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Defining the optimal formulation and schedule of a candidate toxoid vaccine against *Clostridium difficile* infection: A randomized Phase 2 clinical trial☆

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ABSTRACT

Background: Clostridium difficile, a major cause of nosocomial and antibiotic-associated diarrhea, carries a significant disease and cost burden. This study aimed to select an optimal formulation and schedule for a candidate toxoid vaccine against *C. difficile* toxins A and B.
Methods: Randomized, placebo-controlled, two-stage, Phase 2 study in a total of 661 adults aged 40–75 years. Stage I: low (50 μg antigen) or high (100 μg antigen) dose with or without aluminum hydroxide (AlOH) adjuvant, or placebo, administered on Days 0–7–30. Stage II: Days 0–7–30, 0–7–180, and 0–30–180, using the formulation selected in Stage I through a decision tree defined a priori and based principally on a bootstrap ranking approach. Administration was intramuscular. Blood samples were obtained on Days 0, 7, 14, 30, 60 (Stage I and II), 180, and 210 (Stage II); IgG to toxins A and B was measured by ELISA and in vitro functional activity was measured by toxin neutralizing assay (TNA). Safety data were collected using diary cards.
Results: In Stage I the composite immune response against toxins A and B (percentage of participants who seroconverted for both toxins) was highest in the high dose + adjuvant group (97% and 92% for Toxins A and B, respectively) and was chosen for Stage II. In Stage II the immune response profile for this formulation through Day 180 given on Days 0–7–30 ranked above the other two administration schedules. There were no safety issues.

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Conclusions: The high dose + adjuvant (100 μg antigen + AlOH) formulation administered at 0–7–30 days elicited the best immune response profile, including functional antibody responses, through Day 180 and was selected for use in subsequent clinical trials.

1. Introduction

Clostridium difficile (*C. difficile*) is a gram-positive, spore-forming anaerobe, which causes colonic mucosal injury and inflammation by the release of toxins A (a 308 kDa enterotoxin) and B (a 270 kDa cytotoxin) [1]. While carriage of the organism may be asymptomatic, *C. difficile* is a major cause of nosocomial and antibiotic-associated diarrhea in Europe and North America, and severe cases can lead to pseudomembranous colitis and toxic megacolon [2,3]. *C. difficile* infection (CDI) imposes a significant burden of disease [4] and infection rates have increased substantially over recent years. In the US alone, recent data suggest almost 500,000 infections and approximately 29,000 deaths in 2011 [5]. As well as its importance as a nosocomial infection, *C. difficile* is also increasingly present in the community [6] and the financial burden is high, being estimated at over $7 billion annually in Europe [7] and the US [8,9] combined.

Given the limitations of current treatment options, the high rates of recurrence [8,10–12], and with *C. difficile* spores tolerating most disinfection procedures [13], combatting CDI by targeted disease prevention is ideal. Toxins A and B both damage colonic cells, and studies have suggested a relationship between the immune response to these toxins and protection against CDI [14–18]. No vaccine is available against CDI, however a bivalent, toxoid vaccine to stimulate immunity to toxins A and B and negate their harmful effects is currently being developed [19]. Phase 1 data have shown good tolerability and a strong immune response to both toxins in adults including the elderly [20]. Crucial to the continued clinical development of this candidate vaccine is a robust assessment of a range of formulations and dosing schedules. This study was conducted to identify the formulation and schedule to be used in later phase clinical studies. As such, Stage I of the study was designed to assess the safety and immunogenicity of four formulations (high or low dose of antigen, with or without adjuvant) administered in the same schedule and Stage II assessed two further schedules using the formulation selected in Stage I.

2. Methods

2.1. Study design and participants

This randomized, placebo controlled, Phase 2 study was conducted in two stages in 39 centers in the USA. In Stage I a range of doses (low [50 μg total antigen] or high [100 μg total antigen]) and formulations (with or without aluminum hydroxide adjuvant) were assessed for a candidate *C. difficile* vaccine using the same administration schedule. In Stage II two additional schedules of administration were assessed using the formulation selected after Stage I. In Stage I the investigators, participants, outcome assessors, and laboratory personnel were blinded to the formulation administered. Stage II was open-label (except for laboratory personnel). Local independent ethics committees approved the study protocol and amendments. The study was conducted according to the applicable local and national requirements, Good Clinical Practice and applicable International Conference on Harmonization guidelines, and conformed to the principles of the Declaration of Helsinki (Edinburgh revision, October 2000).

Each participant signed an informed consent form prior to enrolment. Vaccine administration took place between 27 October 2010–15 June 2011 (Stage I) and 15 November 2011–23 July 2012 (Stage II) (ClinicalTrials.gov NCT01230957).

To be eligible, participants were aged between 40 and 75 years (stratified into equal groups aged 40–64 years and 65–75 years in each stage) and considered to be at risk of *C. difficile* infection based on (i) impending hospitalization within 60 days of enrolment, or (ii) current or impending (within 60 days of enrolment) residence in a long-term care facility or rehabilitation facility. The main exclusion criteria were: pregnancy or lack of effective contraception, participation in another clinical study, non-study vaccination (other than influenza or pneumococcal vaccines) in the previous 4 weeks, previous vaccination against *C. difficile*, current or prior episode of CDI, receipt of blood products in the previous 3 months, congenital or acquired immunodeficiency or receipt of anti-cancer chemo- or radiotherapy in the previous 6 months, >2 weeks corticosteroid therapy in the previous 3 months, seropositivity for HIV/hepatitis B/hepatitis C, anticipated or current kidney dialysis, hypersensitivity to any vaccine component, bleeding disorder contraindicating intramuscular (IM) injection, chronic disease or addiction that could interfere with study procedures, history of diverticular intestinal bleeding, surgery for gastrointestinal malignancy in the previous 3 months.

