

Toxicology Review of Ebola Zaire Vaccine

BLA: 125690/0

Type and date of submission: Pre-submission; October 24, 2018

Sponsor: Merck Sharp & Dohme

Product: Ebola Zaire (rVSVΔ G-ZEBOV-GP, Live, Attenuated)

Cross references: None

Proposed use: Active immunization of at-risk individuals 18 years of age and older to protect against Ebola disease caused by Zaire Ebola virus

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Précis

Three toxicity study reports, submitted in support the licensure, are reviewed: (1) repeat-dose toxicity study in mice, (2) repeat-dose toxicity study in cynomolgus macaques and (3) developmental toxicity in rats. Other toxicity studies (an exploratory neurovirulence study in cynomolgus Macaques, a preliminary viremia and immunogenicity study in rats and an in vivo biodistribution and persistence in cynomolgus macaques) are not reviewed as they are exploratory or preliminary in nature and not dedicated toxicity studies.

In the repeated toxicity study in mice, three groups of animals (30/sex/group) received two biweekly intramuscular injections of either saline control or 0.2 mL zVSV-ZEBOV-GP at dose of either 2×10^6 or 2×10^7 PFU. After the treatment, half of animals/group was followed by a 4-week recovery period. Two biweekly intramuscular vaccine administrations at doses up to 2×10^7 PFU were well tolerated in animals. The findings were expected local irritation and immunogenic (spleen and iliac lymph node) to vaccine administrations and not considered adverse. After a 30-day recovery period, these findings were either absent or at a lower frequency, indicating on-going recovery.

In the repeated toxicity study in cynomolgus macaques, total of 14 males and 14 females were assigned to three groups of animals received two biweekly intramuscular injections of either saline control (2/sex/group) or 1 mL zVSV-ZEBOV-GP (6/sex/group) at dose of either 3×10^6 or 1×10^8 PFU. After the vaccine treatment, 2/sex/group was followed by a 4-week recovery period. Two biweekly intramuscular vaccine administrations at doses up to 1×10^8 PFU were well tolerated in animals. The findings were expected local (inflammation) and immunogenic (inguinal lymph node) responses to vaccine administrations and not considered adverse. After a 30-day recovery period, these findings were either absent or at a lower frequency, indicating on-going recovery.

In the developmental toxicity study, female rats (22/group) were administered intramuscularly of phosphor buffer or zVSV-ZEBOV-GP 28 days and 7 days prior to mating, on days 6 of gestation and day 7 of lactation at dose of 5.28×10^7 PFU (0.22 mL). An additional 22 females received a single same dose only on gestation day 6. There were no vaccine-related mortalities or clinical or necropsy observations in the dams or in the pups. It had no impact on mating and fertility parameters, ovarian and uterine examination, or natural delivery or litter observation parameters in the dams.

There were no fetal external, soft tissue, or skeletal abnormalities attributed to vaccine administration. Postnatal development as measured by acoustic (auditory) startle, air righting, and pupil constriction, and functional observational battery parameters in the pups were unaffected.

Toxicology Study Review

Title and study number: Toxicity and local tolerance study following intramuscular administration of vesicular stomatitis Ebola virus vaccine in mice with 30-day recovery (Study # 100027546-08100)

Performing laboratory: (b) (4)

Initiation date: May 4, 2015

Amended final report date: January 23, 2017

Batch/lot number of test article: 001-10-14

Animal species and strain: (b) (4) mouse

Breeder/supplier: (b) (4)

Number of animals per sex per group: 30

Age: 13 weeks (males), 9-10 weeks (females)

Body weight range: 20-25 g (males), 15-20 g (females)

Route and site of administration: Intramuscular; thigh

Volume of administration: Two 0.05 mL injections/thigh, 0.2 mL/animal

Frequency of administration and study duration: bi-weekly for 2 doses; 44 days

Dose: 2×10^6 , 2×10^7 PFU

Stability: The stability report is provided in Appendix B.

Means of administration: A syringe fitted with a 27G needle

Report status: Final

Experimental design

Group	Test article	PFU	No. of animals/sex/group/sacrifice
1	Control	0	15
2	rVSV-ZEBOV-GP (b) (4)	2×10^6	15
3	rVSV-ZEBOV-GP	2×10^7	15

Methods:

Endpoint	
Hematology	Not provided
Clinical chemistry	Not provided
Immunogenicity	ELISA

Randomization procedure: The animals were assigned to dose groups using the subject placement function in (b) (4) which ensures similar group body weights by sex.

