

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. N Engl J Med 2010;362:197-205.

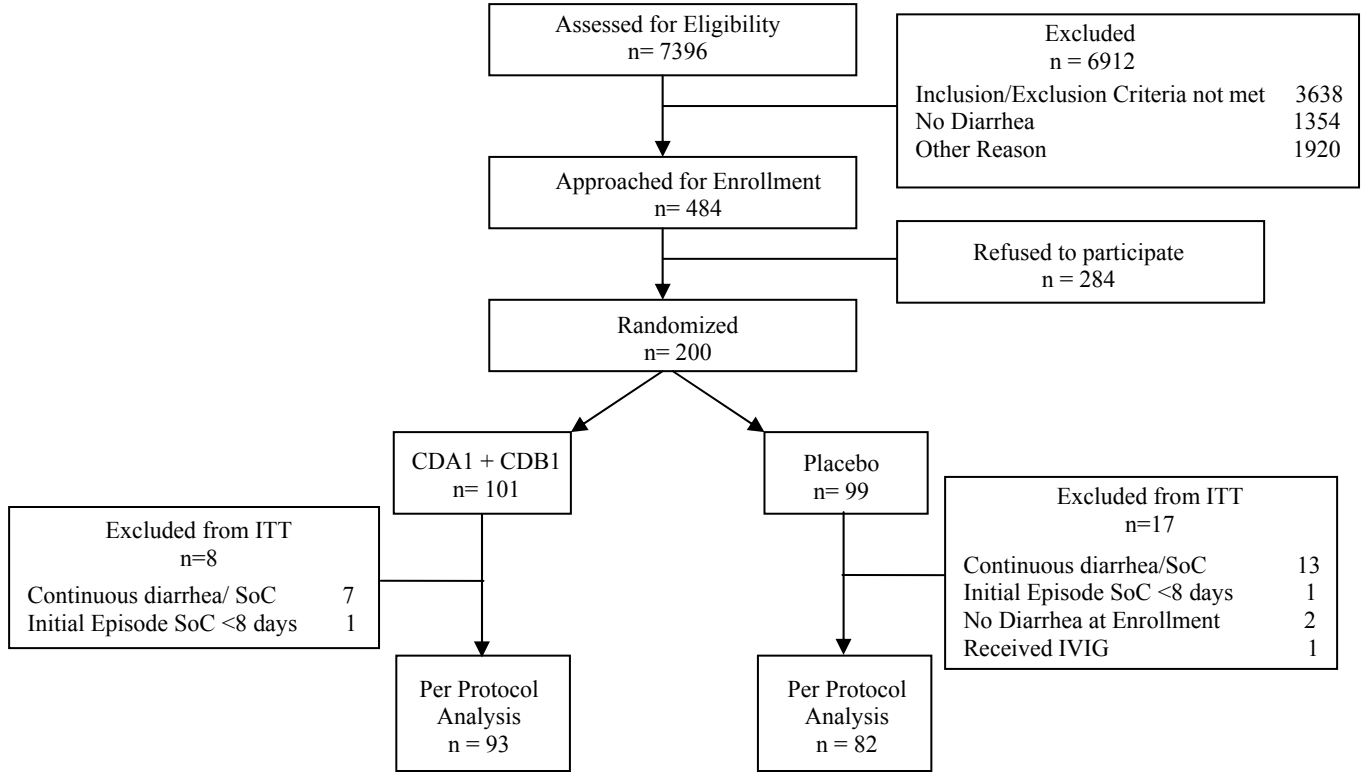
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Supplement to: Treatment with Monoclonal Antibodies to *Clostridium difficile* Toxins A and B

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Supplemental Figure 1. Number of Subjects From Screening Through Analysis



Supplementary Methods

Patient Eligibility

Eligible inpatients and outpatients were ≥ 18 years of age with diarrhea associated with a positive stool test for *C. difficile* toxin(s) in the 14 days prior to enrollment. Diarrhea was defined as 3 or more unformed stools per day for at least 2 consecutive days or more than 6 unformed stools in 1 day. Patients were required to receive standard of care (SoC) of either metronidazole or oral vancomycin for the CDI episode and the patient or legal representative must have read, understood and provided written informed consent and HIPAA authorization.

