

Boosting teenagers with acellular pertussis vaccines containing recombinant or chemically inactivated pertussis toxin: a randomized clinical trial.

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Brief 40 words summary: We compared recombinant versus chemically-detoxified pertussis toxin (PT)-containing vaccines in adolescents primed with 5 doses of acellular pertussis vaccines. Recombinant PT was safe, significantly more immunogenic and reactivated more memory B cells than the comparator licensed vaccine.

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Abstract

Background

Protection induced by acellular pertussis (aP) vaccines is partial and short lived - especially in teenagers - calling for novel immunization strategies.

Methods

We conducted an investigator-driven proof-of-concept randomised-controlled trial in aP-primed adolescents in Geneva to assess the immunogenicity and reactogenicity of a novel recombinant aP (r-aP) vaccine including recombinant pertussis toxin (rPT) and filamentous hemagglutinin (FHA) co-administered with tetanus-diphtheria toxoids, compared to a licensed tetanus-diphtheria-acellular pertussis vaccine containing chemically-detoxified PT (cd/Tdap). The primary immunological endpoints were day28/365 geometric mean concentrations (GMC) of total and neutralizing anti-PT antibodies. Anti-FHA antibodies and PT-specific memory B cells were assessed.

Results

Sixty-two aP-primed adolescents (mean age: 12 years) were randomized and vaccinated with r-aP+Td or cd/Tdap. Reactogenicity, adverse events and baseline GMCs were similar between groups. Day28 PT-neutralizing antibody GMCs were low after cd/Tdap (73.91 IU/ml (95%CI, 49.88-109.52) and approximately 2-fold higher after r-aP+Td (127.68 IU/ml (95%CI, 96.73-168.53), $p=0.0162$). Anti-PT IgG GMCs were also low after cd/Tdap (52.43 IU/ml (95%CI, 36.41-75.50) and two-fold higher after r-aP+Td (113.74 IU/ml (95%CI, 88.31-146.50)) $p=0.0006$). Day28 anti-FHA GMCs were similar in both groups. Day365 anti-PT IgG (but not PT-neutralizing antibody) GMCs remained higher in r-aP+Td vaccinees. PT-specific memory B cells increased significantly after r-aP+Td but not cd/Tdap boosting.

Conclusions

Boosting aP-primed adolescents with r-aP induced higher anti-PT and PT-neutralizing responses than cd/Tdap and increased PT-specific memory B cells. Despite this superior immunogenicity, rPT may have to be given repeatedly, earlier in the immunization schedule and/or with novel adjuvants to exert an optimal influence in aP-primed subjects.

Introduction

Pertussis remains an important cause of infant death worldwide despite high vaccination coverage [1]. Immunization programs started with whole-cell pertussis (wP) vaccines [2]. Chemically inactivated acellular pertussis (aP) vaccines were introduced 50 years later, following the demonstration of their safety and efficacy in infants and toddlers [3]. Most aP immunization schedules include 5 or 6 doses with no apparent reduction of bacterial circulation in older age groups. Pertussis has become increasingly frequent in adolescents – who now represent the majority of cases [4-6]. Although complications are rare, morbidity is significant and infected adolescents/adults may contaminate infants [7]. Pertussis immunity is short-lived in aP-primed vaccinees, its reactivation requiring repeat boosting: in 2014, WHO recommended countries using aP vaccines to consider the need for several booster doses (and/or specific strategies) to prevent infant mortality [8].

The resurgence of pertussis is multifactorial and wP- versus aP-priming likely critical [9-11]. Waning protection of aP-primed vaccinees was first reported over a 5-year period in children [12]. It was subsequently found much more rapid in Tdap-boosted adolescents, declining from 73% to non-significant within 2 years [13]; [4, 5]; [14]. A key factor is the rapid waning of aP-induced PT antibodies, declining to pre-vaccination levels already 5 years post-vaccination [15]. A puzzling observation is that the immunogenicity of aP vaccines, which contain chemically-detoxified pertussis toxoid (cdPT), is higher in infants and children than adolescents [16, 17]: the rise in antibody levels is less after the sixth than fifth dose of aP [18]. We recently proposed [9] that this waning of efficacy may derive from repeat immunizations with cdPT-containing aP vaccines inducing B cells preferentially recognizing cdPT vaccine epitopes instead of epitopes from native PT [19, 20].

The safety and superior immunogenicity of a 9K/129G genetically-detoxified PT (rPT) was demonstrated long ago [21, 22]: rPT has similar immunological and functional properties as native PT and thus higher immunogenicity than cdPT at similar doses [19, 23-25]. BioNet-Asia (BNA) developed a new rPT-expressing *B. pertussis* strain [26]. A Phase I/II randomized controlled trial (RCT) including wP-primed Thai adults indicated similar safety but significantly higher PT seroresponses to the BNA rPT-

containing recombinant aP vaccine (r-aP) than to the cdPT-containing Tdap (cd/Tdap) comparator [27]. This was subsequently confirmed in 450 wP-primed Thai adolescents [28]. Here, we compared the reactogenicity and immunogenicity of BNA r-aP (given with tetanus-diphtheria, r-aP+Td) and cd/Tdap to boost aP-primed Swiss adolescents.

Methods

Study Design and Objectives

The primary objective of this RCT was to test whether adolescents primed and boosted with cdPT-containing aP vaccines would respond better to cd/Tdap or to r-aP+Td, based on Day28 geometric mean concentrations (GMCs) of anti-PT neutralizing antibodies. Secondary objectives included seroresponse rates and GMCs of PT-, FHA, TT- and DT-specific total IgG antibodies, as well as the incidence of AE and SAE during 7 and 28 days, respectively. The durability of vaccine responses was assessed at Day365 through a study extension.