Statistical analysis plan: Data (body weights, organ weights, hematology, coagulation, serum chemistry, and select urinalysis data) collected at (b) (4) using the (b) (4) system was analyzed for test article effects by parametric or nonparametric analysis of variance (ANOVA). For all data, normality was determined by the Shapiro-Wilks test and homogeneity of variances was determined by Levene's test. Data were log-transformed to meet parametric assumptions when necessary. For parametric data determined to be normally distributed and homogenous among groups, an ANOVA F-test was used to determine whether there are differences among the group means. If the ANOVA F-test is significant, then tests for differences between the control and each of the comparison groups was conducted using Dunnett's test, which adjusts for multiple comparisons. For nonparametric data that are not normally-distributed and/or non-homogenous, a Kruskal-Wallis test was used to determine whether there are differences among the group means. If the Kruskal-Wallis test is significant, then tests for differences between the control and each of the comparison groups was conducted using Wilcoxon tests and the Bonferroni-Holm method to correct for multiple comparisons.

The following parameters were evaluated

Parameters	Frequency of Testing
Clinical observations	Twice daily for mortality and moribundity. Twice daily for clinical observations on days of vaccination and once daily on all other days
Injection site observations	Prior to and one and two days after each dose administration
Body weight	Pre-study and on days 1, 2, 7, 14, 15, 16, 21, 28, 35, 42 and 44
Food consumption	Weekly
Body Temperatures	Pre-treatment, prior to each dose, 6, 24 and 48 hours after each dose thru an implantable programmable temperature transponder in the dorsal intrascapular area
Clinical chemistry	Days 16 and 44 by cardiac puncture under CO ₂ /O ₂ anesthesia
Hematology	Same as above
Immune response	Same as above
Viruria	Days 0, 2, and 6
Necropsy	Days 16 and 44
Tissues for histopathology	Same as above

Postmortem procedures: The following tissues were collected at necropsy and microscopically examined. Those tissues marked with an asterisk were weighed.

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
DIGESTIVE	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejunum, ileum), liver*, stomach, gall bladder, pancreas, esophagus, salivary gland (parotid, mandibular, sublingual), Peyer's patch	
RESPIRATORY	Lung with bronchi, trachea,	Nasal cavity
CARDIOVASCULAR	Heart*, aorta	
IMMUNOLOGIC/ HEMATOPOIETIC	Bone with bone marrow (femur) bone marrow (sternum), lymph node (mesenteric, mandibular, Iliac), spleen*, thymus*	
UROGENITAL	Epididymes, kidneys*, urinary bladder, ureters, ovaries*, prostate, testes*, seminal vesicles, uterus, cervix, vagina, oviduct	Fallopian tubes
NEUROLOGIC	Brain*, spinal cord, sciatic nerve	
HORMONAL	Adrenals, mammary gland, thyroid with parathyroid gland, pituitary gland	
OTHER	Skeletal muscle, skin, sciatic nerve, tongue, eyes with optic nerves, Harderian glands, injection sites	
GROSS LESIONS	None	

Results:

Mortality: Six deaths occurred during pre-test and during the study that were not attributed to vaccination. Four animals died during administration of CO₂/O₂ and IPTT implantation. No gross findings or lesions were observed. An investigation was performed on the CO₂/O₂ source tank with no conclusive findings by the supplier. One animal not placed on study was found dead on Day 42. A necropsy was performed with

no findings. One animal in Group 3 was found dead on Day 6 and a necropsy performed. No gross findings were observed. Thus, the cause of death was not determined.

Clinical observations: One group 2 and 7 group 3 animals were thin on a various day between day 33 and day 39. One group 3 animal was thin on days 34-36. One group 3 animal was observed hunched posture on day 40.

Food consumption: There were no changes in food consumption attributed to the control article or vaccine administration.

Body weight: Slight decreased body weights were observed in males on day 28 (5%), day 35 (8%) and day 44 (4%).

Body temperature: All animals on study maintained normal body temperatures post vaccination on days 0 through 44 when compared to each individual baseline temperature.

Injection site observations: All injection site irritations had resolved by two days post administration with erythema observed more frequently than edema. Slight erythema was observed in 3 group 3 animals on day 1. One of them was also observed with edema.