In Stage I, participants were randomized to one of five groups and received three doses of vaccine (low or high dose with or without adjuvant) or placebo at Days 0–7–30 (see Table 1). One of these formulations was then selected for Stage II, based on immunogenicity. In Stage II, additional participants were randomized to receive the selected vaccine formulation according to two alternative vaccination schedules: Days 0–7–180, or 0–30–180. All injections were intramuscular (IM), ideally into alternate arms for sequential vaccinations.

2.2. Vaccines

The investigational *C. difficile* toxoid vaccine was a formalin-inactivated, highly purified preparation of toxoids A and B, and presented as a freeze-dried preparation that was reconstituted with diluent (either adjuvant or water for injection) prior to IM injection (0.5 mL). The adjuvant was 400 μg AlOH provided as 1600 μg/mL in water for injection. Placebo was 0.9% saline.

2.3. Serology

Blood samples were collected on Days 0, 7, 14, 30, 60 (all participants), 180, and 210 (all participants in Stage II and those in Stage I who received the formulation selected for Stage II), and analyzed for (i) serum anti-toxin IgG to *C. difficile* toxins A and B (enzyme-linked immunosorbent assay [ELISA]), and (ii) anti-toxin A and B neutralizing capacity (toxin neutralizing assay [TNA]). All analyses were conducted centrally at the Sponsor's Global Clinical Immunology laboratory.

For the ELISA, full-length *C. difficile* toxin A or toxin B was used to coat ELISA plates. All controls, reference, and samples were added to the microtiter plates, incubated at 37 °C followed by incubating with goat anti-human IgG conjugated to horseradish peroxidase.

Table 1
Summary of seroconversion rates measured by ELISA (Day 0–210) (PP set).

	Stage I					Stage II	
Toxin Day	Group 1 (*N* = 69)	Group 2 (*N* = 68)	Group 3 (*N* = 64)	Group 4 (*N* = 71)	Group 5 (*N* = 38)	Group 6 (*N* = 61)	Group 7 (*N* = 57)
Toxin A							
Day 7	NA	NA	10.6 (4.4;20.6)	NA	NA	5.0 (1.0;13.9)	8.8 (2.9;19.3)
Day 14	36.2 (25.0;48.7)	30.9 (20.2;43.3)	27.7 (17.3;40.2)	26.8 (16.9;38.6)	0.0 (0.0;9.3)	37.7 (25.6;51.0)	29.8 (18.4;43.4)
Day 30	74.3 (62.4; 84.0)	48.5 (36.2;61.0)	67.7 (55.0;78.8)	60.6 (48.3;72.0)	5.3 (0.6;17.8)	67.2 (54.0;78.7)	50.9 (37.3;64.4)
Day 60	94.2 (85.8;98.4)	95.6 (87.6;99.1)	97.0 (89.5;99.6)	90.1 (80.7;95.9)	7.9 (1.7;21.4)	65.6 (52.3;77.3)	91.2 (80.7;97.1)
Day 180	NA	NA	84.8 (73.9;92.5)	NA	NA	50.8 (37.7;63.9)	71.9 (58.5;83.0)
Day 210	NA	NA	81.8 (70.4;90.2)	NA	NA	100.0 (94.1;100.0)	100.0 (93.7;100.0)
Toxin B							
Day 7	NA	NA	12.1 (5.4;22.5)	NA	NA	24.6 (14.5;37.3)	17.5 (8.8;29.9)
Day 14	57.1 (44.8;68.9)	45.6 (33.5;58.1)	57.6 (44.8;69.7)	58.9 (46.8;70.3)	2.6 (0.1;13.8)	68.9 (55.7;80.1)	59.6 (45.8;72.4)
Day 30	75.7 (64.0;85.2)	60.3 (47.7;72.0)	71.2 (58.8;81.7)	67.1 (55.1;77.7)	7.9 (1.7;21.4)	85.2 (73.8;93.0)	73.7 (60.3;84.5)
Day 60	85.7 (75.3;92.9)	82.4 (71.2;90.5)	92.4 (83.2;97.5)	93.2 (84.7;97.7)	13.2 (4.4;28.1)	85.2 (73.8;93.0)	89.5 (78.5;96.0)
Day 180	NA	NA	74.2 (62.0;84.2)	NA	NA	62.3 (49.0;74.4)	71.9 (58.5;83.0)
Day 210	NA	NA	69.7 (57.2;80.4)	NA	NA	93.4 (84.1;98.2)	93.0 (83.0;98.1)
Composite[a]							
Day 7	NA	NA	4.5 (1.0;12.7)	NA	NA	5.0 (1.0;13.9)	3.5 (0.4;12.1)
Day 14	29.0 (18.7;41.2)	29.4 (19.0;41.7)	24.6 (14.8;36.9)	25.4 (15.8;37.1)	0.0 (0.0;9.3)	36.1 (24.2;49.4)	22.8 (12.7;35.8)
Day 30	62.9 (50.5;74.1)	38.2 (26.7;50.8)	46.2 (33.7;59.0)	50.7 (38.6;62.8)	2.6 (0.1;13.8)	62.3 (49.0;74.4)	42.1 (29.1;55.9)
Day 60	85.5 (75.0;92.8)	82.4 (71.2;90.5)	90.9 (81.3;96.6)	87.3 (77.3;94.0)	7.9 (1.7;21.4)	60.7 (47.3;72.9)	84.2 (72.1;92.5)
Day 180	NA	NA	68.2 (55.6;79.1)	NA	NA	37.7 (25.6;51.0)	54.4 (40.7;67.6)
Day 210	NA	NA	62.1 (49.3;73.8)	NA	NA	93.4 (84.1;98.2)	93.0 (83.0;98.1)

Data are percentage of participants with a ≥4-fold increase from Day 0 (95% CI).
N = number of participants included in the PP set (not necessarily the number of participants available for a particular timepoint).
NA = not applicable (group not selected for Stage II).
[a] Composite indicates when a participant demonstrated a ≥4-fold increase for both toxins.