Study Population

An investigator-driven single-center, phase II, observer blind, RCT was conducted at the Children's Hospital of Geneva, University Hospitals of Geneva, Switzerland, between October 2016 and March 2017. Written informed consent was obtained from participants before enrolment. Ethical approval was obtained from Ethics Commission of the Canton of Geneva, Switzerland (CCER 2016- 00500) and from Swissmedic. The clinical study was funded by the Center for Vaccinology of the University of Geneva and conducted in accordance with International Conference on Harmonisation Good Clinical Practice guidelines.

Participants

Adolescents were recruited through private pediatricians and flyers. Inclusion criteria were: age between 11 and 15 years; documented history of aP immunization (5 doses); absence of clinically significant health problems as determined by medical history and physical examination; negative urinary pregnancy test at enrolment and willingness to take reliable birth control measures for female participants who had reached menarche; non-lactation; ability to comply with the study protocol, assent and parental consent to participate in the study. Main exclusion criteria included: fewer than 5 documented

doses of aP vaccines, a last dose of aP in the previous 5 years or of Td in the previous 2 years; PCR-confirmed pertussis infection less than 10 years previously; previous anaphylactic reaction to a vaccine component; any vaccination in the 30 days preceding study inclusion. Vaccination history was retrieved from vaccination records. Eligible participants were randomized to r-aP (Pertagen[®]) with contralateral Td (Td-pur[®]) or the comparator cd/Tdap (Boostrix[®]) vaccines. Group allocation was performed on a 1:1 basis generated by computer randomization, with 10 block size, concealed in sequentially labelled opaque envelopes. Each participant was assigned a unique treatment number that corresponded to her treatment allocation. Only the unblinded immunizing nurses had access to the treatment allocation.

There were two initial study visits: Visit 1 for inclusion, randomization, venous bleed and vaccination, and Visit 2 for safety evaluation, blood draw, and study termination. A 3rd visit was subsequently added on Day365 to assess the durability of vaccine responses. The study was observer-blind given the administration of one or two vaccines: the immunizing nurses and participants were aware of the number/type of vaccines administered while the other investigators remained blinded.

Study vaccines

Pertagen[®] (Lot No: PE25002-2A1) was developed and produced by BioNet-Asia Co., Ltd. (Thailand). A single dose contains 5 µg of rPT (PTgen) and 5 µg of FHA. Td-pur[®] (GlaxoSmithKline AG) contains 20 IU of tetanus toxoid (TT) and 2 IU of diphtheria toxoid (DT). The cd/Tdap comparator (Boostrix[®], GlaxoSmithKline AG) contains 8 µg of cdPT, 8 µg of FHA, 2.5 µg of Pertactin, 20 IU of TT and 2 IU of DT. All vaccines, adsorbed on aluminium hydroxide, were administered intramuscularly as 0.5-ml solution with a 21-gauge/25-mm-long needle.

Safety assessment

Following vaccination, subjects were observed during 30 minutes for immediate reactions. Diary cards recorded solicited local (pain, redness and swelling) and systemic (fever, headache, fatigue, arthralgia, chills, malaise, myalgia and vomiting) reactions during 7 days after vaccination, and adverse events (AEs) and serious adverse events

(SAEs) for 28 days. Causality of AEs and SAEs to study vaccines was determined by the investigators according to ICH guidelines, as specified in the protocol.

Immunogenicity assessment

Blood samples were taken at baseline, Day28 and Day365 after vaccination. The primary immunological endpoint was the PT-neutralizing GMCs. Secondary endpoints included seroresponses (defined by ≥ 4 -fold antibody increases) and GMCs of PT-FHA, TT and DT-specific IgG antibodies. Antigen-specific memory B cells (BMEM) were assessed on D0, D28 and D365.

Antibody assays

Functional anti-PT antibodies were quantified at BNA (Thailand) by measuring in a validated assay the PT neutralizing concentration in Chinese Hamster Ovary (CHO) cells. Serial serum dilutions were incubated with native PT (JN1H-5) as described [27, 30]. The highest dilution of complete neutralization (defined as the absence of cell clustering of crystal violet stained CHO cells) was considered the end-point dilution. This concentration was reported as IU/ml based on the relative activity of the WHO International Standard Pertussis Antiserum (Human) 06/140. Seroresponse rates were defined as the proportion of subjects with ≥ 4 -fold increase in concentration at Day28 compared to baseline. Samples with concentrations below the assay cut-off were attributed a concentration of the cut-off to allow for statistical analyses.

Anti-PT and anti-FHA IgG antibody concentrations were measured by standardized enzyme linked immunosorbent assays (ELISA) at the Center for Vaccinology (Geneva) using plates coated with BNA's purified rPT or FHA as described [27]. Responses to BNA rPT strongly correlate ($R^2=0.9837$, not shown) to those assessed with native PT (nPT), as recommended for use in PT-specific assays [28, 29, 31, 32]. The limit of detection was defined at 1 IU/ml. Anti-TT and anti-DT IgG concentrations were determined in the same laboratory using SERION ELISA classic (Virion/Serion, Germany) [27] with a 0.05 IU/ml cut-off. Seropositivity was defined as >0.1 IU/ml. Samples with concentrations below the cut-off were attributed a concentration of the cut-off to allow for statistical analyses.

