

**Toxicology Review of Respiratory Syncytial Virus mRNA 1345 Vaccine
(Final Report)**

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File: BLA 125796, original submission

Product: Respiratory Syncytial Virus Vaccine

Reviewer: Nabil Al-Humadi
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
4.2.3.7 Other toxicity studies

Type and date of submission: Original, June 29th, 2023

Sponsor: Moderna Tx, Inc. (b) (4)

Proposed indication: Active immunization for the prevention of lower respiratory tract disease (LRTD) (b) (4) caused by respiratory syncytial virus (RSV) in adults 60 years of age and older.

Division name: OVRD/DVRPA

Proprietary name: mRESVIA (em res' vee ah)

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Introduction:

RSV, a member of the *Orthopneumovirus* genus, family *Pneumoviridae*, order *Mononegavirales*, is an enveloped virus with a single-stranded negative-sense RNA genome of 15.2kb. It is a major cause of respiratory infection in both infants and older adults. RSV infection follows a seasonal pattern causing illness primarily in the cooler months of the year in temperate regions and during the wet season in tropical countries with seasonal rainfall (1). RSV has 2 subgroups, A and B (either can cause severe disease), which co-circulate.

In infants, less than 1 year of age, respiratory syncytial virus (RSV) is the leading cause of hospitalization. Globally, RSV causes more than 34 million new acute respiratory illness and up to 200,000 deaths each year (2). In immunosuppressed children, immunosuppressed adults, and elderly, RSV causes severe respiratory illness (3-5).

RSV is transmitted primarily via aerosolized droplets from the sneeze, cough, or breath of an infected person, or via contamination of environmental surfaces with infectious secretions. Viral replication ensues following the introduction of RSV into the nose or upper respiratory tract. This replication is primarily in the ciliated cells of the respiratory epithelium, causing an irrepressible local immune response dictated by viral antigen load. It induces a narrowing of the airways through immune cell infiltrates, epithelial desquamation, mucus production, and bronchiolar edema (6). Also, it causes an increase in resistance, characterizing the acute bronchiolitis observed in infants (7). Within several days of RSV infection, upper respiratory symptoms typically begin. While the infection may remain confined to the upper respiratory tract, the virus may descend to the lower respiratory tract in infants, young children, the immune compromised, and older adults, leading to wheezing, bronchiolitis and potentially hospitalization, mechanical ventilation, and even death.

The G and F glycoproteins of the envelope are the target antigens of protective neutralizing antibodies. However, the F protein is more conserved across strains and antigenic subgroups (RSV A and RSV B). In infants who are at high risk, passive immunotherapy with palivizumab successfully prevented RSV-related LRTI. High titers of neutralizing antibody targeting the RSV F glycoprotein are sufficient for protection against severe disease despite being RSV naïve.

The Sponsor is using its mRNA-based platform to develop mRNA-1345, a novel LNP-encapsulated mRNA-based vaccine, against RSV. mRNA vaccines are being evaluated for their ability to induce immune responses against infectious pathogens including (b) (4)

and the 2019 novel coronavirus (SARS-CoV-2; NCT04283461).

Summary of previous repeat dose toxicity study with similar test article:

The previous rat repeat-dose toxicity profile reported in the GLP studies at IM doses ranging from 9 to 150 µg/dose administered once every 2 weeks for up to 6 weeks is generally consistent and considered to be representative of mRNA vaccines formulated in the same SM-102 LNP matrix, differing only by the encapsulated mRNA sequence(s).

In this study all doses administered were tolerated. Test article-related in-life observations reported at ≥ 9 $\mu\text{g}/\text{dose}$ included reversible or reversing erythema and edema at the injection site. Also, transient increases in body temperature at 6 hours post-dose returning to baseline 24 hours post-dose were reported. The lowest NOAEL determined across the aggregate of the completed studies was 89 $\mu\text{g}/\text{dose}$.

At ≥ 9 $\mu\text{g}/\text{dose}$, test article-related, generally dose-dependent, clinical pathology changes were reported. Hematology changes included increases in white blood cells, neutrophils, and eosinophils and decreased lymphocytes levels. Coagulation changes included increases in fibrinogen and activated partial thromboplastin time. Clinical chemistry changes included decreases in albumin, increases in globulin, and a corresponding decrease in albumin/globulin ratio. By the end of the 2-week recovery period, clinical pathology changes generally reversed or were reversing. At ≥ 9 $\mu\text{g}/\text{dose}$, test article-related transient cytokine increases were reported at 6 hours post-dose including interferon γ protein, monocyte chemoattractant protein, and macrophage inflammatory protein alpha. By the end of the 2-week recovery period, cytokine changes were generally reversing.

At ≥ 9 $\mu\text{g}/\text{dose}$, postmortem test article-related and generally dose-dependent changes in organ weights and macroscopic and microscopic findings were reported. Spleen, liver, and adrenal gland weight increases were reported. By the end of the 2-week recovery period, organ weight changes were generally reversing. Macroscopic changes included skin thickening at the injection site and enlarged lymph nodes. By the end of the 2-week recovery period, injection site changes completely recovered, and lymph node changes were recovering. Microscopic changes included mixed cell inflammation at the injection site; increased cellularity and mixed cell inflammation in the inguinal, iliac, and popliteal lymph nodes; decreased cellularity in the splenic periarteriolar lymphoid sheath; increased myeloid cellularity in the bone marrow; and hepatocyte vacuolation and Kupffer cell hypertrophy in the liver. By the end of the 2-week recovery period, microscopic changes were generally reversing.

Genotoxicity and mutagenicity

SM-102, the custom-manufactured lipid used in the mRNA-1345 LNP formulation, was evaluated in genotoxicity studies (bacterial reverse mutation [(b) (4)] test and an (b) (4) test in human peripheral blood lymphocytes) as an individual agent using a standard (b) (4) approach ((b) (4)). In the (b) (4) test and the (b) (4) test, the results were negative.

In addition, SM-102 was evaluated in a GLP-compliant (b) (4) test using an mRNA-based vaccine formulated in SM-102-containing LNPs ((b) (4)). In male rats at both 24 and 48 hours and in female rats at 48 hours only, SM-102 induced statistically significant increases in MIEs. However, there was no clear dose response, and the increases were generally weak and associated with minimal bone marrow toxicity.

Pre-IND meeting was held for this IND on January 27, 2020.

Non-clinical

Sponsor Question 3:

Does FDA agree that the proposed nonclinical package, consisting of an aggregate of previously completed GLP repeat-dose toxicology studies in rats evaluating mRNA vaccines formulated in the Sponsor's standard proprietary SM-102-containing LNP matrix would be acceptable to fulfill the 21 CFR 312.23(8) requirement for toxicity assessment for the new mRNA-1345 vaccine?

Does the agency agree that the same approach would be acceptable for future mRNA vaccines developed using the Sponsor's technology platform in the same standard proprietary LNP matrix where the only difference is the mRNA sequence(s)?

