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Original article

Safety and immunogenicity of the epicutaneous reactivation of pertussis toxin immunity in healthy adults: a phase I, randomized, double-blind, placebo-controlled trial

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ABSTRACT

Objectives: Protection induced by acellular vaccines can be short, requiring novel immunization strategies. Objectives of this study were to evaluate safety and capacity of a recombinant pertussis toxin (PTgen) -coated Viaskin® epicutaneous patch to recall memory responses in healthy adults.

Methods: This double-blind, placebo-controlled randomized trial (Phase I) assessed the safety and immunogenicity of PTgen administered on days 0 and 14 to healthy adults using Viaskin® patches applied directly or after epidermal laser-based skin preparation. Patch administration was followed by Boostrix®dTpa on day 42. Antibodies were assessed at days 0, 14, 28, 42 and 70.

Results: Among 102 volunteers enrolled, 80 received Viaskin-PT (Viaskin-PT 25 µg ($n = 25$), Viaskin-PT 50 µg ($n = 25$), laser + Viaskin-PT 25 µg ($n = 5$), laser + Viaskin-PT 50 µg ($n = 25$)), Viaskin-placebo ($n = 10$) or laser + Viaskin-placebo ($n = 2$). Incidence of adverse events was similar across groups (any local event: 21/25 (84.0%), 24/25 (96.0%), 4/5 (80.0%), 24/25 (96.0%), 8/10 (80.0%), 10/12 (83.0%), respectively). Direct application induced no detectable response. On day 42, PT-IgG geometric mean concentrations were significantly higher following laser + Viaskin-PT 25 µg and 50 µg (139.87 (95% CI 87.30–224.10) and 121.76 (95% CI 95.04–156.00), respectively), than laser + Viaskin-placebo (59.49, 95% CI 39.37–89.90). Seroresponse rates were higher following laser + Viaskin-PT 25 µg (4/5 (80.0%), 95% CI 28.4–99.5) and 50 µg (22/25 (88.0%), 95% CI 68.8–97.5) than laser + Viaskin-placebo (0/12 (0.0%), 95% CI 0.0–26.5).

Conclusions: Viaskin-PT applied after laser-based epidermal skin preparation showed encouraging safety and immunogenicity results: anti-PT booster responses were not inferior to those elicited by Boostrix®dTpa. This study is registered at ClinicalTrials.gov (NCT 03035370) and was funded by DBV Technologies. **O. Chatzis, Clin Microbiol Infect 2020;■:1**

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Introduction

Pertussis remains an important cause of infant death worldwide [1]. Adolescents and young adults represent the majority of cases [2], serving as a reservoir for transmission to young children [3,4].

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Pertussis toxin (PT) antibody titres rapidly decline following chemically detoxified PT-containing aP vaccines [5,6], requiring regular boosters [7]. Optimized aP vaccines should at least include a detoxified PT retaining the epitope specificity of wild-type PT [8]. A *Bordetella pertussis* strain expressing genetically detoxified PT (PTgen) was generated by BioNet-Asia [9]. PTgen-containing acellular pertussis (aP) vaccines were evaluated in phase I/II and II/III randomized controlled trials in whole-pertussis primed adults and adolescents, demonstrating similar safety with significantly higher antigen-binding (PT-IgG) and neutralizing PT antibody responses compared with chemically detoxified PT-containing Tdap comparators [10,11]. This persisted 1 year later [12]. In aP-primed adolescents also, stronger anti-PT responses were elicited than by the Tdap comparator [13].

Epicutaneous delivery directly targets antigens to Langerhans cells [14]. An epicutaneous patch (Viaskin®, DBV Technologies, Paris, France), successfully tested clinically for desensitization to peanut allergy [15], showed that in mice a single application of PTgen-Viaskin® efficiently recalled memory responses primed by chemically detoxified PT-containing aP vaccine [16].

Objectives of this study were to evaluate the safety and capacity of a PTgen-coated Viaskin® epicutaneous patch to recall memory responses in healthy adults.

Methods

Study design and population

This phase I dose-escalation, double-blind, randomized controlled trial evaluated the safety and immunogenicity of Viaskin® in healthy adults aged 18–40 years; participants received genetically detoxified PT (Viaskin-PT) or placebo (Viaskin-placebo). The study was conducted at the Geneva University Hospital, Switzerland, between September 2016 and April 2018.

Healthy, non-pregnant participants with a documented history of pertussis immunization were recruited from the community through local advertising (flyers); entry criteria can be found in the Supplementary material (Table S1). Participants were enrolled by study investigators and randomized 5:1 to vaccine or placebo using a computer-generated randomization sequence developed by a statistician not otherwise involved in the study; assignments were concealed from investigators by sealed, opaque envelopes until the end of the study. Participants in cohort 1 were randomly assigned to Viaskin-PT 25 µg ($n = 25$) or Viaskin-placebo ($n = 5$), and in cohort 2 to Viaskin-PT 50 µg ($n = 25$) or Viaskin-placebo ($n = 5$), both on intact skin; those in cohorts 3 and 4 were randomized to Viaskin-PT 25 µg ($n = 5$) or Viaskin-placebo ($n = 2$) and to Viaskin-PT 50 µg ($n = 25$) or Viaskin-placebo ($n = 10$), respectively, on epidermal laser-based prepared skin (Fig. 1).

