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Toxicology Review of mRNA-1010 Vaccine

From: (b) (6)

Through: (b) (6)

To: (b) (6)

File: BLA 125869, original submission

Product name: mRNA-1010 Vaccine

Reviewer: (b) (6)

BLA sections reviewed:

4.2.3.2 Repeat dose toxicity studies

4.2.3.3. Genotoxicity studies

4.2.3.5 Reproductive and developmental toxicity studies

Type and date of submission: Original, December 05th, 2025.

Sponsor: Moderna Tx, Inc. 325 Binney Street, Cambridge MA 02142

Proposed indication: Active immunization for the prevention of influenza disease caused by influenza virus subtypes A and B represented in the vaccine in persons 50 years of age and older.

Division name: OVRD/DCTR

A pre-BLA meeting was held on August 22, 2025.

Proprietary name: MFlusiva

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Précis

Study number 1: The sponsor submitted in amendment 43 a study report of a GLP combined perinatal/postnatal developmental and reproductive toxicity of mRNA-1010 by intramuscular route in the (b) (4) rat. The sponsor provided in amendment 55 in response to our information request of their historical data including external, visceral and skeletal abnormalities of the testing facility. In the GLP embryo-fetal developmental toxicity study, two groups of 44 female rats were administered intramuscularly control article (Tris and sucrose), 100 ug of mRNA-1010 in 0.2 mL of (b) (4) mM Tris and (b) (4) g/L sucrose solution 28 days and 14 days prior to mating and on gestation days 1 and 13. The animals in each group were divided into delivery and Cesarean cohorts. There were no effects on mating performance, female fertility or any postnatal development following natural delivery reported in the study. It did not produce any fetal external, visceral, or skeletal malformations.

Study number 2: Animals were randomized and assigned to 10 different groups. Each group consisted of 5/sex/group. Animals were dosed with mRNA 1010 by IM injection with a final injection volume of 200 µL of X on study days 1 and 22. Injections site findings and immunological responses were reported.

Study number 3: Animals were randomized and assigned to 4 different groups. Each group consisted of 15 or 10/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL of X on study days 1 and 22. Injections site findings and immunological responses were reported.

Study number 4: Animals were randomized and assigned to 4 different groups. Each group consisted of 15/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL on study days 1, 15, and 29. Injections site findings and immunological responses were reported.

Study number 5: Animals were randomized and assigned to 4 different groups. Groups 1 and 4 consisted of 15/sex/group and groups 2 and 3 consisted of 10/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL on study days 1, 15, and 29. Injections site findings and immunological responses were reported.

Study number 6: Animals were randomized and assigned to 4 different groups. Groups consisted of 5/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL on study days 1, 15, 29, and 43. Injections site findings, inflammatory responses, and immunological responses were reported.

Study number 7: Animals were randomized and assigned to 4 different groups. Groups consisted of 5/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL on study days 1, 15, 29, and 43. Injections site findings and inflammatory responses were reported.

Study number 8: Animals were randomized and assigned to 4 different groups. Groups 1 and 4 consisted of 15/sex/group and groups 2 and 3 consisted of 10/sex/group. Animals were dosed with (b) (4) by IM injection with a final injection volume of 200 µL on study days 1, 15, and 29. Injections site findings, inflammatory responses, and immunological responses were reported.

Study number 9: Animals were randomized, acclimated for 14 or 15 days, then assigned to one of 4 groups. Groups 1 and 4 consisted of 15/sex/group and groups 2 and 3 consisted of 10/sex/group. Animals were dosed (b) (4) by IM injections with a final injection volume of 200 µL on study days 1, 15, and 29. Injections site findings, inflammatory responses, and immunological responses were reported.

In Vivo toxicology studies

Study number 1: (b) (4) Test in Rat.

Study number 2: (b) (4) mRNA in SM102-Containing Lipid Nanoparticles: (b) (4)
(b) (4) Assay in the Rat.

In Vitro toxicology studies

Study number 1: SM-102 Bacterial Reverse Mutation Test in (b) (4)

Study number 2: SM-102 (b) (4) Test in Human Peripheral Blood Lymphocytes.

Study number 3: (b) (4) (PEG 2K-DMG) and (b) (4); Bacterial Reverse Mutation Test in (b) (4).

Study number 4: (b) (4) (PEG 2K-DMG) and (b) (4)
(b) (4) Test in Human Peripheral Blood Lymphocytes.

It is concluded that no evidence of genotoxic activity in these (b) (4) tests was reported when tested in accordance with regulatory guidelines.

Introduction:

The test article (mRNA-1010) is an LNP-encapsulated Trivalent Influenza Vaccine (TIV) mRNA-based vaccine encoding the full-length, membrane-bound influenza HA glycoproteins of the three seasonal influenza strains recommended annually by the WHO for cell- or recombinant-based vaccines: A/H1N1, A/H3N2, and B/Victoria-lineage. With an RNA content of 12.5 µg per strain per dose (37.5 µg total RNA per dose), RNA corresponding to each strain is present at an equal RNA mass ratio (1:1:1). The RNA encodes native A/H1N1 and A/H3N2 strain HA antigens. For the B/Victoria-lineage strain, the RNA encoding the HA antigen includes 2 stabilizing point mutations.

Forming RNA-containing LNPs, each RNA is encapsulated in a mixture of 4 lipids: SM-102, PEG2000-DMG, DSPC, and cholesterol. The SM-102-based LNP technology for mRNA-1010 is the same proprietary LNP system employed in Moderna's licensed mRNA vaccines. Which include mRNA-1273 (Spikevax™), mRNA-1345 (mRESVIA™), and mRNA-1283 (mNEXSPIKE™) currently authorized in the US and Canada.

mRNA-based vaccine platform leverages the principle and observation that cells *in vivo* can take up mRNA, translate it, and then express the encoded antigens. The delivered mRNA does not enter the cellular nucleus, does not interact with the genome, is nonreplicating, transiently expressed, and does not persist in the body. mRNA is delivered through encapsulation within a proprietary LNP to protect mRNA from rapid degradation in plasma and serum by ribonucleases and to aid in mRNA uptake by cells.

Following cellular uptake, the mRNA serves as a template for the synthesis of the intended proteins. The HA proteins encoded by mRNA-1010 will be translated and then expressed on the cell surface. The membrane-bound HA glycoproteins of the encoded influenza strains are recognized by immune cells as a foreign antigen, eliciting immune responses, which contribute to protection against influenza.

mRNA-1010 is chemically similar to naturally occurring mammalian mRNA with the exception that the uridine nucleoside normally present is fully replaced with N1-methylpseudouridine, a naturally occurring pyrimidine base found in mammalian transfer RNAs (1, 2). This replacement minimizes indiscriminate recognition of the mRNA by pathogen-associated molecular pattern receptors (3). In the mRNA, the cap structure used is identical to the natural mammalian Cap1 structure (4, 5).

In response to changes in circulating viruses (and associated regulatory authority and advisory organization recommendations for vaccines), the mRNA-based manufacturing platform facilitates rapid updating of the targeted viral strains with reliable manufacture of the updated vaccines at commercial scale.

Seasonal strain update(s) for the mRNA-1010 vaccine will be implemented in alignment with the WHO recommendations as subsequently endorsed by regulatory authorities.

At the time of the clinical study conduct, the influenza strains contained in the vaccines were consistent with WHO recommendations for the relevant region(s). The RNAs included in the mRNA-1010 vaccine will continue to be updated to reflect current recommendations for seasonal influenza vaccine strains.

The sponsor submitted one DART study and one non-GLP, one GLP repeat dose toxicity study evaluating the candidate vaccine mRNA-1010, seven additional repeat dose toxicity studies evaluating other mRNA vaccines using the same LNP formulation, and six genotoxicity studies evaluating the LNP.

Studies reviewed in this BLA:

Reproductive Toxicology Study

A GLP compliant prenatal/postnatal developmental and reproductive toxicity study of mRNA-1010 by the intramuscular route in the (b) (4) rat (Study No. 20323624).

General Toxicology Studies

- 1- A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1010, (b) (4) by Intramuscular Injection in (b) (4) Rats. Test Facility Study No. 20287009.
- 2- A 3-Week Study of mRNA-1010.6 by Intramuscular Administration in (b) (4) Rat with a 2-Week Recovery Period. Test Facility Study No. 20457846.
- 3- A 1-Month (3 doses) Study of (b) (4) by Intramuscular Injection in (b) (4) Rat with a 2-Week Recovery Period. Test Facility Study No. 5002033.
- 4- A 6-Week (4 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002034.
- 5- (b) (4): A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats with a 2-Week Recovery Period. Test Facility Study No. 5002045.
- 6- A 6-Week (4 doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002158.
- 7- A 1 Month (3 doses) Intramuscular Injection Vaccine Study of (b) (4) in (b) (4) Rats with a 2-Week Recovery Period. Test Facility Study No. 5002231.
- 8- A 1-Month (3 Doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period. Test Facility Study No. 5002400.

- 9- A 1-Month (3 Doses) Study of (b) (4) by Intramuscular Injection in (b) (4) Rat with a 2-Week Recovery Period. Study number: 5002033.

In Vivo Genotoxicology Studies

- 1- (b) (4) mRNA: (b) (4) Test in Rat. Test Facility Study No. 9800399.
- 2- (b) (4) mRNA in SM102-Containing Lipid Nanoparticles: (b) (4) Assay in the Rat. Test Facility Study Number AF87FU.125012NGLPICH.BTL.

In Vitro Genotoxicology Studies

- 1- (b) (4) (PEG 2K-DMG) and (b) (4) Bacterial Reverse Mutation Test in (b) (4). Test Facility Study No. 9601035.
- 2- (b) (4) (PEG 2K-DMG) and (b) (4) Test in Human Peripheral Blood Lymphocytes. Test Facility Study No. 9601036.
- 3- SM-102 Bacterial Reverse Mutation Test in (b) (4) Test Facility Study No. 9601567.
- 4- SM-102 (b) (4) Test in Human Peripheral Blood Lymphocytes. Test Facility Study No. 9601568.

Toxicology study reviews

Study number 1: A GLP Compliant Prenatal/postnatal Developmental and Reproductive Toxicity Study of mRNA-1010 by the Intramuscular Route in the (b) (4) Rat. Study number: 20323624.

Type and date of submission: Amendment 43; January 19, 2023, and Amendment 55; May 12, 2023.

Related/referred products: Pre-IND (b) (4)

Proposed indication for use: Prevention of influenza disease caused by seasonal influenza A and influenza B subtype viruses covered by the vaccine

Performing laboratory: (b) (4) Initiation date: December 15, 2021

Report date: December 30, 2022

Batch/lot number of test article: DH-08499.1

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: 210-259 g

Route and site of administration: Intramuscular in the quadriceps alternating each dosing occasion

Volume of administration: 0.2 mL

Frequency of administration and study duration: 28 and 14 days prior to mating and on gestation days 1 and 13; study duration 10 weeks

Dose/animal: 100 ug

Stability: The Sponsor provided to the testing facility documentation of the identity, strength, purity, composition, and stability for the test and control article(s). A Certificate of Analysis or equivalent documentation is provided in appendix 3.

Report status: Final

Experimental design

Group	Test Material	Dose (ug)*	Dose Volume (mL)	No. Females Cesarean Cohort	No. Females Delivery Cohort
1	Control article**	0	0.2	22	22
2	mRNA-1010	100	0.2	22	22

*: 0.5 mg/mL, **: (b) (4) mM Tris, (b) (4) g/L sucrose, (b) (4)

Table 1: Experimental design (Study no. 20323624)

Randomization procedure: Females were randomized assigned to groups using a computer-based randomization procedure based on body weight.

Statistical analysis plan: Levene's test will be used to assess the homogeneity of group variances. The groups will be compared using an overall one-way ANOVA F-test when Levene's test is not significant or the Kruskal-Wallis's test when it is significant. When the overall F-test or Kruskal-Wallis's test is found to be significant, then pairwise comparisons will be conducted using Dunnett's or Dunn's test, respectively. For non-parametric, the groups will be compared using an overall Kruskal-Wallis test. When the overall Kruskal-Wallis test is found to be significant, then the above pairwise comparisons will be conducted using Dunn's test. A Fisher's exact test will be used to conduct pairwise group comparisons of interest.

The following parameters were evaluated

	Frequency or parameters of testing
F0 generation	
Viability	Twice daily
Clinical observations	Once weekly during acclimation, daily on not dosing days, prior to and 6- and 24-hours following each dosing, daily beginning on GD0

	Frequency or parameters of testing
Body weights	After arrival, weekly thereafter, on days of dosing and on gestation days 0, 1, 6, 10, 13, 15, 18, 21 and 25 if necessary and on lactation days 1, 4, 7, 10, 14, 18 and 21
Food consumption	Weekly during premating dose period until cohabitation and on gestation days 0, 1, 6, 10, 13, 15, 18, 21 and 25 if necessary and on lactation days 1, 4, 7, 10 and 14
Estrous cycle examinations	Vagina cytology samples were collected 14 days during the mating until confirmation of GD 0
Cohabitation/mating	Maximum of 7 days
Pregnancy and parturition (natural delivery cohort)	Duration of gestation, abnormalities of behavior, pup viability at birth, and litter size
Scheduled euthanasia	GD 21 for Cesarean cohort LD 21/GD25 for natural delivery cohort
Ovarian and uterine examinations (Cesarean cohort)	Gravid uterine weight, number of corpora lutea, implantation sites, placenta, live and dead fetuses, and early and late resorptions on GD 21
Pup viability	Twice daily
Weight, number, and sex of pups	PNDs 1, 4, 7, 10, 14, 18, and 21
Clinical observations	Daily
<u>Developmental observations</u>	
Pinna unfolding	PND 1
Surface righting reflex	PND 1
Hair growth	PND 7
Eye opening	PND 12
Auditory startle reflex	PND 13
Pupil reflex	PND 21
Cesarean cohort	
Late resorption	Gestation day 21
Dead fetuses	Gestation day 21
Fetal body weight	Gestation day 21
Fetal external and morphological (skeletal and visceral) examinations	Gestation day 21

	Frequency or parameters of testing
Antibody analysis (Serum)	F0: Day 1, day 15, GD 1 and 13 and GD 21 (Cesarean cohort) or LDs 13 and 21 thru the vena cava F1: GD 21 thru decapitation or carotid blood collection, LDs 13/thru cardiac puncture and LD 21 thru inferior vena cava
Antibody analysis (milk)	F0: LDs 13 and 21 (if possible)

Table 2: Parameters evaluated (Study no. 20323624)

Results:

Mortality: All F0 generation rats survived to scheduled euthanasia

Clinical observation: The rats administered mRNA-1010 during premating were observed swelling at the injection site (left hindlimbs) in all females as early as SD 2 and continued intermittently until SD 38. Some treated females were observed swelling hindlimb (left or right) between GD1 and GD 4. They were observed 6- and 24-hours post dose. In addition, some females that had thin fur cover during the gestation period was increased (19 vs 8 in the controls). No clinical sings or injection site reaction during the lactation period were reported.

Body weight: There were no effects on body weight during premating, gestation and lactation periods.

Food consumption: There were no mRNA-1010-related effects on food consumption.

