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A phase 1 randomized study assessing safety and immunogenicity of two 3-dose regimens of a *Clostridium difficile* vaccine in healthy older Japanese adults

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ABSTRACT

Background: Clostridium difficile infection (CDI) is a major global cause of nosocomial and community-acquired infections. Despite potentially severe or fatal complications and frequent recurrence, no preventive vaccine is currently available. This randomized, observer-blinded, placebo-controlled phase 1 study in older Japanese adults evaluated safety and immunogenicity of an investigational *C difficile* vaccine containing a mixture of genetically detoxified and chemically inactivated toxoids, A and B.

Methods: Healthy Japanese adults aged 65 to 85 years were randomized in a 3:3:2 ratio to receive 100 or 200 μg of *C difficile* vaccine or placebo, respectively, at 0, 1, and 6 months (month regimen) or 1, 8, and 30 days (day regimen). The primary objective was safety evaluation. Vaccine immunogenicity, the secondary objective, was determined by assessing toxin A– and toxin B–specific neutralizing antibody levels in human sera.

Results: Local reactions were reported by up to 33.3% of subjects per dose in the month regimen; percentages were generally higher in the 200-μg group. Such reactions were all mild or moderate in severity and generally transient. No adverse events in the month regimen led to subject withdrawal, and no serious adverse events were considered vaccine related. Further enrollment and dosing in the day regimen were discontinued after 3 subjects in the 100-μg group reported severe redness after dose 2. In the month regimen study arm, immune responses as measured by toxin-neutralizing antibody geometric mean concentrations, geometric mean fold rises, and proportions of subjects achieving prespecified fold rises were generally higher in the 200-μg group, peaked at month 7, and remained elevated at month 12.

Conclusions: The *C difficile* vaccine candidate was safe, well tolerated, and immunogenic when administered to healthy older Japanese adults at 0, 1, and 6 months. Results support continued development of the vaccine for the prevention of CDI.

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1. Introduction

Clostridium difficile is a gram-positive, spore-forming, anaerobic bacillus that is a major cause of nosocomial diarrhea globally, including in Japan [1–7]. A smaller but increasing proportion of infections are community-associated [2,3,8] (ie, presumed to be acquired outside of healthcare facilities [9]). In Japan, community-associated cases are an estimated 7%–8% of all *C difficile* cases [5]. Risk factors for *C difficile* infection (CDI) include increasing age, comorbidities, contact with healthcare settings, recent antibiotic use, and proton pump inhibitor treatment [1,2,10].

C difficile can produce 3 toxins [11], but toxins A and B are the principal virulence factors for CDI [12]. Although individuals may asymptomatically harbor *C difficile*, symptoms of infection range

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from mild to severe diarrhea that may progress to pseudomembranous colitis, toxic megacolon, intestinal perforation, and death [1,3,13]. PCR ribotype 027 isolates, including highly virulent, drug-resistant strains, have been responsible for increasing rates and severity of CDI in some countries [1,2]; however, ribotype 027 isolates have been only sporadically identified in Japan [6,14].

Globally, the burden of CDI is increasing, including in the United States, where approximately 453,000 infections and nearly 29,000 associated deaths occurred in 2011 [3]; and in Europe, where an estimated 172,000 cases occurred per year in 2011–2012 [7]. Available data indicate that CDI may also be a significant nosocomial pathogen in Japan, with hospital-associated CDI incidence estimated at 0.8 to 3.3 per 10,000 patient-days in 2010–2013 [4–6]. These estimates are lower than those for the United States (6.4–7.9/10,000 patient-days in 2006 [15]) and Europe (4.1/10,000 patient-days in 2008 [1]), but true CDI incidence rates in Japan may be higher owing to underdiagnosis [6,16]. Additionally, CDI mortality rates reported in Japan vary by study (6.9%–15% within 30 days CDI treatment completion) [4–6], in one case exceeding that reported in a large US study (9.3%) [3].

Antibiotics are generally the first-line treatment for primary *C difficile* infections including in Japan [2,17,18]. Importantly, a key challenge to treating CDI is recurrence, which occurs in up to 25% of patients [3,19]. Severe complications such as toxic megacolon or peritonitis, may be treated surgically, but mortality rates associated with these surgeries can be very high [2]. A recent type of therapy used in cases of recurring *C difficile* infections is fecal microbiota transplantation (FMT), which has been shown to resolve recurrent CDI in up to 94% of patients [20,21]. However, regulatory classification of FMT is complex: the procedure is unapproved in the United States and regulations are varied in the Member States in the European Union [22,23]. In Japan, FMT is not widely used as a treatment for CDI [17,18] and is generally not used or recommended to prevent primary infection [24].

Current preventative strategies for primary CDI focus on infection control and antimicrobial stewardship and include guidelines on such practices as hand hygiene, disposable equipment use, and surface disinfection [25]. Despite these measures, increasing CDI disease burden and incidence [1,2,26,27], widespread environmental contamination and transmission within healthcare facilities and the community [2,28], and emergence of highly virulent, drug-resistant strains [1,2], coupled with an aging population [29], make the development of preventive approaches fundamental to combating CDI in Japan and globally. However, currently, there is no vaccine approved to prevent CDI.