Viaskin® patches were applied on days 0 and 14 for 48 hours each. Boostrix® dTpa (GlaxoSmithKline, Belgium, UK) was administered to all volunteers on day 42 (Fig. 1), to confer full immune protection against other antigens including the Boostrix® vaccine.

The protocol was approved by the Ethics Committee of the Canton of Geneva, Switzerland (CCER 2016-01178), authorized by Swiss Agency of Therapeutic Products, and registered at <http://www.clinicaltrials.gov> NCT03035370. The study followed the principles of the Declaration of Helsinki and was conducted in accordance with International Conference on Harmonization Good Clinical Practice Guidelines and local regulations. Volunteers were compensated for their participation (the full study protocol can be accessed upon request).

Study vaccines and skin application

Genetically detoxified PT (PTgen) was manufactured according to Good Manufacturing Practice requirements by BioNet-Asia

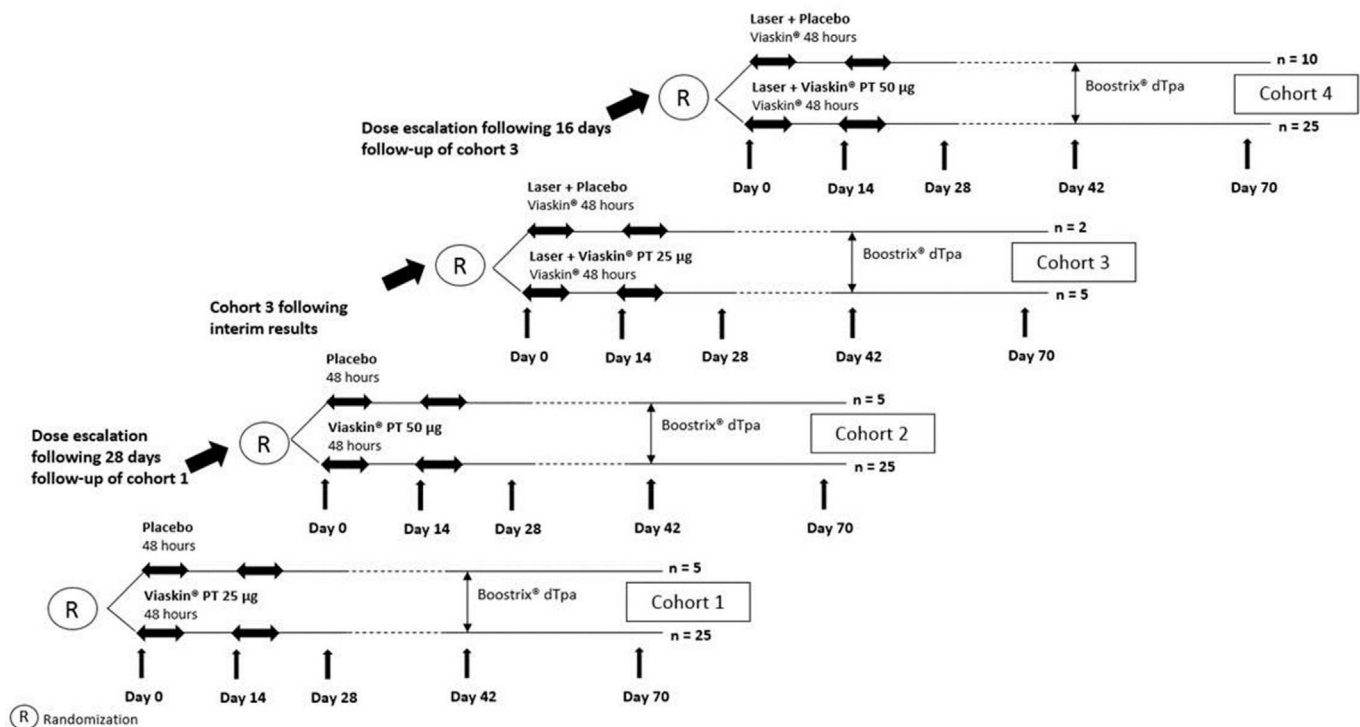


Fig. 1. Study flow. The study was rolled out in two sequences: cohorts 1 and 2 and cohorts 3 and 4. Each sequence evaluated the safety and immunogenicity of Viaskin-PT 25 µg and Viaskin-PT 50 µg versus Viaskin-placebo on intact skin (cohorts 1 and 2) or on controlled epidermal laser-based prepared skin (cohorts 3 and 4). Viaskin patches were applied at days 0 and 14 for 48 hours. Boostrix® dTpa was administered to all participants at day 42. Safety was evaluated within 48 hours after each patch application and throughout the study. The immunogenicity was assessed by the titration of anti-PT IgG antibodies by ELISA and neutralization assay at days 0, 14, 28, 42 and 70.