Quantification of antigen-specific memory B cells

The cultured ELISpot assay was performed as previously described to detect antigen-specific memory B cells (BMEM) [33]. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation and cultured for 5-6 days with *S. aureus* Cowan strain (1:5000, Calbiochem, UK), CpG ODN 2006 (1.7 µg/ml, Invivogen, US and pokeweed mitogen (83 ng/ml, Sigma, Switzerland). Harvested cells were seeded and incubated overnight onto antigen-coated 96-well plates (Millipore, Switzerland). Bound antibody quantification was performed by incubation with alkaline phosphatase-conjugated goat anti-human IgG (Calbiochem, UK) and the alkaline phosphatase substrate kit (Bio-Rad, Switzerland).

Dried plates were read in Oxford using an automated ELISpot reader (AID ELR03, AID Diagnostika, Strassberg, Germany) and spot numbers manually corrected to exclude artefacts. Antigen-specific BMEM were calculated as the mean of 3 wells minus the mean spot count from PBS control wells and expressed as geometric BMEM frequencies per million cultured PBMCs. Values below the lower limit of detection (LLD) of the assay were replaced by a value half the LLD (=1).

Statistical analyses

Statistical analyses were performed by the Center of Excellence for Biomedical and Public Health Informatics (BIOPHICS), Thailand using Statistical Analysis System (SAS) version 9.4. The sample size for this proof-of-concept study was based on practical considerations and not on a formal statistical power calculation. Sixty volunteers were planned for randomization, with 30 in each group. The safety analysis included all randomized subjects who had received a dose of study vaccine. The overall percentage of subjects with at least one spontaneously reported AE, with date of onset up to Day28 after vaccination were tabulated with exact 95%CI, by type of AE; by severity; and by causality. They were displayed by vaccine group as both frequencies and percentages on the ITT data set. The seroresponse rates and GMCs were calculated with exact 95%CI. The difference between pre- and post- GMCs or BMEMs within a group was assessed using the paired T test or Wilcoxon signed rank test, depending on the distribution of data. The difference between groups was assessed by either Chi-square or Fisher's exact test for categorical variables, by the student t-test or Mann-

Whitney U test for continuous variables and by the student independent t-test or Wilcoxon rank sum test for BMEMs. $P \leq 0.05$ was considered to be statistically significant.

Results

Seventy subjects were screened, and 62 were enrolled between October 2016 and March 2017, vaccinated and included in the safety analysis. Two adolescents were excluded from the per protocol immunogenicity analysis as they inadvertently received Hepatitis B and HPV vaccines during the study period (Figure 1). Demographic and baseline characteristics were similar in the two groups (see Table 1). The mean age was 12 years, with 52% males and 92% Caucasian subjects. The mean interval since the last Td booster differed by 6 months between the two groups, but the interval (76 months) since the last pertussis booster was similar. On D365, 56/60 subjects attended a follow-up visit (Figure 1).

Safety

No immediate reactions were reported. Reactogenicity is described in Table 2. Injection site pain was the main reported reaction. A higher incidence of pain was reported following two injections (r-aP+Td) than one (cd/Tdap) (94% versus 74%, $p=0.0382$). Pain was mostly mild in severity (eTable 1) and resolved without sequelae. Other local reactions were rare and mostly mild in severity (Table 2 and eTable 1).

Systemic reactions were few, mild and similarly distributed between groups (Table 2 and eTable 1). Fatigue, headache, and myalgia were the most frequently reported. All reactions were transient and resolved spontaneously within a few days. During the 28-day study period, AEs (including reactogenicity) were reported by 97% and 94% of subjects (eTables 2 and 3) in r-aP+Td group and cd/Tdap group, respectively, with similar frequencies. Two volunteers reported pain or swelling lasting for 11 days: both were from the r-aP+Td group but these reactions occurred on the Td-injected arm. All AEs were transient and resolved without sequelae. No SAEs were reported.

B cell-mediated responses in aP-primed adolescents

Baseline IgG antibody GMCs were comparable except for slightly lower anti-FHA antibodies and higher anti-DT antibodies in the r-aP+Td group (Table 3). Baseline anti-

PT antibodies were low, undetectable in most adolescents despite 5 prior doses of DTaP. A few subjects had high anti-PT concentrations suggesting recent undiagnosed or asymptomatic pertussis exposure.

GMCs increased significantly in both groups one month after boosting (Table 3 and Figure 2). Despite its lower antigenic content, r-aP+Td elicited almost two-fold higher PT-neutralizing GMCs than cd/Tdap (127.68 versus 73.91 IU/ml, $p=0.0162$), as well as 2-fold higher anti-PT IgG GMCs (113.74 versus 52.43 IU/ml, respectively, $p=0.0006$). This resulted in significantly higher Day28/Day0 GMC ratios (Table 3). Despite the lower FHA content of r-aP than cd/Tdap and the lower D0 anti-FHA GMC in the r-aP+Td group, the Day28 anti-FHA IgG GMCs reached similar levels.

Seroresponse rates for PT neutralizing antibodies were higher in adolescents boosted with r-aP+Td than cd/Tdap ($p=0.0489$), but with overlapping 95%CI (Table 3). Due to their lower baseline, FHA-IgG response rates were higher following r-aP+Td than cd/Tdap (97% versus 72%, $p=0.011$). All subjects responded to TT and DT, with similar GMCs for both vaccine groups (Table 3).