FDA response to sponsor question 3

Yes, we agree that submission of the proposed nonclinical package to your future IND [consisting of an aggregate (summary) of previously completed GLP repeat- dose toxicology studies in rats evaluating mRNA vaccines formulated in your SM- 102-containing LNP matrix] would be acceptable to allow us to make a determination if there is adequate information about the pharmacological and toxicological studies of the investigational drug on the basis of which it may concluded that it is reasonably safe to conduct the proposed clinical investigation (21 CFR 312.23(a)(8)) and to justify that no additional toxicology studies will be needed for this product for this phase 1 study. Please include a cross-reference to the IND(s) where the details of completed toxicology studies are available in the composite summary that you plan to submit to your IND. In addition, submission of the same summary information from the aggregate of previously completed nonclinical studies along with your rationale for not conducting additional toxicological studies would be acceptable for future INDs for future mRNA products using the same platform in the same LNP matrix assuming no new clinical or product issues arise that would warrant further non-clinical evaluation.

Please note that DART studies will need to be completed and submitted to support evaluation of your candidate vaccines in pregnant women. We recommend you provide the DART protocol(s) for our review and feedback prior to study initiation.

Sponsor Response	IND/MF Location
The Sponsor will proceed as proposed and provide an aggregate summary of the GLP repeat-dose toxicology studies conducted with the SM-102-containing LNP matrix in Type V Master File# (b) (4) and provide cross-reference information to the INDs where the details of the completed toxicology studies may be found in Section 2.6.6.	MF# (b) (4) Section 2.6.6
The Sponsor plans to conduct DART studies prior to evaluating mRNA-1345 in pregnant women.	

Sponsor question 4:

Does FDA agree that the Sponsor's proposed nonclinical studies will support a phase 1 clinical study in healthy adults and followed by RSV-seropositive children between 12 and 36 months of age, and specifically, that preclinical studies to address the potential risk of vaccine-enhanced respiratory disease can be conducted after the start of the phase 1 study in adults and seropositive children, but prior to start of a subsequent study in RSV seronegative children?

FDA response to sponsor question 4

Regarding the first part of your question, the proposed studies listed under “Sponsor’s Position” (including evaluation of immunogenicity in mice and rats, aggregate of previous repeat dose toxicology studies, a biodistribution study of a related mRNA vaccine ((b) (4)) and studies evaluating the genotoxic potential of SM-102) will be sufficient to support phase 1 clinical studies in healthy adults and RSV-seropositive children between 12 and 36 months of age.

Regarding the second part of your questions, preclinical studies to assess the potential risk of vaccine-enhanced respiratory disease may be completed while the phase 1 study in adults and the phase 1 study in RSV-seropositive children are ongoing and prior to the start of a subsequent study in RSV-seronegative children.

Sponsor Response	IND/MF Location
<p>The Sponsor has provided results from the following studies:</p> <ul style="list-style-type: none"> a. Immunogenicity of mRNA-1345 in mice (Study 3823-1) b. Immunogenicity and toxicity study of mRNA-1345 in rats (Study 2308-121) c. Aggregate summary of the GLP repeat-dose toxicology studies conducted with the SM-102-containing LNP matrix d. Biodistribution study of a related mRNA vaccine ((b) (4)) (Study 5002121) e. Studies evaluating the genotoxic potential of SM-102 	<p>Section 2.6.2 Section 2.6.2 and 2.6.7 MF# (b) (4)</p>
<p>The Sponsor will provide results from preclinical studies to assess the potential risk of vaccine-enhanced respiratory disease prior to the start of a subsequent study in RSV-seronegative children.</p>	N/A

Sponsor question 5:

Does FDA agree that preclinical studies in mice and (b) (4) rats demonstrating that mRNA-1345 induces (1) a high titer of neutralizing antibodies, (2) a high ratio of neutralizing to binding antibody, (3) a non-Th2-biased response, and (4) an absence of (b) (4) rat enhanced lung pathology after RSV challenge at a dose-level with suboptimal neutralizing antibody would be sufficient pre-clinical evidence to enable clinical development of mRNA-1345 in RSV- naïve infants and toddlers?

FDA response to sponsor question 5:

Data obtained from testing candidate RSV vaccines in mice and (b) (4) rats may be used to provide preliminary evidence of safety in differentiating the properties of the candidate RSV vaccine, mRNA-1345, from those seen following immunization with (b) (4) preparations. Please note that there is no requirement to use these two animal models for non-clinical testing and other animal models may be used for the comparisons outlined above. Irrespective of the animal model used for the test, experiments should be designed to include specific control groups to facilitate data interpretation as outlined in the (b) (4)

Please keep in mind the following parameters when designing your tests:

- Regarding the testing in mice as described in Section 3.2.2.2.1 and as outlined in Table 15 in the briefing package: Please include groups given live RSV intranasally as a

negative control for responses associated with enhanced disease and a comparator group given a non-RSV mRNA vaccine as a control for the manufacturing process.

- Regarding the planned evaluation of antibody responses in mice (cited above) and (b) (4) rats as described in Section 3.2.2.2.2. and as outlined in Table 16 of the briefing package please note the following: When evaluating the ratio of neutralizing antibody to binding antibody please determine the titer of anti-RSV IgG binding antibodies using RSV post-fusion F protein (b) (4).
- Regarding the planned evaluation in (b) (4) rats:
 - Please indicate why only female rats will be used for the proposed immunization and challenge study.
 - We note that the two-dose regimen will be evaluated using a series of 8, 10-fold dilutions of mRNA-1345 while single doses of mRNA-1345 will be evaluated using only one high dose [0.3ug] and one low dose [0.03ug]. For appropriate assessment of study results, you will need to show that the vaccine elicits an immune response in some animals in each group while also permitting virus breakthrough in the lung after challenge. If you do not have data to support these assumptions, please consider expanding the single dose series to achieve these goals and increase the likelihood for a successful challenge test.
- Immune complexes [RSV antigen, IgG, and complement] were observed in the lungs of the two children who died following immunization with (b) (4) and subsequent RSV infection. Immune complexes were also detected in lungs of mice immunized with (b) (4) prior to challenge but not present in lungs from mice infected with RSV prior to re-challenge. While this test is not required, please consider testing for immune complex deposition in the lungs of mice after immunization and challenge if the safety assessment of mRNA-1345 versus (b) (4) derived from other studies need additional support.

We recommend that you submit the protocols for the animal studies intended to evaluate the evidence of vaccine-associated ERD for our review and feedback prior to study initiation.

Meeting discussion for sponsor question 5:

Moderna indicated that they could submit the animal study protocol for review prior to initiation of the study. Moderna informed us that they have already planned for the start of the study on February 24, 2020, and that it would be helpful if the Agency can provide comments on their animal study protocol before February 24, 2020. Moderna committed to submit their animal-study protocol by February 03, 2020, and FDA agreed to provide feedback prior to study start date.

Post meeting note:

Moderna submitted the animal-study protocol to the FDA on February 3, 2020, and comments were provided to Moderna on February 20, 2020.