Co., Ltd. (Bangkok, Thailand). Viaskin-PT contained a dry deposit of 25 µg or 50 µg of PTgen on its occlusive chamber (see [Supplementary material, Fig. S1](#)). Viaskin-placebo consisted of a similar formulation without PTgen. Viaskin-PT 25 µg, Viaskin-PT 50 µg and Viaskin-placebo were manufactured by AMATSI DBI (Idron, France), and labelled, packaged and released by CREA-PHARM (Bordeaux, France). Patches were applied on the inner forearm and secured for 48 hours (± 4 hours) by Tegaderm® adhesive tape.

In cohorts 3 and 4, controlled epidermal laser-based skin preparation (a single laser beam) was applied just before Viaskin® application on days 0 and 14. The CE-marked P.L.E.A.S.E.® Professional Laser developed by Pantec Biosolutions AG (Ruggell, Liechtenstein) was used for skin preparation. Settings (fluence: 4.9 J/cm²/pore, 8%, depth: 60 µm) were selected to microporate only the stratum corneum without affecting the epidermal layer (<http://www.clinicaltrials.gov> NCT02988739).

Safety assessment

After each patch application, participants were observed for 90 minutes. Diary cards recorded solicited local and systemic reactions for 14 days after each application. Adverse events and serious adverse events were recorded through the entire study period; reactogenicity events were solicited through day 42.

An independent Data and Safety Monitoring Board (DSMB) reviewed two interim safety analyses once all participants of cohorts 1 and 3 had reached day 28 (visit 5) and day 16 (visit 4), respectively, before dose escalation to Viaskin-PT 50 µg.

Immunogenicity assessment

Serum samples were assessed at days 0, 14, 28, 42 and 70 ([Fig. 1](#)). Anti-PT IgG antibodies were measured by standardized ELISA in the Centre for Vaccinology (Geneva, Suisse), using PTgen-coated plates as described previously [10]. Seroresponse was defined as a four-fold increase in antibody titres on day 42 compared with baseline, seropositivity as anti-PT ≥ 5 IU/mL and seroconversion rate as the percentage of participants with anti-PT ≥ 5 IU/mL on day 42 if baseline anti-PT were < 5 IU/mL.

Functional anti-PT antibodies were quantified at Bertin Pharma Laboratory (Fontenay-aux-Roses, France) by measuring PT neutralizing titres in Chinese hamster ovary cells, as described elsewhere [10]. The PT neutralizing titre was reported as IU/mL on the relative activity of the WHO International Standard Pertussis Antiserum (Human) 06/140. Samples with titres below the assay cut-off were attributed a titre of half of the cut-off to allow for statistical analyses.

Statistical analyses

The sample size was based on clinical and practical considerations and not on a formal power calculation. Categorical variables are described by counts and percentages, and continuous variables by means and standard deviations or median and interquartile ranges (IQR) for non-normal distributions. Differences between Viaskin-PT and Viaskin-placebo were assessed at day 42 by Wilcoxon's rank sum test (IgG concentration) or Fisher's exact test (seroconversion status and seroresponse rate). When applicable, two-sided tests at the 5% significance level were used with no multiplicity adjustment.

Statistical analyses were performed by DBV Technologies, Paris, France using the Statistical Analysis System SAS® Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA). The statistical analysis plan is provided by request.

Results

Study volunteers and demographics

One hundred and two volunteers were enrolled and received Viaskin-PT ($n = 80$) or Viaskin-placebo ($n = 22$; [Figs. 1, 2](#)). Vaccines were administered in two successive periods: (i) Viaskin-PT 25 µg ($n = 25$), Viaskin-PT 50 µg ($n = 25$), Viaskin-placebo ($n = 10$); and (ii) Laser + Viaskin-PT 25 µg ($n = 5$), Laser + Viaskin-PT 50 µg ($n = 25$) and Laser + Viaskin-placebo ($n = 12$). There were 56 deviations from protocol (see [Supplementary material, Table S2](#)), none of which led to exclusions from per-protocol analyses. All participants attended the last study visit and were included in safety and immunogenicity analyses.

Median age was 21.9 (IQR 20.9–23.1) years ([Table 1](#)). The mean interval since last pertussis vaccination was similar among groups (median of 14.6 (IQR 13.2–17.8) years).

Safety

No vaccine-associated serious adverse events were reported (see [Supplementary material, Table S3](#)). One hospitalization occurred at day 43 after Viaskin-PT 25 µg application on intact skin, for an ectopic pregnancy despite a hormonal intrauterine device. No severe or serious treatment-emergent adverse events were reported (see [Supplementary material, Table S3](#)).