Circulating PT-specific BMEM were few at baseline (4.3/million PBMCs). A significant 2-fold increase was observed on D28 after r-aP+Td but not cd/Tdap (Figure 3). FHA- and TT- (but not DT-) BMEM increased significantly and similarly in both groups after boosting (Figure 3).

Persistence of vaccine responses

As protection depends upon the durability of effector/memory responses, these were assessed on D365. Despite their decline, PT-IgG GMCs remained significantly higher following raP/+Td (GMC 26.87, 95%CI 19.51-37.00) than cd/Tdap (GMC 15.75, 95%CI 10.22-24.27, $p=0.047$) (Figure 2 and Table 3). PT-neutralizing GMCs no longer differed between groups (Figure 2).

FHA-, TT- and DT-IgG GMCs declined to similar levels in both groups. Seroresponse rates of PT-neutralizing antibodies remained higher following r-ap+Td than cd/Tdap (79% versus 39%, $p=0.006$) (Table 3). The numbers of PT-BMEM also remained

higher on D365 than at baseline following r-aP+Td ($p=0.047$), but not cd/Tdap ($p=0.448$), most r-aP+Td vaccinees now having detectable PT-BMEM (Figure 3).

Discussion

In this RCT, we report that a new rPT-containing vaccine was well tolerated and elicited 2-fold stronger anti-PT neutralizing and binding antibody responses than a 6th dose of cd/Tdap in aP-primed adolescents.

Antibody titers to PT and FHA were low at baseline. This indicates the lack of induction and/or persistence of long-lived antibody-secreting plasma cells 6 years after a 5th dose of DTaP. Despite the lower content of PT and FHA in r-aP than cd/Tdap, a higher increase of anti-PT neutralizing and binding antibodies were observed following r-aP+Td boosting. Thus, r-aP was more immunogenic than cd/Tdap in adolescents previously immunized with 5 doses of cd-PT-containing DTaP. However, anti-PT antibodies only reached two-fold higher GMCs following r-aP+Td than cd/Tdap boosting. One year later, antibody titers had declined, although anti-PT IgG GMCs remained higher in r-aP+Td than cd/Tdap vaccinees. Thus, a single dose of alum-adsorbed r-aP+Td is unlikely to overcome the limited immunogenicity and short-lived effectiveness of cd/Tdap in aP-primed adolescents [4-6]. However, memory B cells were better induced and more sustained following r-aP+Td, which could suggest the much needed increased boostability.

Our RCT has limitations. First, the small number of participants of this “proof-of-concept” study prevents the assessment of the influence of ethnic diversity and limits its statistical power. However, this sample size was sufficient to reach significant differences in most parameters assessed. . Second, we did not compare T cell responses, which may contribute to protection – or lack thereof. However, although rPT induces larger Th1 responses than cdPT [34, 35], both vaccines are adsorbed on aluminium salts and generate preferential Th2 responses. Last, our protocol did not include giving a booster dose of r-aP on D365, which would have enabled us to formally assess boostability.

We are concerned by the transient increased of PT-specific antibody responses in aP-primed vaccinees, as FHA is not specific of *Bordetella pertussis* and most strain do no longer express pertactin. Neutralizing antibody responses to r-PT boosting were significantly lower than reported in Thai wP-primed adolescents [28]. This could be accounted for by differences in ethnicity, pertussis exposure, number of previous immunizations, influence of Td co-administration, etc. However, priming is key to vaccine efficacy and a growing body of evidence suggests that the change from wP to aP vaccines is responsible for the more rapid waning of pertussis immunity [5, 36, 37] and the progressive decline of PT-boostability: one month after cd/Tdap boosting, PT- and FHA-BMEM were significantly fewer in aP- than wP-primed adolescents and declined rapidly [37]. It is thus encouraging that PT-specific memory B cells were better induced/recalled and possibly better persisted following r-aP+Td than cd-Tdap boosting.

Conclusions and perspectives

Boosting with rPT rather than cdPT only transiently increased PT-neutralizing responses in cd/DTaP-primed and boosted adolescents. This raises concern given the increasing size (mostly in developed countries) of the cd/DTaP-primed cohort, which will soon reach child-bearing age and require effective pertussis boosters during pregnancy.

Potential solutions for aP-primed adolescents/adults include the administration of repeat doses of r-aP. Antibodies were lower following a second dose of adult cd/Tdap [38]. An interesting hypothesis is that this may reflect interference of concomitant Td administration. If such, repeat doses of r-aP without Td could further increase PT antibody and memory B cell responses. Others have suggested to take advantage of the cyclic nature of pertussis outbreaks to provide boosters in periods of higher risks rather than based on age [39], although this may prove challenging. Alternatively, rPT could be combined with Th1/Th17-inducing adjuvants inducing higher inflammatory responses to enhance its immunogenicity and boostability. Last, aP-primed memory B/T cells could perhaps be recalled by a live mucosal vaccine mimicking natural exposure [40]. Otherwise, rPT may have to be used earlier in the pediatric

immunization schedule to exert its full immunogenicity, before too many cdPT-specific memory B cells are elicited. Research should be rapidly oriented in these directions.

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Disclosure of interest

Pailinrut Chinwangso, Indrajeet Kumar Poredi, Wassana Wijagkanalan, Jane Spiegel, Hong Thai Pham and Simonetta Viviani are employees of BioNet-Asia Co., Ltd., the manufacturer of the investigational product.

Pitchaya Boonrak and Saranath Lawpoolsri are employees of Center of Excellence for Biomedical and Public Health Informatics (BIOPHICS) which is a contractor of BioNet-Asia and was responsible for data analysis of this study.