The current study focuses on a *C difficile* vaccine candidate containing a mixture of genetically and chemically detoxified bacterial toxoids, A and B. In a previous first-in-human study conducted in the United States, this vaccine was evaluated in healthy adults aged 50 to 85 years at various doses (50, 100, or 200 μg) given at 0, 1, and 6 months with or without aluminum hydroxide [30]. In that study, the candidate vaccine elicited robust immune responses after the second dose that further increased after the third dose. The vaccine was generally safe and well tolerated and was associated with fewer local reactions when administered with aluminum hydroxide. In a subsequent phase 2 study (NCT02117570), healthy adults aged 50 to 85 years were administered 100 or 200 μg of the toxoid-alone vaccine formulation at 1, 8, and 30 days; vaccinations in this study were stopped after 7 of 162 subjects experienced grade 3 injection site erythema following dose 2.

The study described here assessed the safety and immunogenicity of the aluminum-hydroxide containing formulation of this *C difficile* vaccine in generally healthy older Japanese adults given at 100 or 200 μg under a 1-, 8-, 30-day or 0-, 1-, 6-month schedule; it was conducted before inclusion of Japanese subjects in the subsequent global phase 3 study (NCT03090191).

2. Methods

2.1. Study design and participants

This randomized, observer-blinded, placebo-controlled phase 1 study (NCT02725437) was conducted at 2 centers in Japan from February 2016 to February 2017. Subjects received *C difficile* vaccine or placebo at either 0, 1, and 6 months (month regimen) or 1, 8, and 30 days (day regimen). For each regimen, subjects were randomized using an interactive response technology system in a 3:3:2 ratio to receive 100 μg or 200 μg (total toxoid amount) of the *C difficile* vaccine or placebo, respectively.

The study was conducted in accordance with general ethical principles in the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All local regulatory guidelines were followed. The Institutional Review Board at Hakata Clinic (Fukuoka, Japan) approved the final protocol for both study sites.

Generally healthy Japanese adults aged 65 to 85 years who provided written informed consent were included. Men able to father children agreed to use a highly effective contraceptive method through 28 days after the last vaccine dose, and women were of nonchildbearing potential. Subjects with comorbidities determined to be stable were eligible. Additional inclusion criteria were availability, including by phone, and willingness to participate for the study duration. Exclusion criteria are listed in the Supplementary Appendix.

In each regimen, the study aimed to enroll 24 subjects into each vaccine dose group and 16 subjects in each placebo group, for a total of 128 subjects.

2.2. Interventions

At days 1, 8, and 30 (day regimen) or day 1, month 1 (day 30), and month 6 (month regimen), subjects were injected with aluminum-hydroxide containing *C difficile* vaccine (100 or 200 μg) or placebo (saline) in the upper deltoid muscle. Lot numbers and manufacturers are given in Supplementary Appendix Table S1.

2.3. Safety assessments

The primary objective was to assess safety and tolerability of the vaccine administered at 100 and 200 μg under both regimens as measured by local reactions, systemic events, adverse events (AEs), and serious AEs (SAEs). The safety population, including all subjects who received ≥ 1 dose of the investigational product, was considered separately for each regimen.

Local reactions (redness, swelling, and pain at the injection site) and systemic events (vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, new or worsening joint pain, and fevers) were recorded in an electronic diary (e-diary) for 14 days following each injection (or for 7 days after dose 1 in the day regimen). Local reactions and most systemic events (vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain) were categorized as absent, mild, moderate, severe (based on subject report), or grade 4 (based on investigator assessment of the subject according to predefined grading scales). Fevers were evaluated based on axillary temperature and classified according to a predefined grading scale: mild (37.5 °C–38.4 °C), moderate (38.5 °C–38.9 °C), severe (39.0 °C–40.0 °C), or grade 4 (>40.0 °C).

AEs were recorded through 1 month after dose 3 for both regimens and were coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Study investigators assessed whether a given AE met SAE criteria and assessed whether AEs and SAEs were related to the investigational product. Acute reactions occurring within 30 min of vaccination (immediate AEs) and newly diagnosed chronic medical conditions (NDCMCs) were also required to be recorded. Blood samples were collected for hematology and blood chemistry analysis at day 15, day 30, day 37, month 6, and day 187 for the month regimen study arm and days 8, 15, 30, and 37 for the day regimen study arm.

2.4. Immunogenicity assessments

Immunogenicity evaluation was a secondary objective assessed by measuring both toxin A– and toxin B–specific neutralizing antibody levels in human sera [30]. For the month regimen study arm, serum samples were collected on day 1 (before dose 1), day 15, day 30 (before dose 2), day 37, month 2, month 6 (before dose 3), day 187 (after dose 3), month 7 (primary time point), and month 12. For the day regimen study arm, serum samples were collected on day 1 (before dose 1), day 8 (before dose 2), day 15, day 30 (before dose 3), day 37 (primary time point), month 2, month 4, and month 7.