Viremia/Viruria: At the Day 16 necropsy, there were detectable levels in whole blood of the rVSVZEBOV-GP vaccine in only two group 3 animals. In urine, on day 2 there were detectable levels in one group 1 male and two group 3 males and one group 3 female. On day 6, only one group 3 female exhibited with detectable levels.

Immune response: Titers were observed in all animals from groups 2 and 3 on days 16 and 44. No titers were observed on either day for group 1.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Glutamate dehydrogenase (not determined) Gamma-glutamyl transferase Aspartate aminotransferase Alanine aminotransferase Sorbitol dehydrogenase (not determined)
B) HEPATOBILIARY		Total bile acids (not determined)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
		Alkaline phosphatase Total bilirubin
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin Globulin (calculated) A/G Ratio Cholesterol Creatine kinase Cholinesterase (not determined) Total protein Triglyceride Lactate dehydrogenase

Table of Clinical Chemistry Results

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT	NOT OF NOTE
RED BLOOD CELLS		Hematocrit Hemoglobin conc. Mean corp. Hb. Mean corp. Volume Mean corp. Hb. conc. Total erythrocyte count Reticulocyte
WHITE BLOOD CELLS		Total leukocytes Neutrophil Monocyte Lymphocyte Eosinophil Basophil Large unstained cells (not determined)
CLOTTING POTENTIAL		Platelet count

Table of Hematology Results

Organ weights: Higher spleen weights were reported in groups 2 (11-14%) and 3 (26-33%) on day 16.

Gross pathology: Enlargement of the iliac lymph nodes was noted in eight group 2 females and 7 group 3 females. One group 3 female had also enlarged lumbar lymph nodes on day 16. There was no other test item-related finding.

Microscopic findings: Findings (lymphoid hyperplasia) on day 16 were limited to iliac lymph nodes and spleen. Lymphoid hyperplasia resolved by recovery termination on day 44. The incidences of these findings are summarized below.

Day 16	1M	2M	3M	1F	2F	3F
Iliac lymph node Lymphoid hyperplasia	0/13	13/15	14/14	2/14	13/15	10/15
Spleen Lymphoid hyperplasia	0/15	5/15	9/14	2/15	5/15	6/15

Microscopic findings noted at day 16 were observed at reduced incidences and/or severity at the end of recovery period on day 44 as shown below:

Day 44	1M	2M	3M	1F	2F	3F
Iliac lymph node Lymphoid hyperplasia	0/15	2/15	2/15	4/14	5/14	2/15
Spleen Lymphoid hyperplasia	0/15	2/15	1/15	2/15	1/15	2/15

Test article related effects
Local irritation at the injection sites (erythema and edema) and immunogenic response in the spleen and iliac lymph node (lymphoid hyperplasia). All findings were typical and expected local inflammatory/immunogenic reaction to vaccine administrations. They were completely or partially reversible after a 30-day treatment-free period.

Assessment

There were no biologically significant zVSV-ZEBOV-GP-related effects on clinical observations, mortality, body weights, body temperature, clinical chemistry and hematology. Higher spleen weights and enlargement of iliac lymph nodes were reported in both groups. These gross observations along with their corresponding to microscopic findings of lymphoid hyperplasia were expected immunogenic response to the vaccine administration.

Robust antibody titers on days 15 and 44 were indicative of an active delivery of the test articles in the treated animals.

Based on overall evaluation of the toxicity study report, it can be concluded that two bi-weekly intramuscular injections of zVSV-ZEBOV-GP at doses up to 2×10^7 PFU were well tolerated in mice.

GLP study deviations or amendments: Minor deviations occurred during the conduct of the study were documented in the raw data. These deviations did not affect the quality and integrity of the study. All were minor and considered unlikely to impact on the study outcome.

Investigators Brochure: Not applicable

Title and study number: Toxicity and local tolerance study following intramuscular administration of vesicular stomatitis Ebola virus vaccine in cynomolgus macaques with 30-day recovery (Study # 100027546-08200)

Performing laboratory: (b) (4)

Initiation date: May 5, 2015

Amended final report date: January 23, 2017

Batch/lot number of test article: 001-10-14

Animal species and strain: Cynomolgus macaques

Breeder/supplier: (b) (4)

Number of animals used: 14 males and 14 females in total

Age: 2-4 years

Body weight range: 2.174-4.243 kg

Route and site of administration: Intramuscular; alternating thigh

Volume of administration: 1 mL/thigh

Frequency of administration and study duration: bi-weekly for 2 doses; 44 days

Dose: 3×10^6 , 1×10^8 PFU

Stability: The stability report is provided in Appendix B.