Estrous cycle, mating, and fertility: There were no test article-related effects on the estrus cycling (number of estrous cycles and cycle length). Mating occurred in 95 % in the control group and 100 % the mRNA-1010 group. The fertility and pregnancy indices of 77.3% were slight lower than the control group (93% and 88%, respectively). However, they were within the test facility historical range of 70-100 %.

Ovarian and uterine observations and litter observations in Cesarean cohort: Pregnancy was confirmed in 21 and 17 females in the control and treated groups, respectively. There were no mRNA-1010-related effects on ovarian/uterine examination or litter parameters. All parameters (corpora lutea, uterus weight, placental weights, implantations, pre-implantation loss, post implantation loss, fetal sex ratio, litter sizes, live and dead fetuses and fetal weight) were similar between the control and mRNA-1010 groups or within the historical range of the testing facility.

Fetal external, visceral, and skeletal examinations: There were no test article-related fetal external and visceral malformations or variations or skeletal malformations or variations. Finding of short snout was limited to two fetuses in a single litter. The only skeletal malformation observed was a small premaxilla in a single fetus in the 100 µg/dose group. Skeletal examination also revealed delays in skeletal ossification (incomplete or unossified) and structural changes in the development of the ribs, skull bones, and vertebrae. Overall skeletal abnormalities

(malformations and variations) that were observed were not considered mRNA-1010 related because: 1) the observations were also reported in the control group; 2) the number of affected fetuses/litters was similar to or higher in the control group; 3) the observation was limited to a single fetus/litter; and/or 4) the incidences were within the historical control range of the testing facility.

Nature delivery: There was no vaccine-related effect on parturition and gestation. Eighteen pregnant females and 17 pregnant females in the control group completed delivery. The gestation length (21.6 days) and implantation sites per litter were comparable among both groups. Slightly higher stillborn pups/litter (4.26 % vs 0% in control) and percentage of post implantation loss per litter (6.62 % vs 3.8 % in control) were within historical range of the testing facility.

Litter observations: There were no mRNA-1010-related effects on any litter parameter including the viability index, lactation index, and sex ratio on day 21 postpartum.

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

Pup weights and sex ratio: There were no mRNA-1010-related changes in pup body weight and sex ratio.

Pup reflex and physical development: There were no vaccine-related effect on pinna detachment, surface righting, hair growth, eye opening, auditory startle, or pupil constriction.

Pup necropsy findings: There were no pup macroscopic observations.

Antibody evaluations: Following dose administration of mRNA-1010 to F0 females, a robust IgG antibody response against 2021/2022 Northern Hemisphere influenza strains H1[A/Wisconsin], H3[A/Cambodia], B-Victoria[B/WA19], and B-Yamagata [B/Phuket13] was reported on SD 15 continuing through LD 21. A robust IgG antibody response was reported in F1 fetuses on GD 21, and F1 pups on LD 13 and 21. A robust IgG antibody was reported in F0 maternal milk on LD 13 and 21.

Assessment:

Administration of mRNA-1010 twice during the premating period (28 and 14 days prior to mating) and twice during the gestation period (GDs 1 and 13) to female rats was well tolerated. It did not result in any effects on estrus cycling, mating and maternal systemic toxicity, pregnancy, and fertility index. There was no test article related effect on fetal weight, visceral and skeletal malformations and variations or postnatal development reported in the study.

Antibody titers were reported in all females 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. Further, antibody titers also reported in maternal milk on LD 13 and LD 21.

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.

Study number 2: A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1010, (b) (4) by Intramuscular Injection in (b) (4) Rats.
Study number: 20287009.

Type and date of submission: Original; May 27, 2021

Pre-IND meeting: No

Sponsor: ModernaTX, Inc.

Product: mRNA-1010

Related/referred products: Pre-IND (b) (4)

Proposed indication for use: Prevention of influenza disease caused by seasonal influenza A and influenza B subtype viruses covered by the vaccine

The sponsor referred and submitted 6 toxicity data of 5 other vaccines (mRNAs (b) (4)) using the similar platform to justify for the clinical study of mRNA-1010.

The study results of them are summarized below:

Study Title (Test Article)	Species/ Strain Sex/Number per Group	Doses (µg)/ Duration of Dosing (Route of Admin)	Noteworthy Findings
(b) (4): A 1-month (3 doses) intramuscular injection toxicity study of (b) (4) in (b) (4) rats with a 2-week recovery period	Rats/(b) (4) 10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 13, 65, 129/ 3 doses over 1 month (every 2 weeks) (IM)	No mortalities occurred during the study and no (b) (4) -related changes in ophthalmology assessments or organ weights were observed. At ≥ 13 µg/dose, dose dependent changes in clinical signs, clinical pathology parameters, and cytokine levels were consistent with an inflammatory response at the injection site. Dose-dependent target organ effects were limited to the injection site, tissues surrounding lymph nodes regional to the injection site spleen, and liver of animals given (b) (4) . At the end of the recovery period, all changes were partially or fully recovered. The animals were well-tolerated based on the low severity findings, the low magnitude of the clinical pathology changes, and the almost complete recovery from macroscopic findings.

Study Title (Test Article)	Species/ Strain Sex/Number per Group	Doses (µg)/ Duration of Dosing (Route of Admin)	Noteworthy Findings
A 1-Month (3 doses) intramuscular injection vaccine study of (b) (4) in (b) (4) rats with a 2-week recovery period	Rats/(b) (4) 10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 10, 50, 100/ 3 doses over 3 months (every 4 weeks) (IM)	There were no unscheduled deaths in this study. There were no changes in food consumption and ophthalmology. There were no major decreases in body weight and no deleterious changes in hematology, coagulation, or clinical chemistry. Starting at 10 µg/dose, generally dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, and cytokines were consistent with an inflammatory response. Dose-related target organ effects were observed at the injection site, the bone marrow, the inguinal, mesenteric, iliac and popliteal lymph nodes, adrenal gland, liver, spleen, and thymus. At the end of the 2-week recovery period, all changes were fully recovered. The animals were well-tolerated up to 100 ug.
A 6-week (4 doses) intramuscular injection toxicity study of (b) (4) in (b) (4) rats followed by a 2-week recovery period	10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 8.9, 27, 89/ 4 doses over 6 weeks (every 2 weeks) (IM)	No (b) (4) -related mortalities, no major decreases in body weight, and no changes in food consumption. No deleterious changes in hematology, coagulation, or clinical chemistry. Dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, cytokine levels, and acute phase protein levels, along with a slight body temperature increase in the 89 µg/dose group consistent with a systemic inflammatory response. Dose-related target organ effects limited to the injection site, the bone marrow, the inguinal and popliteal lymph nodes, the connective tissue surrounding the sciatic nerve, and the spleen. At the end of the 2-week recovery period, changes at the injection site, bone marrow, perineurial tissue, spleen, and acute phase proteins recovered. Perinodal inflammation in the popliteal and inguinal lymph nodes were fully recovered.
A 6-week (4 doses) intramuscular injection toxicity study of (b) (4) in (b) (4) rats followed by a 2-week recovery period	10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 9.6, 29, 96/ 4 doses over 6 weeks (every 2 weeks) (IM)	No mortality, no changes in body weight, and no changes in food consumption. No changes in hematology, coagulation, or clinical chemistry parameters. Changes in clinical signs (edema/erythema) at the injection site, clinical pathology parameters, and cytokine/acute phase protein levels along with slight increase in body temperature was consistent with a systemic inflammatory response. Dose-dependent target organ effects were limited to the injection site, the tissues surrounding the sciatic nerve, the popliteal and inguinal lymph nodes, the spleen, the bone marrow, and the liver. At the end of the recovery period, changes noted at the injection site, lymph nodes, sciatic nerve, and liver were partially recovered.

Study Title (Test Article)	Species/ Strain Sex/Number per Group	Doses (µg)/ Duration of Dosing (Route of Admin)	Noteworthy Findings
			Histopathologic changes in the bone marrow and spleen were fully recovered.
A 1-month (3 doses) Study of (b) (4) by intramuscular injection in (b) (4) rat with a 2-week recovery period	10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 10, 50, 150/ 3 biweekly doses over 1 month (IM)	No (b) (4)-related mortalities, no meaningful decreases in body weight or food consumption, and no ophthalmic findings. No deleterious changes in hematology, coagulation, or clinical chemistry parameters. Dose-dependent changes in clinical signs at the injection site, clinical pathology parameters, and cytokines consistent with an inflammatory response at the injection site were noted. Dose-related target organ effects were limited to the injection site; the bone marrow; the inguinal, popliteal, and/or ileac lymph nodes; the connective tissue surrounding the sciatic nerve; the spleen; and the liver. At the end of the 2-week recovery period, changes at the injection site, popliteal lymph nodes, sciatic nerve, and liver (organ weight) were partially recovered. Changes in the bone marrow, inguinal and iliac lymph nodes, spleen, and liver (histopathology findings) were fully recovered.
A 1-month (3 doses) intramuscular injection toxicity study of (b) (4) in (b) (4) rats followed by a 2-week recovery period	10/sex/group in main study + 5/sex/group in recovery study (control and high dose only)	0, 10, 30, 96/ 3 doses over 5 months (every 6 weeks) (IM)	There were no mortalities and no (b) (4)-related changes in body weight, food consumption, and ophthalmology findings the study. Starting at 10 µg/dose, dose-dependent clinical signs (swelling/firmness/redness/scabs) at the injection site, changes in clinical pathology parameters and cytokine concentrations, and increase in body temperature were observed and were consistent with an inflammatory reaction. Dose-dependent inflammatory effects were observed at the injection site, liver, spleen, and seminal vesicle. Microscopic findings, reported in the iliac, inguinal, and popliteal lymph nodes or their perinodal tissue; perineural tissue of the sciatic nerve; spleen (extramedullary hematopoiesis); and bone marrow, were secondary response, or a reactive response to the injection site inflammation. At the end of the 2-week recovery period, all changes were partially or fully recovered. Most of the observed changes were related to local inflammation at the injection site or the subsequent generalized inflammation. None of the changes were considered adverse, and body weight and food consumption were not affected at any dose.

Table 3: Previous studies result for mRNAs (b) (4). (Study no. 20287009). Sponsor provided.

In the study, intramuscular administration of two doses (three weeks apart) of mRNA-1010 at doses of 30, 60 and 100 ug were used. The study was not GLP compliant. The animals were euthanized and discarded two weeks later, and no necropsy (organ weight, gross pathology, and

microscopic examinations) was performed. It results in strong binding antibody responses against matching influenza virus HAs as measured in the custom (b) (4) indicating an active delivery of the vaccine to the animals. The highest dose of 100 µg/dose mRNA-1010 resulted in highest measurable antibody levels for all four antigens. The animals tolerated well with typical local reaction and expected inflammatory and/or immunogenic responses.

Toxicology review

Title and study number: A non-GLP repeat dose immunogenicity and toxicity study of mRNA-1010, (b) (4) by intramuscular injection in (b) (4) rats
(Study#20287009)

Performing laboratory: (b) (4)

Initiation date: January 21, 2021

Report date: April 29, 2021

Batch/lot number of test article: DH-05575 (mRNA-1010); (b) (4)

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

Breeder/supplier: (b) (4)

Number of females per group per phase: 5

Age: 6-8 weeks

Body weight range: 175-350 g (males) or 150-275 g (females)

Route and site of administration: Intramuscular in the quadriceps (hind leg), left on day 1 and right on day 21

Volume of administration: 0.2 mL

Frequency of administration and study duration: 2 tri-weekly doses; 36 days

Dose/animal: 30, 60, and 100 µg

Stability: A summary of analysis was provided to the testing facility and is presented in appendix 2.

Means of administration: Not specified

Report status: Final

Experimental design

Group	Test Material	Dose (µg)*	Dose Volume** (mL)	No. Males	No. Females
1	Control vehicle	0	0.2	5	5
2	mRNA-1010	30	0.2	5	5
3	mRNA-1010	60	0.2	5	5
4	mRNA-1010	100	0.2	5	5
5	(b) (4)	30	0.2	5	5
6		60	0.2	5	5
7		100	0.2	5	5

Group	Test Material	Dose (ug)*	Dose Volume** (mL)	No. Males	No. Females
8	(b) (4)	30	0.2	5	5
9		60	0.2	5	5
10		100	0.2	5	5

*: Three concentrations: 150, 300, and 500 ug/mL

**: Feasible dosing volume

Table 4: Experimental design (Study no. 20287009). Sponsor provided.

Methods

Endpoints	Methodology
Hematology	Not specified
Clinical chemistry	Not specified
Immunogenicity	VaxArray Influenza SeroAssay

Randomization procedure: Animals were randomized and assigned to 10 groups. The randomization procedure was not provided.

Statistical analysis plan: For body weight, hematology and clinical chemistry, if the control group has a sample size less than 3, no inferential statistics will be calculated. If an endpoint and/or parameter within a given collection interval have the same value across all experimental units, no inferential statistics will be calculated. Otherwise, for endpoints and/or parameters where all groups with sample sizes of 3 or greater are included, Levene's test will be used to assess the homogeneity of group variances.

The groups will be compared using an overall one-way ANOVA F-test if Levene's test is not significant or the Kruskal-Wallis test if it is significant. If the overall F-test or Kruskal-Wallis test is found to be significant, then pairwise comparisons will be conducted using Dunnett's or Dunn's test, respectively.

The following parameters were evaluated

	Frequency or parameters of testing
Mortality	Twice daily
Cage side observations	Daily
Detailed clinical observations	6 hours post dose and weekly during none dosing weeks

	Frequency or parameters of testing
Injection site observations	Day 1: immediately, 6- and 24-hours post dose Days 3-7: daily Weeks 2 and 3: weekly Day 21: immediately, 6- and 24-hours post dose Days 24-28: daily Week 5: once
Body weights	Days 5 and 1 prior to the first dosing, weekly thereafter
Clinical chemistry*	Day 23 venipuncture if possible or abdominal aorta and/or vena cave under anesthesia
Hematology **	Same as above
Immunogenicity	Same as above
Coagulation	Not performed
Necropsy	Euthanized by carbon dioxide and discarded on day 36 without necropsy
Tissue for histopathology	Not performed

*Clinical chemistry parameters monitored

Table 5: Parameters evaluated (Study no. 20287009)

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyl transferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin ^a	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

Table 6: Clinical chemistry parameters evaluated (Study no. 20287009)

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red blood cell distribution width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	
Reticulocyte count (absolute)	

Platelet count	Large unstained cells (absolute)
	Other cells (as appropriate)

** Hematology parameters monitored

Table 7: Hematology parameters monitored (Study no. 20287009)

Results:

Mortality: All animals survived to termination.

Clinical observation (cage side and detailed clinical observations): There were no mRNA-1010-related clinical observation. One animal in (b) (4) (100 ug dose) group was observed bruise at the right hindlimb and was due to the injection procedure. One animal in (b) (4) (100 ug dose) group was observed swollen right hindlimb correlated with severe edema. This single incidence was attributable to (b) (4).

Injection site observations: There was transient dose-dependent mRNA-1010-related edema and erythema on days 1 and 22. All observations of edema resolved within 4 days after each administration. Similarly, there was transient (b) (4)-related edema and erythema. All observations of edema and erythema resolved within 4 days of each administration.

Body weight: There were no test article-related effects throughout the study.