Immunogenicity populations were as follows: the evaluable immunogenicity population was assessed for the month regimen and was the primary population for analysis. This population included subjects who were eligible at randomization; received all 3 study vaccinations correctly according to randomization; had blood drawn at month 7 within 20 to 45 days after receipt of dose 3, with the resulting toxin neutralization assay providing ≥ 1 valid and determinate result; and had no major protocol violations. The modified intent-to-treat population was defined for both regimens and included all subjects with ≥ 1 valid and determinate assay result.

Specific immunogenicity endpoints included (1) toxin A– and toxin B–specific neutralizing antibody levels, expressed as geometric mean concentrations (GMCs); (2) geometric mean fold ratios (GMFRs) in neutralizing antibody levels from baseline at available time points; and (3) proportions of subjects with $\geq$ 4-, $\geq$8-, $\geq$16-, and $\geq$ 32-fold rises in neutralizing antibody levels from baseline at available time points.

2.5. Statistical analysis

As the study was intended to be descriptive, the sample size was not chosen based on statistical hypothesis test considerations. Safety endpoints were reported as percentages of subjects along with exact 95% CIs calculated using the Clopper-Pearson method. For immunogenicity analysis, antibody levels below the lower limit of quantitation (LLOQ; defined as 158.0 and 249.5 neutralization units/mL for toxins A and B, respectively) were assigned a value of 0.5 $\times$ LLOQ. For binary immunogenicity endpoints, exact 95% CIs were calculated using the Clopper-Pearson method. For continuous immunogenicity endpoints, 95% CIs were calculated from log-transformed assay results using a Student t distribution; these were then back-transformed to the antilog scale.

3. Results

3.1. Subjects

Overall, 62 of 64 enrolled subjects in the month regimen completed the study (Fig. 1). Enrollment and dosing under the day regimen were discontinued after 3 subjects in the 100-μg group reported severe redness after dose 2; 2 of these subjects also reported severe swelling. These events met a predefined stopping rule, which limited subject numbers in the day regimen, particularly at dose 3 (Fig. 1). However, 35 of 36 subjects in the day regimen completed the study follow-up requirements. In the month regimen, there were no withdrawals owing to AEs, but 1 subject each in the 100-μg and 200-μg groups withdrew between doses 2 and 3 because they were no longer willing to participate in the study. One subject in the day regimen study arm withdrew after 100-μg dose 3 owing to a diagnosis of acute myeloid leukemia unrelated to the vaccine; the subject later died following disease-related complications. Subject demographics were similar across groups in both regimens (Table 1).

3.2. Safety

In the month regimen study arm, local reactions were reported by 0.0% to 12.5%, 4.3% to 12.5%, and 20.8% to 33.3% of subjects per dose in the placebo, 100-μg, and 200-μg groups, respectively (Fig. 2**A**). Injection site pain was the most commonly reported local reaction in the month regimen study arm, reported by a maximum of 29.2% of subjects (after dose 1 in the 200-μg group). Local reactions were generally reported more frequently in the 200-μg group, mostly because of more frequent injection site pain. All reported local reactions were mild to moderate in severity, and incidence did not increase with subsequent doses. Reported local reactions lasted a median of 1 to 3 days in each group after each dose.

In the day regimen study arm, relatively few subjects reported local reactions after dose 1 (**Supplementary Appendix Figure S1A**), with pain at the injection site being the most common local reaction after this dose (up to 28.6% of subjects). However, 54.5% and 36.4% of subjects in the 100-μg group in the day regimen study arm reported redness and swelling, respectively, after dose 2. Three subjects reported severe redness (ie, >10 cm), 2 of whom also reported severe swelling, triggering activation of a predefined stopping rule. All other local reactions reported under the day regimen were mild or moderate in severity. Redness reported after dose 2 had a median duration of 10 and 2 days for the 100- and 200-μg groups, respectively; for the 3 subjects with severe redness, median duration after dose 2 was 4 to 9 days. The median duration of any swelling after dose 2 was 6 days in the 100-μg group; swelling was not reported in the other groups.

In the month regimen study arm, systemic events were reported by 12.5% to 25.0%, 8.3% to 20.8%, and 12.5% to 30.4% of subjects per dose in the placebo, 100-μg, and 200-μg groups, respectively (Fig. 2**B**). Only 1 fever was reported (mild fever [37.5 °C–38.4 °C] after dose 3 in the 200-μg group). The most commonly reported systemic events varied by dose group: for the 200-μg group, fatigue was reported by a maximum 26.1% of subjects (after dose 3); for the 100-μg group, new or worsening muscle pain was reported by a maximum 16.7% of subjects (after dose 1); and for the placebo group, diarrhea (after doses 1 and 3) and new or worsening muscle pain (after dose 3) were reported by a maximum 12.5% of subjects. All systemic events were mild or moderate in severity and occurred with similar frequency across doses. Systemic events had a median duration of 1 to 8 days with the exception of 1 subject in the 100-μg group reporting new or worsening joint pain after dose 1, lasting 17 days. No subjects in the month regimen study arm reported using antipyretic medication.