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FIGURE LEGENDS

Figure 1: Consort Flow Diagram

Footnote: aP: acellular pertussis vaccine, r-aP+Td: acellular pertussis vaccine containing recombinant pertussis toxin (PT) and Filamentous Hemagglutinin (FHA), Td: Tetanus-diphtheria vaccine; cd/Tdap: combination vaccine containing tetanus and diphtheria toxoids, and acellular pertussis vaccine with chemically-detoxified PT.

Figure 2: Antibody responses to r-aP+Td or cd/Tdap.

GMCs/GMTs of PT-IgG (a), PT neutralizing antibodies (b), FHA-IgG (c), TT (d) and DT-IgG (e) with 95% confidence intervals, at baseline (d0), 28 days (d28) and 365 days (d365) following r-aP+Td (open circles) or cd/Tdap (black circles).

Figure 3: Memory B cell response to r-aP+Td or cd-Tdap.

Number of specific antibody secreting cells (ASCs) for PT (a), FHA (b), TT (c) and DT (d) by million PBMCs measured at baseline (d0), 28 (d28) and 365 (d365) days following r-aP+Td (white box left) or cd/Tdap (grey box right) boosting. Median and 95th are shown.

Table 1: Demographic and clinical characteristics of study participants at baseline

Subject status	r-aP + Td	cd/Tdap	Total	P-value
	(N=31)	(N=31)	(N=62)	
Age (years)				0.7280 ^[5]
-Mean (SD)	12.29 (1.42)	12.19 (1.49)	12.24 (1.45)	
-Min/Max	11 – 15	11 – 16	11 - 16	
Gender: n (%)				1.0000 ^[2]
-Male	16 (51.61)	16 (51.61)	32 (51.61)	
-Female	15 (48.39)	15 (48.39)	30 (48.39)	
Ethnicity: n (%)				
-African	3 (9.68)	3 (9.68)	6 (9.68)	1.0000 ^[3]
-Asiatic	2 (6.45)	0 (0.00)	2 (3.23)	0.4918 ^[3]
-Caucasian	29 (93.55)	28 (90.32)	57 (91.94)	1.0000 ^[3]
-Hispanic	3 (9.68)	2 (6.45)	5 (8.06)	1.0000 ^[3]
-Other	1 (3.23)	0 (0.00)	1 (1.61)	1.0000 ^[3]
BMI	30 ^[1 missing data of height]	31	61	0.1209 ^[5]
-Mean (SD)	18.30(2.97)	19.26(3.22)	18.79(3.11)	
-Min/Max	14.23 - 27.20	14.47 - 29.76	14.23 - 29.76	

Subject status	r-aP + Td	cd/Tdap	Total	P-value
	(N=31)	(N=31)	(N=62)	
Vaccination history:				
Time since last dose of diphtheria and tetanus vaccine (Months) ^[a]				0.0266 ^{[4]*}
-Mean (SD)	68.13 (11.02)	74.55 (11.73)	71.34 (11.74)	
-Min/Max	48 – 100	54 - 107	48 - 107	
Time since last dose of pertussis vaccine (Months) ^[a]				0.3381 ^[5]
-Mean (SD)	76.35 (17.98)	76.26 (12.47)	76.31 (15.35)	
-Min/Max	61 – 117	59 - 107	59 - 117	

All results are given for 62 subjects (none missing).

[a]: Time since last dose = Time interval between the last dose of vaccine received and the date of the first visit. [1] No statistic was considered. [2] Overall p-value (2-sided) based on Chi-square test. [3] Overall p-value (2-sided) based on Fisher's exact test. [4] P-value based on Independent T test. [5] P-value based on Wilcoxon rank sum test.

* P-value ≤ 0.05 is considered statistically significant.

Table 2: Local and systemic adverse events between Day 0 and Day 7 post vaccination by vaccine group

	Day 0 – Day 7				
	r-aP ^a	Td ^a	cd/Tdap	r-aP	Td
	(N=31)	(N=31)	(N=31)	vs cd/Tdap	vs cd/Tdap
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	P-value ^b	P-value ^c
Injection site local reactions					
Pain	29 (93.55) (84.90-100.00)	26 (83.87) (70.92-96.82)	23 (74.19) (58.79-89.60)	0.0382 ^{[2]*}	0.3493 ^[2]
Redness	1 (3.23) (0.00-9.45)	0 (0.00) (0.00-0.00)	2 (6.45) (0.00-15.10)	1.0000 ^[3]	0.4918 ^[3]
Swelling	5 (16.13) (3.18-29.08)	7 (22.58) (7.86-37.30)	3 (9.68) (0.00-20.08)	0.7071 ^[3]	0.1672 ^[2]
Other injection site local reactions					
Red papule	0 (0.00) (0.00-0.00)	0 (0.00) (0.00-0.00)	1 (3.23) (0.00-9.45)	1.0000 ^[3]	1.0000 ^[3]
Pruritus	1 (3.23) (0.00-9.45)	0 (0.00) (0.00-0.00)	0 (0.00) (0.00-0.00)	1.0000 ^[3]	_ ^[1]