Means of administration: A syringe fitted with a 25G needle

Report status: Final

Experimental design

Group	Test article	PFU	No. of animals/sex/group Sacrifice day 16	No. of animals/sex/group Sacrifice day 44
1	Control	0	2	0
2	rVSV- ZEBOV- GP (b) (4)	3×10^6	4	2
3	rVSV- ZEBOV- GP	1×10^8	4	2

Methods:

Endpoint	
Hematology	Not provided
Clinical chemistry	Not provided
Coagulation	Not provided
C reactive protein	ELISA
Immunogenicity	ELISA

Randomization procedure: The animals were assigned to dose groups using the subject placement function in (b) (4) which ensures similar group body weights by sex.

Statistical analysis plan: All appropriate quantitative in-life data collected at (b) (4) using the (b) (4) system were analyzed for significance by parametric or non-parametric analysis of variance (ANOVA) depending on the nature of the data. If the normality assumption was violated for the untransformed data, then the same models were fitted to assess the model assumption of normality for the base-10 log-transformed data. If the normality assumption was violated for both the untransformed and the base-10 log-transformed data, then Kruskal-Wallis tests were performed to compare the distributions of titers between the groups at each study time.

The following parameters were evaluated

Parameters	Frequency of Testing
Clinical observations	Twice daily for mortality and moribundity. Twice daily (once before and three hours after vaccination) for clinical observations on days of vaccination and once daily on all other days
Injection site observations	Prior to and one and two days after each dose administration
Body weight	Pre-study and on days 1, 2, 7, 14, 15, 16, 21, 28, 35, 42 and 44
Food consumption (qualitative)	Weekly
Body Temperatures	Pre-treatment, prior to each dose, 6, 24 and 48 hours after each dose thru an implantable programmable temperature transponder in the dorsal intrascapular area and the right hip
Clinical chemistry	Days 0, 16 and 44
Hematology	Same as above
Coagulation	Same as above
Immune response	Days 0, 14, 16, 28 and 44
C reactive protein	Days 2, 7, 14 and 21
Viremia	Days 0, 1, 2, 5, 14, 15, 16 and 19
Viruria	Same as above
Necropsy	Days 16 and 44

Tissues for histopathology	Same as above
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Postmortem procedures: The following tissues were collected at necropsy and microscopically examined. Those tissues marked with an asterisk were weighed.

SYSTEM	ORGAN COLLECTED	ORGAN NOT COLLECTED
DIGESTIVE	Large intestine (cecum, colon, rectum); small intestine (duodenum, jejunum, ileum), liver*, stomach, gall bladder, pancreas, esophagus, gut associated lymphoid tissue, salivary gland (mandibular)	
RESPIRATORY	Lung with bronchi, trachea,	Nasal cavity
CARDIOVASCULAR	Heart*, aorta	
IMMUNOLOGIC/ HEMATOPOIETIC	Bone (femur), bone with bone marrow (sternum), bone marrow (rib), lymph node (mesenteric, mandibular, ingular), spleen*, thymus*	
UROGENITAL	Epididymes, kidneys*, urinary bladder, ovaries*, prostate, testes*, seminal vesicles, uterus* with cervix, vagina	Fallopian tubes
NEUROLOGIC	Brain*, spinal cord, sciatic nerve	
HORMONAL	Adrenals, mammary gland, thyroid with parathyroid gland, pituitary gland	
OTHER	Skeletal muscle, skin, sciatic nerve, tongue, eyes with optic nerves, injection sites, distal interphalangeal joint, right hand, right foot	
GROSS LESIONS	None	

Results:

Mortality: All animals survived until scheduled termination.

Clinical observations: There were no observations that were contributed to the control or vaccine administration.

Food consumption: There were no changes in food consumption.

Body weights: There were no changes in body weights.

Body temperature: All animals on study maintained normal body temperatures post vaccination on days 0 through 16 when compared to each individual baseline temperature.

Injection site observations: One group 1 and one group 3 animals exhibited slight erythema. One group 1 animal also observed with edema.