Sponsor Response	IND Location
The Sponsor will provide results from preclinical studies to assess the potential risk of vaccine-enhanced respiratory disease prior to the start of a subsequent study in RSV-seronegative children.	Section 2.4

Proposed clinical study:

The clinical study is a phase 1, randomized, observer-blind, placebo-controlled, dose-escalation study in adult and pediatric populations. Both, an internal safety team (IST) and an independent data safety monitoring board (DSMB) will oversight the study and pause rules will be implemented. This study will be initiated in healthy adults, then proceed to enroll healthy children who screen seropositive to RSV by microneutralization assay. Enrollment of the pediatric cohorts depends on the safety profile of the adult cohorts (cohorts 1, 2, and 3).

Healthy adult participants (≥ 18 to ≤ 49 years of age) will be enrolled in 4 adult cohorts and randomized via an interactive response technology (IRT). In the adult cohorts' numbers 1, 2, and 4, 3 escalating dose levels, administered in a single dose of mRNA-1345 (50 μg , 100 μg , or 200 μg) or placebo will be evaluated. In addition, an adult cohort number 3 at the middle dose level administered in a 3-dose series of mRNA-1345 (100 μg) or placebo will be evaluated. Enrolled and randomized via IRT, two pediatric cohorts of RSV-seropositive children (≥ 12 to ≤ 36 months of age) will be included. In the pediatric cohorts' numbers 5 and 6, 2 escalating dose levels of mRNA-1345 (30 μg and 100 μg) or placebo administered in a 3-dose series will be evaluated in RSV-seropositive children ≥ 12 to ≤ 36 months of age.

Adult cohorts:

Receiving 1 injection of mRNA-1345 or placebo, seventy-five adults will be randomized across 3 cohorts (25 in each of cohorts 1, 2, and 4) in a 4:1 ratio. In cohort number 3, a total of 25 adults will be randomized in a 4:1 ratio to receive 3 injections of mRNA-1345 or placebo 56 days apart (day 1, day 57, and day 113).

Adults will be enrolled in a dose-escalation fashion as follows:

Adult Cohorts	Injections ^a	N mRNA-1345	N Placebo
Cohort 1 50 μg dose level	1	20	5
Cohort 2 ^b 100 μg dose level	1	20	5
Cohort 3 ^b 100 μg dose level	3	20	5
Cohort 4 200 μg dose level	1	20	5
Total		80	20
		100	

- ^a All participants will receive an injection on day 1. Participants assigned to cohort 3 will receive the second injection on day 57 and the third injection on day 113.
- ^b Cohorts 2 and 3 will enroll in parallel.

Table 1: Summary of treatment in the adult cohorts, sponsor provided.

Pediatric cohorts:

Across 2 cohorts (30 each in cohorts 5 and 6), sixty children will be randomized in a 1:1 ratio to receive 3 injections of mRNA-1345 or placebo, 56 days apart (day 1, day 57, and day 113).

RSV-seropositive children will be enrolled in a sequential dose-escalation fashion as follows:

Pediatric Cohorts	Injections ^a	N mRNA-1345	N Placebo
Cohort 5 30 µg dose level	3	15	15
Cohort 6 100 µg dose level		15	15
Total		30	30
		60	

^a All participants will receive their first injection on day 1, second injection on day 57, and the third injection on day 113.

Table 2: Summary of treatment in the pediatric cohorts

Dosing in cohort 1 (50 µg of mRNA-1345 or placebo) will commence first, and upon review of safety data by the IST through 7 days post injection, dosing in cohorts 2 and 3 will commence in parallel. Subsequently, DSMB review of safety data through 1 month post last injection from all adult participants across the 50 µg/dose and 100 µg/dose levels will trigger enrollment for the highest adult dose level cohort (200 µg/dose, cohort 4) and the lowest pediatric dose level (30 µg/dose, cohort 5) in parallel.

RSV-seropositive children will be randomized sequentially into 2 increasing dose levels of mRNA-1345. The DSMB will review safety data through 1 month post last injection in the 30 µg/dose level pediatric cohort 5 prior to start of dosing in the 100 µg/dose level pediatric cohort 6.

Studies reviewed in this BLA:

- 1- A GLP Combined Perinatal/Postnatal Developmental and Reproductive Toxicity Study of mRNA-1345 by the Intramuscular Route in the (b) (4) Rat. Study number: 20312409.
- 2- A GLP Repeat Dose Toxicity Study of mRNA-1345 by Intramuscular Administration in (b) (4) Rats. Study number: 1021-9921.
- 3- A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1345 by Intramuscular Injection in (b) (4) Rats. Study number: 2308-121.
- 4- A 1 month (3 doses) Intramuscular Injection Vaccine Study of (b) (4) in (b) (4) Rats with a 2-Week Recovery Period (Study # 5002231) (Reviewed by Nabil).
- 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. Study number: 5002045.

- 6- 6-week Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period (Study # 5002034) (Reviewed by Joe).
- 7- 6-Week Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Followed by a 2-Week Recovery Period (Study # 5002158) (Reviewed by Joe).
- 8- 1-Month (3 doses) Intramuscular Injection Toxicity Study of (b) (4) in (b) (4) Rats Following by a 2-Week Recovery Period (Study #5002400) (Reviewed by Joe).
- 9- A 1-Month (3 doses) Study of (b) (4) by Intramuscular Injection in (b) (4) Rat with a 2-Week Recovery Period (Study no. 5002033) (Reviewed by Andrew).
- 10- T Cell Immunogenicity of mRNA-1345 in Mice. Study number: 3797-1
- 11- Immunogenicity of mRNA-1345, (b) (4) in (b) (4) Mice Relative to ERD Controls. Study number X-107.

Genotoxicology studies:

- 1- (b) (4) mRNA: (b) (4) Test in Rat (Study # 9800399) (Reviewed by Joe).
- 2- (b) (4) mRNA: (b) (4) Test in Rat. (Study no. 9800399) (Reviewed by Andrew).
- 3- SM-102 Bacterial Reverse Mutation Test in (b) (4) (Reviewed by Claudia). Study No.: 9601567
- 4- SM-102 (b) (4) Test in Human Peripheral Blood Lymphocytes (Reviewed by Claudia). Study no.: 9601568
- 5- (b) (4) mRNA in SM102-Containing Lipid Nanoparticles: (b) (4) Assay in the Rat. Study number: AF87FU.125012NGLPICH.BTL
- 6- (b) (4) (PEG 2K-DMG) and (b) (4); Bacterial Reverse Mutation Test in (b) (4). Study No.: 9601035
- 7- (b) (4) (PEG 2K-DMG) and (b) (4); In Vitro Mammalian Cell Micronucleus Test in Human Peripheral Blood Lymphocytes. Study no.: 9601036

Toxicology study reviews

Study number 1: A GLP Combined Perinatal/Postnatal Developmental and Reproductive Toxicity Study of mRNA-1345 by the Intramuscular Route in the (b) (4) Rat.
Study number: 20312409.