Relatively few subjects in the day regimen study arm reported systemic events (**Supplementary Appendix Figure S1B**). Most events were mild in severity, but 1 subject in the 200-μg group reported severe fever, fatigue, and new or worsening joint pain after dose 2. Systemic events reported in the day regimen study arm had median durations of 1 to 4 days with the exception of 1

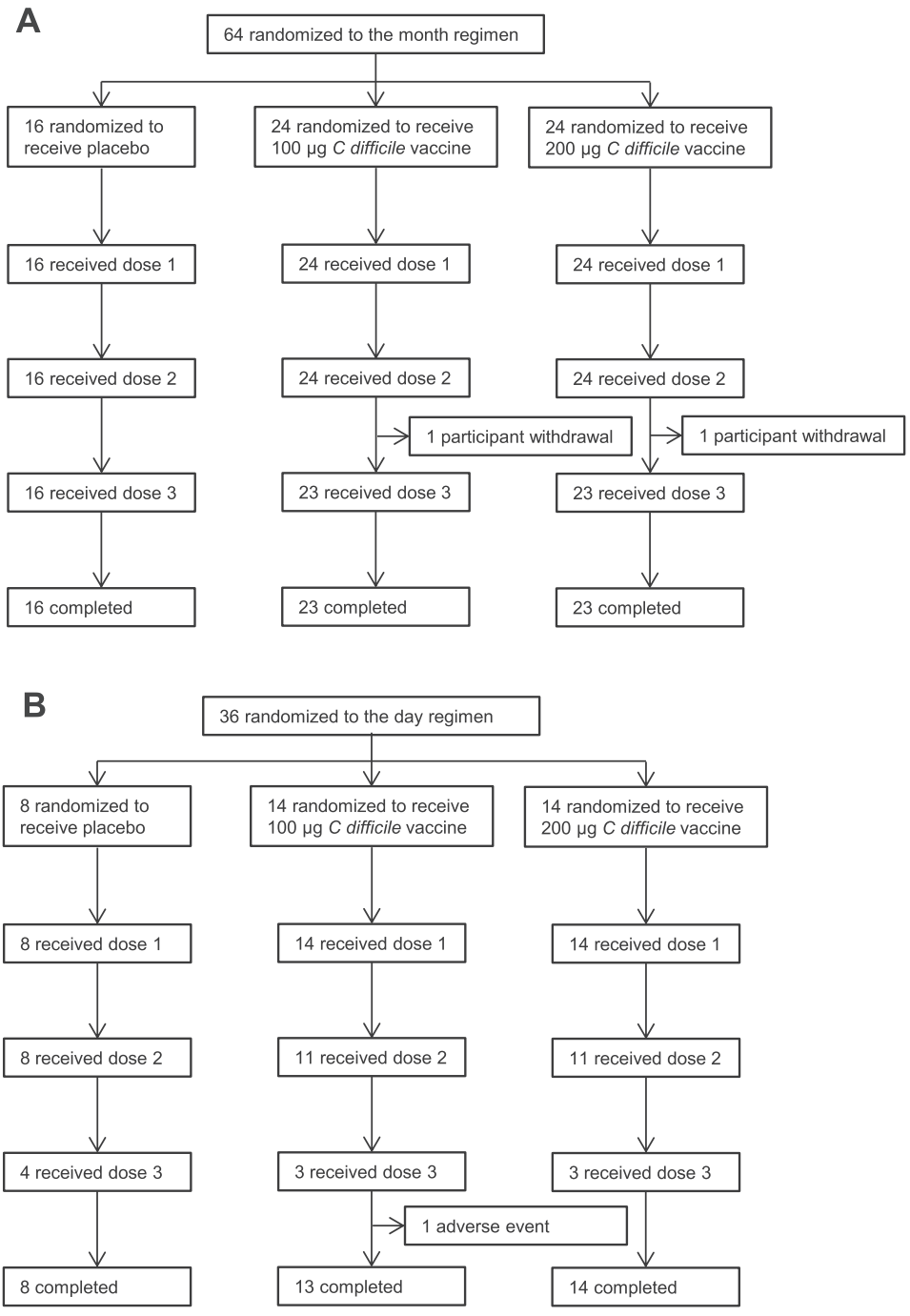

Fig. 1. CONSORT diagram with subject dispositions in the **(A)** month and **(B)** day regimen study arms. Further enrollment and vaccination in the day regimen study arm was discontinued after 3 subjects in the 100-µg *C difficile* group reported severe redness after dose 2; study completion for this regimen indicates completion of study follow-up procedures. *C difficile* = *Clostridium difficile*.

subject in the 100-µg group who reported a mild headache lasting 8 days after dose 3.