Hematology: Twenty-four hours post administration on day 23, mRNA-1010 produced higher neutrophil counts (range: 5.06x-7.37x control) and minimally higher eosinophil counts (range 1.12x-2.20x control). These changes, along with changes in albumin and globulin concentrations, were consistent with an inflammatory and/or immune response as indicated by the injection site observations (swelling, edema, and/or erythema), and were considered mRNA-1010-related. It also produced lower lymphocyte counts (0.36-0.63x control).

(b) (4)

Clinical chemistry: All three vaccines produced similar minimally lower albumin concentration and minimally higher globulin, resulting in lower albumin/globulin ratios (0.52-0.73x control). These changes, along with higher leukocyte counts were consistent with an inflammatory response as expected.

HA antibody titer analysis: In the control group, there was no quantifiable antibody response in any animal. At day 35, all animals administered mRNA-1010 had HA antibody responses. The highest dose of 100 µg/dose resulted in the highest measurable antibody levels of the 3 dose levels, indicating an active delivery of the vaccine to the animals.

Organ weights, gross pathology, and microscopic examinations: Not performed.

Assessment

There were no biologically significant treatment-related effects on clinical signs mortality, body weights, and blood chemistry. Edema and erythema at the injection site were reported in all mRNA-1010 treated animals with more prominent in the high dose groups. They were resolved at least 4 days after each dosing. Dose-independent elevations of eosinophil and neutrophil, indicative of inflammation, were reported in all dose levels. Lower lymphocyte count was reported. However, organ weights and macroscopic and microscopic examinations were not performed/evaluated in the study.

GLP study deviations or amendments: This non-GLP study is not within the scope of regulations. Minor protocol amendments during the study were documented, acknowledged by the study director and assessed for impact. None of them influenced the quality, integrity or interpretation of the results and conclusions.

Conclusion

The safety of the proposed clinical study of mRNA-1010 is supported by the overall nonclinical toxicity data of 5 other similar mRNA-based vaccine mRNA-LNP matrix encapsulating mRNA constructs encoding for different antigens and the limited toxicity data of mRNA-1010 from a non-GLP immunogenicity and toxicity study.

Study number 3: A 3-Week Study of mRNA-1010.6 by Intramuscular Administration in (b) (4) Rat with a 2-Week Recovery Period. Study number: 20457846

Performing laboratory: (b) (4)

Study initiation date: August 14th, 2023

Final report date: April 26th, 2024

Test article batch/lot:

Table of test article identification

	Test Article
Identification:	mRNA-1010.6
Alternate Identification:	mRNA-1010.6 Drug Product
Lot No.:	8527100102
Expiration Date:	08 Mar 2024
Purity:	90%
Storage Conditions:	(b) (4), Protected from Light
Provided by:	(b) (4)

Table 8: Test article identification (Study no. 20457846). Sponsor provided.

Table of vehicle control identification

	Vehicle Control
Identification:	(b) (4) mM Tris (b) (4) g/L sucrose, (b) (4)
Lot No.:	DH-78686.1
Expiration Date:	03 Mar 2024
Storage Conditions:	(b) (4), Protected from Light
Provided by:	Sponsor

Table 9: Vehicle control identification (Study no. 20457846). Sponsor provided.

Animal species and strain: (b) (4) rats, (b) (4)**Breeder/supplier:** (b) (4)**Number of animal per group and sex:** 15 or 10/sex/group**Age:** 10 weeks**Body weight range:** Males weighed between 295 and 407 g and females weighed between 175 and 286 g.**Route and site of administration:** Intramuscular injection**Volume of injection:** 200 µL/dose**Frequency of administration and study duration:** Days 1 and 22. Study duration was 36 days.**Dose:** See study design below**Stability:** Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. Expiration dates were reported above.**Means of administration:** Intramuscular injection**Report status:** Final**Experimental design:**

Animals were randomized and assigned to 4 different groups. Each group consisted of 15 or 10/sex/group. Animals were dosed by IM injection on study days 1 and 22. The details of the study design are listed in the following table:

Table of experimental design

Group No.	Test Article	Dose Level (µg/dose)	Dose Volume (mL)	Dose Concentration (µg/mL)	Main Study		Recovery Study	
					No. of Males	No. of Females	No. of Males	No. of Females
1	Vehicle Control	0	0.2	0	10	10	5	5
2	mRNA-1010.6	5		25	10	10	-	-
3		10		50	10	10	-	-
4		18		90	10	10	5	5

Table 10: Experimental design (Study no. 20457846). Sponsor provided.

Methods:**Randomization procedure:** Yes**Statistical analysis plan:** Yes.**The following parameters were evaluated:**

General In-life Assessments – Main Study, Recovery Animals

Parameter	Population(s)	Frequency (minimum required)	Comments
Mortality/Cageside Observations	All Surviving Animals	At least twice daily (morning and afternoon) beginning upon arrival through termination/release ^{a,b}	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings. Animals will be observed for morbidity, mortality, injury, and availability of food and water. Any animals in poor health will be identified for further monitoring and possible euthanasia.
Post Dose Observations	All Main Study Animals	On dosing days at 1 and 6 hours postdose. ^c	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Detailed Clinical Observations	All Main Study Animals	Once pretest (day -1) and then once weekly thereafter throughout the study and at 24 hours postdose. ^c	Animals are removed from the cage. Pretest interval will be conducted on all animals received/transferred.

Parameter	Population(s)	Frequency (minimum required)	Comments
Individual Body Weights	All Animals	Within 3 days of arrival, Day -1, and once weekly during the terminal and recovery periods.	Not collected from animals found dead.
Food Consumption	All Main Study Animals	Weekly. ^d	Quantitatively measured
Ophthalmic Examinations	All Main Study Animals	Pretest (all animals) and all survivors in groups 1 and 4 examined again at week 4 and at week 8 and end of recovery (if treatment-related findings exist at week 4)	See details in section below.
Injection Site Observations	All Main Study Animals	On dosing days at 6- and 24-hours post dose and scored daily until resolution	The presence (present/not present) of erythema and/or edema will be recorded for individual animals at the injection site. Animals will be removed from their cages for these observations.

^a Procedures on alternate animals will be conducted per testing facility SOP.

^b Except on days of receipt and necropsy where frequency will be at least once daily.

^c For observations that cannot be attributed to an individual animal due to social housing (e.g., watery feces), the observation will be noted to each animal in the socialized group.

^d For observations of reduced appetite that cannot be attributed to an individual animal due to social housing, the observations will be noted for each animal in the socialized group.

Table 11: In life assessment (Study no. 20457846). Sponsor provided.

Clinical pathology sample collection

Group Nos.	Time Point(s)	Hematology	Coagulation	Clinical Chemistry
1-4	Prior to necropsy (day 23 Main Study Animals and day 36 for recovery necropsy)	X	X	X
Unscheduled Euthanasia (when possible)	See the unscheduled euthanasia section of this protocol.			
Target Volume:	-	1 mL	1.2 mL	1.3 mL
Fasting:	Free access to drinking water but will be fasted overnight (at least 8 hours) prior to blood collection.			

Group Nos.	Time Point(s)	Hematology	Coagulation	Clinical Chemistry
Anticoagulant:	-	K2EDTA	Sodium Citrate	Serum Gel Separator
Processing:	-	None	Plasma	Serum

X = Sample to be collected; - = Not applicable; Blood sample collection method: Vena cava after isoflurane inhalation.

Table 12: Clinical pathology sample collection (Study no. 20457846). Sponsor provided.

Antibody titer evaluation sample collection

Group Nos.	Collection Intervals		
	Pretest (All Animals)	Day 22 24 hours post dose (Main Study Animals)	Day 36 Prior to Termination (Recovery Animals)
1-4	X	X	X

X = Sample to be collected

Table 13: Antibody titer evaluation sample collection (Study no. 20457846). Sponsor provided.

Terminal procedures are summarized in the following tables:

Unscheduled deaths

Group No.	Necropsy Procedures			Histology Processing	Microscopic Evaluation
	Necropsy	Tissue Collection	Organ Weights		
Found Dead or Unscheduled Euthanasia	X	Full List ^a	-	Full List ^a	Full List ^a

X = Procedure to be conducted; - = Not applicable.

'Histology processing' = Embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

^a See tissue weighing, collection, processing and evaluation table (Attachment A) for list of tissues applicable to each procedure.

Table 14: Terminal procedures [unscheduled deaths] (Study no. 20457846). Sponsor provided.

Main study animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1 & 4	23	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a
2 & 3	23	X	Full List ^a	Full List ^a	Full List ^a	Gross Lesions Target Tissues ^b

X = Procedure to be conducted.

'Histology processing' = Embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

^a See tissue weighing, collection, processing and evaluation table (Attachment A) for list of tissues applicable to each procedure.

^b Any target tissues identified by the study pathologist during microscopic evaluation of full list animals will be communicated to the study director, then processed, evaluated and reported in non-full list animals.

Table 15: Terminal procedures [main study animals] (Study no. 20457846). Sponsor provided.

Recovery animals

Group No.	Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
		Necropsy	Tissue Collection	Organ Weights		
1, 4	36	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a

X = Procedure to be conducted.

'Histology processing' = Embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin.

^a See tissue weighing, collection, processing and evaluation table (Attachment A) for list of tissues applicable to each procedure.

Table 16: Terminal procedure [recovery animals] (Study no. 20457846). Sponsor provided.

Postmortem procedures:

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Animal ID	-	X	-	-
Artery, aorta	-	X	X	X
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X ^a	-	-
Bone marrow, sternum	-	X	X	X
Bone, femur	-	X (1)	X (1)	X (1)
Bone, sternum	-	X	X	X
Brain	X	X	X	X
Epididymis	X (2)	X (2) ^b	X (2)	X (2)
Esophagus	-	X	X	X
Eye	-	X (2) ^b	X (2)	X (2)
Ganglion, dorsal root, lumbar	-	X	-	-
Gland, adrenal	X (2)	X (2)	X (2)	X (2)
Gland, clitoral	-	X (2)	-	-
Gland, coagulating	-	X (2)	-	-
Gland, Harderian	-	X (2)	X (1)	X (1)
Gland, lacrimal	-	X (2)	-	-
Gland, mammary	-	X	X	X
Gland, parathyroid	-	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X	X
Gland, preputial	-	X (2)	-	-
Gland, prostate	X	X	X	X
Gland, salivary, parotid	-	X (2)	-	-
Gland, salivary, sublingual	-	X (2)	-	-
Gland, salivary, mandibular	-	X (2)	X (1)	X (1)

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Gland, seminal vesicle	-	X (2)	X (2)	X (2)
Gland, thyroid	-	X (2)	X (2)	X (2)
Gland, thyroid/parathyroid	X (2)	-	-	-
Gland, Zymbal's	-	X (2)	-	-
Gut-associated lymphoid tissue	-	X	X	X
Heart	X	X	X	X
Joint, femorotibial	-	X (1)	X (1)	X (1)
Kidney	X (2)	X (2)	X (2)	X (2)
Large intestine, cecum	-	X	X	X
Large intestine, colon	-	X	X	X
Large intestine, rectum	-	X	X	X
Larynx	-	X	-	-
Liver	X	X	X	X
Lung	-	X	X	X
Lymph node(s) draining administration site(s): iliac (maintain orientation)	-	X (2)	X (2)	X (2)
Lymph node(s) draining administration site(s): inguinal (orientation maintained)	-	X (2)	X (2)	X (2)
Lymph node, mandibular	-	X (2)	X (1)	X (1)
Lymph node, mesenteric	-	X	X	X
Muscle, skeletal (masseter)	-	X (2)	X (1)	X (1)
Nerve, optic	-	X (2) ^b	X (2)	X (2)
Nerve, sciatic	-	X (2)	X (1)	X (1)
Nerve, tibial	-	X (2)	-	-
Ovary	X (2)	X (2)	X (2)	X (2)
Oviduct	-	X (2)	-	-
Pancreas	-	X	X	X
Site, injection, intramuscular (both)	-	X (2)	X (2)	X (2)
Skin	-	X	X	X
Small intestine, duodenum	-	X	X	X
Small intestine, ileum	-	X	X	X
Small intestine, jejunum	-	X	X	X
Spinal cord, cervical	-	X	X	X
Spinal cord, thoracic	-	X	X	X
Spinal cord, lumbar	-	X	X	X
Spleen	X	X	X	X

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Stomach	-	X	X	X
Testis	X (2)	X (2) ^b	X (2)	X (2)
Thymus	X	X	X	X
Tongue	-	X	X	X
Trachea	-	X	X	X
Ureter	-	X (2)	-	-
Urinary bladder	-	X	X	X
Uterus/Cervix	X	X	X	X
Vagina	-	X	X	X
Macroscopic abnormalities ^c	-	X	X	X

X = Procedure to be conducted. - = Not applicable. (1) = One side. (2) = Both sides.

^a Bone marrow smears will be collected from the humerus at scheduled and unscheduled necropsies (for possible examination). Smears will not be collected from animals that are found dead or from animals that were euthanized moribund and then stored in the refrigerator prior to necropsy. Bone marrow smears will be allowed to air dry and are not fixed in (b) (4).

^b Eyes and optic nerves will be preserved in (b) (4) fixative. Testes and epididymides will be preserved in modified (b) (4) fixative.

^c Macroscopic abnormalities in the tissues listed, and in any other tissues, will be sampled, processed and evaluated histologically.

Table 17: Postmortem procedure (Study no. 20457846). Sponsor provided.

Results:

No test article-related mortality was reported.

Clinical chemistry, hematology, and coagulation:

Clinical chemistry

In groups 2, 3, and 4 males and females, mRNA-1010.6-related, generally dose-responsive decreases in albumin (range: 0.95x – 0.88x mean control) and increases in globulin concentration (range: 1.08x – 1.18x mean control) with resultant decreases in A/G (range: 0.88x – 0.77x mean control) were reported on day 23. These changes in serum protein parameters were consistent with an inflammatory and/or acute phase response.

In groups 3 and 4 males, mRNA-1010.6-related increases in mean cholesterol concentration (range: 1.20x – 1.25x mean control) were reported on day 23.

At the end of the recovery period on day 36, decreases in albumin concentration in males and females and decreases in A/G in females mostly resolved in group 4. All remaining mRNA-1010.6-related alterations in clinical chemistry parameters had resolved in group 4 males and females.

On day 23, one group 4 male exhibited mild increases in aspartate aminotransferase (AST; individually, 2.86x mean control) and alanine aminotransferase (ALT; individually, 2.15x mean control) activity.

In group 4 females, there were trends for increases in mean creatinine (1.19x mean control) and urea nitrogen concentrations (1.16x mean control) on day 23. Trends for increases in phosphorous (up to 1.13x mean control) and/or potassium concentrations (up to 1.10x mean control) in group 4 males and females were also reported. These changes were of small magnitude of difference and of inconsistent effect among cohort group members.

Remaining fluctuations among individual and mean clinical chemistry values were considered sporadic, consistent with biologic variation, and/or negligible in magnitude, and not related to test article administration.