Performing laboratory: (b) (4)

Study initiation date: August 20, 2021

Final report date: May 12, 2022

Test article batch/lot:

	Test Article
Identification:	mRNA-1345
Batch/Lot No.:	DHM-72202
Expiration Date:	08 Jul 2022
Physical Description:	White to off-white liquid (dispersion)

	Test Article
Supplies Stock Concentration	0.482 mg/mL ^a
Density:	Assumed 1 g/mL
Storage Conditions (temperature set to maintain):	-20°C (Frozen), protected from light, protected from moisture
Provided by:	Sponsor

^a Test article was prepared using actual concentration of 0.482 mg/mL.

Table 3: Test article identification (Study no. 20312409).

	Control Article
Identification:	20 mM Tris, 87 g/L Sucrose, (b) (4) mM Sodium Acetate, (b) (4)
Alternate Identification:	DHM-73733, mRNA-1345 Diluent Buffer
Batch/Lot No.:	DHM-73733
Expiration Date:	02 Feb 2022
Physical Description:	Clear, colorless liquid
Density:	Assumed 1 g/mL
Storage Conditions (temperature set to maintain):	(b) (4)
Provided by:	Sponsor

Table 4: Control article identification (Study no. 20312409).

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

Breeder/supplier: (b) (4)

Number of animals per group and sex: 44 females/group

Age: 70 days

Body weight range: 210 – 254 grams

Route and site of administration: Intramuscular (IM) injection into the quadriceps on the hindlimbs; alternating on each dosing occasion.

Volume of injection: 0.2 mL

Frequency of administration and study duration: Administered by intramuscular (IM) injection during the premating period (28 and 14 days prior to mating) and on gestation days (GDs) 1 and 13.

Dose: 0 or 96 µg/dose

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. It has been stated by the sponsor that “For the time periods covered by the parameters of this study, homogeneity and stability were demonstrated for all samples related to bulk test article, control article, test article formulation, and antibody analysis”. It has also been stated that “Stability of the test article formulations will not be determined in this study”.

Means of administration: Intramuscular (IM)

Report status: Final report

Experimental design:

Animals were randomized and assigned to 2 different groups. Animals, 44 females/group, were dosed by intramuscular (IM) injection during the premating period (28 and 14 days prior to mating) and on gestation days (GDs) 1 and 13. The dose levels were 0 and 96 µg/dose and administered at a dose volume of 0.2 mL/dose. The details of the study design are listed in the following table:

Group No.	Test Material	Dose Level (µg/dose)	Dose Concentration (mg/mL)	Fixed Dose Volume (µL/dose)	No. of Females ^a	
					Cohort 1 (Caesarean-Sectioning Phase)	Cohort 2 (Natural Delivery Phase)
1	Control Article ^b	0	0	200	22	22
2	mRNA-1345	96	0.482	200	22	22

^a Cohort 1 females were allocated to the Caesarean-sectioning phase. Cohort 2 females were assigned to the natural delivery (parturition) phase.

^b 20 mM Tris, 87 g/L Sucrose, (b) (4) mM Sodium Acetate, (b) (4).

Table 5: Experimental design (Study no. 20312409).

F0 generation females assigned to cohort 1 were Caesarean sectioned on GD 21 and those assigned to cohort 2 were allowed to naturally deliver their litters after completion of the 21-day postpartum period. All F0 generation rats were euthanized on GD 21 (cohort 1) or lactation day (LD) 21 (cohort 2). All F1 generation pups assigned to cohort 2 were euthanized on day 13 or 21 postpartum.

Methods:

Randomization procedure: Yes

Statistical analysis plan: Yes.

The following parameters were evaluated:

Parameter	Population	Frequency (minimum required)	Comments
Viability	All animals	<ul style="list-style-type: none"> At least twice daily 	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Clinical Observations: General	All animals	<ul style="list-style-type: none"> At least once weekly during the acclimation period Before each dose is administered Daily on each non-dose administration day (except 24-hour postdose) Daily beginning on GD 0 through the day of euthanasia 	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.

Parameter	Population	Frequency (minimum required)	Comments
Clinical Observations: Postdose Observations	F0 females	<ul style="list-style-type: none"> Approximately 6 hours and 24 hours post-dose) on dosing days 	Animals will be removed from their cages for observations. Time intervals for postdose observations may be adjusted if deemed appropriate by the study director during the course of the study. Such adjustments will be documented in the raw data.
Maternal Observations	•	•Daily during the postpartum period (cohort 2).	Maternal observations will be recorded.
Individual Body Weights	□	<ul style="list-style-type: none"> On the day after arrival and at least once weekly during the premating dose period, including on the days of dosing On SDs 1, 8, 15, 22, and 28 On GDs 0, 3, 6, 9, 12, 15, 18, 21, and 25 (if necessary) Lactation Day (LD) 1, 4, 7, 10, 14, 18, and 21 (Cohort 2) 	Animals may be weighed more often, if necessary, to monitor health status.
Food Consumption	•	<ul style="list-style-type: none"> Once weekly during the premating dose period until cohabitation. On GDs 0, 3, 6, 9, 12, 15, 18, 21, and 25 (if necessary) On LDs 1, 4, 7, 10, and 14 	<p>Food consumption will be quantitatively measured per cage, where appropriate. During cohabitation, when two rats occupy the same nesting box with one food jar, replenishment of the food jars will be documented. Individual values will not be recorded or tabulated.</p> <p>Food consumption will not be tabulated after day 14 postpartum, when it is expected that pups will begin to consume maternal food.</p>

Parameter	Population	Frequency (minimum required)	Comments
Estrous Cycle Evaluations	•	<ul style="list-style-type: none"> 14 consecutive days before the initiation of cohabitation and then until spermatozoa are observed in a smear of the vaginal contents and/or a copulatory plug is observed in situ during the cohabitation period 	Estrous cycles will be evaluated by examining the vaginal cytology of samples obtained by vaginal lavage
Cohabitation/Mating	□	<ul style="list-style-type: none"> Maximum of 7 days 	Within each dose group, rats will be assigned to cohabitation (i.e., pairing), one breeder male per one female. The cohabitation period will consist of a maximum of 7 days. Females observed with spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed in situ will be at GD 0 and assigned to individual housing. Females not mated after completion of the 7-day cohabitation period will be considered to be at GD 0 on the last day of cohabitation, assigned to individual housing and will be euthanized 25 days after the end of the cohabitation period (for rats that do not deliver a litter) or continued on study to be assigned to cohort 2 as needed (for rats that do deliver a litter) at the discretion of the study director.
Natural Delivery Observations		<p>Female rats will be evaluated for:</p> <ul style="list-style-type: none"> Adverse clinical signs observed Duration of gestation (GD 0 to the time the first pup is observed) Litter size (defined as all pups delivered) Pup viability at birth 	-

Table 6: In-life procedures, observations, and measurements for F0 generation, sponsor provided (Study no. 20312409).