Adverse events were reported by 37.5%, 16.7%, and 18.8% of subjects in the 200-µg, 100-µg, and placebo groups in the month regimen study arm, respectively (Table 2). Adverse events were most commonly categorized as infections and infestations (including bronchitis, cystitis, pharyngitis, rhinitis, and upper respiratory tract infections) and musculoskeletal and connective tissue disorders (including osteoarthritis, arthralgia, lumbar spinal stenosis, pain in an extremity, and spondylolisthesis). None of the AEs in this regimen led to subject withdrawal. No immediate AEs were reported during the study. One subject in the 200-µg group reported 2 NDCMCs of lumbar spinal stenosis and spondylolisthesis. Three SAEs were reported by 2 subjects in the 200-µg group (lumbar vertebral fracture, lumbar vertebral stenosis, and osteoarthritis); none of these were considered related to the vaccine.

In the day regimen, AEs were reported by 21.4%, 50.0%, and 0% of subjects in the 200-µg, 100-µg, and placebo groups, respectively, with the most commonly reported AEs categorized as general disorders and administration site conditions (data not shown). Related AEs were reported by 14.3% and 35.7%, of subjects in the

Table 1
Demographic characteristics of subjects in the month and day regimen study arms.

	Month Regimen Vaccine Group				Day Regimen Vaccine Group			
	Placebo (n*=16)	100 μg *C diff* (n*=24)	200 μg *C diff* (n*=24)	Total (N*=64)	Placebo (n*=8)	100 μg *C diff* (n*=14)	200 μg *C diff* (n*=14)	Total (N*=36)
Sex, n† (%)								
Female	9 (56.3)	10 (41.7)	14 (58.3)	33 (51.6)	5 (62.5)	9 (64.3)	8 (57.1)	22 (61.1)
Male	7 (43.8)	14 (58.3)	10 (41.7)	31 (48.4)	3 (37.5)	5 (35.7)	6 (42.9)	14 (38.9)
Race, n† (%)								
Asian	16 (100.0)	24 (100.0)	24 (100.0)	64 (100.0)	8 (100.0)	14 (100.0)	14 (100.0)	36 (100.0)
Racial designation, n† (%)								
Japanese	16 (100.0)	24 (100.0)	24 (100.0)	64 (100.0)	8 (100.0)	14 (100.0)	14 (100.0)	36 (100.0)
Age at randomization, y								
Mean (SD)	69.9 (4.01)	68.8 (3.50)	70.8 (4.18)	69.8 (3.93)	73.0 (3.16)	72.7 (3.93)	73.8 (2.86)	73.2 (3.32)
Median	69.0	68.0	70.5	69.0	72.5	72.0	74.0	73.0
Min, max	65, 78	65, 75	65, 79	65, 79	69, 77	65, 81	69, 80	65, 81
Baseline C diff serostatus,‡ n† (%)								
Seronegative								
Toxin A–/toxin B–	16 (100.0)	21 (87.5)	20 (83.3)	57 (89.1)	6 (75.0)	11 (78.6)	8 (57.1)	25 (69.4)
Seropositive								
Toxin A+/toxin B–	0 (0.0)	2 (8.3)	1 (4.2)	3 (4.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Toxin A–/toxin B+	0 (0.0)	1 (4.2)	3 (12.5)	4 (6.3)	2 (25.0)	3 (21.4)	5 (35.7)	10 (27.8)
Toxin A+/toxin B+	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	1 (2.8)
Toxin A and/or toxin B not evaluated								
Toxin A not evaluated	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Toxin B not evaluated	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Toxin A and toxin B not evaluated	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

C diff = *Clostridium difficile* vaccine; LLOQ = lower limit of quantitation.
* N = total number of subjects in the specified group.
† n = number of subjects with the specified characteristic.
‡ Baseline serostatus (before dose 1 on day 1) defined based on LLOQ value for toxin A–specific or toxin B–specific neutralizing antibody level, with "+" indicating neutralizing antibody level ≥ LLOQ and "–" indicating neutralizing antibody level < LLOQ.

200-μg and 100-μg groups, respectively; these concerned injection site reactions and systemic events of diarrhea and headache. One AE (acute myeloid leukemia in the 100-μg group) led to withdrawal, and one NDCMC (arteriosclerosis coronary artery) was reported in the 200-μg group; both of these were categorized as SAEs, but neither was considered vaccine-related. No immediate AEs were reported in the day regimen. There was 1 related severe or life-threatening AE reported in the 100-μg group (injection site redness and swelling).

Sporadic abnormalities in hematology and blood chemistry results were observed for all groups in both the month and day regimen study arms. No clinically relevant shifts were observed after any dose or through 6 months after dose 3 in any group, with the exception of abnormalities associated with acute myeloid leukemia in the subject in the day regimen previously discussed. Most shift abnormalities were mild, several were moderate, and none were severe or grade 4.