When Viaskin patches were applied to intact skin, local adverse reactions were generally mild (see [Supplementary material, Table S4](#)). Erythema was frequent in both Viaskin-placebo participants (10/10, 100.0%) and Viaskin-PT 25 µg or 50 µg (22/25 (88.0%) and 24/25 (96.0%)), located peripherally (site of the adhesive tape) and not centrally (site of antigen deposit). Pruritus and pain were less frequent.

Most volunteers receiving epidermal laser skin preparation experienced generally mild adverse reactions at the application site. Again erythema was most frequently observed in Laser + Viaskin-placebo participants (11/12, 91.7%) and Laser + Viaskin-PT 25 µg or 50 µg (5/5 and 25/25, both 100.0%) participants; it was mild in all cases but one. Other local reactions (pruritus, dryness) were also generally mild: (see [Supplementary material, Fig. S2, Table S4](#)).

Systemic solicited adverse events were frequent—9/10 (90.0%), 19/25 (76.0%), 21/25 (84.0%) in Viaskin-placebo, Viaskin-PT 25 µg and Viaskin-PT 50 µg, respectively; 10/12 (83.3%), 5/5 (100.0%) and 24/25 (96.0%) in Laser + Viaskin-placebo, Laser + Viaskin-PT 25 µg and Laser + Viaskin-PT 50 µg, respectively. Headache, asthenia, abdominal pain, myalgia and subjective fever were the most frequently reported systemic reactions (see [Supplementary material, Table S5](#)). All reactions were transient and resolved without sequelae.

Immunogenicity

ELISA anti-PT IgG antibody responses

Baseline IgG antibody geometric mean concentrations (GMCs) were low and comparable among groups ([Fig. 3a](#)).

Application of the Viaskin-PT patches without skin preparation (Cohorts 1 and 2) did not induce detectable anti-PT immune responses (see [Supplementary material, Table S6](#)).

Following skin preparation, day-42 anti-PT IgG GMCs were significantly higher in the Viaskin-PT 25 µg ($n = 5$; 33.24 IU/mL (95% CI 9.59–115.23); $p = 0.003$) and Viaskin-PT 50 µg ($n = 25$; 57.00 IU/mL (95% CI 41.39–78.52); $p < 0.001$) groups compared with the Viaskin-placebo group ($n = 12$; 4.03 IU/mL (95% CI 2.56–6.37)) ([Fig. 3](#), data not shown for Viaskin-PT 25 µg). One-month (day 70)