Parameter	Frequency (minimum required)	Comments
Viability	Litters will be observed for dead pups at least twice daily and the pups in each litter will be counted at least once daily	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Clinical Observations: General Appearance	<ul style="list-style-type: none"> At least once daily. 	Clinical observations may be recorded more frequently than cited. Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Individual Body Weights	PND 1, 4, 7, 10, 14, 18, and 21 postpartum.	Animals may be weighed more often, if necessary, to monitor health status.
Reflex and Physical Development:	<ul style="list-style-type: none"> Pinna unfolding beginning on PND 1 (until both unfolded) Hair growth beginning on PND 7 Eye opening beginning on PND 12 (until both open) Auditory startle reflex beginning on PND 13 Surface righting reflex beginning on PND 1 (ability to right in 5 seconds) 	Pups will be evaluated daily until criterion is achieved or until scheduled euthanasia. A pup that reaches the criteria will no longer be tested.
	<ul style="list-style-type: none"> Pupil constriction on PND 21. 	Evaluated once.

PND = Postnatal day

Table 7: In-life procedures, observations, and measurements for F1 generation (cohort 2), sponsor provided (Study no. 20312409).

Group Nos.	Cohort	Time Points					
		SD 1 ^a	SD 15 ^a	GD 1 ^a	GD 13 ^a	GD 21 ^b	LD 21 ^b
1-2	1	X	X	X	X	X	-
1-2	2	X	X	X	X		X
Unscheduled euthanasia (when possible) and dams with no surviving pups		X					
Method/Comments:		Jugular vein (SD 1, SD 15, GD 1 and GD 13 in-life blood collections) or via the vena cava while under isoflurane/oxygen anesthesia (GD 21 and LD 21 terminal blood collections). If necessary, in-life blood samples may be collected from an alternate site (lateral tail vein); if so, the alternate site will be documented in the raw data. Additional blood samples may be obtained (e.g. due to sample quality) if permissible sampling frequency and blood volume are not exceeded. Blood will be collected from unscheduled euthanized animals, when possible.					
Target Blood Volume (mL):		1.2 mL					
Anticoagulant:		None, in SST					
Special Requirements:		None					
Processing:		Serum					

X = Sample to be collected; - = Not applicable, SST = Serum separator tube, SD = Study day; GD = Gestation day; LD = Lactation day

^a Sample collected prior to dose administration.

^b Terminal blood sample collection.

Table 8: Antibody serum sample collection; Maternal serum samples (cohorts 1 and 2), sponsor provided (Study no. 20312409)

Terminal procedures are summarized in the following tables:

Group No.	Scheduled Euthanasia Day	Necropsy Procedures				Histology Processing and Microscopic Evaluation	Fetal Evaluations
		Ovarian/ Uterine Examination	Necropsy	Tissue Collection ^a	Organ Weights		
1	GD 21	X	X	X	X ^b	-	X
2		X	X	X	-	-	-
Unscheduled Deaths		X	X	X	-	-	-

X = Procedure to be conducted; - = Not applicable; GD = Gestation day

^a See tissue collection and evaluation table – F0 generation scheduled euthanasia, attachment A and tissue collection and evaluation table – F0 and F1 generation – unscheduled euthanasia, attachment B for list of tissues applicable to each procedure.

^b The gravid uterus and the placentae will be weighed for all rats that survive to scheduled euthanasia.

Table 9: Necropsy procedures for F0 generation females - cohort1, sponsor provided (Study no. 20312409)

Group No.	Scheduled Euthanasia Day	Necropsy Procedures				Histology Processing and Microscopic Evaluation
		Ovarian/ Uterine Examination	Necropsy	Tissue Collection ^a	Organ Weights	
1	LD 21	X	X	X	-	-
2						
Unscheduled Deaths		X	X	X	-	-
Dams that did Not Deliver	GD 25	X	X	X	-	-
Dams with No Surviving Pups	b	X	X	X	-	-

X = Procedure to be conducted; - = Not applicable; GD = Gestation Day; LD = Lactation Day

^a See tissue collection and evaluation table– F0 generation scheduled euthanasia, attachment A and tissue collection and evaluation table – F0 and F1 generation – unscheduled euthanasia, attachment B for list of tissues applicable to each procedure.

^b On the day the observation is made.

Table 10: Necropsy procedures for F0 generation females-cohort 2, sponsor provided (Study no. 20312409)

Ovarian and uterine examinations

Cohort 1

The reproductive tract will be dissected from the abdominal cavity. The gravid uterus will be weighed. The uterus will be opened, and the contents will be examined. The fetuses will be removed from the uterus and placed in individual containers. Each placenta will be weighed. The following will be examined and/or recorded:

- 1- Corpora lutea
- 2- Implantation sites
- 3- Placentae (size, color or shape) – any abnormalities will be recorded

- 4- Live and dead fetuses
- 5- Early and late resorptions

Cohort 2

The reproductive tract will be dissected from the abdominal cavity. The number and distribution of implantation sites will be recorded.

Fetal examinations for cohort 1

Representative photographs of external, visceral and skeletal abnormalities will be taken for illustration of, or consultation on, observations (unless otherwise requested by the study director). Photographs will not be included in the report but will be retained as electronic images and archived with the raw data. Abnormalities will be classified as malformations, variations, or incidental.

Examination	Procedure
Aborted/Conceptuses <i>in utero</i>/Delivered Conceptuses	Examined for external, visceral, and/or skeletal abnormalities to the extent possible
Late Resorptions	Examined for external abnormalities to the extent possible, and discarded without further examination
Dead Fetuses	Examined for external, visceral, and/or skeletal abnormalities to the extent possible
Body Weights	Recorded for each live fetus.
Sex	Each fetus will be externally sexed.
External	All fetuses will be examined for external abnormalities.
Visceral	Approximately one-half of the fetuses in each litter will be examined for visceral abnormalities by using a modification of the microdissection technique of Staples. Each fetus will be fixed in Bouin's solution, and the heads will subsequently be examined by free-hand sectioning. The head slices of normal fetuses will be discarded following examination; head slices from fetuses with observations will be retained in 70% ethanol. The decapitated carcasses will not be retained.
Skeletal	The remaining fetuses (approximately one-half of the fetuses in each litter) will be retained in alcohol and examined for skeletal abnormalities after staining with alizarin red S. Following examination, skeletal preparations will be retained in glycerin with thymol added as a preservative.

Table 11: Fetal examinations for cohort 1, sponsor provided (Study no. 20312409)

Postmortem procedures:

Tissue	Weigh ^a	Collect	Histology	Microscopic Evaluation ^{b,c}	Comment
Artery, aorta	-	X	X	X	-
Bone marrow smear	-	X	-	-	Two bone marrow smears will be collected from the femur 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 are allowed to air dry and are not fixed in (b) (4).
Bone marrow	-	X	X	X	-
Bone, femur	-	X	X	X	-
Bone, sternum	-	X	X	X	-
Brain	X	X	X	X	Retain olfactory lobes with head.
Epididymides	X	X	X	X	Paired examination.
Esophagus	-	X	X	X	-
Eyes	-	X	X	X	Paired examination; Preserve in (b) (4) fixative. Retain with optic nerve.
Gland, adrenal	X	X	X	X	Paired examination.
Gland, harderian	-	X	X	X	Only 1 required for microscopic examination.
Gland, mammary	-	X	X	X	For males, examine only if present in routine section of skin.
Gland, parathyroid	-	X	X	X	Examine only if present in the routine section of thyroid.
Gland, pituitary	X	X	X	X	-
Gland, prostate	X	X	X	X	-
Gland, salivary	-	X	X	X	Only 1 required for microscopic examination.
Gland, seminal vesicle	-	X	X	X	Paired examination.
Gland, thyroid	X	X	X	X	Paired examination
Gut-associated lymphoid tissue	-	X	X	X	Examine only if present in routine section of intestine.
Heart	X	X	X	X	-
Joint, femorotibial	-	X	-	-	-
Kidneys	X	X	X	X	Paired examination.
Large intestine, cecum	-	X	X	X	-
Large intestine, colon	-	X	X	X	-
Large intestine, rectum	-	X	X	X	-
Larynx	-	X	X	X	Collect but do not prepare slide and do not examine.
Liver	X	X	X	X	-
Lungs	X	X	X	X	-
Lymph node, mandibular	-	X	X	X	Only 1 required for examination.
Lymph node, mesenteric	-	X	X	X	-