After exposure to peroxidase enzyme substrate, plates were read by an ELISA plate reader using SoftMax Pro software. The average optical density (OD) value for the plate blank was subtracted from all the ODs within each plate. The concentration of antibodies in serum was then derived by extrapolating from a standard curve. The reference standard consisted of pooled human plasma from previously immunized subjects. The reference was assigned a value of 100 EU/mL, thus quantitative results were reported in EU/mL.

For the TNA, serial dilutions of sera were mixed with *C. difficile* challenge toxin and incubated with cultured Vero cells that are sensitive to the toxin. The OD of each plate was read at 562 nm (signal) and 630 nm (background). The 50% specific signal was determined by SoftMax Pro software for each plate. The titer of neutralizing antibodies for the sample tested is the reciprocal final dilution corresponding to the 50% specific signal. The 50% signal was the OD value of the cell control plus one half the difference between the toxin control and cell control. The titer was determined by interpolation of the dilution corresponding to the 50% specific signal.

2.4. Reactogenicity and safety

Pre-defined (solicited) adverse events (AEs) were automatically considered to be related to vaccination (adverse reactions [ARs]): solicited injection site (pain, erythema, swelling) and systemic (fever, headache, malaise, myalgia, arthralgia) ARs were collected on the day of vaccination and for 6 days after each vaccination. Unsolicited AEs were collected for 30 days after each vaccination. Serious adverse events (SAEs) were collected throughout the study. The relationship of unsolicited AEs and SAEs to vaccination was assessed by the investigator.

2.5. Biological safety

In Stage II only, blood and urine samples were taken on Days 0, 14, and 210 for hematology (hemoglobin, white blood cells [WBCs], lymphocytes, neutrophils, eosinophils, platelets), serum biochemistry (sodium, potassium, glucose, creatinine, albumin, liver function tests), and urinalysis (protein, glucose, red blood cells, WBCs). All analyses were done at local laboratories.

2.6. Statistical analyses

All analyses were descriptive, with no hypotheses tested. The primary objectives were to describe the safety profile and the immune response elicited by the *C. difficile* toxoid vaccine candidate, with the aim of selecting a vaccine formulation and dosing schedule for further clinical development. A per protocol (PP) analysis set was defined for immunogenicity based on meeting inclusion/exclusion criteria, receipt of the correct number of doses, receipt of the correct formulation administered according to the protocol and in the proper time window, provision of pre- or post-dose 1 serology samples according to protocol-defined time windows, protocol deviations, lack of a valid serology result pre- or post-dose 1. A safety analysis set, including all participants who received at least one trial vaccination, was used for the safety analyses. For the main parameters, 95% confidence intervals (CIs) of point estimates were calculated using the normal approximation for quantitative data and the exact binomial distribution for proportions [21].

For the selection of the formulation for Stage II, a bootstrap analysis was done using the data to Day 60 from Stage I. A similar analysis was done for the selection of the optimal schedule using the data through Day 210 in Stage II. A pre-specified ranking/selection analysis based on the immunogenicity outcomes was applied to choose the best formulation in Stage I and the best schedule in Stage II. Geometric mean concentrations (GMCs) of IgG against each toxin (measured by ELISA) were ranked both separately and as a composite (A and B). For Stage I, the probability of a treatment group being ranked number 1 was determined based on the Day 60 GMCs. In the absence of one treatment group reaching 80% probability, ranking was applied to Day 30 and Day 14 data. Similarly for Stage II, the probability of a schedule being ranked number 1 was determined based on the Day 60 GMCs. In the absence of one schedule reaching 80% probability, ranking was

applied to Day 14 and Day 210 data. The formulation selection was based primarily on the ELISA IgG results, with TNA results being supportive, and the immune response in the older age stratum (65–75 years of age) participants was also considered in the choice of formulation.

Being a descriptive study, the sample size calculation was not hypothesis-driven. With 100 participants planned per vaccine group and 50 in the placebo group there was a 95% probability of detecting an AE with a true incidence of 3.0% and 5.8%, respectively.

3. Results

3.1. Participants studied

A total of 455 participants were enrolled into Stage I (101 participants in Groups 1, 2, and 3; 102 participants in Group 4; 50 participants in Group 5), and 206 enrolled into Stage II (103 in each of Group 6 and Group 7) (Fig. 1). Of these, 416 (91.4%) participants completed Stage I to Day 60 (89 [88.1%], 95 [94.1%], 94 [93.1%], 90 [88.2%] and 48 [96.0%] participants in Groups 1, 2, 3, 4 and 5, respectively) and 168 (81.6%) participants completed Stage II (89 [86.4%] and 79 [76.7%] in Groups 6 and 7, respectively). Age, ethnicity, and gender were comparable in each group. There were no important differences between the two age ranges in terms of safety and immunogenicity and so only the pooled data for the age range 40–75 years are presented.

The majority of participants (597 [90.5%]) had a planned hospital stay at enrolment and of these the majority (509 [85.3%]) were admitted to hospital prior to receiving the third dose of study vaccine.

3.2. Serology

3.2.1. Stage I

The GMC responses (ELISA) for Groups 1–5 on Days 0, 14, 30, and 60 are presented in Fig. 2 (data presented in Supplementary Table 1) and seroconversion rates (percentage of participants with a ≥4-fold increase from Day 0) are presented in Table 1. Following vaccination on Days 0, 7 and 30, there was a steady increase in GMC in Groups 1–4 (active vaccine groups) peaking at Day 60 for toxins A and B. At Day 60, for toxin A, Group 3 (100 μg + AlOH) showed the highest GMC response (91.5 EU/mL) and also the highest seroconversion rate (97%); for toxin B, GMC was highest in Group 4 (high dose, no adjuvant) (156.8 EU/mL) while seroconversion rate remained highest in Group 3 (92%). The composite seroconversion rate for toxins A and B (i.e. the percentage of participants who seroconverted for both toxins) was highest in Group 3 (91% at Day 60).