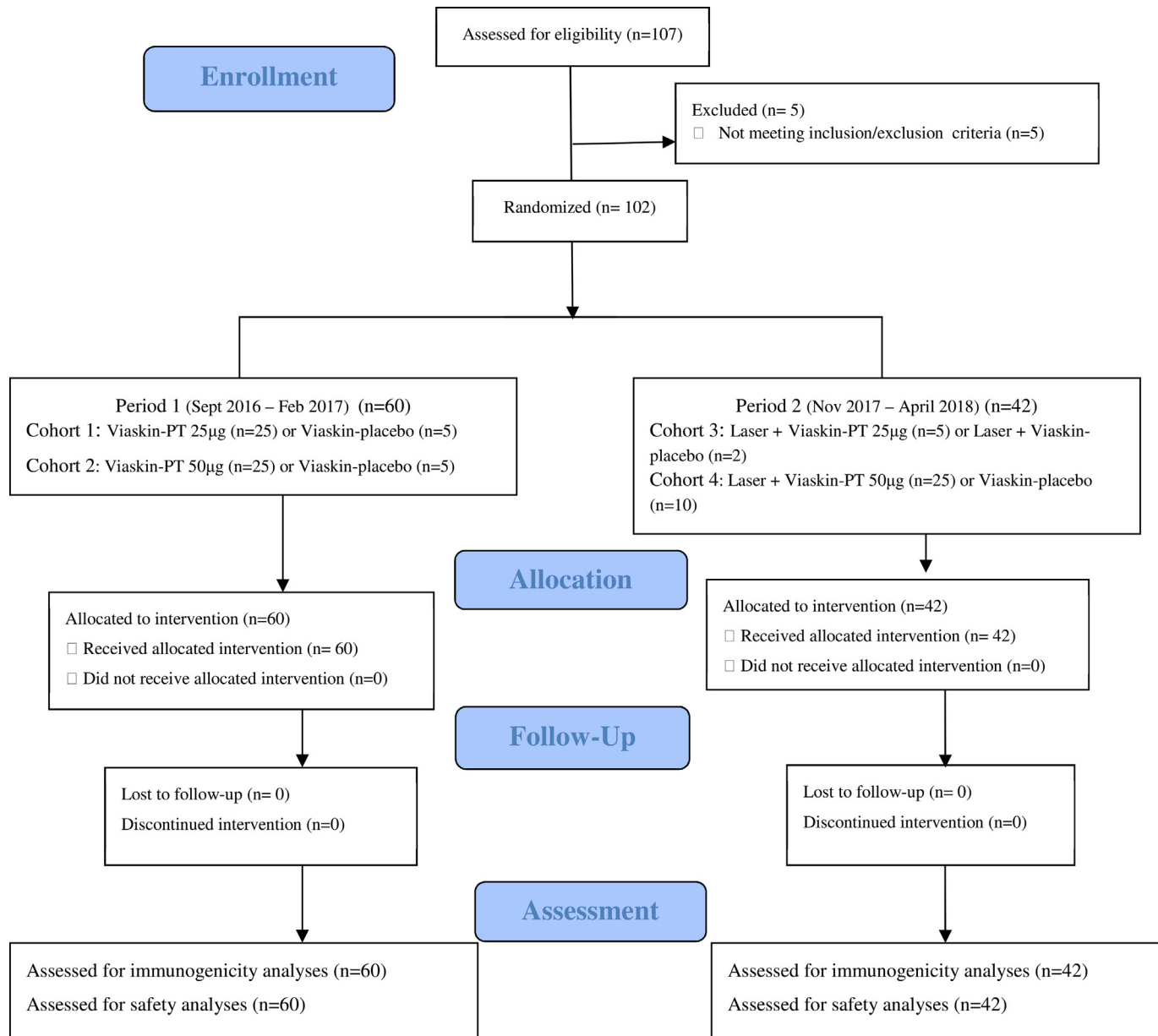


Fig. 2. Study flow chart.

after administration of Boostrix® dTpa, GMCs anti-PT IgG antibodies were similar in the three groups (Fig. 3a and see [Supplementary material, Table S6](#)).

The day 42 reverse cumulative distribution of anti-PT IgG antibody responses to two applications of Viaskin-PT 25 µg or 50 µg following skin preparation was similar to that elicited by Boostrix® on day 70 (Figs. 3c,d, data not shown for Viaskin-PT 25 µg).

After skin preparation, all seronegative volunteers seroconverted by day 42 (see [Supplementary material, Table S7](#)). Seroresponse was reached by day 42 for 4/5 (80.0%) recipients of Viaskin-PT 25 µg and 22/25 (88.0%) recipients of Viaskin-PT 50 µg, but no Viaskin-placebo recipient (Table 2). At day 70 (1 month after Boostrix®), GMCs were not further increased in Viaskin-PT recipients and were not different from those of Viaskin-placebo (Fig. 3a).

PT-neutralizing antibodies

PT-neutralizing antibodies were assessed in cohorts 3 and 4 (skin preparation). Baseline PT-neutralizing GMTs were lower in the Laser + Viaskin-PT 50 µg than in the Laser + Viaskin-placebo group (36.0 IU/mL (95% CI 26.4–49.0) versus 63.0 IU/mL (95% CI 39.6–100.3) (Table 3).

One month after the second Viaskin application (day 42), anti-PT neutralizing GMTs were higher in Laser + Viaskin-PT 50 µg (121.76 IU/mL, 95% CI 95.04–156.0) and Laser + Viaskin-PT 25 µg (139.87 IU/mL, 95% CI 87.30–224.10) compared with placebo recipients (59.49 IU/mL, 95% CI 39.37–89.9) (Fig. 3b, Table 3). One month after Boostrix® dTpa (day 70), GMTs of anti-PT neutralizing antibodies were similar in all groups (Table 3).

Reverse cumulative distributions of PT-neutralizing antibody titres after two doses of Viaskin-PT 50 µg (Figs. 3e,f) or Viaskin-PT

Table 1
Demographics at baseline by study arm

Treatment groups		Viaskin-placebo (n = 10)	Viaskin-PT 25 µg (n = 25)	Viaskin-PT 50 µg (n = 25)	Laser + Viaskin-placebo (n = 12)	Laser + Viaskin-PT 25 µg (n = 5)	Laser + Viaskin-PT 50 µg (n = 25)
Age (years) at consent	Mean (SD)	21.2 (1.8)	22.2 (2.3)	22.5 (3.1)	22.3 (2.0)	21.9 (1.6)	22.8 (2.2)
	Median	21.7	21.7	21.9	22.0	21.9	22.0
	Q1; Q3	19.9; 21.7	20.7; 22.7	20.9; 22.9	21.0; 23.5	20.9; 22.9	21.0; 24.0
	Min; Max	18.7; 24.9	18.7; 28.7	18.9; 33.9	20.0; 26.1	19.9; 23.9	20.0; 28.0
Gender, n (%)	Female	5 (50.0%)	15 (60.0%)	16 (64.0%)	8 (66.7%)	3 (60.0%)	19 (76.0%)
	Male	5 (50.0%)	10 (40.0%)	9 (36.0%)	4 (33.3%)	2 (40.0%)	6 (24.0%)
Ethnicity, n (%)	Black	0 (0.0%)	1 (4.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
	Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
	Caucasian	10 (100.0%)	23 (92.0%)	22 (88.0%)	9 (75.0%)	5 (100.0%)	21 (84.0%)
	Hispanic	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0 (0.0%)	1 (4.0%)
Last documented anti-PT vaccination (years)	Other	0 (0.0%)	1 (4.0%)	2 (8.0%)	2 (16.7%)	0 (0.0%)	1 (4.0%)
	Mean (SD)	15.2 (2.5)	16.5 (3.7)	15.8 (4.0)	14.0 (2.5)	14.4 (0.9)	14.8 (3.0)
	Median	14.5	15.4	14.7	14.1	14.6	14.6
	Q1; Q3	14.0; 15.7	13.9; 19.6	13.5; 18.2	12.4; 15.4	14.0; 14.7	13.0; 16.0
	Min; Max	12.0; 20.8	10.4; 23.7	10.0; 26.6	10.5; 19.7	13.0; 15.5	10.0; 20.9

Table 2
Seroreponse rate defined by the percentage of volunteers with at least four-fold increase from baseline of anti-PT IgG antibody titres at Days 42 and 70

Treatment groups		Viaskin-placebo (n = 10)	Viaskin-PT 25 µg (n = 25)	Viaskin-PT 50 µg (n = 25)	Laser + Viaskin-placebo (n = 12)	Laser + Viaskin-PT 25 µg (n = 5)	Laser + Viaskin-PT 50 µg (n = 25)
Seroreponse rate at Day 42, n (%)							
n		10	25	25	12	5	25
<4-fold increase		10 (100.0%)	24 (96.0%)	24 (96.0%)	12 (100.0%)	1 (20.0%)	3 (12.0%)
≥4-fold increase		0 (0.0%)	1 (4.0%)	1 (4.0%)	0 (0.0%)	4 (80.0%)	22 (88.0%)
95% CI (%) ^a		0.0–30.8	0.1–20.4	0.1–20.4	0.0–26.5	28.4–99.5	68.8–97.5
p value versus placebo ^b			>0.999	>0.999		0.002	<0.001
Seroreponse rate at Day 70, n (%)							
N		10	25	25	12	5	25
<4-fold increase		2 (20.0%)	7 (28.0%)	5 (20.0%)	1 (8.3%)	1 (20.0%)	3 (12.0%)
≥4-fold increase		8 (80.0%)	18 (72.0%)	20 (80.0%)	11 (91.7%)	4 (80.0%)	22 (88.0%)
95% CI (%) ^a		44.4–97.5	50.6–87.9	59.3–93.2	61.5–99.8	28.4–99.5	68.8–97.5
p value versus placebo ^b			>0.999	>0.999		0.515	>0.999

^a Using the Clopper–Pearson method.^b p value is based on Fisher's exact test.**Table 3**
Geometric mean titres of pertussis toxin-specific neutralizing antibodies

Treatment groups		Laser + Viaskin-placebo (n = 12)	Laser + Viaskin-PT 25 µg (n = 5)	Laser + Viaskin-PT 50 µg (n = 25)
GMT at day 0 (IU/mL)				
Mean		63.03	69.93	35.98
Mean 95% CI		39.60–100.32	26.21–186.58	26.40–49.03
GMT at day 14 (IU/mL)				
Mean		59.51	121.76	87.33
Mean 95% CI		36.44–97.18	59.26–250.17	60.64–125.75
GMT at day 42 (IU/mL)				
Mean		59.49	139.87	121.76
Mean 95% CI		39.37–89.90	87.30–224.10	95.04–156.00
GMT at day 70 (IU/mL)				
Mean		100.05	160.67	106.00
Mean 95% CI		54.51–183.64	100.28–257.42	78.70–142.77

Geometric mean titres (GMT) are calculated using a log10 transformation. Mean confidence intervals are calculated using Student's law.

25 µg measured at day 42 were similar to those elicited by Boostrix® dTpa at day 70.

Discussion

We show here that anti-PT IgG binding and neutralizing antibody titres increased following the application of Viaskin-PT and did not further increase after Boostrix® dTpa, suggesting that

maximal 'boostability' had been achieved; this was reached with a favourable safety profile but required epidermal laser-based skin preparation.

Murine studies suggested that vaccine delivery to the epidermis might be potent [16,17]. Indeed, application of Viaskin-PT on intact murine skin is sufficient to reactivate vaccine-induced pertussis immunity [16]. In our two first human cohorts, however, the application of Viaskin-PT on intact skin elicited little to no response.