Tissue	Weigh ^a	Collect	Histology	Microscopic Evaluation ^{b,c}	Comment
Gross Lesions	-	X	X	X	-
Muscle, skeletal	-	X	X	X	-
Nerves, optic	-	X	X	X	Examine only if present in the routine section of the eye. Preserve in (b) (4) fixative. Retain with eyes.
Nerve, sciatic	-	X	X	X	Only 1 required for microscopic examination
Nerve, tibial	-	X	-	-	-
Ovaries	X	X	X	X	Paired examination.
Pancreas	-	X	X	X	-
Site, administration	-	X	X	X	Appropriate sites for collection based on dosing regimen and route of administration.
Skin	-	X	X	X	Inguinal area
Small intestine, duodenum	-	X	X	X	-
Small intestine, ileum	-	X	X	X	-
Small intestine, jejunum	-	X	X	X	-
Spinal cord	-	X	X	X	Examine one transverse and one longitudinal section from each of the following areas cranial cervical, mid-thoracic, lumbar (intumescence)
Spleen	X	X	X	X	-
Stomach	-	X	X	X	-
Testes	X	X	X	X	Paired examination; Preserve in (b) (4) fixative.
Thymus	X	X	X	X	-
Tongue	-	X	X	X	-
Trachea	-	X	X	X	-
Urinary bladder	-	X	X	X	-
Uterus/cervix	X	X	X	X	-
Vagina	-	X	X	X	-

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

^a Paired organs will be weighed together

^b At the discretion of the study pathologist, findings for extraneous tissues (non-protocol tissues that may be present on a slide as a result of collection of protocol tissues) will be recorded when observed.

^c Efforts will be made to evaluate all protocol-required tissues microscopically; however, it is not always feasible for every protocol-required tissue to be present on every slide. Protocol-required tissues that are not examined will be documented in the histopathology data and the impact of these missing tissues on the study will be documented in the pathology report.

Table 12: Tissue collection and preservation – F0 generation, sponsor provided (Study no. 20312409)

Tissue	Weigh	Collect	Comment
Gross lesions/masses	-	X	All animals.
Heart	-	X	Animals found dead or euthanized before scheduled termination.
Kidneys	-	X	Animals found dead or euthanized before scheduled termination.
Liver	-	X	Animals found dead or euthanized before scheduled termination. .

Tissue	Weigh	Collect	Comment
Lungs	-	X	Animals found dead or euthanized before scheduled termination.
Spleen	-	X	Animals found dead or euthanized before scheduled termination.
Stomach	-	X	Animals found dead or euthanized before scheduled termination.

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

Table 13: Tissue collection and preservation – F1 generation, sponsor provided (Study no. 20312409)

Results:

All F0 generation rats assigned to cohorts 1 and 2 survived to scheduled euthanasia.

Maternal clinical observations

Premating

In group 2, primarily injection site reactions that were considered secondary to the route of administration of the test article, were reported. These reactions included limited usage of the hindlimbs, swollen hindlimbs, skin scabbing most notably along the left and/or right hindlimb and thin fur cover along the left and/or right forelimb and/or forepaw. Limited usage of and swollen hindlimbs were the most frequently reported injection site reactions and both findings were reported in all females during the premating period. Limited usage of one or both hindlimbs was reported on one or more occasion between SD 2 and SD 18 and swollen hindlimbs (left and/or right) were reported on one or more occasion between SD 1 and the end of the premating period.

Gestation

In group 2, primarily injection site reactions that were considered secondary to the route of administration of the test article, were reported. These reactions included limited usage of the hindlimbs, swollen hindlimbs, skin scabbing most notably along the left and/or right hindlimb and thin fur cover along the left and/or right forelimb and/or forepaw. In addition, there was a low incidence of lack of pinch reflex (females 2501 and 2543) and a skin abrasion on the hindlimb (females 2504 and 2516) in females that also had limited usage of the hindlimbs and swollen hindlimbs.

Lactation

No test article-related clinical observations during the lactation period were reported.

Body weights and body weight gains

Premating

No test article-related effect on body weight were reported.

In group 2, mean body weight gain was 86.3% and 75.3% of controls for the intervals of study day (SD) 1 to 8 and SD 15 to 22, respectively. Mean body weight gain in group 2 was similar to or higher than the control group during non-dosing weekly intervals (123.4% to 216.7% of controls) and for the overall premating period of 104.7% of controls for the interval of SD 1 to

28 (104.7% of controls). The reductions in mean body weight gain during the pre-mating period might be interrelated to localized effects at the injection site (e.g., swollen, and limited usage of the hindlimbs) reported during the same interval. All other differences, regardless of statistical significance, were considered unrelated to test article because the observations were transient and did not persist.

Gestation

No test article-related effects on mean maternal body weight, adjusted body weight, or mean gravid uterine weight during the gestation period were reported.

Test article-related reductions in mean maternal body weight gain were reported at each tabulated interval from GD 0 to GD 9 (73.7% to 88.9% of controls). Statistical significance ($p \leq 0.01$) was achieved for the interval of GD 0 to 3. Mean maternal body weight gain was similar to or higher than controls for the remainder of the gestation period and for the overall interval of GD 0 to 21 (99.1% of controls). Mean adjusted body weight gain was significantly ($p \leq 0.05$) reduced (81.7% of controls). This reduction might be interrelated to localized effects at the injection site (e.g., swollen and limited usage of the hindlimbs) reported during the same interval. In group 2, mean gravid uterine weight was significantly ($p \leq 0.05$) increased (110.3% of controls). This increase was considered secondary to an increase in the mean number of fetuses and live fetuses that occurred in this group. The increase was not considered related to mRNA-1345 because a similar increase in the number of live pups delivered did not occur in the dams allowed to naturally delivery their litters. The increase represented normal variation in litter size that is found in this strain of rat (historical control data).

All other differences, regardless of statistical significance, were considered unrelated to test article treatment because the observation was transient and did not persist.