	Day 0 – Day 7				
	r-aP ^a	Td ^a	cd/Tdap	r-aP	Td
	(N=31)	(N=31)	(N=31)	vs cd/Tdap	vs cd/Tdap
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	P-value ^b	P-value ^c
Systemic reactions					
Objective fever	1(3.23) (0.00-9.45)	1(3.23) (0.00-9.45)	0(0.00) (0.00-0.00)	1.0000 ^[3]	1.0000 ^[3]
Headache	7(22.58) (7.86-37.30)	7(22.58) (7.86-37.30)	4(12.90) (1.10-24.70)	0.3186 ^[2]	0.3186 ^[2]
Fatigue	10(32.26) (15.80-48.71)	10(32.26) (15.80-48.71)	7(22.58) (7.86-37.30)	0.3930 ^[2]	0.3930 ^[2]
Myalgia	5(16.13) (3.18-29.08)	5(16.13) (3.18-29.08)	7(22.58) (7.86-37.30)	0.5202 ^[2]	0.5202 ^[2]
Arthralgia	4(12.90) (1.10-24.70)	4(12.90) (1.10-24.70)	1(3.23) (0.00-9.45)	0.3539 ^[3]	0.3539 ^[3]
Chills	3(9.68) (0.00-20.08)	3(9.68) (0.00-20.08)	0(0.00) (0.00-0.00)	0.2377 ^[3]	0.2377 ^[3]

	Day 0 – Day 7				
	r-aP^a	Td^a	cd/Tdap	r-aP	Td
	(N=31)	(N=31)	(N=31)	vs cd/Tdap	vs cd/Tdap
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	P-value^b	P-value^c
Sweats	1 (3.23) (0.00-9.45)	1 (3.23) (0.00-9.45)	1 (3.23) (0.00-9.45)	1.0000 ^[3]	1.0000 ^[3]
Subjective fever	2 (6.45) (0.00-15.10)	2 (6.45) (0.00-15.10)	4 (12.90) (1.10-24.70)	0.6713 ^[3]	0.6713 ^[3]
Nausea	2(6.45) (0.00-15.10)	2(6.45) (0.00-15.10)	4(12.90) (1.10-24.70)	0.6713 ^[3]	0.6713 ^[3]
Vomiting	0(0.00) (0.00-0.00)	0(0.00) (0.00-0.00)	1(3.23) (0.00-9.45)	1.0000 ^[3]	1.0000 ^[3]
Abdominal pain	1(3.23) (0.00-9.45)	1(3.23) (0.00-9.45)	2(6.45) (0.00-15.10)	1.0000 ^[3]	1.0000 ^[3]
Diarrhea	1(3.23) (0.00-9.45)	1(3.23) (0.00-9.45)	1(3.23) (0.00-9.45)	1.0000 ^[3]	1.0000 ^[3]

	Day 0 – Day 7				
	r-aP ^a	Td ^a	cd/Tdap	r-aP	Td
	(N=31)	(N=31)	(N=31)	vs cd/Tdap	vs cd/Tdap
	n (%)	n (%)	n (%)	P-value ^b	P-value ^c
	(95% CI)	(95% CI)	(95% CI)		
Other systemic reactions					
Dysmenorrhea	1 (3.23)	1 (3.23)	0 (0.00)	1.0000 ^[3]	1.0000 ^[3]
	(0.00-9.45)	(0.00-9.45)	(0.00-0.00)		

a : r-aP and Td were given concomitantly to the same subjects on different arms.

b : Comparison between arms injected with r-aP vs cd/Tdap

c : Comparison between arms injected with Td vs cd/Tdap

Note:

[1] No statistic was considered

[2] Overall p-value (2-sided) based on Chi-square test

[3] Overall p-value (2-sided) based on Fisher's exact test

* P-value ≤ 0.05 is considered statistically significant.

Table 3: Seroresponse rates and GMCs of antibody to PT (total IgG or neutralizing), FHA, diphtheria and tetanus toxoids, measured at baseline, 28 and 365 days after booster vaccination with either r-aP+ Td vaccines or cd/Tdap vaccines.

	r-aP + Td						cd/Tdap						raP+Td vs Tdap	raP+Td vs Tdap	raP+Td vs Tdap
	N=31	N=31	N=28	D0-D28	D0-D365	D28-D365	N=29	N=29	N=28	D0-D28	D0-D365	D28-D365	D0	D28	D365
	Day 0	Day 28	Day 365	P-value	P-value	P-value	Day 0	Day 28	Day 365	P-value	P-value	P-value	P-value	P-value	P-value
GMCs	IU/ml (95% CI)	IU/ml (95% CI)	IU/ml (95% CI)				IU/ml (95% CI)	IU/ml (95% CI)	IU/ml (95% CI)						
PT IgG antibodies	3.79 (2.86-5.02)	113.74 (88.31-146.50)	26.87 (19.51-37.00)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	5.19 (3.40-7.91)	52.43 (36.41-75.50)	15.75 (10.22-24.27)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.2339 [2]	0.0006 [3]*	0.0465 [3]*
GMC ratios		30.04 (21.68-41.63)	6.79 (4.60-10.02)					10.11 (7.21-14.17)	2.96 (1.98-4.43)					<0.0001 [3]*	0.0036 [3]*
PT neutralizing antibodies	7.02 (5.32-9.28)	127.68 (96.73-168.53)	36.82 (26.90-50.40)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	8.38 (5.45-12.88)	73.91 (49.88-109.52)	25.43 (17.86-36.20)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.8791 [2]	0.0162 [2]*	0.1324 [2]
GMC ratios		18.18 (13.28-24.87)	5.31 (3.66-7.70)					8.82 (5.83-13.36)	2.98 2.06-4.31					0.0057 [3]*	0.0356 [2]*
FHA IgG antibodies	36.43 (25.59-51.86)	459.27 (358.12-588.97)	140.80 (105.68-187.59)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	52.10 (31.53-86.08)	382.96 (293.90-498.99)	138.25 (102.11-187.16)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.2330 [3]	0.3099 [3]	0.9216 [2]
GMC ratios		12.61 (8.81-18.04)	3.82 (2.84-5.15)					7.35 (4.86-11.12)	2.66 (1.78-3.98)					0.0476 [3]*	0.2758 [2]
TT IgG antibodies	0.70 (0.49-0.99)	12.32 (9.91-15.31)	4.39 (3.45-5.59)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.46 (0.28-0.75)	10.57 (8.47-13.18)	3.83 (2.95-4.98)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.1535 [3]	0.3167 [3]	0.4370 [3]
DT IgG antibodies	0.20 (0.14-0.28)	1.77 (1.13-2.77)	0.46 (0.34-0.63)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.11 (0.08-0.15)	3.18 (1.79-5.64)	0.55 (0.44-0.71)	<0.0001 [1]*	<0.0001 [1]*	<0.0001 [1]*	0.0214 [2]*	0.0508 [2]	0.3269 [3]
Seroresponse rates		N (%) (95% CI)	N (%) (95% CI)					N (%) (95% CI)							

