of PCVs by providing broad coverage against all serotypes and can be produced with less manufacturing complexity and cost. The non-encapsulated whole-cell vaccine candidate, PATH-wSP, was shown to be well tolerated based on local and systemic reactogenicity profiles in this Phase 1 study in healthy adult participants, inducing both T- and B-cell immune responses. Multiple vaccinations did not result in escalating reactogenicity, which can sometimes be seen with other whole cell vaccines such as whole cell pertussis [16].

Both SPn-specific immunologic activity as well as functional (protection) activity was demonstrated most consistently at the highest dose of PATH-wSP tested (0.6 mg). Specific pneumococcal proteins known to be involved in the pathogenicity of SPn were shown to have PATH-wSP antibody-mediated immune responses. No one specific antibody response to a single antigen was identified in all recipients, although both PspA and Ply antibody responses were detected in 75% of the 0.6-mg vaccinated participants. A platform of SPn-specific assays may be needed to fully characterize the response to a whole-cell pneumococcal vaccine. In addition, similar to preclinical findings, PATH-wSP vaccination stimulated *in vitro* IL-17 responses from PBMC, with the 0.6-mg dose providing the best response. Another compelling feature of this Phase 1 trial was the demonstration of functional protective antibodies using both a PPA model and a pneumolysin toxin neutralization assay (Ply-nAb). Since 0.6 mg of PATH-wSP was the

only dosage that induced consistent measureable immune responses over baseline, an optimal dose in adults was not likely identified in this study. Further dose escalation is warranted to achieve a response in all participants (or at least in those with low baseline antibody levels).

Trial limitations included small sample size, limited validated assays, and no established immune markers for an SPn whole cell vaccine. At the time of this writing, a Phase 2 trial to assess dose-escalation to 1 mg (adults) and age de-escalation in toddlers with and without co-administration of expanded program on immunization vaccine boosters is underway in Kenya. Given the potential for this vaccine to impact SPn carriage (via IL-17A responses), an exploratory carriage study in these same toddlers is being conducted. Further age de-escalation to infants is planned with a goal to develop a cost-effective vaccine capable of protecting against SPn carriage, pneumonia, and IPD.

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394 **Table 1. Demographics and treatment compliance**

	Treatment 1 (0.1 mg) N=10	**Treatment 2 (0.3 mg) N=10**	**Treatment 3 (0.6 mg) N=10**	**Placebo N=12**	**Total N=42**
Sex (N) female/male	3/7	4/6	7/3	8/4	22/20
Age (years) mean (standard deviation [SD]) **N (%)**	28.9 (5.4)	25.2 (6.1)	29.9 (6.8)	28.5 (7.0)	28.1 (6.4)
Weight (kg) mean (SD) **N (%)**	83.3 (17.8)	94.1 (26.2)	78.5 (21.4)	83.2 (26.1)	84.7 (23.1)
Systolic/diastolic blood pressure mean mmHg (SD) at baseline **N (%)**	114 (14.0)/71 (5.7)	116 (11.5)/71 (11.4)	109 (12.4)/74 (10.7)	118(10.0)/75 (8.7)	
Ethnicity (N) and race Hispanic or Latino/ Non-Hispanic or Non-Latino (total)	2/8	3/7	2/8	2/10	9/11
Black or African American (n/total)	2/10	2/10	4/10	2/12	10/42
White/Caucasian (n/total)	7/10	7/10	5/10	10/12	29/42
Other (n/total)	1/10	1/10	1/10	0/12	3/42
Vaccinations completed N (%) At least 1	10 (100.0)	10 (100.0)	10 (100.0)	12 (100.0)	42 (100.0)
At least 2	9 (90.0)	8 (80.0)	9 (90.0)	10 (83.3)	36 (85.7)
All 3	9 (90.0)	8 (80.0)	9 (90.0)	9 (75.0)	35 (83.3)
Completed day 84 visit N (%)	9 (90.0)	5 (50.0)	9 (90.0)	9 (75.0)	32 (76.2)
Completed 6-month safety phone call N (%)	7 (70.0)	4 (40.0)	8 (80.0)	6 (50.0)	25 (59.5)
Lost to follow-up at day 84 N (%)	1 (10.0)	5 (50.0)	1 (10.0)	3 (25.0)	10 (23.8)
Total lost to follow-up by 6-month phone call N (%)	3 (30.0)	6 (60.0)	2 (20.0)	6 (50.0)	17 (40.5)

395

396 **Table 3. Antibody responses to eight selected pneumococcal proteins in placebo and 0.6 mg**
397 **PATH-wSP vaccinees**

		Antigen (gene locus number or name)								
	N	0191	0785	1031	1119	1479	1500	1942	Ply	Individuals with at least 2-fold rise in IgG
Placebo	3	0	0	0	0	0	0	0	0	0
600 µg	8	5	3	4	0	2	3	1	6	8/8

Abbreviation**:** Ply = pneumolysin; IgG = immunoglobulin G.

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Table 4 Passive transfer of protection

Treatment Group	**Placebo**			**600 µg PATH-wSP**								
Subject No.	**074**	**101**	**119**	**100**	**107**	**108**	**113**	**115**	**120**	**128**	**135**	**146**
MST - Pre	31	209	30	31	138	46	56	30	140	114	50	>336
MST - Post	29	150	26	286	>336	138	193	119	>336	138	>336	>336
(Dilution)	(1:50)	(1:50)	(1:50)	(1:50)	(1:10)	(1:10)	(1:10)	(1:50)	(1:50)	(1:100)	(1:100)	(1:50)
P value test	NS	NS	NS	*	NS	*	*	*	*	NS	*	NS

Abbreviation: MST-Pre = median survival time pre-vaccination (hours); MST-Post = median survival time post-vaccination. NS = Post MST not significantly different from Pre MST; * $p < 0.05$ (using 2-tailed Wilcoxon 2-sample rank test). Dilution = dilution of serum transferred.

403 **Figure Legends**

404 **Figure 1.** Immunoglobulin G (IgG) responses following vaccination with PATH-wSP measured
405 by *S. pneumoniae* whole cell antigen (SPWCA) enzyme-linked immunosorbent assay (ELISA)
406 and Antibodies in Lymphocyte Supernatant (ALS) assays. A. Pre and post dose 3 serum IgG
407 immune responses measured by SPWCA ELISA. B. Pre and post dose 3 IgG responses measured
408 from cultured peripheral blood mononuclear cells using the ALS assay.

409 **Figure 2.** Immunoglobulin G responses following vaccination with PATH-wSP measured by
410 pneumolysoid and pneumococcal surface protein A enzyme-linked immunosorbent assays.

411 **Figure 3.** IL-17 production in peripheral blood mononuclear cells following *in vitro* stimulation
412 with *S. pneumoniae* whole cell antigen (SPWCA).

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