Viremia/Viruria:

Detection was observed in the blood by day 1 and carried through day 2 in eleven of the twelve group 2 and all group 3 animals. By the day 5, all group 2 animals were negative and two males and two females in group 3 were with detectable level. By day 15, a single female in group 3 was positive. Detection in urine was not observed until day 5 from a single male and single female in group 3. Urine samples from all animals were negative except to one group 3 female and one group 1 male exhibited detection on day 15 and day 14, respectively.

Immune response: Anti-EBOV GP IgG levels increased in groups 2 and 3 through day 16 for the core animals and through day 28 for recovery animals. At day 44 levels were still higher than those at day 16. Titers were not observed in all animals from group 1 except one.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Glutamate dehydrogenase (not determined) Gamma-glutamyl transferase Aspartate aminotransferase Alanine aminotransferase Sorbitol dehydrogenase (not determined)
B) HEPATOBILIARY		Total bile acids (not determined)

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
		Alkaline phosphatase Total bilirubin
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	C-reactive protein Day 2, Group 2* Group 3* Fibrinogen Day 16 Group 3, 1.8-1.9X	Albumin Globulin (calculated) A/G Ratio Cholesterol Creatine kinase Cholinesterase (not determined) Total protein Triglyceride Lactate dehydrogenase

Table of Clinical Chemistry Results

*: The significance of these finding was not clear as a small number of animals also had high CRP level on day 0

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT	NOT OF NOTE
RED BLOOD CELLS		Hematocrit Hemoglobin conc. Mean corp. Hb. Mean corp. Volume Mean corp. Hb. conc. Total erythrocyte count Reticulocyte
WHITE BLOOD CELLS		Total leukocytes Neutrophil Monocyte Lymphocyte Eosinophil Basophil Large unstained cells (not determined)
CLOTTING POTENTIAL		Activated partial thromboplastin time Prothrombin Fibrinogen Platelet count

Table of Hematology Results

Organ weights: There were no organ weight changes.

Gross pathology: There was no test item-related finding.

Microscopic findings: The test article-related findings were limited to the injection sites and regional draining lymph nodes (inguinal). Inflammation at the injection sites was characterized by the presence of neutrophils, macrophages, lymphocytes, and plasma cells between skeletal muscle fibers or in the subcutis with or without myofiber degeneration or regeneration (fragmented, shrunken, or basophilic myofibers). Complete resolution of inflammation at right and left injection sites was evident in all animals at recovery termination on day 44. Lymphoid hyperplasia in the inguinal lymph node was characterized by an increase in size and/or number of lymphoid follicles within the cortex with germinal center formation or by the presence of well-formed follicles in the cortex with or without expansion of the paracortex. At recovery termination on day 44, lymphoid hyperplasia remained present in all males and in one low dose female. The incidences of these findings are summarized in the next table.

Day 16	1M	2M	3M	1F	2F	3F
Inguinal lymph node Lymphoid hyperplasia	0/2	3/4	4/4	0/2	3/4	2/4
Left injection site Inflammation	0/2	3/4	1/4	0/2	1/4	0/4
Right injection site Inflammation	0/2	2/4	4/4	0/2	4/4	3/4

Microscopic findings noted at day 16 were observed at reduced incidences and/or severity at the end of recovery period on day 44 as shown below:

Day 44	1M	2M	3M	1F	2F	3F
Inguinal lymph node Lymphoid hyperplasia	0/0	2/2	2/2	0/0	1/2	0/2

Test article related effects
Local irritation at the injection sites (erythema, edema and inflammation) and immunogenic response in the inguinal lymph node (lymphoid hyperplasia). All findings were typical and expected local inflammatory/immunogenic reaction to vaccine administrations. Lymphoid hyperplasia was still present in males after a 30-day treatment-free period.

Assessment

There were no biologically significant zVSV-ZEBOV-GP-related effects on clinical observations, mortality, body weights, body temperature, coagulation, hematology, gross pathology and organ weights. Elevated fibrinogen along with less clear significant elevation of C-reactive protein was reported in high dose group. Microscopic findings were limited to the injection sites (inflammation) and inguinal lymph node (lymphoid hyperplasia). These findings were typical local and/or immunogenic response to the vaccine administration.

Robust antibody titers on days 15 and 44 were indicative of an active delivery of the test articles in the treated animals.

Based on overall evaluation of the toxicity study report, it can be concluded that two bi-weekly intramuscular injections of zVSV-ZEBOV-GP at doses up to 1×10^8 PFU were well tolerated in cynomolgus macaques.