Lactation

No test article-related effect on mean maternal body weight during the lactation period were reported. Mean maternal body weight gain was 74.2% of controls for the interval of lactation day (LD) 1 to 21. Although not statistically significant, mean maternal body weight gain was less than that of the control animals during some intervals (LD 1 to 4: -31.9%; LD 4 to 7: -24.7%), and significantly greater than control animals at other intervals (LD 7 to 10: +201.9%). There was a loss of mean maternal body weight gain of -8.3 g and -7.2 g in group 2 when compared with a loss of -5.5 g and -1.6 g in group 1 for the intervals of LD 14 to 18 and LD 18 to 21, respectively.

Food consumption

Premating

No test article-related effect on food consumption were reported.

Gestation

In group 2, reductions in mean maternal food consumption for the interval of gestation day (GD) 0 to 3 ($p \leq 0.01$; 8.8% of controls) were reported.

Lactation

In group 2, mean maternal food consumption was 95.3% of controls for the interval of LD 1 to 14. Mean maternal food consumption was reduced at each tabulated interval (91.4% to 92.7% of controls) from LD 1 to 14 (exception LD 7 to 10). These reductions corresponded to reductions in mean maternal body weight gain at the same intervals.

Estrous cycle evaluation, mating, and fertility

In group 2, the mean number of days to mating was statistically significantly ($p \leq 0.01$) increased (3.0 days) when compared with group 1 (2.2 days). Because the difference was within one estrus cycle (approximately 4.2 days) with no effect on estrous cycling or fertility, this increase was not considered adverse. The mean number of estrous cycles (2.2 days) and mean cycle length (4.21 days) in group 2 was similar to group 1. Similarly, mating and fertility indices (97.7%) and pregnancy indices (95.5%) were similar to group 1.

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Pairing (Litter: A)		Group 1	Group 2
Group Size - Females		44	44
Number of Cycles d-13→d0 [k]	Mean	2.0	2.2
	SD	0.4	0.7
	N	44	44
Mean Cycle Lengths (Days) d-13→d0 [k1]	Mean	4.16	4.21
	SD	0.49	0.64
	N	44	43
	%Diff	-	1.11

[k] - Kruskal-Wallis & Dunn; [k1] - Dunn

Table 14: Summary of estrous cycling: F0 generation, sponsor provided (Study no. 20312409)

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Pairing (Litter: A)		Group 1	Group 2
Group Size - Females		44	44
Paired - Females	N+ve	44	44
Mated Females	N+ve	43	43
Pregnant	N+ve	42	42
Pre-coital Interval (Days) [k]	Mean	2.2	3.0 **
	SD	0.9	1.0
	N	43	43
	%Diff	-	38.7
Pregnant No Confirmed Mating [f]	N+ve	0	0
Confirmed Mating Days 1-7 [f]	N+ve	43	43
	%	100.0	100.0
Female Mating Index [f]	%	97.7	97.7
	ProA	43/44	43/44
Female Fertility Index [f]	%	97.7	97.7
	ProA	42/43	42/43
Female Pregnancy Index [f]	%	95.5	95.5
	ProA	42/44	42/44

[k] - Dunn: ** = $p \leq 0.01$; [f] - Fisher's Exact

Table 15: Summary of reproductive performance: F0 generation, sponsor provided (Study no. 20312409)

Macroscopic observations

No test article-related macroscopic observations were reported during necropsy examination.

Ovarian and uterine examinations and litter observations (cohort 1)

Pregnancy was confirmed in 21 or 22 females in the 0 and 96 µg/dose groups, respectively.

No test article effect on ovarian, uterine, or litter parameters were reported. No test article effect on the litter means values for corpora lutea, implantation sites, percent pre-implantation loss, litter size, live and dead fetuses, early and late resorptions, percent post-implantation loss, fetal sex ratio, and mean fetal weights (male, female, and combined) were reported. They were similar to the control group or were within the historical range of the testing facility (historical control data). With the exception of females 2502 and 2509, there were no abnormalities detected in any placenta examined.

In group 2, the mean number of fetuses and number of live fetuses (15.5 compared with 13.8 in group 1) and live male fetuses were significantly ($p \leq 0.05$) increased when compared with the group 1 due to an increase in the mean number of corpora lutea (18.3 compared with 16.4 in group 1) and implantation sites ($p \leq 0.01$; 16.4 when compared with 14.6 in group 1). In cohort 2, no differences were reported in the mean number of implantation sites. The increase represented normal variation in litter size that is found in this strain of rat (historical control data). The mean placental weight was also reduced (7.6% when compared with group 1).

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Group Size - Females		22	22
Number of Females Pregnant	N+ve	21	22
	%	95.5	100.0
Female with Live Fetuses	N+ve	21	22
	%	100.0	100.0
Female with Resorptions	N+ve	13	12
	%	61.9	54.5
Female with all Nonviable	N+ve	0	0
	%	0.0	0.0
Terminal Euthanasia	N+ve	22	22
	%	100.0	100.0
Unscheduled Death/Euthanasia	N+ve	0	0
	%	0.0	0.0
Found Dead	N+ve	0	0
	%	0.0	0.0
Unscheduled Euthanasia	N+ve	0	0
	%	0.0	0.0
Aborted	N+ve	0	0
	%	0.0	0.0
Delivered	N+ve	0	0
	%	0.0	0.0

Table 16: Summary of maternal performance and mortality: F0 generation-Cohort 1, sponsor provided (Study no. 20312409)

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Female with Live Fetuses	N+ve	21	22
	%	100.0	100.0
Number of Corpora Lutea [k]	Mean	16.4	18.3
	SD	3.1	3.2
	N	21	22
	%Diff	-	11.8
Number of Implantations [k]	Mean	14.6	16.4**
	SD	2.5	1.9
	N	21	22
	%Diff	-	12.6
Pre-implantation Loss (%) [k]	Mean	10.40	9.07
	SD	10.81	10.23
	N	21	22
	%Diff	-	-12.78
Total Number of Resorptions [k]	Mean	0.8	0.9
	SD	0.9	1.0
	N	21	22
	%Diff	-	6.7
Number of Early Resorptions [k]	Mean	0.8	0.8
	SD	0.9	1.0
	N	21	22
	%Diff	-	1.1
Number of Late Resorptions [k]	Mean	0.0	0.0
	SD	0.0	0.2
	N	21	22
	%Diff	-	-
Total Number of Fetuses [k]	Mean	13.8	15.5*
	SD	3.1	1.9
	N	21	22
	%Diff	-	13.0
Number of Live Fetuses [k]	Mean	13.8	15.5*
	SD	3.1	1.9
	N	21	22
	%Diff	-	13.0
Number of Live Male Fetuses [k]	Mean	6.8	8.3*
	SD	2.2	2.1
	N	21	22
	%Diff	-	21.5
Number of Live Female Fetuses [k]	Mean	7.0	7.3
	SD	2.8	2.0
	N	21	22
	%Diff	-	4.6
Number of Dead Fetuses [k]	Mean	0.0	0.0
	SD	0.0	0.0

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Post-implantation Loss (%) [k]	N	21	22
	%Diff	-	-
	Mean	6.89	5.21
	SD	12.16	6.22
Live Male Fetus/Litter (%) [k]	N	21	22
	%Diff	-	-24.34
	Mean	50.70	53.05
	SD	13.87	12.39
Mean Fetal Weight all (g) [G]	N	21	22
	%Diff	-	4.63
	Mean	5.843	5.631
	SD	0.567	0.349
Mean Fetal Weight males (g) [G]	N	21	22
	%Diff	-	-3.617
	Mean	5.954	5.773
	SD	0.606	0.317
Mean Fetal Weight females (g) [G]	N	21	22
	%Diff	-	-3.035
	Mean	5.730	5.467
	SD	0.510	0.378
Live Mean Placental Weight (g) [G]	N	21	22
	%Diff	-	-4.588
	Mean	0.544	0.503
	SD	0.106	0.064
	N	21	22
	%Diff	-	-7.618
	Mean		
	SD		

[k] - Dunn: * = $p \leq 0.05$; ** = $p \leq 0.01$

Table 17: Summary of ovarian and uterine examinations and litter observations: F0 generation - Cohort 1, sponsor provided (Study no. 20312409).