Table of mRNA-1010.6-related clinical chemistry changes

Group	1		2		3		4	
Dose (ug/dose) ^a	0		5		10		18	
Sex	M	F	M	F	M	F	M	F
ALB								
Day 23	3.11	3.53	0.95x	0.94x	0.95x	0.93x	0.93x	0.88x
Day 36	3.06	3.68	NE	NE	NE	NE	0.95x	0.95x
GLOB								
Day 23	2.64	2.80	1.11x	1.08x	1.16x	1.09x	1.18x	1.10x
Day 36	2.70	2.86	NE	NE	NE	NE	—	—
A/G								
Day 23	1.19	1.26	0.84x	0.88x	0.82x	0.85x	0.77x	0.81x
Day 36	1.14	1.32	NE	NE	NE	NE	—	0.92x
CHOL								
Day 23	67.1	84.8	—	—	1.20x	—	1.25x	—
Day 36	62.6	82.4	NE	NE	NE	NE	—	—
CL								
Day 23	103.1	104.2	—	—	—	—	—	0.98x
Day 36	102.0	103.0	NE	NE	NE	NE	—	—

M – Males; F – Females; NE – Not evaluated.

ALB - Albumin; GLOB - Globulin; A/G - Albumin to globulin ratio; CHOL - Cholesterol; CL - Chloride.

A dash (—) indicates absence of an mRNA-1010.6-related change. Numerical values (Groups 2-4) indicate fold change of the treated group mean value relative to the control group (Group 1) mean value.

^a Dose administered via IM injection on days 1 and 22.

Table 18: mRNA-1010.6-related clinical chemistry changes (Study no. 20457846). Sponsor provided.

Hematology

In groups 2, 3, and 4 males and females, mRNA-1010.6-related increases in white blood cell (range: 1.44x – 1.59x mean control), neutrophil (range: 2.32x – 5.73x mean control), and/or

eosinophil counts (range: 2.08x – 3.92x mean control) were reported on day 23. Increases in neutrophil and eosinophil counts generally followed a dose-responsive pattern. Increases in white blood cell, neutrophil, and/or eosinophil counts were consistent with an inflammatory and/or acute phase response.

In group 4 males, mRNA-1010.6-related decreases in reticulocyte count (0.79x mean control) were reported on day 23. This might be secondary to the inflammatory and/or acute phase response.

At the end of the recovery period on day 36, all mRNA-1010.6-related alterations in hematology parameters had resolved in group 4 males and females.

On day 23, in groups 3 and 4 males and groups 2, 3, and 4 females, there were trends for decreases in lymphocyte and/or monocyte counts, and in group 4 males and groups 3 and 4 females, there were trends for decreases in platelet counts. These findings were not considered mRNA-1010.6-related due to the small magnitude of difference in mean values, inconsistent effect among cohort group members, overlap of absolute values with the range of concurrent control values, absence of a dose response, lack of correlative findings in related clinical pathology parameters, and/or biologic variability inherent to these parameters.

Remaining fluctuations among individual and mean hematology values, regardless of statistical significance, were considered sporadic, consistent with biologic variation, and/or negligible in magnitude, and not related to test article administration.

Table of mRNA-1010.6-related hematology changes

Group	1		2		3		4	
Dose ($\mu\text{g}/\text{dose}$) ^a	0		5		10		18	
Sex	M	F ^b	M	F	M	F	M	F ^b
WBC ($10^3/\mu\text{L}$)								
Day 23	9.525	7.759	—	—	—	—	1.44x	1.59x
Day 36	10.742	7.963	NE	NE	NE	NE	—	—
NEUT ($10^3/\mu\text{L}$)								
Day 23	1.663	1.068	2.32x	3.00x	2.48x	4.60x	4.27x	5.73x
Day 36	1.740	1.878	NE	NE	NE	NE	—	—
EOS ($10^3/\mu\text{L}$)								
Day 23	0.059	0.060	2.53x	2.35x	2.08x	2.57x	3.92x	3.20x
Day 36	0.122	0.100	NE	NE	NE	NE	—	—
RETIC ($10^3/\mu\text{L}$)								
Day 23	194.66	151.47	—	—	—	—	0.79x	—
Day 36	200.46	178.98	NE	NE	NE	NE	—	—

M – Males; F – Females; NE – Not evaluated.

WBC - White blood cell count; NEUT – Neutrophil count; EOS – Eosinophil count; RETIC – Reticulocyte count.

A dash (—) indicates absence of a test article-related change. Numerical values (Groups 2-4) indicate fold change of the treated group mean value relative to the control group (Group 1) mean value.

Bolded values reflect statistically significant values at $p \leq 0.05$ or $p \leq 0.01$.

^a Dose administered via IM injection on days 1 and 22.

^b Specimen clotting limited evaluation of recovery female data to 4 of 5 control females and 3 of 5 mRNA-1010.6-treated females at 18 ug/dose.

Table 19: mRNA-1010.6-related hematology changes (Study no. 20457846). Sponsor provided.

Coagulation

In groups 2, 3, and 4 males and females, mRNA-1010.6-related, dose-responsive increases in fibrinogen concentration (range: 2.06x – 3.03x mean control) were reported on day 23 and were consistent with an inflammatory and/or acute phase response.

At the end of the recovery period on day 36, increases in mean fibrinogen concentration were considered partially resolved in group 4 females (1.52x mean control). The increase in mean fibrinogen concentration in group 4 females on day 36 was attributed to one of two females. All remaining mRNA-1010.6-related alterations in coagulation parameters had resolved in group 4 males and females.

Table of mRNA-1010.6-related coagulation changes

Group	1		2		3		4	
Dose ($\mu\text{g}/\text{dose}$) ^a	0		5		10		18	
Sex	M	F ^b	M	F	M	F	M	F ^b
FIB (mg/dL)								
Day 23	276.1	213.7	2.06x	2.32x	2.33x	2.65x	2.70x	3.03x
Day 36	282.2	214.7	NE	NE	NE	NE	—	1.52x ^c
APTT (sec)								
Day 23	15.91	12.83	—	1.19x	1.08x	1.19x	1.17x ^d	1.24x
Day 36	16.50	15.17	NE	NE	NE	NE	—	—

M – Males; F – Females; NE – Not evaluated.

FIB - Fibrinogen; APTT - Activated partial thromboplastin time.

A dash (—) indicates absence of an mRNA-1010.6-related change. Numerical values (Groups 2-4) indicate fold change of the treated group mean value relative to the control group (Group 1) mean value.

Bolded values reflect statistically significant values at $p \leq 0.05$ or $p \leq 0.01$.

^a Dose administered via IM injection on days 1 and 22.

^b Specimen clotting limited evaluation of recovery female data to 3 of 5 control females and 2 of 5 mRNA-1010.6-treated females at 18 ug/dose.

^c Fold change attributable to Animal No. 4501 (individually, 2.08x mean control)

^d Fold change accentuated by Animal No. 4013 (individually, 1.58x mean control)

Table 20: mRNA-1010.6-related coagulation changes (Study no. 20457846). Sponsor provided.

Systemic toxicity:

No treatment-related, mortality, clinical observation, body weight, ophthalmic changes, macroscopic pathology, or organ weights were reported.

In groups 2, 3, and 4 males and females, mRNA-1010.6-related transient effects on food consumption were reported. Females appeared to be more affected than the males with decreases (up to -30% and -11% in females and males, respectively) in food consumption during some

weekly measurements when compared to controls. The noted effects were resolved completely or were trending towards recovery within a week or two weeks following each dose. There were no mRNA-1010.6-related effects on body weights.

Organ weights

No mRNA-1010.6-related effects on body weights were reported. A decreased absolute liver weight was reported in group 4 recovery study females. All other differences in organ weight parameters were interpreted as incidental and/or within normal range of variation.

Microscopic evaluations

Day 23

Injection sites

At injection site 2 (evaluated 24 hours after injection), findings consisted of minimal to marked mixed cell inflammation in the subcutis and/or muscle in groups 2, 3, and 4 males and females. The mixed cell inflammation was composed mainly of polymorphonuclear cells and few mononuclear cells.

At injection site 1 (evaluated 22 days after injection), findings consisted of a slightly increased incidence when compared to controls of minimal mononuclear cell infiltrates in the subcutis and/or muscle in groups 3 and 4 males and females. Additionally, mixed cell inflammation at a lower incidence and severity compared to injection site 2 (evaluated 24 hours after injection) was reported in groups 3 and 4 males only.

Minimal mononuclear cell infiltration and myofiber necrosis at the injection sites were reported in some group 1 animals. These findings were attributed to the intramuscular injection administration.

Iliac and inguinal lymph nodes

In groups 2, 3, and 4 males and females, mRNA-1010.6-related increased lymphocyte cellularity was reported in the iliac lymph nodes and in the inguinal lymph nodes of groups 3 and 4 female animals. This was considered a secondary, reactive change to the injection site mixed cell inflammation. Although these findings were present in control animals, there was a dose-related increase in the incidence and severity of the findings, particularly in the iliac lymph nodes.

Sciatic nerve

In groups 3 and 4 males and in groups 2, 3, and 4 females, mRNA-1010.6-related minimal to marked mixed cell inflammation involving the perineural sheath of the sciatic nerve was reported. Perineural involvement was considered as an extension of the injection site inflammation.

Spleen

In groups 3 and 4 females and in groups 2, 3, and 4 males, mRNA-1010.6 related findings in the spleen included a minimal decreased cellularity of lymphocytes in the periarteriolar lymphocyte sheaths (PALS). The decreased cellularity might be due to the presence of apoptosis. In groups 3 and 4 females and in groups 2, 3, and 4 males, minimal to mild increased extramedullary

hematopoiesis was reported. This finding was also attributed to a secondary, reactive response to the injection site mixed cell inflammation.

Sternum bone marrow

In groups 3 and 4 females and in groups 2, 3, and 4 males, minimal to mild increased hematopoiesis, characterized primarily by myeloid cells, was reported in the bone marrow, sternum.

Adrenal gland

An increased incidence of minimal to mild cortical vacuolation was reported in groups 2, 3, and 4 males. This finding was characterized by the presence of cortical cells containing prominent intracytoplasmic clear vacuoles of varying size and noted to be above the background incidence of intracytoplasmic lipid vacuoles that are normally reported in the adrenal gland.

Table of incidence and severity of mRNA-1010.6-related findings- Main study

Sex		Males				Females			
Group		1	2	3	4	1	2	3	4
Dose (µg/dose)		0	5	10	18	0	5	10	18
Site, Injection (1A)	No. Examined	10	10	10	10	10	10	10	10
Infiltrate, mononuclear cell, muscle	No. Affected minimal	2	0	1	3	2	0	0	2
		2	0	1	3	2	0	0	2
Infiltrate, mononuclear cell, subcutis	No. Affected minimal	0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0
Inflammation, mixed cell, subcutis	No. Affected minimal mild moderate	0	0	4	0	0	0	0	0
		0	0	2	0	0	0	0	0
		0	0	1	0	0	0	0	0
		0	0	1	0	0	0	0	0
Site, Injection (1B)	No. Examined	10	10	10	10	10	10	10	10
Infiltrate, mononuclear cell, muscle	No. Affected minimal	1	0	3	4	2	0	2	3
		1	0	3	4	2	0	2	3
Infiltrate, mononuclear cell, subcutis	No. Affected minimal	0	0	0	0	0	0	1	0
		0	0	0	0	0	0	1	0
Inflammation, mixed cell, muscle	No. Affected mild	0	0	1	0	0	0	0	0
		0	0	1	0	0	0	0	0
Inflammation, mixed cell, subcutis/muscle	No. Affected mild	0	0	1	0	0	0	0	0
		0	0	1	0	0	0	0	0
Site, Injection (1C)	No. Examined	10	10	10	10	10	10	10	10
Infiltrate, mononuclear cell, subcutis	No. Affected minimal	0	0	0	1	0	0	0	0
		0	0	0	1	0	0	0	0

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (μ g/dose)	0	5	10	18	0	5	10	18
Inflammation, mixed cell, No. Affected subcutis	0	0	1	1	0	0	0	0
minimal	0	0	1	0	0	0	0	0
mild	0	0	0	1	0	0	0	0
Inflammation, mixed cell, No. Affected subcutis/muscle	0	0	1	0	0	0	0	0
minimal	0	0	1	0	0	0	0	0
Site, Injection (2A) No. Examined	10	10	10	10	10	10	10	10
Infiltrate, mononuclear No. Affected cell, subcutis	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Inflammation, mixed cell, No. Affected muscle	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Inflammation, mixed cell, No. Affected subcutis	0	0	4	3	0	0	6	4
minimal	0	0	1	0	0	0	2	0
mild	0	0	3	3	0	0	2	1
moderate	0	0	0	0	0	0	2	2
marked	0	0	0	0	0	0	0	1
Inflammation, mixed cell, subcutis/muscle No. Affected	0	9	2	5	0	9	4	3
minimal	0	2	0	0	0	1	0	0
mild	0	4	0	2	0	5	2	2
moderate	0	3	1	3	0	3	1	1
marked	0	0	1	0	0	0	1	0
Site, Injection (2B) No. Examined	10	10	10	10	10	10	10	10
Infiltrate, mononuclear No. Affected cell, subcutis	0	0	1	0	0	0	0	0
minimal	0	0	1	0	0	0	0	0
Inflammation, mixed cell, No. Affected muscle	0	0	0	0	0	3	0	0
minimal	0	0	0	0	0	1	0	0
mild	0	0	0	0	0	2	0	0
Inflammation, mixed cell, No. Affected subcutis	0	0	3	1	0	1	6	4
minimal	0	0	1	0	0	0	3	0
mild	0	0	1	1	0	0	2	1
moderate	0	0	1	0	0	1	1	2
marked	0	0	0	0	0	0	0	1
Inflammation, mixed cell, No. Affected subcutis/muscle	0	10	3	9	0	6	4	4
mild	0	6	0	1	0	4	1	1

Sex		Males				Females			
Group		1	2	3	4	1	2	3	4
Dose ($\mu\text{g}/\text{dose}$)		0	5	10	18	0	5	10	18
	moderate	0	3	3	6	0	2	2	2
	marked	0	1	0	2	0	0	1	1
Site, Injection (2C)	No. Examined	10	10	10	10	10	10	10	10
Inflammation, mixed cell, No. Affected muscle		0	0	2	0	0	1	1	0
	mild	0	0	2	0	0	1	0	0
	moderate	0	0	0	0	0	0	1	0
Inflammation, mixed cell, No. Affected subcutis		0	0	4	0	0	1	1	2
	minimal	0	0	3	0	0	1	0	0
	mild	0	0	0	0	0	0	1	0
	moderate	0	0	1	0	0	0	0	1
	marked	0	0	0	0	0	0	0	1
		0	0	0	0	0	0	0	0
Inflammation, mixed cell, No. Affected subcutis/muscle		0	2	2	8	0	1	7	8
	minimal	0	1	0	1	0	0	0	0
	mild	0	1	1	1	0	1	6	4
	moderate	0	0	1	3	0	0	1	3
	marked	0	0	0	3	0	0	0	1
		0	0	0	0	0	0	0	0
Lymph Nodes, Iliac	No. Examined	10	9	10	10	9	9	10	10
Cellularity, increased, lymphocyte	No. Affected	2	7	4	8	2	7	7	9
	minimal	1	3	2	1	2	2	0	1
	mild	1	4	2	1	0	3	6	4
	moderate	0	0	0	4	0	2	0	4
	marked	0	0	0	2	0	0	1	0
Lymph Nodes, Inguinal	No. Examined	10	10	10	10	10	10	10	10
Cellularity, increased, lymphocyte	No. Affected	2	2	1	2	2	2	4	4
	minimal	1	2	0	1	2	1	3	2
	mild	1	0	1	1	0	1	1	1
	moderate	0	0	0	0	0	0	0	1
Nerve, Sciatic	No. Examined	10	10	10	10	10	10	10	10
Inflammation, mixed cell, No. Affected perineural		0	0	8	5	0	1	7	10
	minimal	0	0	3	2	0	1	5	4
	mild	0	0	3	2	0	0	2	4
	moderate	0	0	2	1	0	0	0	1
	marked	0	0	0	0	0	0	0	1
		0	0	0	0	0	0	0	0

Sex		Males				Females			
Group		1	2	3	4	1	2	3	4
Dose ($\mu\text{g}/\text{dose}$)		0	5	10	18	0	5	10	18
Spleen	No. Examined	10	10	10	10	10	10	10	10
Cellularity, decreased, lymphocyte, PALS	No. Affected minimal	0	3	6	6	2	3	6	9
		0	3	6	6	2	3	6	9
Hematopoiesis, increased	No. Affected minimal mild	0	3	3	5	0	0	2	2
		0	3	3	4	0	0	2	2
		0	0	0	1	0	0	0	0
Bone Marrow, Sternum	No. Examined	10	10	10	10	10	10	10	10
Hematopoiesis, increased	No. Affected minimal mild	0	1	1	2	0	0	3	2
		0	1	1	1	0	0	3	2
		0	0	0	1	0	0	0	0
Glands, Adrenal	No. Examined	10	10	10	10	10	-	-	10
Vacuolation, increased, cortex	No. Affected minimal mild	0	4	2	6	0	-	-	0
		0	3	2	6	0	-	-	0
		0	1	0	0	0	-	-	0

Table 21: Microscopic evaluation main study (Study no. 20457846). Sponsor provided.