3.3. Immunogenicity

In the month regimen study arm, 62 of 64 subjects were included in the evaluable immunogenicity population; the remaining 2 subjects were excluded because they did not receive all 3 vaccine doses. Immune responses as measured by GMCs of toxin A– and toxin B–specific neutralizing antibodies rose after dose 2 and further increased after dose 3 in the 100- and 200-μg groups (Fig. 3). Responses peaked at month 7, remained elevated at month 12, and were generally highest in the 200-μg group. Apart from one subject in the 100-μg dose group, all other subjects who received active vaccine had neutralizing antibody levels ≥ LLOQ for both toxin A and B by month 7 (data not shown). Analyzing results by baseline serostatus indicated that most subjects who were initially seronegative for either toxin developed neutralizing antibody levels reaching or exceeding prespecified thresholds following vaccination; in the 200-μg dose group, this was true for 100% of initially seronegative subjects at month 7 for both toxins (data not shown). In the day regimen study arm, there were limited data as a result of the triggering of the stopping rule, as described above; therefore, inferences regarding immunogenicity could not be made at day 30 or beyond.

Analyzing GMFRs of toxin A– and toxin B–specific neutralizing antibodies in the month regimen study arm indicated a pattern similar to that for GMCs (Fig. 4), with responses peaking at month 7, remaining elevated at month 12, and being generally higher in the 200-μg group. Likewise, proportions of subjects with ≥ 4-, ≥8-, ≥16-, and ≥ 32-fold rises from baseline in antibody levels generally followed similar patterns (data not shown).

4. Discussion and conclusions

The globally increasing burden of CDI [1,2,26,27], coupled with an aging population [29], highlights an urgent need for improved measures for CDI prevention and treatment. However, no vaccine is currently approved to prevent CDI and its potentially severe complications. The current study focuses on a *C difficile* vaccine candidate containing a mixture of genetically detoxified and chemically inactivated toxoids, A and B, that was developed to broadly protect against clinically relevant *C difficile* strains, including hypervirulent strains, by inducing toxin-neutralizing antibody responses.

In this current study, the *C difficile* candidate vaccine was generally safe and well tolerated in older Japanese adults at 0, 1, and 6 months. Local reactions were generally more frequent in the