For TNA, GMTs are presented in Fig. 2 and in Supplementary Table 2, and seroconversion rates are presented in Table 2. These data at Day 60 tended to favor Group 3 for toxin A and Group 4 for toxin B (however, for the elderly participants these data were preferable in Group 3 [for participants 65–75 years of age, the Day 60 Toxin A GMT in Group 3 was 568.8 1/dil (95% CI: 352.5; 918.0) and Group 4 was 372.1 1/dil (95% CI: 178.5; 775.9) and the Day 60 Toxin B GMT in Group 3 was 289.0 1/dil (95% CI: 116.1; 719.4) and in Group 4 was 288.1 1/dil (95% CI: 91.2; 910.2]). Seroconversion rate data at Day 60 were highest in Group 3 for toxin A (97.0%) and similar for toxin B in Groups 3 and 4 (63.6% and 67.1%, respectively) (the composite seroconversion rate was also similar on Groups 3 and 4, being 62.1% and 64.4%, respectively).

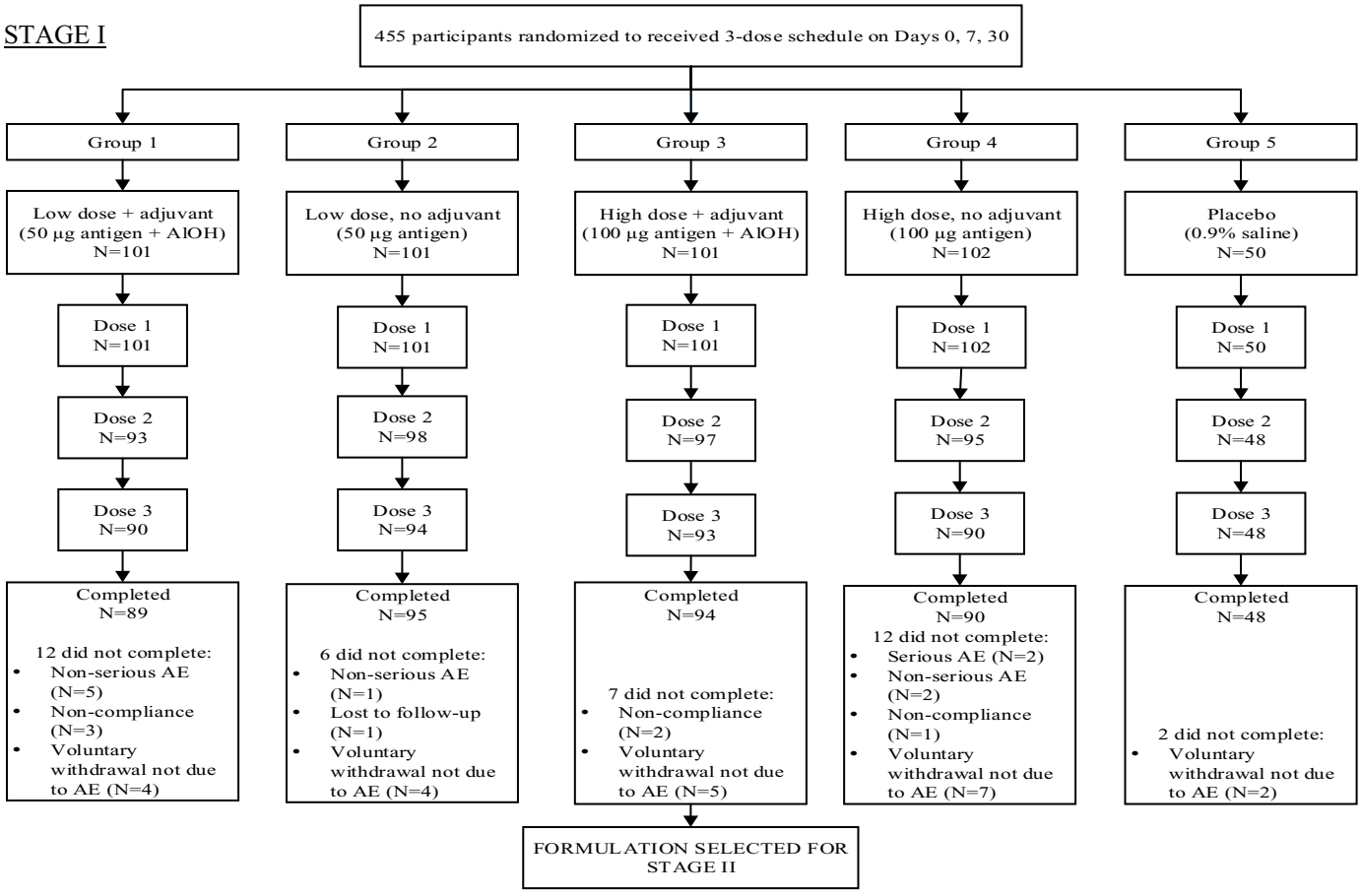

Fig. 1. Participant disposition.