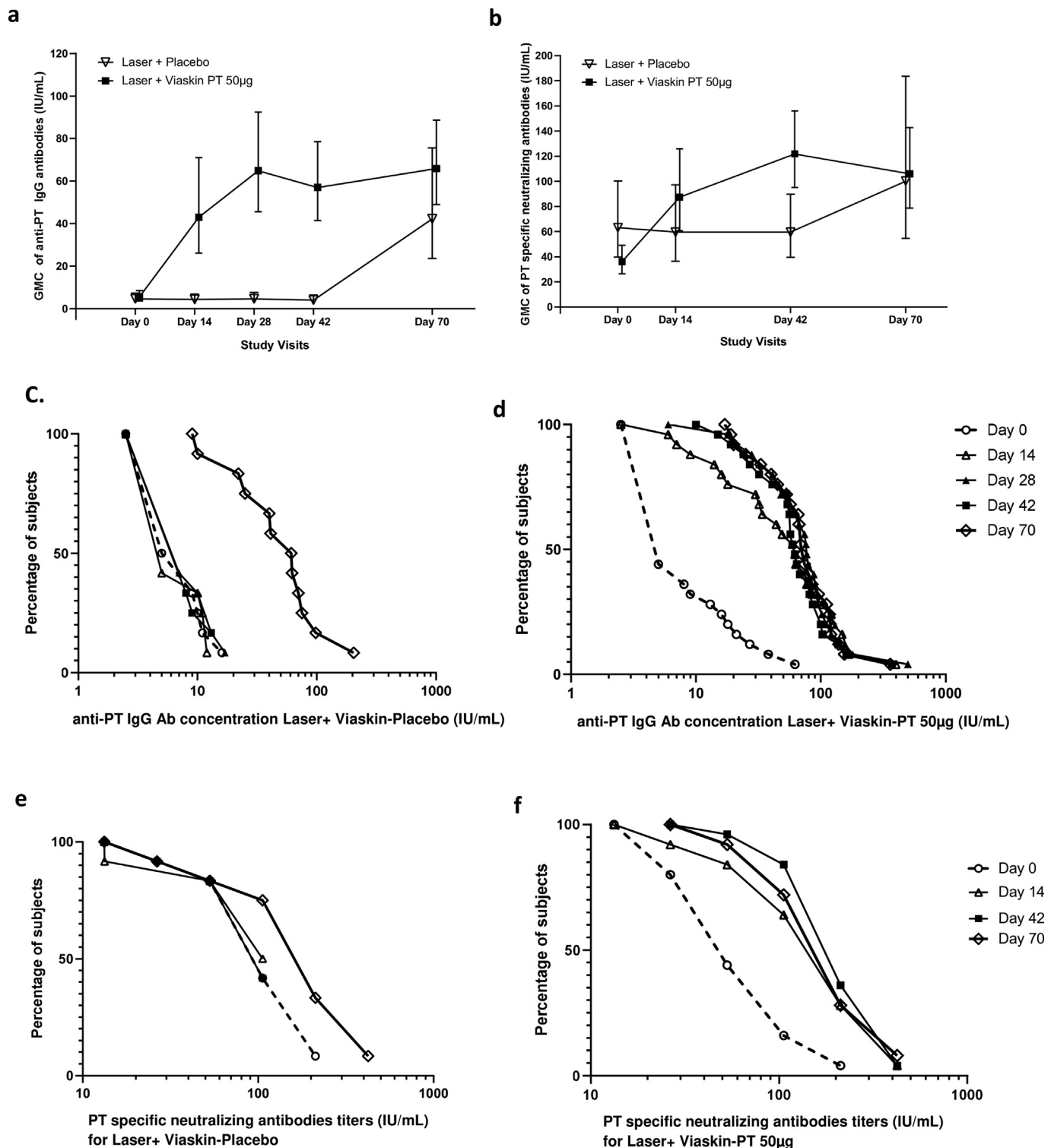


Fig. 3. Immunological response following Viaskin patch vaccination procedure. (a) ELISA PT-IgG for Laser + Viaskin-Placebo ($n = 12$) and Laser + Viaskin 50 µg ($n = 25$) measured at Days 0, 14, 28, 42 and 70 and (b) PT neutralizing antibodies Geometric Mean Concentration (GMC) for Laser + Viaskin-Placebo ($n = 12$) and Laser + Viaskin 50 µg ($n = 25$) measured at Days 0, 14, 28, 42 and 70. Results are expressed in IU/mL with 95% CI for each time-point. (c–f) Reverse cumulative distribution (RCD) curves of anti-PT IgG antibodies measured at Days 0, 14, 28, 42 and 70 for ELISA and at Days 0, 14, 42 and 70 for neutralizing assay. The percentage of participants is distributed among the cut-off values titres of ELISA anti-PT IgG antibodies for (c) Laser + Viaskin-Placebo ($n = 12$), (d) Laser + Viaskin-PT 50 µg ($n = 25$), and neutralizing antibodies for (e) Laser + Viaskin-Placebo ($n = 12$) and (f) Laser + Viaskin-PT 50 µg ($n = 25$).