GLP study deviations or amendments: Minor deviations occurred during the conduct of the study were documented in the raw data. These deviations did not affect the quality

and integrity of the study. All were minor and considered unlikely to impact on the study outcome.

Investigators Brochure: Not applicable

Title and study number: A reproductive toxicity study of V920 (rVSV-ZEBOV-GP) administered by intramuscular injection in (b) (4) rats (Study#20084143)

Performing laboratory: (b) (4)

Initiation date: January 11, 2017

Final report date: December 1, 2017

Batch/lot number of test article: 001 10 14

Animal species and strain: (b) (4) rat

Breeder/supplier: (b) (4)

Number of females per group per phase: 22

Age: 69 days

Body weight range: 196-243 g

Route and site of administration: Intramuscular in the right and left hindlimb

Volume of administration: 0.11 mL/limb, 0.22 mL/animal

Frequency of administration and study duration: Groups 1 and 2, 28 (DS 1) and 7 (DS 22) days prior to mating, gestation day (GD) 6 and lactation day (LD) 7; or Group 3, GD day 6

Dose/animal: 5.28×10^7 PFU

Stability: The sponsor provided to the testing facility documentation of the identity, strength, purity, composition and stability for the test article. A certificate of analysis or equivalent is presented in appendix 2.

Means of administration: Appropriate needle and syringe

Report status: Final

Experimental design

Group	Test Material	Dose (PFU)	Dose volume (mL)	Dose frequency	No. Females A	No. Females B
1	PBS control	0	0.22	DS 1, DS 22, GD 6 and LD7	22	22
2	V920*	5.28×10^7	0.22	Same as above	22	22
3	V920*	5.28×10^7	0.22	GD 6	22	22

A= Ovarian/uterine examination observations on GD 21

B= Natural delivery observations

*: also known as rVSV-ZEBOV-GP

Randomization procedure: Females were randomized assigned to groups. The randomizing stratification system was not specified.

Statistical analysis plan: Proportional data was analyzed using Fisher's test. Continuous data were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance, when appropriate (i.e., Bartlett's Test was not significant). If the Analysis of Variance was significant, Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate (i.e., Bartlett's Test was significant), the Kruskal-Wallis Test was used. In cases where the Kruskal-Wallis Test was statistically significant, Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. Count data (e.g., number of corpora lutea) was evaluated using the procedures described above for the Kruskal-Wallis Test and Dunn's Method of Multiple Comparisons.

The following parameters were evaluated

	Frequency or parameters of testing
F0 generation	
Clinical observations and survival	Twice daily for viability Prior to and 1-2 hour after each dose and daily for clinical observation
Body weights	Twice weekly prior to cohabitation and daily during gestation and lactation periods and the day of scheduled euthanasia
Gravid uterine weights (subgroup A)	Gestation day 29
Food consumption	Twice weekly prior to cohabitation, days of dose administration and GDs 6, 9, 12, 15, 18, 20 and 25
Mating	Daily
Cohabitation	Seven consecutive days
Natural delivery observations (Subgroup B on duration of gestation, litter size and pup viability)	
F1 generation	
Sub group B	
Litter viability and deaths	Twice daily
Clinical observations	Daily
Body weights	Days 1, 4, 7, 10, 14 and 21 postpartum (PP)
Developmental observations	
Functional observational battery	Days 19, 20 or 21 PP
Acoustic startle	Day 13 PP
Air righting	Day 14 PP
Pupil constriction	Day 21 PP
Ophthalmological examinations	Days 18-20 PP
Sub group A	
Ovarian and uterine contents and macroscopic lesions	Gestation day 21
Fetal body weight	Gestation day 21

Fetal morphological examination	Gestation day 21
Viremia and immunogenicity evaluation (both subgroups)	F0: Pre-dose, GDs 7 and 21 (subgroup A) or LD 21(subgroup B) thru the jugular vein F1: GD21 (subgroup A) thru the umbilical cord, LD 21 PP (subgroup B) thru the vena cava

Results:

Mortality: There was no V920-related mortality. One in group 2 was euthanized in DS 29 due to fractured palate. One group 2 was found dead on GD 7 following blood sample collection. These deaths were not considered to be V920 related.

Clinical observation: There were no test article-related clinical observations during the study period.

Body weight: There were no V920-related effects on body weights, gravid uterine weights. Transient weight gains were observed during pre-mating period (dosing days 15 to 21) in group 3 and on GDs 9-12 and GDs 15-18 in group 2.