Day 36

Injection site 1 and 2 findings of mononuclear cell infiltrates in group 4 males and females were of low incidence and minimal severity and indicative of recovery. The other mRNA-1010.6-related findings were partially resolved in the lymph nodes, perineural region of the sciatic nerve, spleen, and bone marrow based on the low incidence of animals affected and minimal severity. Effects in the adrenal glands were fully recovered.

Table of incidence and severity of microscopic findings – Recovery sacrifice

Sex		Males		Females	
Group		1	4	1	4
Dose ($\mu\text{g}/\text{dose}$)		0	18	0	18
No. Examined		5	5	5	5
Site, Injection (1A)	No. Examined	5	5	5	5
Infiltrate, mononuclear cell, muscle	No. Affected minimal	0	0	1	1
		0	0	1	1
Site, Injection (1B)	No. Examined	5	5	5	5
Infiltrate, mononuclear cell, muscle	No. Affected minimal	1	2	0	1
		1	2	0	1
Site, Injection (2A)	No. Examined	5	5	5	5

Sex		Males		Females	
Group		1	4	1	4
Dose (µg/dose)		0	18	0	18
No. Examined		5	5	5	5
Infiltrate, mononuclear cell, muscle	No. Affected minimal	0	1	0	0
		0	1	0	0
Infiltrate, mononuclear cell, subcutis	No. Affected minimal	0	1	0	0
		0	1	0	0
Site, Injection (2B)	No. Examined	5	5	5	5
Infiltrate, mononuclear cell, muscle	No. Affected minimal	0	1	0	1
		0	1	0	1
Infiltrate, mononuclear cell, subcutis	No. Affected minimal	0	1	0	1
		0	1	0	1
Site, Injection (2C)	No. Examined	5	5	5	5
Infiltrate, mononuclear cell, muscle	No. Affected minimal	0	1	0	0
		0	1	0	0
Lymph Nodes, Iliac	No. Examined	5	5	5	5
Cellularity, increased, lymphocyte	No. Affected minimal mild moderate	0	2	1	1
		0	1	1	0
		0	0	0	1
		0	1	0	0
Lymph Nodes, Inguinal	No. Examined	5	5	5	5
Cellularity, increased, lymphocyte	No. Affected minimal	0	0	1	0
		0	0	1	0
Nerve, Sciatic	No. Examined	5	5	5	5
Inflammation, mixed cell, perineural	No. Affected minimal mild	0	1	0	1
		0	0	0	1
		0	1	0	0
Spleen	No. Examined	5	5	5	5
Hematopoiesis, increased	No. Affected minimal	0	1	0	1
		0	1	0	1
Bone Marrow, Sternum	No. Examined	5	5	5	5
Hematopoiesis, increased	No. Affected minimal	0	0	0	1
		0	0	0	1

Table 22: Microscopic evaluation recovery phase (Study no. 20457846). Sponsor provided.

Body temperature:

Not measured

Antibody analysis

A total of 12 predose samples and 10 postdose control samples had IgG titers above the Lower Limit of Quantitation (LLOQ).

Following two immunizations of mRNA-1010.6 on days 1 and 22, groups 2, 3, and 4 showed robust IgG titers for HA H1[A/Wisconsin], H3[A/Darwin], B-Victoria[B/Austria], and B-Yamagata[B/Phuket13] at days 22 and 36.

Table of mean IgG antibodies against 4 strains of influenza titer levels

Group		1		2		3		4	
Dose Level (µg/dose)		0		5		10		18	
	Sex	M	F	M	F	M	F	M	F
H1[A/Wisconsin]	Pretest	<0.3	0.76 ^a	<0.3	2.69 ^a	<0.3	3.15 ^b	<0.3	5.01 ^a
	Day 22	1.73 ^a	0.47 ^a	1008	1558	1171	1929	1379	3376
	Day 36	1.01 ^a	<0.3	N/A	N/A	N/A	N/A	13298	24310
H3[A/Darwin]	Pretest	<0.12	1.51 ^a	<0.12	<0.12	<0.12	<0.12	0.91 ^a	<0.12
	Day 22	<0.12	<0.12	549	1481	670	1560	920	2055
	Day 36	<0.12	<0.12	N/A	N/A	N/A	N/A	7864	9571
B-Victoria[B/Austria]	Pretest	<0.06	<0.06	0.71 ^a	0.46 ^a	0.38 ^b	<0.06	<0.06	<0.06
	Day 22	<0.06	<0.06	4374	7119	2862	7519	4955	9649
	Day 36	1.29 ^b	0.41 ^a	N/A	N/A	N/A	N/A	32666	70257
B-Yamagata[B/Phuket13]	Pretest	<0.08	<0.08	0.48 ^a	<0.08	<0.08	<0.08	<0.08	<0.08
	Day 22	<0.08	<0.08	840	1156	1293	1766	1367	2803
	Day 36	3.31 ^a	<0.08	N/A	N/A	N/A	N/A	9333	12795

^a The reported value is based on a positive result of a single animal and all other animals were below the respective Lower Limit of Quantitation (LLOQ).

^b The reported value is based on an average of positive results of at least 2 animals and all other animals were below the respective Lower Limit of Quantitation (LLOQ).

Note for H1[A/Wisconsin]: “Not detected” antibody titers are reported as “<0.3 Antibody Units/mL” which is derived “<0.003 Antibody Units/mL × 100” Lower Limit of Quantitation (LLOQ) is 0.003 AU/mL. Dilution factor 1:100 is the lowest dilution factor used for these samples.

Note for H3[A/Darwin]: “Not detected” antibody titers are reported as “<0.12 Antibody Units/mL” which is derived “<0.0012 Antibody Units/mL × 100” Lower Limit of Quantitation (LLOQ) is 0.0012 AU/mL. Dilution factor 1:100 is the lowest dilution factor used for these samples.

Note for B-Victoria[B/Austria]: “Not detected” antibody titers are reported as “<0.06 Antibody Units/mL” which is derived “<0.0006 Antibody Units/mL × 100” Lower Limit of Quantitation (LLOQ) is 0.0006 AU/mL. Dilution factor 1:100 is the lowest dilution factor used for these samples.

Note for B-Yamagata[B/Phuket13]: “Not detected” antibody titers are reported as “<0.08 Antibody Units/mL” which is derived “<0.0008 Antibody Units/mL × 100” Lower Limit of Quantitation (LLOQ) is 0.0008 AU/mL. Dilution factor 1:100 is the lowest dilution factor used for these samples.

Table 23: IgG antibody findings (Study no. 20457846). Sponsor provided.

Test article related effects are listed in the table below:

Test article related effects	Effects considered incidental
↑ WBC ↑ Neutrophil ↑ Eosinophil	

Test article related effects	Effects considered incidental
↑ Fibrinogen Injection site findings Iliac and inguinal lymph node Sciatic nerve findings Microscopic spleen extramedullary hematopoiesis findings Adrenal gland findings Immune responses	

Table 24: Test article related effects (Study no. 20457846).

Assessment:

No treatment-related, mortality, clinical observation, body weight, ophthalmic changes, macroscopic pathology, or organ weights were reported.

White blood cells (WBCs) (also called leukocytes or leucocytes) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.¹ The increase in WBC might be related to the immune response induced by the test article treatment.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The increase in neutrophils might be related to the immune responses initiated by the test article treatment.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Test article-related mixed cell inflammation in the subcutis and/or muscle were reported. The mixed cell inflammation was composed mainly of polymorphonuclear cells and few mononuclear cells. Mononuclear cell infiltrates in the subcutis and/or muscle was also reported. Mixed cell inflammation was reported in groups 3 and 4 males only.

Test article-related increased lymphocyte cellularity was reported in the iliac lymph nodes and in the inguinal lymph nodes. This was considered a secondary, reactive change to the injection site

¹ Maton, D., Hopkins, J., McLaughlin, Ch. W., Johnson, S., Warner, M. Q., LaHart, D., & Wright, J. D., Deep V. Kulkarni (1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall. [ISBN 0-13-981176-1](#).

mixed cell inflammation. Although these findings were present in control animals, there was a dose-related increase in the incidence and severity of the findings, particularly in the iliac lymph nodes.

Test article-related mixed cell inflammation involving the perineural sheath of the sciatic nerve was reported. Perineural involvement was considered as an extension of the injection site inflammation.

Test article-related findings in the spleen included decreased cellularity of lymphocytes in the periarteriolar lymphocyte sheaths (PALS). The decreased cellularity might be due to the presence of apoptosis. Test article-related increased extramedullary hematopoiesis was also reported.

Test article-related increased incidence of cortical vacuolation was reported in the adrenal gland. This finding was characterized by the presence of cortical cells containing prominent intracytoplasmic clear vacuoles of varying size and noted to be above the background incidence of intracytoplasmic lipid vacuoles that are normally reported in the adrenal gland.

Following two immunizations of mRNA-1010.6 on days 1 and 22, test article-related robust IgG titers for HA H1[A/Wisconsin], H3[A/Darwin], B-Victoria[B/Austria], and B-Yamagata[B/Phuket13] were reported at days 22 and 36.

Based on the overall findings in this study, it can be concluded that in (b) (4) rats, treatment by IM injection on study days 1, 15, and 29 with mRNA-1010 caused changes in hematology parameters, and the cellularity of adrenal glands, iliac and inguinal lymph nodes, and spleen. Also, it caused injection site and sciatic nerve inflammation and immune responses.

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

Conclusion

Transient mRNA-1010.6-related edema was reported at the injection site, which resolved within two days post-each-dose. There are transient mRNA-1010.6-related effects on food consumption with no effects on body weights. Clinical pathology findings were indicative of a systemic inflammatory and/or immune response. Microscopic target organs included the injection site, iliac and/or inguinal lymph nodes, spleen and sternum bone marrow. Following the recovery period, the anatomic and clinical pathology findings were all fully or partially recovered. Antibody analyses showed a robust non-dose-dependent antibody titer response against all 4 analytes (HA H1[A/Wisconsin], H3[A/Darwin], B-Victoria[B/Austria], and B-Yamagata[B/Phuket13]) on the last day of dosing, as well as 2 weeks post the last dose. The highest tested dose of 18 µg was established as NOAEL.

95 pages determined to be not releasable: (b)(4)

(b) (4)

Study number 2: (b) (4) *mRNA in SM102-Containing Lipid Nanoparticles:* (b) (4)
 Assay in the Rat. Study number:
 AF87FU.125012NGLPICH.BTL.

Objective:

The objective of this study was to evaluate the test article for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in rat bone marrow.

Method:

(b) (4) rats were received from (b) (4) on (b) (4). The age at time of initiation, as well as the body weights and days of acclimation, of the rats assigned to the study groups at randomization are indicated below:

Study	Sex	Body Weight Range at Randomization (grams)	Age at Initiation (weeks)	Days of Acclimation
Definitive (Main)	Male	167.7 to 175.9	6	7
	Female	140.8 to 149.3		
Definitive (TK)	Male	163.2 to 179.8	6	7
	Female	138.4 to 151.4		

Table 107: Experimental design, sponsor provided (Study no. AF87FU.125012NGLPICH.BTL).

Male and female rats were dosed 0.32, 1.07, or 3.21 mg/kg or 6.0., 20, or 60 mg/kg, mRNA or SM102 lipid, respectively. Animals were treated by intravenous injection with a 5 mL/kg dose volume. Two and six hours after dosing, plasma samples were collected for mRNA quantification and cytokine analysis, respectively. Clinical observations and body temperatures were monitored before and after dosing. Twenty-four and forty-eight hours after dosing, animals were euthanized, and bone marrow were collected and processed for the (b) (4) assay.

Results:

(b) (4) assay

No test article-related mortality or clinical observations were reported. At the high dose level (3.21/60 mg/kg), increased temperatures in both males and females were reported from 1-2 hours post dose to 8 hours post dose and met the protocol-specified parameters for hyperthermia ($\geq 1^{\circ}\text{C}$ increase for at least 4.5 hours).