Patients were excluded if they had a history of chronic diarrheal illness from other causes such as ulcerative colitis or Crohn's disease; had a catastrophic life-threatening illness at the time of enrollment as measured by a score of 4 using a modified Horn's index^{1,2}; had surgery planned to manage severe CDI colitis within 24 hours of enrollment; or had received another investigational agent within previous 30 days. Women who were pregnant, unwilling to undergo pregnancy testing if of child-bearing potential or were breastfeeding were also excluded. In addition, patients were excluded for any other condition that in the opinion of the investigator would jeopardize the safety or rights of the patient participating in the study or make it unlikely the patient could complete the study.

Monoclonal Antibody Development and Purification

CDA1 and CDB1 were derived using recombinant DNA technology, expressed in Chinese Hamster Ovary cell lines and purified by column chromatography, low pH and

nanofiltration. The purified MAbs were characterized by using standard methodologies for determining antibody structure and purity.

Study Definitions for Primary and Secondary Efficacy Assessments

The primary efficacy endpoint of the protocol was recurrent CDI defined as a new diarrhea episode associated with a positive *C. difficile* stool toxin test that occurs after the cessation of diarrhea (< 3 unformed stools/day x 2 consecutive days) and discontinuation of SoC for the initial episode. Less rigorous and more inclusive definitions of recurrence were also analyzed in both intent-to-treat (ITT) and per-protocol (PP) populations.

These additional efficacy endpoint definitions were determined by consensus agreement between the sponsor, independent statistician and the DSMB prior to study unblinding.

The more inclusive categories of diarrhea also analyzed in this study were defined as follows:

Recurrent CDI or SoC: this definition included subjects who met the protocol definition of laboratory documented recurrent CDI plus those subjects who had recurrent diarrhea after resolution of the initial episode and were treated with SoC for presumed CDI in the absence of a diagnostic test for *C. difficile*.

Recurrent diarrhea: included subjects who met the protocol definition of CDI recurrence and those who had recurrent diarrhea after resolution of the initial episode that may or may not have been treated with SoC.

Any diarrhea: included subjects with any new diarrhea episode occurring after the end of the initial diarrhea episode (< 3 unformed stools/day x 2 consecutive days) regardless of SoC treatment.

There were three secondary efficacy assessments defined in the protocol and included time to resolution of initial diarrhea defined as the cessation of diarrhea for at least two consecutive days with the date of resolution being the date of the first day of diarrhea cessation for 2 days. Standard of care treatment failure was defined as a recurrence of diarrhea while on SoC treatment for the initial episode; a change in SoC treatment due to worsening or persistence of diarrhea or the persistence of diarrhea at day 14 of SoC treatment. A severe initial CDI episode was defined as 5 or more unformed stools per day for two consecutive days reported from day 1 until resolution of the initial episode (*i.e.* cessation of diarrhea and off SoC).

Per Protocol Population

The per-protocol population excluded subjects who received continuous SoC or had continuous diarrhea until the end of follow-up in the study; did not receive SoC for at least 8 days to treat the initial CDI episode; had no diarrhea at the time of enrollment; or, received intravenous immune globulin within 6 days of enrollment in the study.

Laboratory Assays

Anti-toxin A and anti-toxin B ELISA

Microtiter plates were coated with either a recombinant fragment of toxin A that binds CDA1 (fragment 4, Exotoxin A residues 1852-2710) or a recombinant fragment of toxin B (fragment 4, Exotoxin B residues 1777-2366) that binds CDB1. Standards were prepared from dilutions of known concentrations of CDA1 and CDB1. Serum samples and standard were incubated on these coated plates and anti-toxin A and B antibodies

were detected using an affinity purified Goat F (ab')₂ Fragment anti-Human IgG, conjugated to alkaline phosphatase. Anti-toxin A and B IgG concentrations in control and test samples were determined by interpolation from the standard curve using a 4-parameter curve fitting algorithm with standards ranging in concentration from 0.274 ng/mL to 200 ng/mL. The lower limit of detection for this assay was 0.5 µg/mL and 0.6 µg/mL for anti-toxin A IgG and anti-toxin B IgG, respectively. Values lower than the limit of detection were reported as ½ of that limit.