Our assumption is that antigen delivery was limited by specific characteristics of human adult skin. Indeed, human skin has lower water content, loses excess water more slowly and possesses a thicker stratum corneum than mouse skin [18]. We therefore implemented in cohorts 3 and 4 a minimally invasive, controlled epidermis-limited skin preparation to potentiate the efficacy of epicutaneous vaccination. Laser-based microporation was selected to provide a reproducible and fully controllable procedure—in contrast to other mechanical methods [19]. We employed an ablative fractional infrared laser used for dermatological treatment [20–22], which may be precisely adjusted to target specific layers of the skin. This markedly improved immunogenicity. Subsequent murine studies showed that the laser beam increases trans-epidermal water loss, enhancing antigen solubilization from Viaskin® and hence the amount of antigen available for delivery [23].

This approach was well tolerated. Local reactions were frequent but mild and observed only following laser skin preparation. Systemic reactions were also common, more frequently following Viaskin-PT 25 µg and 50 µg (5/5 (100.0%) and 24/25 (96.0%), respectively) than Viaskin-placebo (10/12 (83.3%)). These expected self-limited inflammatory reactions did not differ from those elicited by Boostrix®.

Following skin preparation, anti-PT immune responses were already apparent on day 14, but further increased after the second Viaskin-PT. Anti-PT IgG titres reached a plateau after the second Viaskin-PT, without increasing further following Boostrix® dTpa (Fig. 3a). This suggests that memory B-cell reactivation was initiated by the first application and maximally amplified by the second, such that subsequent immunization had no additional effect.

Anti-PT titres did not differ between recipients of Laser + Viaskin-PT 25 µg and 50 µg, although this observation should be considered with caution given the small size of the former group. The amount of antigen required for sufficient delivery to Langerhans cells, migration towards lymph nodes and reactivation of immune memory is unknown. But it is likely that a small fraction of the antigen deposited on the Viaskin® is delivered and sufficient [24], suggesting that the dose of PTgen may be further reduced.

Pertussis toxin IgG titres tended to be even higher in participants vaccinated with Viaskin-PT versus Boostrix® dTpa, suggesting that the combination of epidermal preparation, Viaskin® application and PTgen jointly contributed to maximally reactivate immunity. Correlates of protection against pertussis have not been established [2,25,26]. However, a mono-component PT pertussis vaccine effectively controlled pertussis over 15 years in Denmark [27]. Hence, PT-only epicutaneous booster vaccines may have a role, for example during pregnancy when vaccination against diphtheria and tetanus is not required.

Finally, even recombinant PT may not optimally enhance B-cell and T-cell responses [28]. Hence, epicutaneous administration targeting Langerhans cells with more immunogenic antigens may more effectively recall pre-existing immunity: in murine models the addition of filamentous haemagglutinin to PT further increased anti-PT responses, possibly through bystander T cells [16]. In a recent clinical trial, a higher PT-specific B memory cell response was observed after aP vaccines containing PTgen and filamentous haemagglutinin [13]. This approach could be interesting to deliver antigens when avoidance of adjuvants is preferred.

Our study has limitations. It includes a limited number of participants, as do most phase I first-in-human studies. This limitation was mitigated by comparing two doses of Viaskin-PT, 25 µg and 50 µg, with Viaskin-placebo and a licensed dTpa booster. Second, few volunteers received laser + Viaskin-PT 25 µg, precluding the identification of the minimal PT dose required for boosting.

Altogether, the combination of epidermal laser skin preparation and epicutaneous delivery of PTgen by Viaskin® is capable of reactivating pre-existing PT immunity as efficiently as an intramuscular administration of alum-adsorbed chemically detoxified PT. Although this requires a laser, it obviates the need for injections. The interest in needleless and adjuvant-free vaccination in specific circumstances (e.g. patient compliance, mass vaccination) warrants further evaluation of this novel approach.

Transparency declaration

LM, BP, MRo and PLH were DBV Technologies employees during the course of the clinical trial and own stocks from the company. LM is working as a consultant of DBV Technologies for the writing of the paper. PHB was the CEO of the company (DBV Technologies) during the course of the clinical trial and owns stocks from the company. PHL is a member of the scientific committee of DBV Technologies. CH worked as a contractor for DBV Technologies. WW is employed by BioNet-Asia. HTP is CEO of BioNet-Asia. PHL and CAS received honoraria for expert advice and/or travel fees to advisory boards from DBV Technologies. All other authors declare no conflict of interest.

Authors' contributions

OC, GBR, LM, MRo, CH, PHL, PHB and CAS jointly conceived the study plan and interpreted the results. BP, BL, PLH and WW developed analytical tools. OC, GBR, MRo, MR, AH, AM, GG, CH, HTP and CAS collected data and/or contributed directly to the clinical trial and its monitoring. OC, LM, WW and CAS wrote the manuscript. All authors revised and approved the manuscript. CAS and OC had full access to all data and take responsibility for the integrity of the data and the data analysis.

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Appendix A. Supplementary data

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