Male Body Temperature

Treatment	Sex	Pretreatment				Group Mean Body Temperatures ($^{\circ}\text{C}$)									
			-48hr	-24hr	0hr	Average	0.5 Hr	1 Hr	2 Hr	4 Hr	5 Hr	6 Hr	8 Hr	24 Hr	48 Hr
			Pre-Dose	Pre-Dose	Pre-Dose	Pre-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose	Post-Dose
Vehicle															
24 Hour	M	Mean	37.1	36.8	37.1	37.0	38.1	38.4	37.9	37.4	37.9	37.9	37.4	37.5	N/A
		+/-SD	0.3	0.6	0.3		± 0.4	± 0.4	± 0.4	± 0.2	± 0.4	± 0.3	± 0.3	± 0.3	N/A
		$^{\circ}\text{C}$ Change				--	1.1	1.4	0.9	0.4	0.9	0.9	0.4	0.5	N/A
48 Hour	M	Mean	37.2	37.0	36.9	37.0	38.1	38.1	37.6	36.7	37.4	37.5	37.3	36.9	37.3
		+/-SD	0.2	0.4	0.2		± 0.4	± 0.3	± 0.4	± 0.4	± 0.3	± 0.4	± 0.3	± 0.2	± 0.3
		$^{\circ}\text{C}$ Change				--	1.1	1.1	0.6	-0.3	0.4	0.5	0.3	-0.1	0.3
(b) (4) mRNA in SM102-Containing Lipid Nanoparticles															
0.32/6.0 mg/kg/day															
24 Hour	M	Mean	37.1	37.4	36.8	37.1	37.7	37.9	37.5	37.3	37.9	37.8	37.8	37.3	N/A
		+/-SD	0.3	0.2	0.1		± 0.5	± 0.4	± 0.6	± 0.4	± 0.2	± 0.7	± 0.6	± 0.4	N/A
		$^{\circ}\text{C}$ Change				--	0.6	0.8	0.4	0.2	0.8	0.7	0.7	0.2	N/A
48 Hour	M	Mean	36.8	37.1	36.8	36.9	38.0	37.9	37.7	37.5	37.7	37.3	37.4	36.9	37.5
		+/-SD	0.2	0.1	0.1		± 0.2	± 0.2	± 0.6	± 0.2	± 0.1	± 0.1	± 0.2	± 0.2	± 0.5
		$^{\circ}\text{C}$ Change				--	1.1	1.0	0.8	0.6	0.8	0.4	0.5	0.0	0.6
1.07/20 mg/kg/day															
24 Hour	M	Mean	36.9	36.9	36.9	36.9	37.9	37.9	37.7	37.1	37.7	37.8	37.8	37.1	N/A
		+/-SD	0.3	0.4	0.2		± 0.3	± 0.3	± 0.2	± 0.2	± 0.3	± 0.3	± 0.2	± 0.6	N/A
		$^{\circ}\text{C}$ Change				--	1.0	1.0	0.8	0.2	0.8	0.9	0.9	0.2	N/A
48 Hour	M	Mean	37.4	37.0	37.2	37.2	38.2	38.0	37.8	37.9	37.9	38.0	37.9	37.1	37.0
		+/-SD	0.4	0.3	0.2		± 0.2	± 0.5	± 0.5	± 0.6	± 0.4	± 0.5	± 0.5	± 0.5	± 0.3
		$^{\circ}\text{C}$ Change				--	1.0	0.8	0.6	0.7	0.7	0.8	0.7	-0.1	-0.2
3.21/60 mg/kg/day															
24 Hour	M	Mean	37.0	36.9	37.2	37.0	38.0	37.7	38.1	38.1	38.1	38.5	38.2	37.4	N/A
		+/-SD	0.1	0.2	0.3		± 0.4	± 0.4	± 0.2	± 0.3	± 0.2	± 0.2	± 0.3	± 0.7	N/A
		$^{\circ}\text{C}$ Change				--	1.0	0.7	1.1	1.1	1.1	1.5	1.2	0.4	N/A
48 Hour	M	Mean	37.0	36.9	37.0	37.0	37.9	38.0	38.1	38.1	38.5	38.4	38.2	37.7	36.7
		+/-SD	0.4	0.2	0.2		± 0.3	± 0.4	± 0.4	± 0.7	± 0.8	± 0.7	± 0.7	± 1.2	± 0.6
		$^{\circ}\text{C}$ Change				--	0.9	1.0	1.1	1.1	1.5	1.4	1.2	0.7	-0.3

SD = Standard deviation

N/A - Not Applicable due to study design

 $^{\circ}\text{C}$ Change = Post-treatment temperature - Pretreatment temperature

ND = No data due to mortality.

¹SD = Standard deviation not available due to single surviving animal.⁴SD = No Standard deviation available due to single value reported.

Female Body Temperature

Treatment	Sex	Pretreatment				Group Mean Body Temperatures (°C)									
			-48hr	-24hr	0hr	Average	0.5 Hr	1 Hr	2 Hr	4 Hr	5 Hr	6 Hr	8 Hr	24 Hr	48 Hr
Vehicle															
24 Hour	F	Mean	36.9	36.9	37.2	37.0	37.9	38.5	37.6	37.0	37.9	37.9	38.1	38.1	N/A
		+/-SD	0.1	0.2	0.3		±0.3	±0.2	±0.4	±0.2	±0.4	±0.3	±0.4	±0.4	N/A
		°C Change				--	0.9	1.5	0.6	0.0	0.9	0.9	1.1	1.1	N/A
48 Hour	F	Mean	34.8	34.6	35.1	34.8	36.6	36.6	36.4	34.9	38.0	36.3	35.6	34.9	35.8
		+/-SD	4.3	4.1	4.5		±4.2	±4.3	±4.3	±4.2	±0.4	±4.3	±4.1	±4.3	±4.5
		°C Change				--	1.8	1.8	1.6	0.1	3.2	1.5	0.8	0.1	1.0
(b) (4) mRNA in SM102-Containing Lipid Nanoparticles															
0.32/6.0 mg/kg/day															
24 Hour	F	Mean	36.4	36.2	36.6	36.4	37.9	38.0	37.7	37.7	38.1	37.8	37.9	37.3	N/A
		+/-SD	0.5	0.7	1.0		±0.9	±0.7	±0.8	±0.9	±0.8	±0.8	±0.5	±0.9	N/A
		°C Change				--	1.5	1.6	1.3	1.3	1.7	1.4	1.5	0.9	N/A
48 Hour	F	Mean	35.0	36.5	35.0	35.5	36.6	37.9	36.7	35.9	36.2	35.8	35.7	35.5	35.9
		+/-SD	3.0	1.9	3.3		±2.5	±1.8	±2.7	±2.9	±2.7	±2.8	±2.9	±2.5	±2.8
		°C Change				--	1.1	2.4	1.2	0.4	0.7	0.3	0.2	0.0	0.4
1.07/20 mg/kg/day															
24 Hour	F	Mean	37.6	38.3	38.1	38.0	39.2	39.4	38.8	38.2	39.3	38.8	39.0	38.4	N/A
		+/-SD	1.9	1.8	1.7		±1.7	±1.9	±2.0	±2.0	±1.7	±2.0	±1.9	±1.4	N/A
		°C Change				--	1.2	1.4	0.8	0.2	1.3	0.8	1.0	0.4	N/A
48 Hour	F	Mean	37.0	36.8	37.7	37.2	38.5	38.6	38.0	37.9	38.0	38.1	38.1	37.0	37.1
		+/-SD	0.7	0.5	0.9		±0.8	±0.8	±1.2	±1.0	±0.8	±0.9	±0.8	±0.8	±0.9
		°C Change				--	1.3	1.4	0.8	0.7	0.8	0.9	0.9	-0.2	-0.1
3.21/60 mg/kg/day															
24 Hour	F	Mean	41.4	41.8	41.7	41.6	42.5	42.8	42.9	42.7	42.9	43.0	42.6	41.6	N/A
		+/-SD	1.9	2.0	1.8		±2.1	±2.3	±2.2	±2.1	±2.1	±2.1	±2.3	±2.5	N/A
		°C Change				--	0.9	1.2	1.3	1.1	1.3	1.4	1.0	0.0	N/A
48 Hour	F	Mean	39.1	39.3	39.4	39.3	40.5	40.5	40.6	40.7	40.9	40.7	40.5	39.6	39.3
		+/-SD	1.8	1.8	1.5		±1.9	±2.2	±2.1	±2.1	±2.2	±2.2	±2.1	±2.2	±2.1
		°C Change				--	1.2	1.2	1.3	1.4	1.6	1.4	1.2	0.3	0.0

SD = Standard deviation

°C Change = Post-treatment temperature - Pretreatment temperature

ND = No data due to mortality.

°SD = Standard deviation not available due to single surviving animal.

°SD = No Standard deviation available due to single value reported.

N/A - Not Applicable due to study design

Table 108: Body temperature for males and females, sponsor provided (Study no. AF87FU.125012NGLPICH.BTL).

Bone marrow analysis

A statistically significant reduction in the PCEs/EC ratio was reported in the low dose (0.32/6.0 mg/kg [mRNA/SM102 lipid]) males at 48 hours compared to the vehicle control group. Group variances for the mean of the micronucleus frequency were compared using Levene's test. No significant difference in the group variance ($p > 0.05$) between control and test article groups were reported. Therefore, the parametric approach, ANOVA followed by Dunnett's post-hoc analysis, was used in the statistical analysis of data.

No statistically significant increase in the incidence of MnPCEs was reported in the test article treated groups relative to the vehicle control group (ANOVA followed by Dunnett's post-hoc analysis, $p > 0.05$).

The positive control, (b) (4), induced a statistically significant increase in the incidence of MnPCEs (Student's t-test, $p \leq 0.05$).

The number of MnPCEs in the vehicle control groups did not exceed the historical control range.

The incidence of MnPCEs per 40,000 PCEs scored (4000 PCEs/animal) and the proportion of polychromatic erythrocytes per total erythrocytes are summarized in the following table:

Treatment	Gender	Time (Hrs)	Animals	%PCE (Mean +/- SD)	Toxicity (%)	% MnPCE (Mean +/- SD)	Number of MnPCE/PCE Scored
Vehicle							
0 mg/kg/day	M	24	5	58.3 ± 6.2	---	0.10 ± 0.04	19 /20000
0 mg/kg/day	F	24	5	66.7 ± 5.4	---	0.12 ± 0.04	23 /20000
(b) (4) mRNA in SM102-Containing Lipid Nanopartic							
0.32 mg/kg/day	M	24	5	66.2 ± 4.8*	14	0.11 ± 0.02	22 /20000
0.32 mg/kg/day	F	24	5	68.7 ± 7.2	3	0.10 ± 0.04	20 /20000
1.07 mg/kg/day	M	24	5	61.7 ± 4.6	6	0.10 ± 0.04	20 /20000
1.07 mg/kg/day	F	24	5	64.1 ± 5.7	-4	0.10 ± 0.03	19 /20000
3.21 mg/kg/day	M	24	5	66.3 ± 3.1*	14	0.09 ± 0.03	18 /20000
3.21 mg/kg/day	F	24	5	61.0 ± 7.2	-9	0.11 ± 0.02	21 /20000
(b) (4)							
40 mg/kg/day	M	24	5	27.7 ± 4.3**	-53	3.70 ± 0.47**	740 /20000
Vehicle							
0 mg/kg/day	M	48	5	70.0 ± 4.4	---	0.08 ± 0.02	15 /20000
0 mg/kg/day	F	48	5	61.8 ± 11.3	---	0.10 ± 0.04	20 /20000
(b) (4) mRNA in SM102-Containing Lipid Nanopartic							
0.32 mg/kg/day	M	48	5	57.9 ± 9.2**	-17	0.09 ± 0.02	17 /20000
0.32 mg/kg/day	F	48	5	62.1 ± 7.6	1	0.12 ± 0.03	23 /20000
1.07 mg/kg/day	M	48	5	63.8 ± 3.2	-9	0.09 ± 0.04	17 /20000
1.07 mg/kg/day	F	48	5	64.4 ± 2.5	4	0.13 ± 0.03	25 /20000
3.21 mg/kg/day	M	48	5	67.7 ± 4.7	-3	0.08 ± 0.03	15 /20000
3.21 mg/kg/day	F	48	5	66.9 ± 9.0	8	0.11 ± 0.05	21 /20000

*p < 0.05 or **p < 0.01, One-Way ANOVA with Post-Hoc Dunnett's Test or T-Test

24 Hrs MnPCE male GLM P-value = 0.795, R-sqr = 6.04%

24 Hrs MnPCE female GLM P-value = 0.791, R-sqr = 6.13%

Table 109: Summary of **(b) (4)** analysis, sponsor provided (Study no. AF87FU.125012NGLPICH.BTL).

Based on the above results, **(b) (4)** mRNA in SM102-containing lipid nanoparticles is negative for the induction of micronucleated polychromatic erythrocytes.

Bioanalysis

Due to technical issues with the assay, results were considered to be unreliable and thus not reported.

Cytokine analysis

Administration of **(b) (4)** mRNA in SM102-containing lipid nanoparticles to rats resulted in cytokine increases in IL-6, MCP-1, MIP-1 α , and IP-10 at 6 hours post-dose in one or both sexes at 1.07 or 20 mg/kg (mRNA or SM-102, respectively) and in both sexes at 3.21 or 60 mg/kg (mRNA or SM-102, respectively).

Table of (b) (4) mRNA in SM102-containing lipid nanoparticles: (b) (4)
assay in the rat

Sex: Male			Group6	Group7	Group8	Group9
Day(s) Relative to Start Date						
IL-1 beta (pg/ml)	1(6HPD)	Mean	103.917	140.453	153.613	107.800
		SD	23.9177	35.4193	128.8893	4.5989
		N	3	3	3	3
IL-6 (pg/ml)	1(6HPD)	Mean	1881.760*	2586.950*	3515.173	6766.187
		SD	1425.7395*	2101.1177*	3162.8510	736.3812
		N	3*	3*	3	3
MCP-1 (pg/ml)	1(6HPD)	Mean	1263.717	1703.160	3852.117	3353.137
		SD	271.6087	0.0000	1338.9747	1097.6276
		N	3	3	3	3
TNF- α (pg/ml)	1(6HPD)	Mean	47.440	51.283	104.193	46.857
		SD	2.6710	17.9581	47.9196	2.6656
		N	3	3	3	3
MIP-1 α (pg/ml)	1(6HPD)	Mean	34.410	41.910	83.430	66.620
		SD	0.0000	6.9072	26.7255	27.1412
		N	3	3	3	3
IP-10 (pg/ml)	1(6HPD)	Mean	500.587	527.610	2290.207	3746.320
		SD	63.3960	127.1126	1090.3360	3297.2851
		N	3	3	3	3

*Calculation includes one or more individual value(s) out of linear range

Table 110: Summary of cytokine values in males, sponsor provided (Study no. AF87FU.125012NGLPICH.BTL).

Sex: Female			Group6	Group7	Group8	Group9
Day(s) Relative to Start Date						
IL-1 beta (pg/ml)	1(6HPD)	Mean	112.927	163.327	219.227	118.073
		SD	21.7139	56.2205	91.0994	30.7706
		N	3	3	3	3
IL-6 (pg/ml)	1(6HPD)	Mean	962.830*	2527.237	3543.200	3490.410
		SD	1160.1796*	905.0889	1849.8502	1081.5334
		N	3*	3	3	3
MCP-1 (pg/ml)	1(6HPD)	Mean	1092.177	1319.093	1381.213	5093.663
		SD	219.2132	269.9198	163.1729	3446.7490
		N	3	3	3	3
TNF- α (pg/ml)	1(6HPD)	Mean	47.770	64.807	49.590	50.537
		SD	27.9642	26.8080	8.6063	25.7539
		N	3	3	3	3
MIP-1 α (pg/ml)	1(6HPD)	Mean	48.147	57.197	50.517	126.010
		SD	8.2658	17.4563	6.1914	30.3349
		N	3	3	3	3
IP-10 (pg/ml)	1(6HPD)	Mean	405.460	667.743	2934.433	12355.077
		SD	36.1255	208.3876	296.1695	2842.3394
		N	3	3	3	3

*Calculation includes one or more individual value(s) out of linear range

Table 111: Summary of cytokine values in females, sponsor provided (Study no. AF87FU.125012NGLPICH.BTL).

Conclusion:

(b) (4) mRNA in SM102-containing lipid nanoparticles was concluded to be negative for the induction of micronucleated polychromatic erythrocytes.

In Vitro Tox Studies

Study number 1: (b) (4) **Vaccine:**

SM-102 Bacterial Reverse Mutation Test in (b) (4)

Reviewed by (b) (6) . Study number: 9601567.

Test Article: SM-102; Batch (Lot) No.: (b) (4)

Study No.: 9601567

Date of dose preparation: 22nd September 2016

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Test for induction of reverse mutation.

Strains: (b) (4)

Controls:

Negative control: (b) (4)

(b) (4)

(b) (4)

Table 112: Positive control substance, sponsor provided (Study no. 9601567).

Study design:

Dose No.	Formulation Conc. (µg/mL)	Dose Volume (b) (4)	Final Conc. (b) (4)	Number of Replicates		Number of Strains
				(b) (4)	(b) (4)	
Negative Control	-	100	-	3	3	5
1/ SM-102	19.0 ^b	83.2	1.58	3	3	5
2/ SM-102	58.2 ^b	85.9	5.0	3	3	5
3/ SM-102	184 ^b	85.9	15.8	3	3	5
4/ SM-102	500 ^a	100	50	3	3	5
5/ SM-102	1581 ^a	100	158	3	3	5
6/ SM-102	5000 ^a	100	500	3	3	5
7/ SM-102	15811 ^a	100	1581	3	3	5
8/ SM-102	50000 ^a	100	5000 ^c	3	3	5
Positive controls	d	100	d	3	3	5

a Theoretical concentration; actual concentration may differ slightly due to the limitations of the instruments used.

b Measured concentration

c Test Item was tested at levels up to (b) (4), which is the standard limit dose recommended by regulatory guidelines.

d Dose depends on the test organism, the positive controls and methodology used (see Section 4.5.1.2)

Table 113: Study design, sponsor provided (Study no. 9601567).

Results:

Dose formulation: The five highest formulation concentrations ranging from 500 to 50000 µg/mL met acceptance criteria, with chemical analysis indicating mean achieved concentrations within ±10% of the theoretical concentration for the stock solution and ±15% for lower-level solutions. The lowest three formulation concentrations did not meet acceptance criteria (dose number 1 was +20% of nominal, dose number 2 was +16% of nominal and dose number 3 was +16% of nominal). Analyses of the retention samples were not performed due to the consistency of the results of the duplicate samples. Instead, the results were accepted and the dose volumes of the three lowest test item concentrations to be tested were adjusted accordingly to obtain the concentrations specified in the study plan.

(b) (4)

Incomplete, or absent, (b) (4), or substantial reductions in (b) (4), were not obtained following exposure to SM-102, indicating that the test item was (b) (4) at the levels tested. Precipitation was observed at concentrations ≥ 1581 (b) (4) in the absence of (b) (4) and at concentrations ≥ 500 (b) (4) in the presence of (b) (4).

No substantial increases in (b) (4) numbers were obtained with any of the tester strains, following exposure to SM-102 at any dose level, in either the presence or absence of (b) (4). Therefore, SM-102 was negative for the induction of mutagenicity in this in vitro assay.

Conclusion: SM-102 did not show any evidence of genotoxic activity in this *in vitro* mutagenicity assay when tested in accordance with regulatory guidelines.

Study number 2: SM-102 (b) (4) Test in Human Peripheral Blood Lymphocytes. Reviewed by (b) (6) . Study number: 9601568.

Study no.: 9601568

Conducting laboratory and location: (b) (4)

Date of study initiation (dosing): 15th Sep 2016

Date of study completion: 12th Jan 2017

GLP compliance: Yes

Drug, lot #, and % purity:

Test article: SM-102;

Batch (Lot) No.: (b) (4)

Controls:

Negative control: (b) (4), Batch /Lot No.: (b) (4)

Positive controls (in the Absence of (b) (4)), identification: (b) (4)

Identity: (b) (4)

Positive controls (in the Presence of (b) (4))

Study design: The objective of this study was to determine the potential genotoxicity of SM-102, using an (b) (4) test in human peripheral blood lymphocytes. The highest dose level tested was 500 µg/mL, the maximum dose level recommended by the ICH S2(R1) guideline.

Dose No.	Formulation Conc. (µg/mL) ^a	Final Conc. (µg/mL)	Number of Cultures		
			4 Hours (b) (4)	4 Hours (b) (4)	24 Hours (b) (4)
Negative Control	-	-	2	2	2
1/ SM-102	325	3.25	2	2	2
2/ SM-102	568	5.68	2	2	2
3/ SM-102	995	9.95	2	2	2
4/ SM-102	1740	17.4	2	2	2
5/ SM-102	3050	30.5	2	2	2
6/ SM-102	5330	53.3	2	2	2
7/ SM-102	9330	93.3	2	2	2
8/ SM-102	16300	163	2	2	2
9/ SM-102	28600	286	2	2	2
10/ SM-102	50000	500	2	2	2
(b) (4)	25	0.25	2	-	-
	30	0.30	2	-	-
	1000	10	-	2	-
	1500	15	-	2	-
	10	0.10	-	-	2
	20	0.20	-	-	2

a = Theoretical concentrations; actual concentrations may differ slightly due to the limitations of the instruments used. b = Where the high level = 0.5 mg/mL.

Table 114: Study design, sponsor provided (Study no. 9601568).

Human peripheral blood lymphocytes were (b) (4)

Results:

(b) (4) assessment:

Treatment	Conc. (µg/mL)	Average (b) (4)	(%) (b) (4)	Total No. of (b) (4) examined	% (b) (4)
<i>4 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	1.8	0	2000	0.2
SM-102	163	1.9	-8	2000	0.5
	286	1.9	-8	2000	0.6
	500 ^{ppt}	1.8	0	2000	0.1
(b) (4)	0.25	1.5	43	1482	4.4**
<i>4 hours treatment in the presence of (b) (4)</i>					
(b) (4)	-	1.8	0	2000	0.4
SM-102	163	1.8	-3	2000	0.5
	286	1.8	-1	2000	0.6
	500	1.8	1	2000	0.3
(b) (4)	10	1.4	46	2000	2.2**
<i>24 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	1.7	0	2000	0.4
SM-102	163	1.7	-8	2000	0.2
	286	1.7	-2	2000	0.2
	500 ^{ppt}	1.7	1	2000	0.3
(b) (4)	0.10	1.6	3	2000	2.0**

CBPI = Cytokinesis-Block Proliferation Index.

a = Relative to the vehicle control.

(b) (4)

* p≤0.05, ** p≤0.01 otherwise p>0.05 Fisher's exact test with single-sided probabilities.

ppt = Precipitate visible in the culture medium at the end of treatment

Table 115: SM-102 - summary results and statistical analysis, sponsor provided (Study no. 9601568).

SM-102 did not cause any statistically significant increases in the incidence of (b) (4) cells compared to the concurrent negative control at any experiment.

The incidence of (b) (4) cells for all negative control and test item groups was within the laboratory negative historical control range. The positive controls caused

statistically significant increases in frequency of (b) (4) in each regime of the study, confirming the sensitivity of the test system and the effectiveness of the (b) (4).

Incidental observations:

Precipitation was observed at the end of treatment at 500 µg/mL, in both the 4-hour regime and the 24-hour regime in the absence of (b) (4). No precipitation was observed at the end of treatment in the presence of (b) (4). Cloudy media was observed in the 4-hour regime in the absence of (b) (4) at dose levels ≥ 93.3 µg/mL, in the 4-hour regime in the presence of (b) (4) at the highest dose level, and in the 24-hour regime at dose levels ≥ 286 µg/mL. No cytotoxicity was reported in the assay.

Conclusion: It is concluded that SM-102 did not show any evidence of genotoxic activity in this *in vitro* test for induction of (b) (4), when tested in accordance with regulatory guidelines.

Study number 3: (b) (4) (PEG 2K-DMG) and (b) (4); **Bacterial Reverse Mutation Test in** (b) (4). **Study number: 9601035.**

Study design:

(b) (4) were treated with the test items at a range of concentrations up to 5000 µg (b) (4) (the standard limit dose for this assay), in the presence and absence of a (b) (4), using the (b) (4) version of the bacterial mutation test.

Bacteria were incubated with standard positive controls, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the (b) (4).

Dose No.	Formulation Conc. (µg/mL)	Dose Volume (b) (4)	Final Conc. (b) (4)	No. of Replicates		No. of Strains
				(b) (4)	(b) (4)	
Vehicle	-	100	-	3	3	5
1/ Test item	158	10.0	1.58	3	3	5
1/ Test item	158	31.6	5.0	3	3	5
1/ Test item	158	100	15.8	3	3	5
2/ Test item	500	100	50	3	3	5
3/ Test item	1581	100	158	3	3	5
4/ Test item	5000	100	500	3	3	5
5/ Test item	15811	100	1581	3	3	5
6/ Test item	50000	100	5000†	3	3	5
Positive controls	‡	100	‡	3	3	5

‡ Depends on the test organism, positive control reference item and methodology used.

† Test item was tested at levels up to 5000 µg (b) (4), which is the standard limit dose recommended by regulatory guidelines.

Table 116: Study design - (b) (4) assay (per test item), sponsor provided (Study no. 9601035).

Test and reference items

Test items:

Identification: (b) (4) (PEG 2K-DMG)

Batch/Lot No.: (b) (4)

Receipt Date: 23 Jan 2015

Retest Date: 08 Sep 2015 Physical

Description: White powder

Purity: 97%, dose calculations were corrected for purity

Storage Conditions: (b) (4)

Supplier: (b) (4)

Appearance of Formulation: Clear colorless solution (stock solution)

Identification: (b) (4)

Batch/Lot No.: (b) (4)

Receipt Date: 23 Jan 2015

Retest Date: Oct 2015

Physical Description: Colorless oil, without foreign matter

Purity: 93.9%, dose calculations were corrected for purity

Storage Conditions: (b) (4)

Supplier: (b) (4)

Appearance of Formulation: Clear colorless solution (stock solution)

Vehicle controls

Vehicle for Test Item (b) (4) (PEG 2K-DMG)

Identification: (b) (4)

Supplier: (b) (4)

Batch/Lot No.: (b) (4)

Expiration Date: May 2016

Physical Description: Clear colorless liquid

Purity: 100%; dose calculations were not corrected for purity

Storage Conditions: (b) (4)

Vehicle for test item (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Batch/Lot No.: (b) (4)

Expiration Date: 31 Oct 2015 Physical

Description: Clear colorless liquid

Purity: 100%; dose calculations were not corrected for purity

Storage Conditions: (b) (4)

Positive controls

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: Sep 2017 Physical

Description: White powder

Purity: 99.6%; dose calculations were not corrected for purity

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: 19 Sep 2016

Physical Description: Yellow powder

Purity: 97.6%; dose calculations were corrected for purity and water content

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: 10 Feb 2019 Physical

Description: Yellow-tan powder

Purity: 97.9%; dose calculations were not corrected for purity

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: 20 Mar 2018 Physical

Description: Orange powder

Purity: 99%; dose calculations were not corrected for purity

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: 14 Mar 2019

Physical Description: Yellow powder

Purity: 97.5%; dose calculations were not corrected for purity

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Lot No.: (b) (4)

Expiration Date: Jun 2016

Physical Description: Yellow powder

Purity: 99.9%; dose calculations were not corrected for purity

Vehicle: (b) (4)

Concentration: (b) (4)

Storage Conditions: (b) (4)

Test system

The bacteria used in this study were originally supplied by (b) (4) and was tested for appropriate phenotype characteristics and spontaneous reversion rates; response to diagnostic mutagens is also routinely assessed. The following bacterial strains were used:

(b) (4)

Results

Bacterial mutation test

The mean revertant (b) (4) for the vehicle controls were close to or within the laboratory historical control range. Positive controls (with (b) (4) where required) induced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain, confirming sensitivity of the test system and activity of the (b) (4). Indicating that the test items were non-toxic to the bacteria at the levels tested, incomplete, or absent, (b) (4) of non-revertant bacteria, or substantial reductions in revertant (b) (4), were not obtained following exposure to (b) (4) (PEG 2K-DMG) or (b) (4).

At a concentration of 5000 µg/(b) (4), possible precipitate was reported throughout the (b) (4) treated with (b) (4) (PEG 2K-DMG), with each of the (b) (4) strains, in

the presence of (b) (4). No precipitation was reported on any of the (b) (4) treated with (b) (4) in either the absence or presence of (b) (4). However, an oily residue was reported at the two highest concentrations in both the presence and the absence of (b) (4).

Following exposure to (b) (4) (PEG 2K-DMG) or (b) (4) at any dose level, in the presence or absence of (b) (4), no substantial increases in revertant (b) (4) numbers were reported with any of the tester strains. Therefore, in this *in vitro* assay, (b) (4) (PEG 2K-DMG) and (b) (4) were considered to be negative for the induction of mutagenicity.

Conc.			No. of Revertants					(b) (4) Observations *			Fold
Strain	(µg/(b) (4))	(b) (4)	x ₁	x ₂	x ₃	mean	SD	x ₁	x ₂	x ₃	Response †
(b) (4)	(b) (4)	0	17	24	13	18	6				1.0
	50	0	10	14	14	13	2				0.7
	158	0	14	15	17	15	2				0.9
	500	0	13	13	18	15	3				0.8
	1581	0	6	13	13	11	4				0.6
	5000	0	17	9	8	11	5				0.6
(b) (4)	(b) (4)	0	5	20	12	12	8				1.0
	50	0	13	19	15	16	3				1.3
	158	0	10	18	6	11	6				0.9
	500	0	10	15	18	14	4				1.2
	1581	0	17	8	13	13	5				1.0
	5000	0	13	18	13	15	3				1.2
(b) (4)	(b) (4)	0	36	32	33	34	2				1.0
	50	0	32	33	34	33	1				1.0
	158	0	25	31	36	31	6				0.9
	500	0	27	37	32	32	5				1.0
	1581	0	39	27	28	31	7				0.9
	5000	0	23	42	37	34	10				1.0
(b) (4)	(b) (4)	0	107	97	101	102	5				1.0
	50	0	126	111	122	120	8				1.2
	158	0	100	125	130	118	16				1.2
	500	0	123	113	114	117	6				1.1
	1581	0	85	113	78	92	19				0.9
	5000	0	137	114	132	128	12				1.3
(b) (4)	(b) (4)	0	38	44	44	42	3				1.0
	50	0	29	27	43	33	9				0.8
	158	0	39	46	36	40	5				1.0
	500	0	46	57	37	47	10				1.1
	1581	0	36	38	37	37	1				0.9
	5000	0	36	47	43	42	6				1.0

* Comments on the (b) (4): no comments.

† Fold response in mean revertant compared to concurrent vehicle control.

SD = Standard deviation.

Table 117: (b) (4) (PEG 2K-DMG) - (b) (4) assay in the absence of (b) (4), sponsor provided (Study no. 9601035).

Conc.			No. of Revertants					(b) (4)	Observations *			Fold
Strain	(μg/(b) (4))	(b) (4)	x ₁	x ₂	x ₃	mean	SD	x ₁	x ₂	x ₃	Response †	
(b) (4)	(b) (4)	+	11	13	7	10	3				1.0	
	50	+	19	14	18	17	3				1.6	
	158	+	14	9	17	13	4				1.3	
	500	+	8	14	5	9	5				0.9	
	1581	+	8	14	5	9	5				0.9	
	5000	+	10	7	13	10	3	p	p	p	1.0	
(b) (4)	(b) (4)	+	12	17	11	13	3				1.0	
	50	+	24	17	19	20	4				1.5	
	158	+	15	22	15	17	4				1.3	
	500	+	10	15	15	13	3				1.0	
	1581	+	11	10	8	10	2				0.7	
	5000	+	14	5	5	8	5	p	p	p	0.6	
(b) (4)	(b) (4)	+	40	53	52	48	7				1.0	
	50	+	39	41	41	40	1				0.8	
	158	+	47	43	37	42	5				0.9	
	500	+	53	44	34	44	10				0.9	
	1581	+	37	36	46	40	6				0.8	
	5000	+	51	42	38	44	7	p	p	p	0.9	
(b) (4)	(b) (4)	+	135	123	106	121	15				1.0	
	50	+	135	122	151	136	15				1.1	
	158	+	127	149	137	138	11				1.1	
	500	+	103	112	95	103	9				0.9	
	1581	+	93	107	94	98	8				0.8	
	5000	+	85	86	102	91	10	p	p	p	0.8	
(b) (4)	(b) (4)	+	38	48	38	41	6				1.0	
	50	+	48	53	46	49	4				1.2	
	158	+	65	52	44	54	11				1.3	
	500	+	43	51	48	47	4				1.1	
	1581	+	51	48	57	52	5				1.3	
	5000	+	47	46	44	46	2				1.1	

* Comments on the (b) (4): p = presence of possible precipitate throughout (b) (4)

† Fold response in mean revertant compared to concurrent vehicle control.

SD = Standard deviation.

Table 118: (b) (4) (PEG 2K-DMG) - (b) (4) assay in the presence of (b) (4), sponsor provided (Study no. 9601035).

Strain	Conc.	(b) (4)	No. of Revertants					(b) (4)	Observations *			Fold
	(µg (b) (4))		x ₁	x ₂	x ₃	mean	SD	x ₁	x ₂	x ₃	Response †	
(b) (4)	(b) (4)	0	17	15	8	13	5				1.0	
	50	0	10	13	13	12	2				0.9	
	158	0	11	13	10	11	2				0.9	
	500	0	8	17	14	13	5				1.0	
	1581	0	17	15	18	17	2	r	r	r	1.3	
	5000	0	17	13	9	13	4	r	r	r	1.0	
(b) (4)	(b) (4)	0	14	13	9	12	3				1.0	
	50	0	8	13	10	10	3				0.9	
	158	0	6	11	13	10	4				0.8	
	500	0	11	15	4	10	6				0.8	
	1581	0	8	11	13	11	3	r	r	r	0.9	
	5000	0	17	14	11	14	3	r	r	r	1.2	
(b) (4)	(b) (4)	0	55	47	38	47	9				1.0	
	50	0	37	39	29	35	5				0.8	
	158	0	25	48	44	39	12				0.8	
	500	0	41	43	43	42	1				0.9	
	1581	0	34	50	51	45	10	r	r	r	1.0	
	5000	0	41	33	50	41	9	r	r	r	0.9	
(b) (4)	(b) (4)	0	140	108	114	121	17				1.0	
	50	0	122	109	113	115	7				1.0	
	158	0	99	102	120	107	11				0.9	
	500	0	120	123	85	109	21				0.9	
	1581	0	104	102	140	115	21	r	r	r	1.0	
	5000	0	128	135	94	119	22	r	r	r	1.0	
(b) (4)	(b) (4)	0	32	51	43	42	10				1.0	
	50	0	50	44	34	43	8				1.0	
	158	0	34	43	55	44	11				1.0	
	500	0	47	41	41	43	3				1.0	
	1581	0	46	47	42	45	3	r	r	r	1.1	
	5000	0	48	39	38	42	6	r	r	r	1.0	

* Comments on the (b) (4): r = oily residue on surface of (b) (4)

† Fold response in mean revertant compared to concurrent vehicle control.

SD = Standard deviation.

Table 119: (b) (4) - (b) (4) assay in the absence of (b) (4), sponsor provided (Study no. 9601035).

Strain	Conc. (µg)(b) (4)	(b) (4)	No. of Revertants					(b) (4)	Observations *			Fold Response †
			x ₁	x ₂	x ₃	mean	SD		x ₁	x ₂	x ₃	
(b) (4)	(b) (4)	+	15	15	15	15	0					1.0
	50	+	13	15	13	14	1					0.9
	158	+	22	17	10	16	6					1.1
	500	+	20	15	22	19	4					1.3
	1581	+	20	25	29	25	5	r	r	r		1.6
	5000	+	23	17	24	21	4	r	r	r		1.4
(b) (4)	(b) (4)	+	24	15	18	19	5					1.0
	50	+	9	13	19	14	5					0.7
	158	+	17	14	15	15	2					0.8
	500	+	11	6	11	9	3					0.5
	1581	+	23	13	19	18	5	r	r	r		1.0
	5000	+	15	24	19	19	5	r	r	r		1.0
(b) (4)	(b) (4)	+	62	57	52	57	5					1.0
	50	+	56	51	51	53	3					0.9
	158	+	58	57	66	60	5					1.1
	500	+	50	51	56	52	3					0.9
	1581	+	70	56	44	57	13	r	r	r		1.0
	5000	+	42	70	52	55	14	r	r	r		1.0
(b) (4)	(b) (4)	+	178	144	128	150	26					1.0
	50	+	135	122	125	127	7					0.8
	158	+	144	156	175	158	16					1.1
	500	+	139	140	144	141	3					0.9
	1581	+	144	131	159	145	14	r	r	r		1.0
	5000	+	127	137	137	134	6	r	r	r		0.9
(b) (4)	(b) (4)	+	66	50	41	52	13					1.0
	50	+	47	47	48	47	1					0.9
	158	+	51	65	56	57	7					1.1
	500	+	50	44	47	47	3					0.9
	1581	+	57	44	46	49	7	r	r	r		0.9
	5000	+	69	69	50	63	11	r	r	r		1.2

* Comments on the (b) (4): r = oily residue on surface of (b) (4).

† Fold response in mean revertant compared to concurrent vehicle control.

SD = Standard deviation.

A Low count considered due to normal variation rather than toxicity since not clearly dose-related and not outside normal limits based on historical control values.

Table 120: (b) (4) - (b) (4) assay in the presence of (b) (4), sponsor provided (Study no. 9601035).

Conc. Strain	Treatment (µg (b) (4))	(b) (4)	No. of Revertants			Mean	SD	Fold Response ^a	Fold Response ^b
			x ₁	x ₂	x ₃				
(b) (4)	0.5	0	364	309	369	347	33	26	19
	50	0	608	398	251	419	179	35	34
	1	0	123	111	131	122	10	2.6	3.6
	0.5	0	476	546	514	512	35	4.2	5.0
	0.5	0	218	242	283	248	33	5.9	5.9
	5	+	212	222	194	209	14	14	20
	5	+	153	161	141	152	10	8.0	11
	5	+	566	673	557	599	65	11	12
	5	+	1193	1232	1162	1196	35	8.0	9.9
	20	+	156	169	185	170	15	3.2	4.1

a. Fold response in mean revertant compared to concurrent vehicle control (b) (4).

b. Fold response in mean revertant compared to concurrent vehicle control.

SD = Standard deviation.

Table 121: Positive controls for the (b) (4) assay, sponsor provided (Study no. 9601035).

Conclusion

Following exposure to the test items, in the absence or presence of (b) (4), no substantial increases in the revertant (b) (4) were reported in any strain. Thus, it is concluded that (b) (4) (PEG 2K-DMG) and (b) (4) did not show any evidence of genotoxic activity in this *in vitro* mutagenicity assay.

Study number 4: (b) (4) (PEG 2K-DMG) and (b) (4) Test in Human Peripheral Blood Lymphocytes. Study number: 9601036.

Study design:

In this study, human peripheral blood lymphocytes were treated with the test items at a range of concentrations from 3.25 to 500 µg/mL for 4 hours in the absence and presence of a (b) (4), and continuously for 24 hours in the absence of (b) (4) activation. For each regime, appropriate concurrent vehicle and positive controls were included.

Table of experimental design

Dose No.	Formulation Conc. (µg/mL)	Final Conc. (µg/mL)	No. of Cultures		
			4 Hours (b) (4)	4 Hours (b) (4)	24 Hours (b) (4)
Vehicle*	-	-	2	2	2
1/ Test item	325	3.25	2	2	2
2/ Test item	568	5.68	2	2	2
3/ Test item	995	9.95	2	2	2
4/ Test item	1740	17.4	2	2	2
5/ Test item	3050	30.5	2	2	2
6/ Test item	5330	53.3	2	2	2

Dose No.	Formulation Conc. (µg/mL)	Final Conc. (µg/mL)	No. of Cultures		
			4 Hours (b) (4)	4 Hours (b) (4)	24 Hours (b) (4)
7/ Test item	9330	93.3	2	2	2
8/ Test item	16300	163	2	2	2
9/ Test item	28600	286	2	2	2
10/ Test item	50000	500	2	2	2
(b) (4)	30	0.30	2	-	-
	45	0.45	2	-	-
	60	0.60	2	-	-
(b) (4)	500	5.0	-	2	-
	1000	10	-	2	-
	1500	15	-	2	-
(b) (4)	3.5	0.035	-	-	2
	5.0	0.050	-	-	2
	7.0	0.070	-	-	2

* Vehicle for test item (b) (4) (PEG 2K-DMG) is (b) (4) and for (b) (4) is (b) (4).

Table 122: Experimental design, sponsor provided (Study no. 9601036).

Test items

Identification: (b) (4) (PEG 2K-DMG)

Batch (Lot) No.: (b) (4)

Retest Date: 8 Sept 2015

Receipt Date: 23 Jan 2015

Physical Description: White powder Molecular

Weight: (b) (4)

Purity: 97%; dose calculations were corrected for purity

Supplier: (b) (4)

Storage conditions: (b) (4)

Appearance of Formulation: Clear colorless solution (stock solution)

Identification: (b) (4)

Batch (Lot) No.: (b) (4)

Retest Date: Oct 2015

Receipt Date: 23 Jan 2015

Physical Description: Colorless oil, without foreign matter

Molecular Weight: (b) (4)

Purity: 93.9%, dose calculations were corrected for purity

Correction Factor: A correction factor of (b) (4) was used

Supplier: (b) (4)

Storage Conditions: (b) (4)

Appearance of Formulation: Clear colorless solution (stock solution)

Reference items

Vehicle controls

Vehicle for test item (b) (4) (PEG 2K-DMG)

Identification: (b) (4)

Supplier: Sigma-(b) (4)

Batch/Lot No.: (b) (4)

Expiration Date: May 2016

Physical description: Clear colorless liquid

Purity: 100%; dose calculations were not corrected for purity

Storage Conditions: (b) (4)

Vehicle for test item (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Batch/Lot No.: (b) (4)

Expiration Date: 31 Oct 2015

Physical Description: Clear colorless liquid

Purity: 100%; dose calculations were not corrected for purity

Storage Conditions: (b) (4)

Positive controls

In the absence of (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Batch (Lot) No.: (b) (4)

Expiration Date: Aug 2016

Physical Description: Grey powder

Purity: Dose calculations were not corrected for purity

Storage Conditions: (b) (4)

Vehicle: (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Batch (Lot) No.: (b) (4)

Expiration date: Aug 2015

Physical description: Liquid

Concentration: 10 µg/mL

Storage Conditions: (b) (4)

Vehicle: (b) (4)

In the presence of (b) (4)

Identification: (b) (4)

Supplier: (b) (4)

Batch (Lot) No.: (b) (4)

Expiration date: Mar 2015

Physical Description: White powder

Purity: 102.3%; dose calculations were not corrected for purity

Storage conditions: (b) (4)

Vehicle: (b) (4)

Results

(b) (4) assessment

The vehicle control results were within the laboratory negative historical control range and the positive controls for (b) (4) produced substantial increases in the incidence of (b) (4) (at least twice) compared with the concurrent vehicle controls.

At any experimental point, (b) (4) (PEG 2K-DMG) and (b) (4) did not cause any substantial increases in the proportion of (b) (4) (see tables below). None of the treatment groups produced an incidence of (b) (4) in excess of the upper 99% reported limit for the laboratory negative historical control range.

Treatment	Conc. (µg/mL)	Average (b) (4)	(%) (b) (4)	Total No. of (b) (4) Examined	% (b) (4)
<i>4 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	2.0	0.0	2000	0.50
(b) (4)	163	2.0	0.6	2000	0.50
	286	1.9	5.8	2000	0.55
	500	2.0	-3.7	2000	0.85
(b) (4)	0.45	1.4	56.5	2000	12.50*
<i>4 hours treatment in the presence of (b) (4)</i>					
(b) (4)	-	1.8	0.0	2000	0.20
(b) (4)	163	2.0	-20.4	2000	0.35
	286	1.9	-9.8	2000	0.50
	500	1.9	-8.5	2000	0.55
(b) (4)	10	1.6	26.2	2000	3.65*
<i>24 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	1.7	0.0	2000	0.20
(b) (4)	53.3	1.6	9.3	2000	0.40
	93.3	1.4	45.1	2000	0.20
	163	1.3	60.8	2000	0.60
(b) (4)	0.035	1.9	-28.0	2000	3.55*

¹ (b) (4)

² Relative to the vehicle control.

³ (b) (4)

* = Substantial increase compared to concurrent vehicle control.

Table 123: (b) (4) (PEG 2K-DMG), sponsor provided (Study no. 9601036).

Treatment	Conc. (µg/mL)	Average (b) (4)	(%) (b) (4)	Total No. of (b) (4) Examined	% (b) (4)
<i>4 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	2.0	0.0	2000	0.35

Treatment	Conc. (µg/mL)	Average (b) (4)	(%) (b) (4)	Total No. of (b) (4) Examined	% (b) (4)
(b) (4)	163	2.0	-5.9	2000	0.25
	286	1.9	0.5	2000	0.20
	500	1.9	5.2	2000	0.40
(b) (4)	0.45	1.6	41.5	2000	19.30*
<i>4 hours treatment in the presence of (b) (4)</i>					
(b) (4)	-	1.8	0.0	2000	0.50
(b) (4)	163	1.9	-4.8	2000	0.50
	286	1.9	-6.2	2000	0.30
	500	1.8	-1.5	2000	0.35
(b) (4)	10	1.5	35.8	2000	3.80*
<i>24 hours treatment in the absence of (b) (4)</i>					
(b) (4)	-	1.9	0.0	2000	0.25
(b) (4)	163	1.8	3.9	2000	0.50
	286	1.8	10.2	2000	0.25
	500	1.7	17.8	2000	0.50
(b) (4)	0.035	1.7	17.7	2000	4.75*

¹ (b) (4)

² Relative to the vehicle control.

³ (b) (4)

(b) (4)

* = Substantial increase compared to concurrent vehicle control.

Table 124: (b) (4) summary results, sponsor provided (Study no. 9601036).

Incidental observations

No precipitation was reported at the end of treatment with test item (b) (4) (PEG 2K-DMG). At 500 µg/mL concentration, a change in (b) (4) was reported at harvest in both 4-hour treatment regimes, in the absence and presence of (b) (4). At the end of treatment and at harvest, (b) (4) was reported at concentrations ≥ 286 µg/mL in the 24-hour treatment. The (b) (4) is possibly indicative of (b) (4).

No precipitation was reported at the end of treatment for test item (b) (4). Cloudy medium was reported in the absence of (b) (4) at concentrations ≥ 93.3 µg/mL at the end of treatment in both the 4-hour and the 24-hour regimes, and in the 4-hour regime in the presence of (b) (4) at concentrations ≥ 286 µg/mL at the end of treatment.

Conclusion

In conclusion, (b) (4) (PEG 2K-DMG) and (b) (4) did not (b) (4) in human peripheral blood lymphocytes, with or without an (b) (4).

Overall conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues reported in this BLA.

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