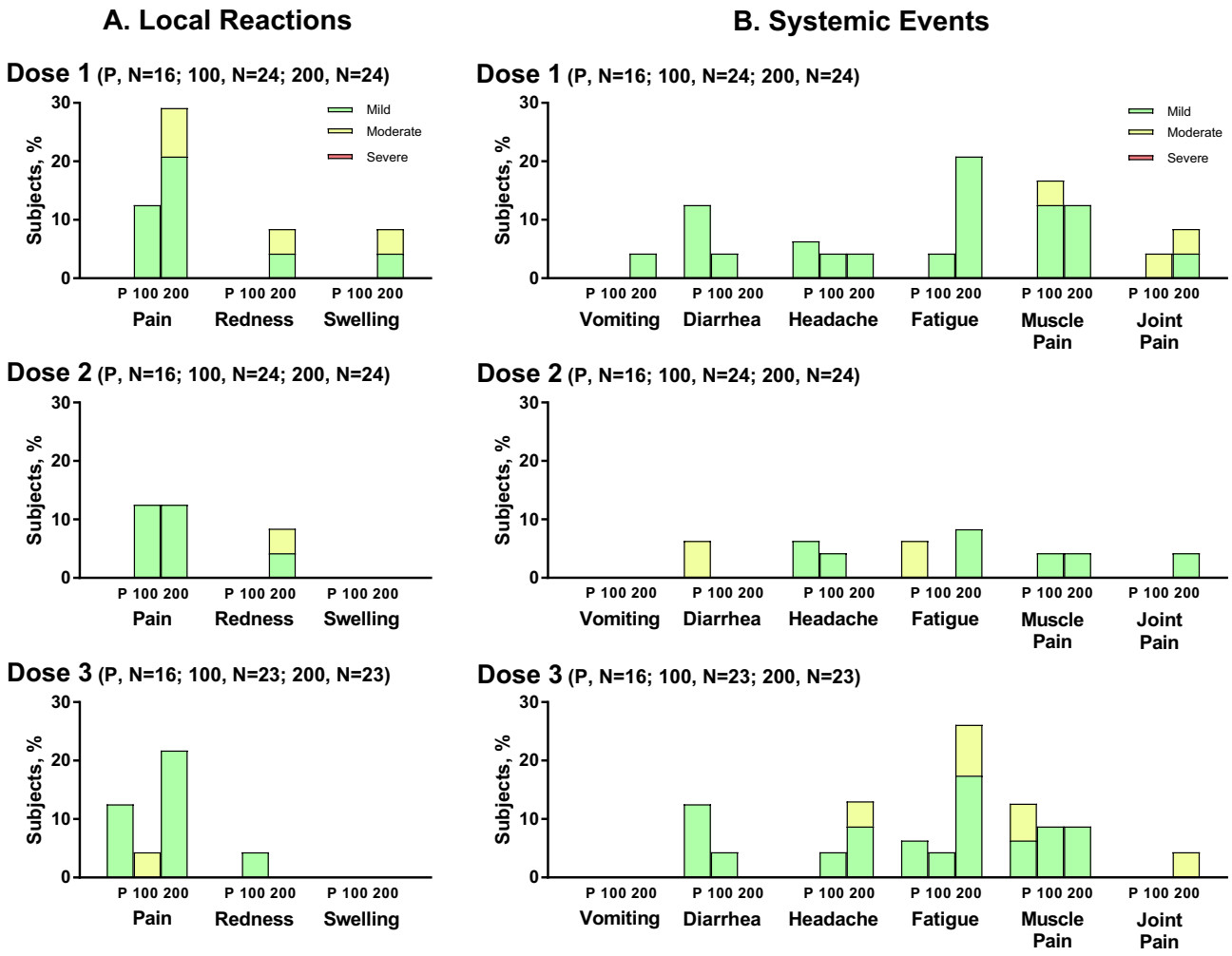

Fig. 2. Percentages of subjects in the month regimen study arm experiencing any **(A)** local reactions* or **(B)** systemic events† by severity after each dose of 100 μg or 200 μg *C difficile* vaccine**.** N = number of subjects in each group who received the indicated dose. *Includes any pain, redness, or swelling at the injection site. †Includes any fever ≥ 37.5 °C (≥99.5°F), vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, or new or worsening joint pain. *C difficile* = *Clostridium difficile*; P = placebo; 100 = 100-μg *C difficile* vaccine; 200 = 200-μg *C difficile* vaccine.

Table 2
Adverse events among subjects in the month regimen study arm.

Category of AE	Month Regimen Vaccine Group								
	Placebo (n* = 16)			100 μg *C diff* (n* = 24)			200 μg *C diff* (n* = 24)		
	n† (%)	(95% CI)	Number of Events	n† (%)	(95% CI)	Number of Events	n† (%)	(95% CI)	Number of Events
AEs	3 (18.8)	(4.0–45.6)	4	4 (16.7)	(4.7–37.4)	6	9 (37.5)	(18.8–59.4)	17
Related to treatment	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
SAEs	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	2 (8.3)	(1.0–27.0)	3
Related to treatment	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
Severe or life-threatening AEs	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	1 (4.2)	(0.1–21.1)	1
Related to treatment	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
Immediate AEs	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
NDCMCs	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	1 (4.2)	(0.1–21.1)	2
AEs leading to withdrawal	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
Related to treatment	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0
Deaths	0 (0.0)	(0.0–20.6)	0	0 (0.0)	(0.0–14.2)	0	0 (0.0)	(0.0–14.2)	0

AE = adverse event; *C diff* = *Clostridium difficile* vaccine; NDCMC = newly diagnosed chronic medical condition; SAE = serious adverse event.
* N = total number of subjects in the specified group.
† n = number of subjects reporting ≥ 1 of the specified events.

200-μg group, largely because more subjects in this group reported injection site pain. No reported SAEs were considered related to the study vaccine. Immune responses were generally higher in the 200-μg versus the 100-μg group, with responses peaking at month 7 and remaining elevated at month 12 compared with baseline. In contrast, dosing and enrollment in the 1-, 8-, 30-day regimen were

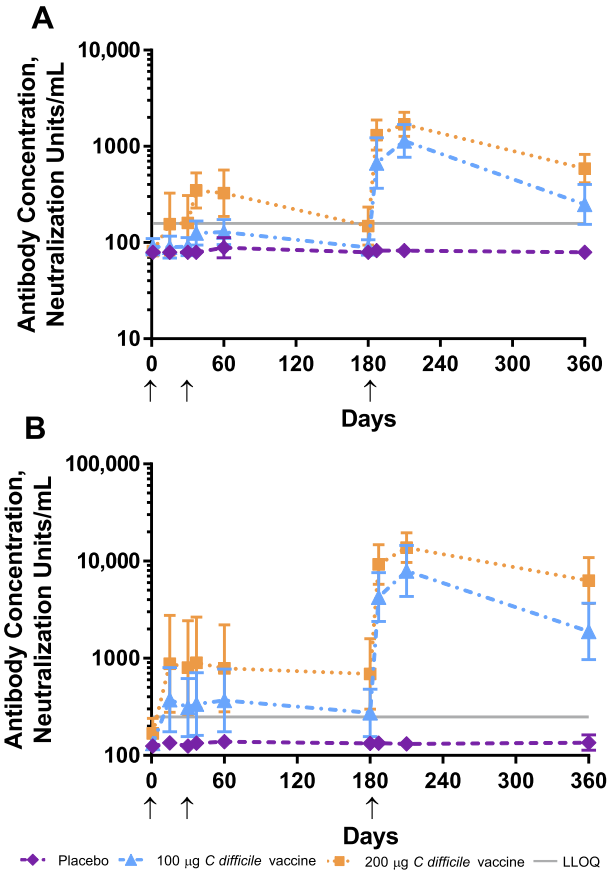

Fig. 3. Geometric mean concentrations for **(A)** toxin A–specific neutralizing antibody and **(B)** toxin B–specific neutralizing antibody for subjects in the month regimen study arm. LLOQs are also shown for each antibody. Error bars are not plotted in cases where 95% CIs were not estimable or in cases where the error bars would be smaller than the height of the symbols. Arrows on the x-axis indicate days on which vaccine was administered. *C difficile* = *Clostridium difficile;* LLOQ = lower limit of quantitation.