PT neutralizing Ab (≥4-fold increase)	30 (97%) (90.6-100)	22 (79%) (63.4-93.8)		23 (79%) (64.6-94.1)	11 (39%) (21.2-57.4)		0.0489 [4] ^a	0.0060 [4] ^a	
PT-IgG (≥4-fold increase)	30 (97%) (90.6-100)	20 (71%) (54.7-88.2)		27 (93%) (83.9-100)	11 (39%) (21.2-57.4)		0.0489 [4] ^a	0.0305 [4] ^a	
FHA-IgG (≥4-fold increase)	30 (97%) (90.6-100)	9 (32%) (14.8-49.4%)		21 (72%) (56.2-88.7)	10 (36%) (18-53.5)		0.0110 [4] ^a	1.0000 [4]	
TT-IgG (>0.1 IU/ml)	30 (97%) (90.6-100)	31 (100%) (100 - 100)	28 (100%) (100 - 100)	28 (97%) (89.9 – 100)	29 (100%) (100 - 100)	28 (100%) (100 - 100)	1.0000 [4]	Ns	Ns
DT-IgG (>0.1 IU/ml)	24 (77%) (62.7-92.1)	31 (100%) (100 – 100)	27 (96%) (89.6-100)	16 (55%) (37.1 – 73.3)	29 (100%) (100 – 100)	28 (100%) (100 - 100)	0.0677 [5]	Ns	1.0000 [4]

GMC: Geometric mean concentration

Ns: No statistic was considered because all subjects had antibody > 0.1 IU/ml at Day28.

[1] P-value based on paired t-test

[2] P-value based on Wilcoxon rank sum test

[3] P-value based on Independent T test

[4] Overall p-value (2-sided) based on Fisher's exact test

[5] Overall p-value (2-sided) based on Chi-square test

*P-value ≤ 0.05 is considered statistically significant.