Food consumption: There were no V920-related effects on food consumption.

Mating and fertility: There were no test article-related effects on fertility parameters (days in cohabitation, fertility index and pregnancy index).

Necropsy: There were no macroscopic observations detected in the rats that survived to scheduled necropsy.

Ovarian and uterine examinations and litter observations: Increased numbers of corpora lutea were seen in groups 2 and 3. However they were within upper end of range of historical control data for the testing facility and litter sizes and numbers of live fetuses were within the range of historical control data. Increased number of early resorptions was reported in group 3 but was within the range of historical control data. Pregnancy rates were comparable. Therefore, these differences were not considered to be V920-related.

F1 generation

Subgroup A (fetus)

Fetal external, visceral and skeletal examinations: There were no test article-related fetal external, visceral or skeletal malformations.

Subgroup B (pup)

Clinical observations: There were no test article-related clinical signs in the pups.

Reflex and physical developmental parameters: There were no test article-related effects on air righting, acoustic startle or pupil constriction.

Functional observational battery: There were no test article-related effects. Slight reduction in hindlimb grip strength was observed in group 3 male but were comparable to the historical control values.

Ophthalmological examinations: There were no test article-related changes in the pups.

Body weight: There were no test article-related effects on pup weight.

Anti-ZEBOV glycoprotein antibody evaluations: Group 2 animals that were administered the test article showed increasingly higher antibody titers against ZEBOV on DS 7 while in group 3 were negative as expected. On GD 21, all samples from groups 2 and 3 demonstrated immune response against ZEBOV glycoprotein. Titers ranged from 12K to 312k in group 2 and 12K to 62 K in group 3. Pooled litter samples collected on GD 21 from F1 fetus in group 2 were positive for antibodies against ZEBOV glycoprotein. Furthermore, on LD 21 all samples from the dams and the F1 generation pups in group 2 showed the presence of anti-ZEBOV antibodies, indicating the active delivery of the vaccine and presence of passive transfer of maternal antibodies.

Viremia: On GD7 and GD 21 no detectable viremia was observed in group 2. On GD 7, the virus was detected in group 3 one day following a single injection on GD 6 but was no longer detectable on GD21.

Administration of V-920 twice during the pre-mating period (28 and 7 days prior to mating), on GD6 and LD7 to female rats was well tolerated. It did not result in any test article-related effects on mating and maternal systemic toxicity. There was no test article related effect on female fertility index, fetal weight, fetal visceral and skeletal malformations and variations and postnatal development.

Antibody titers were reported in all animals receiving the vaccine, indicating an active delivery of the test article to the animals. The titers also reported in the fetuses and pups from the dams receiving the test article, indicating transfer of immunogenicity in utero.

GLP study deviations or amendments: Minor protocol amendments were recorded in the draft report. None of them influenced the quality, integrity or interpretation of the results.

Investigation Brochure: Not applicable.

Assessment

There were no rVSV-ZEBOV-GP related clinical or necropsy observations in the F0 generation dams or in the F1 generation pups. Administration of ZEBOV vaccine had no impact on fertility parameters, ovarian and uterine examination and litter parameters or natural delivery parameters. There were no fetal external, soft tissue or skeletal abnormalities attributed to administration of the vaccine. Postnatal development in the F1 generation pups were unaffected by the vaccine administration.

Recommendation

The BLA has adequate nonclinical toxicology data in support licensure of the vaccine. It is approvable from a toxicological perspective.

Animal developmental study results should be indicated in the appropriate sections of package insert according to the new content and format requirements of the Pregnancy, Lactation and Females and Males of Reproductive Potential subsections of labeling for human prescription drug and biological products. The recommended revisions for them are recommended below:

8.1 Pregnancy

Risk Summary

A developmental toxicity study has been performed in female rats administered 0.22 mL (b) (4) prior to mating, during gestation and lactation. A single human dose is 1 mL. The study revealed no evidence of harm to the fetus or offspring (until weaning) due to (b) (4) [see animal Data].

Animal Data

In a developmental toxicity study female rats received (b) (4) by intramuscular injection 28 days and 7 days prior to mating, on gestation day 7 and on lactation day 7. The dose was 0.22 mL at each occasion (a single human dose is 1 mL). No adverse effects on pre-weaning development up to post-natal day 21 were observed. There were no fetal malformations or variations observed due to the vaccine.

13.1 section is adequate as proposed.

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