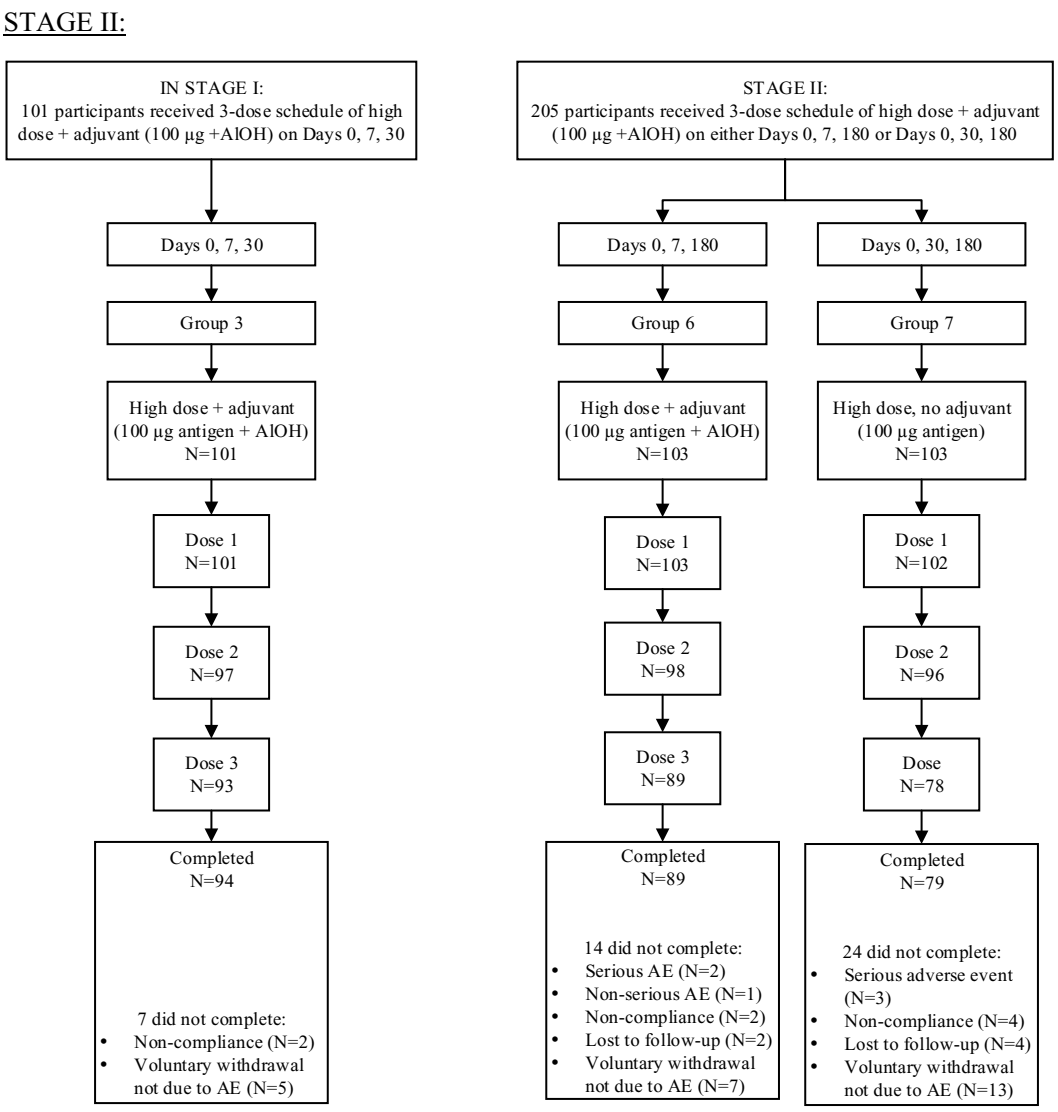

Fig. 1. (*Continued*).

Based on these results, the 100 µg + AlOH formulation (Group 3) was selected as having the best overall immunogenic profile and on this basis was chosen for further evaluation in Stage II.

3.2.2. Stage II

For Group 3 (0, 7, 30 day schedule) the GMCs for both toxins A and B peaked at Day 60, and were higher than for Group 6 (0, 7, 180 vaccination schedule) and Group 7 (0, 30, 180 day schedule) at this time point (the latter two groups having received only the first two doses). In Group 3, GMCs for both toxins at Day 180 continued to rank above Groups 6 and 7, but had decreased by Day 210 whereas in Groups 6 and 7 (both of which had a third vaccination at Day 180) GMCs for both toxins increased markedly from Day 180 to Day 210, being similar in both groups. These data are presented graphically to Day 180 in Fig. 3 (the time period used for the ranking and choice of schedule); the data to Day 210 are presented in Supplementary Table 3. Seroconversion rates for each toxin and the composite seroconversion rate data (Table 1) showed the same trends as the ELISA GMC data, with the highest percentage of seroconverted participants being at Day 60 for Group 3 and Day 210 for Groups 6 and 7.

The TNA GMT data (presented graphically to Day 180 in Fig. 3; data presented to Day 210 in Supplementary Table 4) supported the trends described for the ELISA data, peaking at Day 60 for Group 3, at which point GMTs were higher than for Groups 6 and 7. As for the ELISA data, Group 3 GMT data remained highest at Day 180 (except for Toxin B, which was slightly higher for Group 7 [228.2 versus 175.4 1/dil]), and Groups 6 and 7 were highest by Day 210 (following the third vaccination in these two latter groups at Day 180). The TNA seroconversion data for both toxins and for the composite response also confirmed the evaluation based on the ELISA data, with Group 3 being highest at Day 60 and Groups 6 and 7 being highest at Day 210 (Table 2).

The bootstrap ranking analysis for the ELISA data at Day 60 in Groups 3, 6, and 7 identified Group 3 as having at least 80% probability of being ranked first for toxin B and for the composite data (toxin A and B); toxin A for Group 3 was also ranked higher than Groups 6 or 7.

Overall, the vaccination schedule in Group 3 (0, 7, 30 days) provided the highest immune response up to Day 180.

3.3. Safety and reactogenicity (Stage I and II)

In all groups there were very few immediate AEs (occurring in the 30 min after vaccination). The incidence of solicited injection site and systemic reactions occurring in the 6 days after

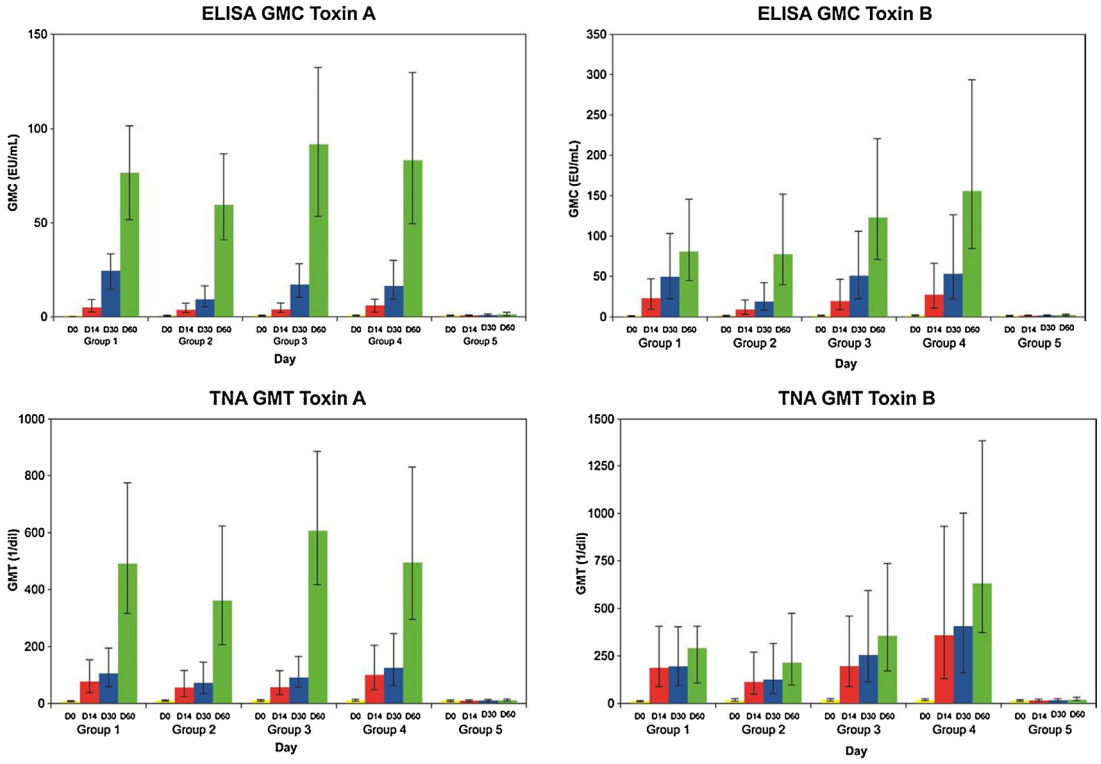

Fig. 2. Stage I: ELISA GMCs and TNA GMTs with associated 95% confidence intervals (Groups 1–5) (PP set).

vaccination was similar in each vaccine group, and higher than for placebo (Table 3). In all groups, pain was the most common injection site reaction and myalgia was the most common systemic reaction; most were Grade 1 in intensity, began within the first 3 days, and had resolved within 3 days.

The incidence of unsolicited AEs after vaccination was comparable between vaccine groups, ranging from 58.8% to 76.0%, and similar to the placebo group (56.0%); those related to the vaccine ranged from 9.8% to 20.6% for the vaccine groups with none in the placebo group.

Table 2
Summary of seroconversion rates measured by TNA (Day 0–210) (PP set).

	Stage I					Stage II	
Toxin Day	Group 1 (*N* = 69)	Group 2 (*N* = 68)	Group 3 (*N* = 66)	Group 4 (*N* = 71)	Group 5 (*N* = 38)	Group 6 (*N* = 61)	Group 7 (*N* = 57)
Toxin A							
Day 7	NA	NA	13.6 (6.4;24.3)	NA	NA	8.2 (2.7;18.1)	10.5 (4.0;21.5)
Day 14	40.0 (28.5;52.4)	30.9 (20.2;43.3)	31.8 (20.9;44.4)	42.5 (31.0;54.6)	0.0 (0.0;9.3)	39.3 (27.1;52.7)	42.1 (29.1;55.9)
Day 30	47.1 (35.1;59.5)	36.8 (25.4;49.3)	40.9 (29.0;53.7)	43.8 (32.2;56.0)	0.0 (0.0;9.3)	49.2 (36.1;62.3)	33.3 (21.4;47.1)
Day 60	91.4 (82.3;96.8)	75.0 (63.0;84.7)	97.0 (89.5;99.6)	82.2 (71.5;90.2)	0.0 (0.0;9.3)	41.0 (28.6;54.3)	82.5 (70.1;91.3)
Day 180	NA	NA	92.3 (83.0;97.5)	NA	NA	44.3 (31.6;57.6)	73.7 (60.3;84.5)
Day 210	NA	NA	87.9 (77.5;94.6)	NA	NA	100.0 (94.1;100.0)	100.0 (93.7;100.0)
Toxin B							
Day 7	NA	NA	18.2 (9.8;29.6)	NA	NA	29.5 (18.5;42.6)	21.1 (11.4;33.9)
Day 14	55.7 (43.3;67.6)	35.3 (24.1;47.8)	50.0 (37.4; 62.6)	52.1 (40.0;63.9)	0.0 (0.0;9.3)	54.1 (40.9;66.9)	56.1 (42.4;69.3)
Day 30	57.1 (44.8;68.9)	41.8 (29.9;54.5)	56.1 (43.3;68.3)	56.2 (44.1;67.8)	0.0 (0.0;9.3)	57.4 (44.1;70.0)	56.1 (42.4;69.3)
Day 60	60.0 (47.6;71.5)	50.0 (37.6;62.4)	63.6 (50.9;75.1)	67.1 (55.1;77.7)	5.3 (0.6;17.8)	57.4 (44.1;70.0)	63.2 (49.3;75.6)
Day 180	NA	NA	53.0 (40.3;65.4)	NA	NA	54.1 (40.9;66.9)	59.6 (45.8;72.4)
Day 210	NA	NA	51.5 (38.9;64.0)	NA	NA	86.9 (75.8;94.2)	89.5 (78.5;96.0)
Composite[a]							
Day 7	NA	NA	7.6 (2.5;16.8)	NA	NA	6.6 (1.8;16.0)	7.0 (2.0;17.0)
Day 14	31.4 (20.9;43.6)	25.0 (15.3;37.0)	22.7 (13.3;34.7)	35.6 (24.8;47.7)	0.0 (0.0;9.3)	32.8 (21.3;46.0)	33.3 (21.4;47.1)
Day 30	38.6 (27.2;51.0)	28.4 (18.0;40.7)	30.3 (19.6;42.9)	32.9 (22.3;44.9)	0.0 (0.0;9.3)	36.1 (24.2;49.4)	29.8 (18.4;43.4)
Day 60	60.0 (47.6;71.5)	47.1 (34.8;59.6)	62.1 (49.3;73.8)	64.4 (52.3;75.3)	0.0 (0.0;9.3)	31.1 (19.9;44.3)	56.1 (42.4;69.3)
Day 180	NA	NA	50.8 (38.1;63.4)	NA	NA	34.4 (22.7;47.7)	47.4 (34.0;61.0)
Day 210	NA	NA	47.0 (34.6;59.7)	NA	NA	86.9 (75.8;94.2)	89.5 (78.5;96.0)