Human anti-human antibody (HAHA) Assay

ELISA plates were coated with CDA1 or CDB1. Study subjects' serum samples were added to the wells and incubated with the immobilized MAb. A molar excess of biotinylated MAb was added, incubated with peroxidase-labelled streptavidin conjugate followed by addition of peroxidase substrate. Bound biotinylated MAb results in color formation directly proportional to the amount bound and is a surrogate for the amount of solid phase bound human anti-human antibody (HAHA) free to react with the biotinylated MAb. The limit of detection for HAHA to CDA1 or CDB1 were determined by assaying control sera from over 80 healthy young adults in experiments with positive (rabbit anti-human CDA1 or rabbit anti-human CDB1) and negative controls (commercial pool of male sera). All samples were assayed in triplicate. The limits of detection were defined as the mean of the control sera's tested + 2 standard deviations (SD).

Supplementary Results

Early Withdrawals during the Study Period

Of the 101 patients in the CDA1+CDB1 group, 9 did not complete the study: 7 died during the study period; 1 was lost to follow-up and 1 withdrew consent. Of the 99 patients in the placebo group, 13 dropped out: 8 died during the study period; 2 were lost to follow-up and 3 withdrew consent. The number of deaths during the study period were not significantly different between the study groups (7 vs 8, $p=0.79$).

Of the 7 deaths in the CDA1+CDB1 group, 1 died of staphylococcal pneumonia and urosepsis; 1 of septic shock; 3 of cardio-respiratory arrest or failure; 1 from systemic embolization and 1 from sudden death day 45 on study. Of the 8 deaths in the placebo group, 1 died of pneumonia, 2 from septic shock/*Clostridium difficile* colitis; 2 from cardio-respiratory arrest or failure; 1 from gastrointestinal bleed and 2 from sepsis.

Supplemental Table 1. Additional Efficacy Analyses by Treatment Assignment

		CDA1+CDB1	Placebo	p-level
Recurrent CDI - % (n)	ITT*	6.9 (7)	25 (25)	0.0004
	PP*	6.5 (6)	29 (24)	<0.0001
Recurrent CDI or SoC - % (n)	ITT	14 (14)	33 (33)	0.0014
	PP	14 (13)	38 (31)	0.0004
Recurrent Diarrhea - % (n)	ITT	28 (28)	50 (49)	0.0022
	PP	29 (27)	56 (46)	0.0004
Any Diarrhea - % (n)	ITT	35 (35)	58 (57)	0.0017
	PP	36 (33)	62 (51)	0.0005

*Intention-to-treat (ITT) population: CDA1+CDB1 = 101; Placebo = 99

Per-protocol (PP) population: CDA1+CDB1 = 93; Placebo = 82

Supplemental Table 2. Demographics and Baseline Characteristics based on Hospitalization Status

	Inpatient (n=102)	Outpatient (n=98)	p-value
Years of Age - Mean	69	59	<0.0001
Female sex - % (n)	62 (63)	70 (69)	0.23
White race - % (n)	86 (88)	88 (86)	0.83
More than one previous CDI - % (n)	30 (31)	31 (30)	1.0
Vancomycin as SoC - % (n)	29 (30)	22 (22)	0.33
Severe at Enrollment - % (n)	45 (46)	35 (34)	0.15
Horn's Index - Mean	2.4	1.8	<0.0001
BI strain of <i>C. difficile</i> - % (n)*	36 (29)	21 (15)	<0.05

*Denominator: Inpatients = 80, Outpatients = 71 due to *C. difficile* not isolated, not typable or missing stool samples

Supplemental Table 3. Adverse Events that were Significantly Different between Treatment Groups during the 84 Day Study Period

MedDRA Preferred Term	CDA1+CDB1 (N=101)	Placebo (N=99)	p-value
% (n)			
Serious Adverse Events			
Hypotension	0	7 (7)	0.01
Dehydration	0	5 (5)	0.03
Non-Serious Adverse Events			
Anorexia	20 (20)	42 (42)	0.0007
Anxiety	1 (1)	8 (8)	0.02
Diarrhea	40 (40)	56 (55)	0.03
Depression	0 (0)	5 (5)	0.03
Insomnia	3 (3)	10 (10)	<0.05