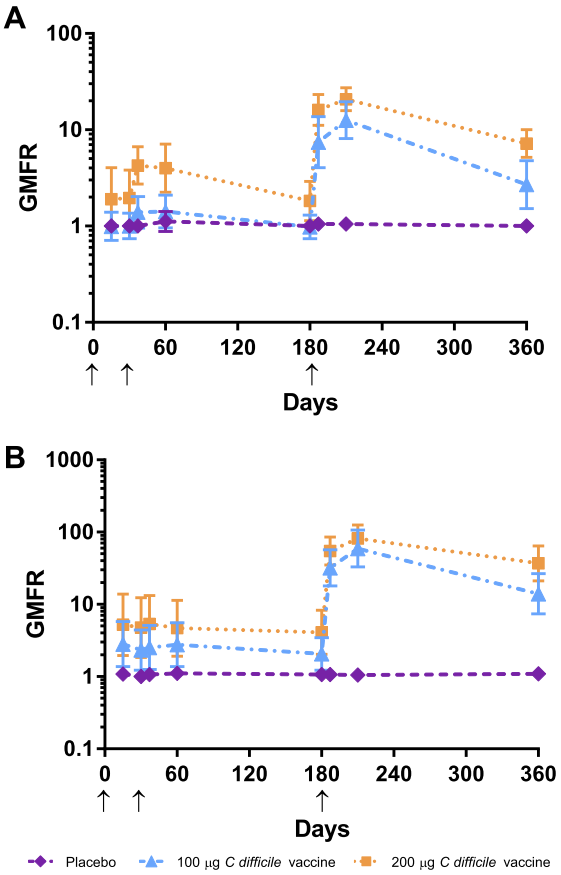

Fig. 4. Geometric mean fold ratios for **(A)** toxin A–specific neutralizing antibody and **(B)** toxin B–specific neutralizing antibody for subjects in the month regimen study arm. Error bars are not plotted in cases where 95% CIs were not estimable or in cases where the error bars would be smaller than the height of the symbols. Arrows on the x-axis indicate days on which vaccine was administered. *C difficile* = *Clostridium difficile*; GMFR = geometric mean fold ratio.

discontinued after 3 subjects reported severe redness following dose 2. A general limitation of the study was small sample sizes, especially after discontinuation of enrollment and dosing in the day regimen study arm.

The pattern of immune responses observed for the month regimen study arm was similar to observations from the first-in-human study of the vaccine in US subjects aged 50 to 85 years, as described earlier (ie, immune responses peaked after dose 3 and remained elevated at month 12) [30]. Of note, almost all subjects in the month regimen study arm who were seronegative at baseline had levels ≥ LLOQ for both toxin A– and toxin B–neutralizing antibodies at month 7. Additionally, results from another ongoing study of the same candidate vaccine in healthy US adults aged 65 to 85 years (NCT02561195) will further inform interpretation of current results. Subjects in the ongoing US study will be followed up for up to 4 years after dose 3 to determine antibody response persistence.

The month regimen was associated with a clinically acceptable safety profile and was selected for a phase 3 study (NCT03090191) [31]. Potential licensure of the *C difficile* vaccine candidate in Japan and other countries would necessitate education regarding disease characteristics as well as development of vaccination guidelines.

Overall, these results support development of this *C difficile* vaccine given as a 200-µg dose at 0, 1, and 6 months for CDI prevention in an older adult population. The results particularly support the safety and immunogenicity of the vaccine in older Japanese adults and continued development of the vaccine in Japan and globally.

5. Role of the sponsor

Pfizer was involved in study design, data collection, data analysis, data interpretation, writing of the study report, and the decision to submit the paper for publication.

6. Authorship

All authors attest they meet the ICMJE criteria for authorship.

Author contributions

RdS, MY, MA, CK, and WG were involved in conception and design of the study. MI and TY participated in data acquisition. MA participated in statistical analysis. RdS, MY, MA, CK, MP, KJ, WG, and CW participated in data analysis and interpretation. All authors were involved in drafting the manuscript or revising it critically for intellectual content.

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Conflicts of interest

MI and TY have no potential conflicts of interest to disclose. All other authors are employees of Pfizer Inc and may hold stock or stock options.

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Data Sharing Statement

Upon request, and subject to certain criteria, conditions and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines and medical devices (1) for indications that have been approved in the US and/or EU or (2) in programs that have been terminated (i.e., development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.014.

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