Data are percentage of participants with a ≥4-fold increase from Day 0 (95% CI).
N = number of participants included in the PP set (not necessarily the number of participants available for a particular timepoint).
NA = not applicable (group not selected for Stage II).
[a] Composite indicates when a participant demonstrated a ≥4-fold increase for both toxins.

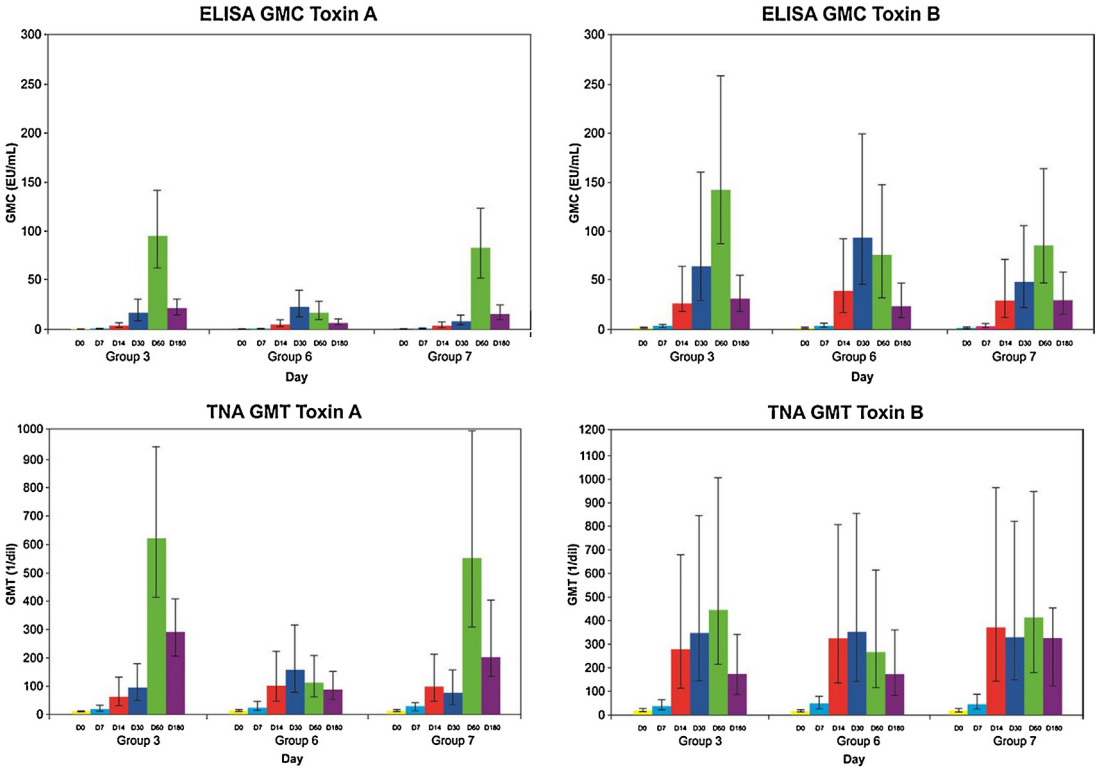

Fig. 3. Stage II: ELISA GMCs and TNA GMTs with associated 95% confidence intervals (Day 0–180) (Groups 3, 6, 7) (PP set).

No vaccine-related SAEs were reported; there were four deaths (one in Group 3, two in Group 6, and one in Group 7) but none was considered to be related to the vaccine.

3.4. Biological safety (Stage II only)

Although there were occasional out of range values that were of clinical significance, these were generally associated with underlying medical conditions and not unexpected for this study population. None was considered to be related to the vaccine, and none was of clinical importance in the context of this study.

4. Discussion

This Phase 2 study was conducted to define the optimal formulation and vaccination schedule for a candidate toxoid vaccine to protect against CDI to be used in Phase 3 clinical development. The dose levels in Stage I (50 and 100 μg) were chosen based on Phase I studies in healthy adult and elderly volunteers [20], in which doses up to 50 μg had been used. The response using the 50 μg dose level had been strong for toxin A but was lower than expected for toxin B, and so this dose was used as a starting dose in the present study, with the 100 μg dose level being included to evaluate whether the increased dose would result in an improved response for toxin B and also to assess whether the higher dose would be associated with a more rapid response. The vaccination schedules chosen for assessment in Stage II were comparatively evaluated based on the goal of achieving an immune response by Day 14 and with priority in the analysis being given to the immune response to Day 60 as this represents typical waiting times for planned, non-emergent, major surgical procedures [22]. The majority of participants were enrolled prior to a planned hospitalization, and most were admitted

Table 3
Solicited injection site and systemic adverse reactions occurring in the 7 days after any dose of vaccine (safety analysis set).