Figure 1

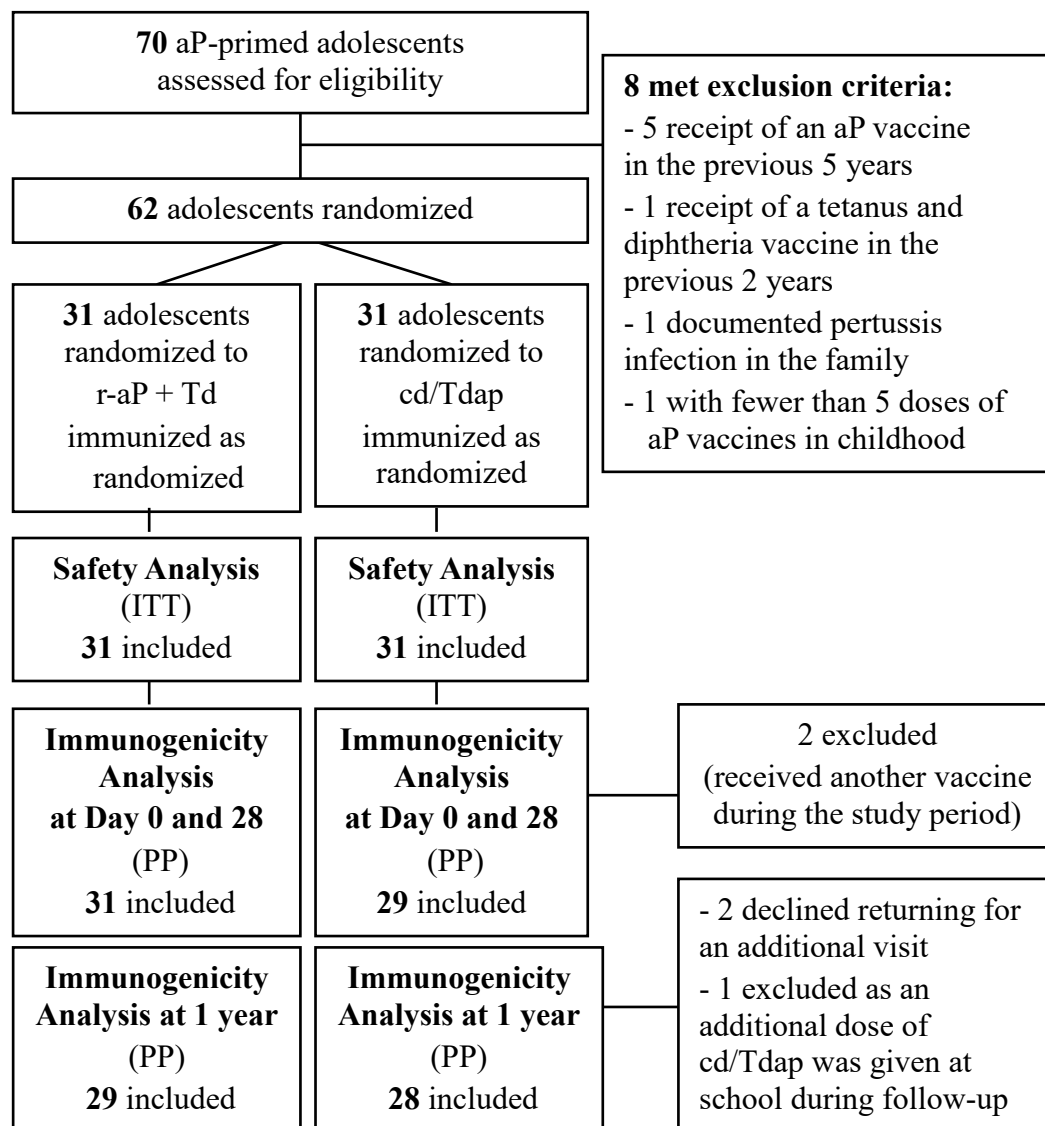


Figure 2:

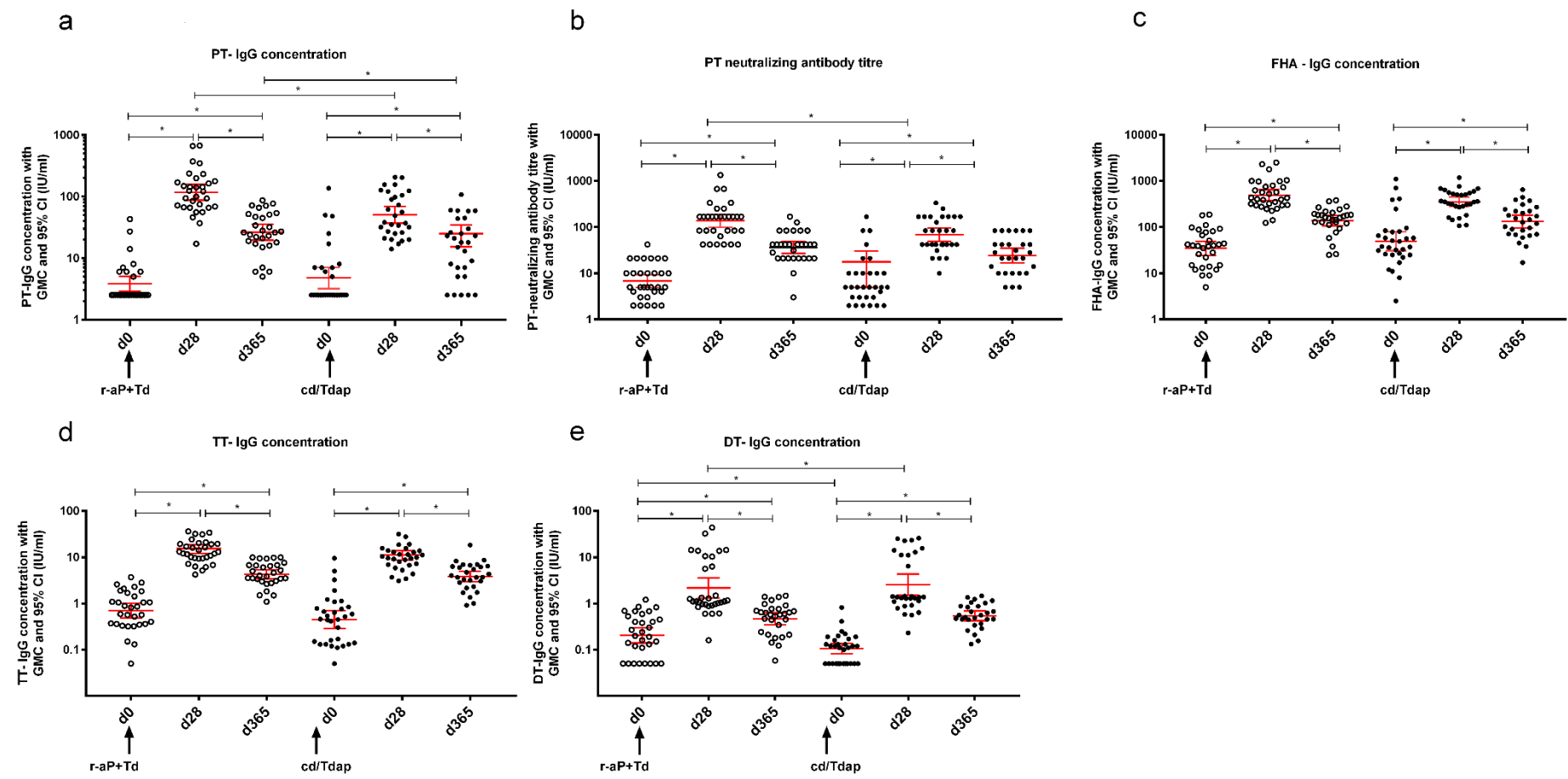


Figure 3:

