Toxicology Review of MenACWY as a Meningitis Vaccine Formulations

BLA: 125300

Sponsor: Novartis Vaccines and Diagnostics, Inc.

Product: Meningococcal ACWY Conjugate Vaccine

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Cross references: BB-IND -b(4)-; DMF -b(4)-; DMF -b(4)-; DMF -b(4)-; DMF -b(4)-; BB-MF -b(4)-

Proposed use: To prevent an invasive disease (e.g., meningitis, sepsis) caused by *Neisseria meningitidis* serogroups A, C, W-135 and Y in infants and older age groups, Novartis is developing a new tetravalent conjugate meningococcal vaccine containing serogroups A, C, W-135 and Y.

Précis: The components of this vaccine are the meningococcal A antigen conjugate and a liquid component containing the meningococcal C, W-135 and Y antigen conjugates. This BLA seeks licensure for use in individuals aged 11 to 55 years. Three pivotal and two supportive clinical studies in the relevant populations, and twelve studies in other populations comprised the content of this BLA. The effect of different doses (-b(4)-;to 10µg) of each serogroup on the immune response and --b(4)---------- were investigated in phase 1 and phase 2. The final composition for a single injection was selected as: 10µg of conjugated oligosaccharide A, with 5µg each of conjugated oligosaccharide C, W, and Y, without adjuvant (10-5-5-5), were based on phases 1 and 2 studies.

In this single (40 µg/0.5 mL dose) and repeated (five 40 µg/0.5 mL dose at two weeks interval) dose toxicology study, New Zealand White rabbits were treated with either control or test articles. Control or test article were administered via intramuscular (gluteal muscle of the hindlimb) injection on study days (SD's) 0, 14, 28, 42, and 56. Terminal sacrifice necropsies were conducted on SD's 2 and 58 for single and repeated dose studies, respectively. Recovery sacrifice necropsies were conducted on SD's 14 and 70 for single and repeated dose studies, respectively.

Introduction:
Although either one can be seen separately, meningitis (inflammation of the meninges) and encephalitis (inflammation of the brain) often are seen simultaneously (meningoencephalitis) in the same animal. In animals with meningoencephalitis, the clinical signs of meningitis often precede the clinical signs of encephalitis and may remain the predominant feature of the illness. Bacteria, viruses, fungi, protozoa, rickettsia, parasite migrations, chemical agents, and idiopathic or immune-mediated diseases are the causes of meningitis, encephalitis, and meningoencephalitis. In ruminants, generally bacterial infections are more common than other causes of

meningitis or encephalitis. In species other than ruminants, especially adult animals, viruses, protozoa, rickettsia, and fungi are as frequent or more frequent causes of meningitis or encephalitis than are bacteria. Some causes of meningitis or encephalitis, eg, certain rickettsia and bacteria, are seasonal.

Infections of the nervous system often are the result of some injury to its protective barriers. In all species, direct extension of bacterial or mycotic infections to the CNS can develop from sinusitis, otitis media or interna, vertebral osteomyelitis, or diskospondylitis; these infections can also be secondary to migrating grass awns or other foreign bodies, deep bite wounds, or traumatic injuries adjacent to the head or spine.

Proposed clinical study: The effect of different doses (-b(4)- to 10µg) of each serogroup of MenACWY on the immune response and --b(4)---------- ---------- ---------- were investigated in phases 1 and 2 studies. --b(4)----------
The final composition for a single injection was selected as: 10µg of conjugated oligosaccharide A, with 5µg each of conjugated oligosaccharide C, W, and Y, without adjuvant (10-5-5-5). The safety and immunogenicity of the final 10-5-5-5 formulation was then evaluated in individuals aged 11 years and over in a series of phase 2 and phase 3 studies.

Toxicology Study Review:

Title and study number: A single and multiple dose intramuscular study in -b(4)---------- rabbits with meningitis vaccine formulations. Study No. 02-2752
Performing laboratory: ----b(4)----------
Study initiation date: March 26th, 2002
Study completion date: March 18th, 2003
Test article batch/lot:
Monovalent meningococcal A antigen lyophilized (MenA): ChB552001011
Trivalent meningococcal CWY antigens in --b(4)---------- (MenCWYP): MenCWYPO1V
Trivalent meningococcal CWY antigens in ---b(4)---------- (MenCWYH): MenCWYH01V
Animal species and strain: ---b(4)---------- rabbits
Breeder/supplier: ----b(4)----------
Number of animal per group and sex:
---b(4)--------: 14/sex (28 total)
--b(4)----------: 34/sex (68 total)
Placed on test (multiple dose): --b(4)-------: 24/sex (48 total)
Age: 13 weeks for --b(4)-------- animals and 12 weeks for --b(4)------ animals
Body weight range:
For ---b(4)----- animals: 2443-2859 males, 2409-2804 females
For --b(4)------- animals: 2777-3545 males, 2781-3898 females

Route and site of administration: intramuscular injection into the gluteal muscle of the hindlimb.
Volume of injection: 0.5 mL
Frequency of administration and study duration: Animals (6/sex/group) received five doses two weeks apart over eight weeks (days 0, 14, 28, 42, and 56). Two days after the final dose, three animals per sex per group were necropsied, and the remaining 3 animals/sex/group were necropsied two weeks after the final administration. Animals were necropsied on day 58 (main study animals) and on day 70 (recovery animals).
Dose: 80 µg/mL for both MenACWY ---b(4)---------- and MenACWY --b(4)----------
Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Test items were provided as single-use vials (one vial per dose). Stability studies were performed by the sponsor of the IND on the same batches of ----b(4)---------- control as used in this study.

Data in the stability analysis report shows that MenA lyo, $MenCW_{135}Y$/-b(4)--- and $MenCW_{135}Y$/-b(4)--- are stable, up to 1 month, at 2-8ºC when stored as --b(4)--------- filled product. Vaccine lot stored under temperature stress conditions, for up to -b(4)---, showed slight variability in the percentage of --b(4)--------, mainly in the formulation --b(4)----------.

Means of administration: Intramuscular injection
Report status: Final

Experimental design:

Group	Treatment	Doses			Number of Animals (#/sex/group)			
					Clinical Pathology		Necropsy	Microscopic pathology Animals /sex
	Test Article	Dose Level (µg)	Dose Volume	Conc. (µg/mL)	Pretest and days 2, 16, 30, 44, and 58. Animals /sex	Day 70 Animals /sex	Days 58 and 70 Animals /sex	
1	-b(4)--- Control	0	0.5 mL	0	6	3	3	6
2	-b(4)--- Control	0	0.5 mL	0	6	3	3	6
3	Men ACYW with --b(4)--------	40	0.5 mL	80	6	3	3	6
4	Men ACYW with --b(4)---------	40	0.5 mL	80	6	3	3[a]	6

[a] one female was euthanized on day 69 for humane, non-treatment related reasons.

Methods:
The following parameters were evaluated: clinical signs (once daily), skin reactions at the intramuscular site of injection (approximately 24 and 48 hours after dosing, once weekly during the non-dosing weeks and prior to necropsy), body weights [weekly and terminally (after fasting)], food consumption (daily), ophthalmoscopy (once during pretest and prior to the days 58 and 70 necropsies), heart rate (once pretest, and on days 21 and 50), body temperature (prior to each dose, two days after each dose and once

weekly during the non-dosing weeks), haematology, coagulation and clinical chemistry (during pretest, two days after each dose, and on day70), gross anatomy at termination, organ weights and histopathology on a selection of tissues. Blood samples for antibody-determination were taken and analyzed (non-GLP) under the responsibility of the sponsor (from all animals at pretest, days 14, 28, 42, and from 3/sex/group at each necropsy).

Parameters	Frequency of Testing
Cageside observation[1]	Twice daily
Clinical observations[2]	Once daily
Body weight	Weekly and terminally (after fasting)
Food consumption	Daily
Body temperature	Prior to each dose, two days after each dose and once weekly during the non-dosing weeks
Ophthalmologic exam	Once during pretest and prior to the days 58 and 70 necropsies
Clinical chemistry*	During pretest, two days after each dose, and on day70
Hematology*	During pretest, two days after each dose, and on day70
Coagulation*	During pretest, two days after each dose, and on day70
Immunological response	From all animals at pretest, days 14, 28, 42, and from 3/sex/group at each necropsy
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Approximately 24 and 48 hours after dosing, once weekly during the non-dosing weeks and prior to necropsy

* (medial auricular artery), (NC = not collected)

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.

[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Postmortem procedures: The following tissues were collected at necropsy. Those tissues marked with an asterisk were weighed. Tissues examined for histology are marked with an '!'.

Organ/Tissue	Collected	Not collected
Adrenal glands	X!*	
Aorta	X!	
Bone (sternum & femur)	X!	
Bone marrow (sternum & femur)	X!	
Brain (cerebrum, cerebellum, medulla and pons)	X!*	
Cervix		X
Colon	X!	
Duodenum	X!	
Epididymides	X!	
Esophagus	X!	
Eyes (optic nerve)	X!	
Fallopian tubes (oviduct)		
Gall bladder	X!	
Gross lesions (if any)		X
Harderian gland (if applicable)		X
Heart	X!*	
Ileum	X!	
Jejunum	X!	
Kidneys	X!	
Lacrimal glands		
Larynx		
Liver	X!*	
Lung (main-stem; bonchi)	X!*	
Lymph nodes (cervical)	X!	
Lymph nodes (mandibular)	X!	
Lymph nodes (mesenteric)		
Mammary glands	X!	
Naso-oropharyngeal cavity (turbinates, nares, soft palate)	X!	
Ovaries	X!*	
Pancreas	X!	
Peyer's patch (if applicable)		X
Pituitary gland	X!	
Prostate	X!	
Rectum	X!	
Salivary glands (mandibular)	X!	

Sciatic nerve	X!	
Skeletal muscle	X!	
Skin	X!	
Spinal cord (cervical, lumbar, thoracic)	X!	
Spleen	X!*	
Testes	X!*	
Thymus	X!*	
Thyroid (w/ parathyroid glands)	X!	
Tongue		X
Ureters		X
Uterus (w/ cervix)	X!	
Urinary bladder	X!	
Vagina	X!	
Zymbal's gland (if applicable)		X

Table of Histology – Tissues listed above were collected, from all animals, and examined microscopically. Any abnormalities, seen during histology processing, not noted during macroscopic examinations, were recorded.

Results:
Morbidity and mortality: one animal (number 4502, group 4) was sacrificed on day 69 for humane reason. This animal did not eat for several days due to a broken jaw. All other animals survived to their scheduled termination.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR B) HEPATOBILIARY	**Lactate dehydrogenase (LDH):** SD pretest F↓1.6 G3, SD 30 M↓3.1 G4, SD 58 M ↓1.6 G3, SD 58 F ↓1.8 G3, **Aspartate aminotransferase (AST)**: SD 58 M ↑1.8 G4, **Alanine aminotransferase (ALT):** SD 58 F ↑1.6 G3, SD 70 F ↓1.6 G3	Glutamate dehydrogenase, sorbitol dehydrogenase Total bile acids
	Alkaline phosphatase (ALP): SD 70 F ↑2.0 G3	Gamma-glutamyl transferase (GGT) Total bile acids Total bilirubin
ACUTE PHASE REACTANTS		C-reactive protein, fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	**Creatine kinase:** SD 2 F ↑9.2 G4, SD 16 M ↑2.5 G3, SD 30 M ↓1.8 G3, SD 30 M ↓12.1 G4, SD 30 F ↑2.6 G3, SD 30 F ↓2.7 G4, SD 58 F ↓2.1 G3, SD 58 F ↓1.6 G4, SD 70 F ↑1.9 G3, SD 70 F ↓9.4 G4 **Total Cholesterol:** SD 44 F ↑1.6 G3, SD 58 F ↑1.6 G3,	Albumin (A) Globulin (G, calculated) or A/G Ratio Cholinesterase Total protein Fasting Triglycerides

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)**)**	NOT OF NOTE

Table of Clinical Chemistry Results

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤1.5)	NOT OF NOTE
RED BLOOD CELLS		Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC) Reticulocytes
WHITE BLOOD CELLS	**Neutrophil count:** SD2 F ↓1.6 G4, SD30 M ↓1.7 G4, SD58 M ↓2.4 G4, SD58 F ↑1.6 G3, **Eosinophils count:** SD16M ↓2.6 G3, SD16M ↓1.6 G4, SD16F ↓1.5 G3, SD16F ↓1.6 G4, SD30F ↓2.3 G3, SD30F ↓3.2 G4, SD44M ↓3.0 G3, SD44F ↓2.2 G3, SD44F ↓2.5 G4, SD58M ↓2.0 G3, SD58M ↓3.0 G4, SD58F ↓1.6 G3, SD58F ↓2.1 G4, SD70M ↑1.9 G4, **Basophils:** SD16M ↓3.3 G3, SD16F ↓3.3 G3, SD30M ↓3.1 G3, SD30M ↓1.7 G4, SD30F ↓3.0 G3, SD30F ↓2.5 G4, SD44M ↓2.7 G3, SD44M ↓1.9 G4, SD44F ↓2.8 G3, SD44F ↓1.8 G4, SD58M ↓2.4 G3, SD58F ↓2.5 G3 **Monocyte count** SD70M ↓1.6 G3, SD70M ↓2.9 G4,	lymphocyte count Macrophage Total Leukocytes (WBC) Large Unstained Cells (LUC)

	SD70F ↑1.7 G4,	
CLOTTING POTENTIAL		Activated partial-thromboplastin time clotting time Platelet count Prothrombin time Mean platelet volume Fibrinogen
OTHERS		Bone marrow cytology

Table of Hematology Results

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in body weight, clinical signs, relative food consumption, ophthalmoscopic parameters, heart rate, body temperature, or organ weight were found. The sporadic occurrences of decreased food consumption and fecal volume were not considered test article-related because they were seen in all treatment groups. Animal number 2004 (group 2) had moderate erythema on day 57 only. Slight edema on day 44 was reported in one animal (number 3001) of group 3.

Lactate dehydrogenase were significantly reduced in group 3 (males and females) on study day (SD) 58. Aspartate aminotransferase (AST) was significantly increased in group 4 males and group 3 females on SD 58. In group 3 females, alanine aminotransferase (ALT) was significantly increased on SD 58 and significantly decreased on SD 70 (recovery). Alkaline phosphatase (ALP) was significantly increased in group 3 females on SD 70. Creatine kinase was significantly increased in groups 4 (SD 2, F) and 3 (SD 16, M; SD 30, F; and SD 70, F). There were significant decrease in creatine kinase levels in groups 3 (SD30, M; SD58, F) and 4 (SD30, M and F; SD 58 and 70, F). Total cholesterol was significantly increased in group 3 (females) on SD's 44 and 58.

Neutrophils were decreased significantly in group 4 on SD's 2 (F), 30 and 58 (M) and increased in group 3, females, only on SD's 16 and 58. Significant decreases in eosinophils count was reported in groups 3 and 4 on SD's 16 (M and F), 30 (F), 44 (M and F), and 58 (M and F). On SD 70, eosinophils were increased in group 4 males and decreased in group 3 females. Basophils were decreased significantly in males and females of group 3 on SD's 16, 30, 44, and 58. The levels of basophils were also significantly decreased in group 4 on SD's 30 (M and F), 44 (M and F), and 58 (M). Monocytes were decreased significantly on SD 70 in male groups 3 and 4, and increased significantly in females group 4.

Discolored lungs were reported in male groups 3 and 4. Abnormal contents in the kidney were observed in one female of group 4 at terminal sacrifice. At recovery sacrifice, discolored lungs and cyst in the ovaries were observed in females group 3.

Organ Weights at Terminal Sacrifice:

SEX		MALES				FEMALES			
GROUPS		1 (CONTROL)	2	3	4	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
BODY WEIGHT (gram)[a]		2986.9	2840.8	2945.3	2863.0	3050.5	3023.2	3068.8	3062.8
BRAIN									
Absolute Weight[a]	gram	10.32	10.14	10.27	9.98	10.84	9.83	10.88	10.59
Per Body Weight[a]	%	0.3458	0.3569	0.3492	0.3489	0.3559	0.3254	0.3551	0.3452
ADRENALS									
Absolute Weight[a]	gram	0.3313	0.3904	0.3390	0.4013	0.2744	0.2771	0.3619	0.3114
Per Body Weight[a]	%	0.0111	0.0137	0.0115	0.0140	0.0090	0.0092	0.0118	0.0102
Per Brain Weight[a]	%	3.2153	3.8526	3.3177	4.0198	2.5585	2.8281	3.3312	2.9579
LUNGS									
Absolute Weight[a]	gram	13.07	12.84	12.60	13.60	12.60	13.11	11.82	12.66
Per Body Weight[a]	%	0.4358	0.4533	0.4272	0.4738	0.4144	0.4347	0.3852	0.4135
Per Brain Weight[a]	%	126.16	126.57	123.39	136.50	116.17	132.83	108.79	119.96
HEART									
Absolute Weight[a]	gram	5.68	6.13	5.41	5.62	5.94	6.07	6.02	5.31
Per Body Weight[a]	%	0.1902	0.2156	0.1837	0.1961	0.1950	0.2010	0.1967	0.1734
Per Brain Weight[a]	%	54.99	60.43	52.89	56.34	54.77	61.78	55.22	50.29*
KIDNEYS									
Absolute Weight[a]	gram	17.30	18.70	17.32	15.81	15.61	16.28	16.53	17.08
Per Body Weight[a]	%	0.5781	0.6582	0.5892	0.5519	0.5119	0.5395	0.5372	0.5561
Per Brain Weight[a]	%	167.27	184.22	168.28	158.49	144.09	165.01	152.45	160.81
LIVER									
Absolute Weight[a]	gram	77.17	75.75	77.17	70.91	62.06	63.76	68.43	69.16
Per Body Weight[a]	%	2.58	2.6612	2.62	2.4773	2.0361	2.1105	2.2274	2.2500
Per Brain Weight[a]	%	746.26	745.98	752.00	710.44	571.80	649.47	630.55	650.95
SPLEEN									
Absolute Weight[a]	gram	0.9873	0.8398	0.8677	0.9967	1.0972	0.9111	1.0820	1.2605
Per Body Weight[a]	%	0.0329	0.0295	0.0294	0.0348	0.0359	0.0302	0.0355	0.0412
Per Brain Weight[a]	%	9.5349	8.2903	8.5347	9.9920	10.2497	9.2630	9.8937	12.0359
TESTES									
Absolute Weight[a]	gram	5.25	4.26	4.31	4.44				
Per Body Weight[a]	%	0.1757	0.1501	0.1463	0.1544				
Per Brain Weight[a]	%	50.78	41.96	42.01	44.60				
THYMUS									
Absolute Weight[a]	gram	3.54	2.14	3.81	2.46	3.61	4.38	3.72	3.61
Per Body Weight[a]	%	0.1188	0.0758	0.1297	0.0857	0.1182	0.1448	0.1219	0.1181*
Per Brain Weight[a]	%	34.34	21.22	37.65	24.78	33.08	44.59	34.14	34.40
OVARIES									
Absolute Weight[a]	gram					0.2094	0.2277	0.3064	0.2101
Per Body Weight[a]	%					0.0069	0.0075	0.0099	0.0068
Per Brain Weight[a]	%					1.9559	2.3039	2.8412	1.9714

Table of organ weight and their normalization (terminal sacrifice). Absolute weights are expressed as mean(grams). *different from controls at $P \leq 0.05$

Organ Weights at Recovery Sacrifice:

SEX		MALES				FEMALES			
GROUPS		1 (CONTROL)	2	3	4	1 (CONTROL)	2	3	4
NUMBER OF ANIMALS		3	3	3	3	3	3	3	3
BODY WEIGHT (gram)[a]		2771.8	2865.4	2776.6	2968.8	3020.7	3010.1	3011.3	2990.7
BRAIN									
Absolute Weight[a]	gram	10.1824	9.5682	10.2122	9.9343	10.2157	10.0414	10.2449	10.2113
Per Body Weight[a]	%	0.3688	0.3339	0.3685	0.3346	0.3385	0.3333	0.3402	0.3415
ADRENALS									
Absolute Weight[a]	gram	0.3719	0.3829	0.3478	0.2694	0.2857	0.3081	0.3418	0.3586
Per Body Weight[a]	%	0.0135	0.0133	0.0126	0.0091	0.0094	0.0103	0.0113	0.0120
Per Brain Weight[a]	%	3.7069	3.9908	3.4377	2.7333	2.7781	3.0913	3.3254	3.5105
LUNGS									
Absolute Weight[a]	gram	12.0385	10.8050	12.0534	11.9526	10.5969	12.1838	11.8491	11.5304
Per Body Weight[a]	%	0.4362	0.3774	0.4370	0.4031	0.3506	0.4052	0.3934	0.3858
Per Brain Weight[a]	%	118.06	113.01	117.72	121.23	103.50	122.00	115.91	112.91
HEART									
Absolute Weight[a]	gram	5.6566	5.5164	5.4460	5.8728	5.9826	6.4005	5.5759	5.8535
Per Body Weight[a]	%	0.2042	0.1927	0.1967	0.1981	0.1972	0.2130	0.1852	0.1957
Per Brain Weight[a]	%	55.78	57.70	53.55	59.33	59.31	64.18	54.54	57.33
KIDNEYS									
Absolute Weight[a]	gram	15.7913	16.0116	15.1955	16.8623	16.4495	16.1138	15.7185	16.3212
Per Body Weight[a]	%	0.5695	0.5584	0.5479	0.5677	0.5457	0.5359	0.5223	0.5454
Per Brain Weight[a]	%	156.25	167.22	149.31	170.10	161.19	161.28	153.36	159.85
LIVER									
Absolute Weight[a]	gram	65.1244	67.0278	62.4089	70.6091	63.6721	64.6329	70.0602	74.3998
Per Body Weight[a]	%	2.3524	2.3370	2.2356	2.3794	2.1091	2.1534	2.3263	2.4880
Per Brain Weight[a]	%	646.27	699.94	611.09	711.50	622.84	650.27	682.27	728.61
SPLEEN									
Absolute Weight[a]	gram	0.8703	0.7686	0.7523	0.7885	1.1760	1.2549	1.0536	0.9056
Per Body Weight[a]	%	0.0311	0.0268	0.0269	0.0266	0.0390	0.0416	0.0350	0.0303
Per Brain Weight[a]	%	8.4490	8.0203	7.4408	7.9326	11.4630	12.4405	10.2778	8.8693
TESTES									
Absolute Weight[a]	gram	4.9025	4.6282	4.2358	4.1665				
Per Body Weight[a]	%	0.1778	0.1617	0.1541	0.1405				
Per Brain Weight[a]	%	48.52	48.41	41.77	41.88				
THYMUS									
Absolute Weight[a]	gram	2.5575	2.2639	2.8608	2.9391	3.6916	3.8712	3.9776	3.2888
Per Body Weight[a]	%	0.0917	0.0792	0.1016	0.0990	0.1219	0.1290	0.1318	0.1101
Per Brain Weight[a]	%	25.21	23.69	27.97	29.83	36.22	38.98	38.52	32.20
OVARIES									
Absolute Weight[a]	gram					0.2307	0.2543	0.2662	0.2533
Per Body Weight[a]	%					0.0076	0.0085	0.0089	0.0085
Per Brain Weight[a]	%					2.2407	2.5455	2.5863	2.4796

Table of organ weight and their normalization (recovery sacrifice). Absolute weights are expressed as mean (grams). *different from controls at $P \leq 0.05$

There was a 19% decrease in heart/brain weight ratios in group 4 females with no macroscopic or microscopic pathology associated with this decrease. Group 4 females also showed statistically significant decreases in mean thymus weight (17%), thymus/body weight ratio (18%) and thymus/brain weight ratio (23%), when compared to their respective control (group 2). These decreases were not considered test article related. No changes in organ weights or ratios were reported in the recovery groups.

Gross Pathology:

Terminal sacrifice

Group	Findings
1M	NF
2M	Discolored injection sites 1 (1/3)* and 2 (1/3)
3M	Discolored lungs (1/3) and injection sites 1 (2/3) and 2 (1/3); Firmness at injection site 1 (1/3)
4M	Discolored lungs and injection sites 1 (1/3); Firmness at injection site 1 (1/3)
1F	Discolored injection site 1 (2/3), cervical LN (1/3), and mediastinal LN (1/3); Enlarged mediastinal LN (1/3)
2F	Discolored injection site 1 (2/3)
3F	Discolored thymus (1/3), injection site 1 (2/3), and injection site 2 (1/3); Edematous injection site 1 (1/3)
4F	Abnormal contents in the kidney (1/3); discolored injection site 1 (2/3)

(NF = no findings); * (number of animals with the observation/total number of animals in the group).

Recovery sacrifice

Group	Findings
1M	Discolored injection site 1 (1/3)*
2M	NF
3M	Discolored injection site 1 (2/3)
4M	Discolored injection site 1 (1/3)
1F	Discolored injection site 1 (1/3)
2F	NF
3F	Discolored lungs 1 (1/3); Cyst in the ovaries (1/3)
4F	NF

(NF = no findings); * (number of animals with the observation/total number of animals in the group).

Microscopic finding are listed below:
Single dose-terminal sacrifice

Groups	Findings
1M	NF
2M	Subcutaneous/muscle hemorrhage (1/2)
3M	NF
4M	Subcutaneous/muscle hemorrhage (2/2)
1F	Subcutaneous/muscle hemorrhage (1/2)
2F	Muscle histiocytic aggregates (1/2)
3F	Subcutaneous/muscle foci of granular amorphous material (1/2); Subcutaneous/muscle hemorrhage (2/2)
4F	Subcutaneous/muscle foci of granular amorphous material (1/2); Subcutaneous/muscle hemorrhage (1/2)

(NF = no findings)

Single dose-recovery sacrifice

Groups	Findings
1M	NF
2M	NF
3M	NF
4M	Muscle histiocytic aggregates (1/2)
1F	NF
2F	Muscle histiocytic aggregates (1/2)
3F	NF
4F	Muscle histiocytic aggregates (1/2)

(NF = no findings)

Multiple dose-injection site 1 (terminal sacrifice)

Groups	Findings
1M	Slight-subcutaneous/muscle foci of granular amorphous material; minimal-myofiber degeneration
2M	Minimal to slight-subcutaneous/muscle hemorrhage; minimal-mixed inflammatory inflitrate
3M	Minimal-subcutaneous mixed inflammatory infiltrate; minimal to slight-subcutaneous/muscle foci of granular amorphous material; minimal to slight-subcutaneous/muscle hemorrhage; minimal to slight-mixed inflammatory inflitrate
4M	Moderate subcutaneous edema; minimal to slight-subcutaneous mixed inflammatory infiltrate; minimal-subcutaneous/muscle foci of granular amorphous material; minimal to marked-subcutaneous/muscle hemorrhage; surface-inflammatory cells/debris; slight-acanthosis; minimal to slight-histiocytic aggregates; minimal to slight-mixed inflammatory infiltrate;

	minimal-myofiber degeneration;
1F	Minimal-subcutaneous/muscle hemorrhage; minimal-subcutaneous/muscle foci of granular amorphous material; minimal to slight-histiocytic aggregates; minimal-mixed inflammatory infiltrate; minimal-myofiber degeneration;
2F	Minimal-subcutaneous/muscle hemorrhage; minimal to slight-subcutaneous/muscle foci of granular amorphous material; minimal to slight-histiocytic aggregates; minimal to slight-mixed inflammatory infiltrate
3F	Slight subcutaneous edema; slight to moderate-subcutaneous mixed inflammatory infiltrate; minimal to slight-subcutaneous/muscle hemorrhage; moderate-subcutaneous/muscle foci of granular amorphous material; minimal epithelium erosion; minimal to moderate-mixed inflammatory infiltrate
4F	Minimal-subcutaneous mixed inflammatory infiltrate; minimal to slight-subcutaneous/muscle hemorrhage; slight-histiocytic aggregates; slight-mixed inflammatory infiltrate; slight-myofiber degeneration

(NF = no findings)

Multiple dose-injection site 1 (recovery sacrifice)

Groups	Findings
1M	Minimal epithelium erosion; slight-histiocytic aggregates
2M	Minimal-subcutaneous/muscle foci of granular amorphous material; minimal to slight-histiocytic aggregates; slight- mixed inflammatory infiltrate
3M	Minimal-subcutaneous/muscle hemorrhage; minimal-mixed inflammatory infiltrate
4M	Minimal-histiocytic aggregates; minimal- mixed inflammatory infiltrate
1F	Minimal-subcutaneous/muscle hemorrhage; slight-histiocytic aggregates; minimal myofiber degeneration
2F	NF
3F	Slight-histiocytic aggregates; minimal-mixed inflammatory infiltrate
4F	Minimal-histiocytic aggregates; minimal-mixed inflammatory infiltrate

(NF = no findings)

Multiple dose-injection site 2 (terminal sacrifice)

Groups	Findings
1M	Minimal-histiocytic aggregates
2M	Slight-subcutaneous/muscle granular amorphous material; slight-histiocytic aggregates
3M	Slight-subcutaneous/hemorrhage; slight-histiocytic aggregates; slight-muscle/mixed inflammatory infiltrate
4M	NF
1F	Slight-histiocytic aggregates
2F	NF
3F	Minimal-subcutaneous/hemorrhage; minimal-histiocytic aggregates; minimal-muscle/mixed inflammatory infiltrate
4F	NF

(NF = no findings)

Multiple dose-injection site 2 (recovery sacrifice)

Groups	Findings
1M	Minimal to slight-histiocytic aggregates
2M	Minimal-histiocytic aggregates; minimal-muscle/mixed inflammatory infiltrate
3M	NF
4M	Minimal-histiocytic aggregates
1F	Slight-histiocytic aggregates
2F	Slight-histiocytic aggregates
3F	NF
4F	Slight-histiocytic aggregates; minimal-muscle/mixed inflammatory infiltrate

(NF = no findings)

Body temperature for doses 1, 2, 3, 4, and 5

Group	SD 0, 2, and 7	SD 14, 16, and 21	SD 28, 30, and 35	SD 42, 44, and 49	SD 56, 58, 63 and 70
1 M & F	0	0	0	0	0
2 M & F	0	0	0	0	0
3 M & F	0	0	0	0	0
4 M & F	0	0	0	0	0

Table of occurrences for body temperature > ºC

Heart rate

Group	Pre-test	SD 21	SD 50
1 M	157	125	121
2 M	158	121	114
3 M	165	119	119
4 M	151	121	116
1 F	167	136	117
2 F	166	139	118
3 F	172	141	119
4 F	163	148	121

Table of heart rates [beats per minute (bpm)].

Local toxicity:
All animals appeared within normal limits in the dermal irritation assessment of the injection sites. One animal (animal number 2004) only reported with moderate erythema on day 57.

Macroscopically, discoloration at the injection site1 and/or 2 was reported in all, except male control, treated groups at terminal sacrifice. Firmness at injection site 1 was reported in male groups 3 and 4. Discolored cervical and mediastinal lymph nodes and enlarged mediastinal lymph node were reported in one female of group 1. Discolored thymus and edematous injection site 1 were reported in females group 3. At recovery sacrifice discolored injection site 1 were reported in males groups 1, 3, and 4 and females group 1.

Microscopically the injection site, in the single dose-treated animals at the terminal sacrifice, showed Subcutaneous/muscle hemorrhage in male groups 2 and 4 and in female groups 1, 3, and 4. Muscle histiocytic aggregates were reported in female group 2. Subcutaneous/muscle foci of granular amorphous material were seen in female groups 3 and 4. At recovery sacrifice, muscle histiocytic aggregates were reported in male group 4 and in female groups 2 and 4.

At the terminal sacrifice of the multiple dose-treated animals at injection site 1, minimal and/or slight-subcutaneous/muscle foci of granular amorphous material were reported in male groups 1, 3, and 4 and in female groups 1, 2, and 3. Minimal-myofiber degeneration was seen in males and females groups 1 and 4. Minimal, minimal to slight, or marked-subcutaneous/muscle hemorrhage was reported in male groups 2, 3, and 4 and female groups 1, 2, 3, and 4. Minimal, minimal to slight, or minimal to moderate-mixed inflammatory infiltrate were reported in male groups 2, 3, and 4 and female groups 1, 2, 3, and 4. Moderate and slight-subcutaneous edema was reported in male group 4 and female group 3, respectively. Surface-inflammatory cells/debris and slight-acanthosis were reported in male group 4. Minimal to slight or slight-histiocytic aggregates was reported in male group 4 and female groups 1, 2, and 4. Minimal epithelium erosion was seen in female group 3.

At the recovery sacrifice of the multiple dose-treated animals at injection site 1, minimal epithelium erosion and slight-histiocytic aggregates were reported in male group 1.

Serology:
In individual rabbits' sera, the IgG total titer anti MenA, MenC, MenW, and MenY PS showed negative results at all the pretest and two days after the first dose sera. Sera collected on day 28 from group 3 showed increased titer levels for all the four antigens. And, very few animals did not give any titer in the sera collected on day 28 in group 4. Sera collected on days 42, 58, and 70 from groups 3 and 4 showed very high titers for all antigens, except for one animal which did not yield any titer for anti MenW pS. There was an increase in the titer after 3 doses compared to the titer induced by 2 doses for all the antigens, mainly in group 4 which received ACWY---b(4)----. There were not a significant increase of the titer for all the antigens in both groups 3 and 4 after two more doses (IV and V doses). The maximum response was induced by 3 doses of the ACYW conjugates vaccines combinations.

(Test article related effects are listed in the table below)

Test article related effects	Effects considered incidental*
↓LDH M&F, ↑AST M&F, ↓creatine kinase, ↑ cholesterol F G3 only, ↑ Neutrophil F G3 and ↓ M G4, ↓ eosinophils, ↓ basophils, ↓ monocytes M G3 & G4 Discoloration at the injection site1 and/or 2	↑↓ALT, ↑ ALP

Assessment:

No treatment-related, mortality, nor any toxicologically relevant changes in body weight, clinical signs, relative food consumption, ophthalmoscopic parameters, heart rate, body temperature, or organ weight were found.

Significant, test article related, decreases in clinical pathology parameters were reported. Lactate deyhdorgenase levels were decreased on SD's 30 (G4) and 58 (G3) in males and on SD 58 in (G3) females. Creatine kinase levels were decreased in males (G 3 & 4) on SD 30 and in females (G4) on SD3 and (G3&4) on SD58. Increases in creatine kinase levels were also seen at different time points (SD16, M, G3; SD2, F, G4; SD30, F, G3; and SD70, F, G3). Neutrophil, eosinophil, basophil, and monocyte counts were decreased significantly at different time points in one or both treated (G's 3 &/or 4) groups in males and/or females (See the systemic toxicity section on page 10). Significant increases in clinical pathology parameters such as: AST (SD 58, M, G4), total cholesterol (SD's 44 & 58, F, G3), neutrophils (SD58, F, G3), eosinophils (SD70, M, G4) and monocytes (SD70, F, G4) were also reported.

Only two instances of dermal irritation (erythema and/or edema) were reported. Animal 2004 (group 2) had moderate erythema on day 57 only. Animal number 3001 (group 3) had slight edema on day 44 only. No other observations or erythema edema were reported in any of the treated group during the study.

Minor inflammatory and degenerative changes at the site of injection were due to administration of Men ACYW in ----b(4)----------, were reported. The reactions were characterized by foci amorphous material, associated with histiocytic aggregates, hemorrhage and inflammatory infiltrate. These observations were resolved completely (single dose phase) or partially (multiple dose phase) at 14-day recovery period.

Based on the overall findings in this study, it can be concluded that in Albino --b(4)---------- rabbits single or repeated intramuscular administration of Men ACYW vaccine had no adverse effects in terms of systemic toxicity and local tolerance at the dose level of 40 ug antigen.

Immunology performed in this study verified that an active dose was administered.

GLP study deviations or amendments: No significant deviations or amendments were recorded that influenced the quality, integrity or interpretation of the results.

Conclusions:
Based on nonclinical toxicity assessments of this study, there are no significant safety issues to preclude the BLA from going into effect.

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