Adverse reaction	Stage I					Stage II	
	Group 1 (*N* = 100)	Group 2 (*N* = 102[a])	Group 3 (*N* = 101)	Group 4 (*N* = 102)	Group 5 (*N* = 50)	Group 6 (*N* = 103)	Group 7 (*N* = 102)
Any solicited reaction	70.1 (60.0;79.0)	69.7 (59.6;78.5)	75.2 (65.7;83.3)	69.8 (59.6;78.7)	43.8 (29.5;58.8)	82.2 (73.3;89.1)	83.0 (74.2;89.8)
Injection site reactions:	53.6 (43.2;63.8)	43.4 (33.5;53.8)	54.5 (44.2;64.4)	58.3 (47.8;68.3)	25.0 (13.6;39.6)	70.3 (60.4;79.0)	66.0 (55.8;75.2)
Pain	52.6 (42.2;62.8)	42.4 (32.6;52.8)	52.5 (42.3;62.5)	57.3 (46.8;67.3)	25.0 (13.6;39.6)	68.3 (58.3;77.2)	65.0 (54.8;74.3)
Erythema	5.2 (1.7;11.6)	3.0 (0.6;8.6)	6.9 (2.8;13.8)	10.4 (5.1;18.3)	0.0 (0.0;7.4)	11.9 (6.3;19.8)	5.0 (1.6;11.3)
Swelling	6.2 (2.3;13.0)	1.0 (0.0;5.5)	5.0 (1.6;11.2)	4.2 (1.1;10.3)	0.0 (0.0;7.4)	9.9 (4.9;17.5)	8.0 (3.5;15.2)
Systemic reactions:	58.8 (48.3;68.7)	57.6 (47.2;67.5)	58.4 (48.2;68.1)	52.1 (41.6;62.4)	37.5 (24.0;52.6)	64.4 (54.2;73.6)	62.0 (51.7;71.5)
Fever	5.3 (1.7;11.9)	6.1 (2.3;12.7)	1.0 (0.0;5.4)	0.0 (0.0;3.8)	0.0 (0.0;7.4)	5.9 (2.2;12.5)	3.0 (0.6;8.5)
Headache	35.4 (25.9;45.8)	27.3 (18.8;37.1)	31.7 (22.8;41.7)	30.2 (21.3;40.4)	18.8 (8.9;32.6)	35.6 (26.4;45.8)	32.0 (23.0;42.1)
Malaise	32.0 (22.9;52.2)	32.3 (23.3;42.5)	33.7 (24.6;43.8)	31.3 (22.2;41.5)	20.8 (10.5;35.0)	30.7 (21.9;40.7)	29.0 (20.4;38.9)
Myalgia	37.1 (27.5;47.5)	33.3 (24.2;43.5)	42.6 (32.8;52.8)	34.4 (25.0;44.8)	20.8 (10.5;35.0)	38.6 (29.1;48.8)	45.0 (35.0;55.3)
Arthralgia	27.8 (19.2;37.9)	20.2 (12.8;29.5)	23.8 (15.9;33.3)	24.0 (15.8;33.7)	12.5 (4.7;25.2)	26.7 (18.4;36.5)	30.0 (21.2;40.0)

Data are number of participants experiencing at least one reaction (95% CI).
N = number of participants included in the safety analysis set (not necessarily the number of participants available for a particular endpoint).
[a] One subject, even though randomized to Group 1, received Group 2 treatment at all 3 vaccine injections.

to hospital within a short interval after enrolment. The effect of the AlOH adjuvant was evaluated as it was considered likely that an adjuvant would be necessary to optimize the immune response, particularly in elderly patients or those with co-morbidities who comprise a large proportion of the population at risk of CDI.

All vaccine formulations and schedules demonstrated a clinically acceptable safety profile, and a comparison of the immune responses based on the formulation (in Stage I) and schedule (in Stage II) resulted in the 100 μg + AlOH formulation and the 0, 7, 30 day schedule being selected for further clinical development of the candidate vaccine. The higher dose and inclusion of the adjuvant improved the response to toxin B, which had been suboptimal in earlier studies [20] and, when given at 0, 7, and 30 days, resulted in good response kinetics in terms of the response at Day 14 and Day 60.

Health-care acquired CDI is most likely to occur between 3 and 5 days after exposure to *C. difficile* spores [23]. Additionally, it has been shown that 85% of nosocomial CDI cases occur within 1 month of discharge from hospital, and the remainder occur within 3 months of discharge [23]. The average waiting time for planned surgery has been estimated at between 2 weeks to 5 months, and so the immune response profile at Day 60 that was sustained through Day 180 was prioritized in the selection of the optimal formulation and schedule. Using these criteria, the formulation and the 0, 7, 30 day schedule selected would optimize protection not only during hospitalization but also after discharge.

The vaccine is being developed to protect patients with chronic underlying medical conditions who are likely to have hospital stays and/or receive antibacterials in the future, and to be given on an out-patient basis prior to hospitalization (e.g. for elective surgery). As such, individuals would ideally be immunized in advance of exposure to *C. difficile* spores using the 0, 7, 30 day schedule, which could begin to provide a protective immune response as early as Day 14 and which may be augmented and sustained after a third dose on Day 30. In this way, protection could be provided to at-risk patients before entering the period of highest risk. Provision of another dose on (Stage I of this study) or after Day 30 (Stage II of this study) results in further augmentation of immune responses, indicating the potential for sustaining immune responses. Additionally, it is likely that vaccination compliance in a real life setting would be better for a shorter schedule, again favoring the 0, 7, 30 day schedule (Groups 1–5) over one that continues to Day 180 (Groups 6 and 7); this was confirmed in the present study with overall completion rates of 91.4% in Groups 1–5 and 81.6% in Groups 6 and 7.

As there is no known immunological correlate of protection against CDI, it is unclear whether the investigational immunologic measures assessed may be predictive of vaccine efficacy. However, the vaccine formulations tested induced both neutralizing and binding antibodies, both of which are considered to be valid options for the choice of formulation and schedule, and the responses for both ELISA and TNA persisted over the course of the study. Continued persistence will be evaluated and defined through extended follow-up.

Based on the results of this Phase 2 study, the adjuvanted high-dose candidate vaccine formulation administered with a 0, 7, 30 day schedule was selected for further clinical evaluation. Vaccine efficacy, including duration of response against the primary (first occurrence) symptomatic CDI episode is currently being assessed in an ongoing, multinational Phase 3 efficacy study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.03.028.

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