Fetal examinations

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification. Fetal evaluations were based on 289 and 342 live GD 21 Cesarean-delivered fetuses in groups 1 and 2, respectively. There were 140 and 165 fetuses examined for visceral abnormalities and 149 and 177 fetuses were examined for skeletal abnormalities and fetal ossification site averages in groups 1 and 2, respectively. No test article-related effect on fetal external, visceral, or skeletal parameters were reported.

External examinations

No test article-related effect on external malformations or variations were reported.

Visceral examinations

No test article-related effect on visceral malformations or variations were reported.

Skeletal examinations

No test article-related effect on skeletal malformations or variations were reported.

All skeletal abnormalities that were reported were considered not test article related because: 1) the observations were limited to the control group; 2) the number of affected fetuses/litters was similar to the control group; 3) the observations were limited to 1 or 2 fetuses/litters in any dose group; and/or 4) the fetal/litter incidences were within the historical control range of the testing facility.

Skeletal ossification counts

No test article-related effect on the mean number of ossification sites per fetus were reported.

The mean number of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral, and caudal), rib pairs, sternum (manubrium, sternal centers, and xiphoid), forelimbs (carpals, metacarpals, digits, and phalanges) or hindlimbs (tarsals, metatarsals, digits, and phalanges) were unaffected by test article treatment.

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
Hyoid [k]	Mean	1.00	0.98
	SD	0.00	0.04
	N	21	22
	%Diff	-	-1.64
Cervical Vertebrae [k]	Mean	7.00	7.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Thoracic Vertebrae [k]	Mean	13.05	13.02
	SD	0.15	0.05
	N	21	22
	%Diff	-	-0.26
Lumbar Vertebrae [k]	Mean	5.95	5.98
	SD	0.15	0.05
	N	21	22
	%Diff	-	0.56
Sacral Vertebrae [k]	Mean	4.00	4.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Caudal Vertebrae [k]	Mean	6.53	6.34
	SD	0.76	0.66
	N	21	22
	%Diff	-	-2.93
Ribs, Paired [k]	Mean	13.04	13.01
	SD	0.13	0.03
	N	21	22
	%Diff	-	-0.22
Manubrium [k]	Mean	1.00	1.00

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Mating (Litter: A)		Group 1	Group 2
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Sternal Centra [k]	Mean	4.00	4.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Xiphoid [k]	Mean	1.00	1.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Carpals [k]	Mean	0.00	0.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	-
Metacarpals [k]	Mean	4.00	4.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Forelimb Digits [k]	Mean	5.00	5.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Forelimb Phalanges [k]	Mean	8.47	8.59
	SD	0.54	0.39
	N	21	22
	%Diff	-	1.42
Tarsals [k]	Mean	0.04	0.00
	SD	0.16	0.02
	N	21	22
	%Diff	-	-87.27
Metatarsals [k]	Mean	4.90	4.88
	SD	0.19	0.17
	N	21	22
	%Diff	-	-0.33
Hindlimb Digits [k]	Mean	5.00	5.00
	SD	0.00	0.00
	N	21	22
	%Diff	-	0.00
Hindlimb Phalanges [k]	Mean	6.82	6.47
	SD	1.20	0.92
	N	21	22
	%Diff	-	-5.13

Table 18: Summary of mean fetal skeletal ossification sites: F0 generation - Cohort 1, sponsor provided (Study no. 20312409).

Natural delivery and litter observations (Cohort 2)

Natural delivery

Of all pregnant females, all dams delivered a live litter. No test article-related effect on the gestation length (mean range 21.7 days/group), gestation index (number of rats with live offspring/number of pregnant rats), mean implantation sites per delivered litter (14.6), percentage of dams with no liveborn pups, mean percentage of stillborn pups/litter (0.36%), live birth index (99.64%), and percentage of post-implantation loss per litter (7.01%) were reported. These changes were similar when compared with the control group or were within the historical control range of the testing facility. All maternal grooming and nesting/nursing activities were normal.

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
Group Size - Females		22	22
Number of Females Pregnant [f]	N+ve	21	20
	%	95.5	90.9
Gestation Length (Days) [k]	Mean	22.1	21.7
	SD	0.9	0.5
	N	21	20
	%Diff	-	-1.8
Gestation Index [f]	%	100.0	100.0
	ProA	21/21	20/20
Females Completing Delivery [f]	N+ve	21	20
Females with Liveborn [f]	N+ve	21	20
Female with no Liveborn Pups [f]	N+ve	0	0
Fem w/ Stillborn Pups [f]	N+ve	1	1
Stillborn Pups/Litter (%) [k]	Mean	0.37	0.36
	SD	1.68	1.60
	N	21	20
	%Diff	-	-2.50
Number Pups Stillborn	Sum	1	1
Number Live Newborn Pups [k]	Mean	15.0	13.5
	SD	2.3	3.3
	N	21	20
	%Diff	-	-9.7
	Sum	314	270
Live Birth Index (%) [k]	Mean	99.63	99.64
	SD	1.68	1.60
	N	21	20
	%Diff	-	0.01
Live Male Pups/Litter (%) Birth [G]	Mean	52.09	55.27
	SD	15.49	12.78
	N	21	20
	%Diff	-	6.11
Implantation Sites - Total [k]	Mean	16.0	14.6
	SD	2.6	3.5
	N	21	20
	%Diff	-	-8.5
Post-implant Loss/Litter (%) [k]	Mean	5.62	7.01
	SD	5.67	7.58
	N	21	20

	%Diff	-	24.83
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Table 19: Summary of natural delivery observations: F0 generation - Cohort 2, sponsor provided (Study no. 20312409).

Observation Type: All Types Sex: Female From Day 0 (Littering (A)) to 21 (Littering)	0 ug/dose Group 1	96 ug/dose Group 2
Grooming of pups - normal		
Number of animals affected	21	20
Number of times recorded	33	26
% of affected animals	100	100
First to last seen	0 - 1	0 - 1
AmntcSacPlcntaUmbilicaRem-norm		
Number of animals affected	21	20
Number of times recorded	33	26
% of affected animals	100	100
First to last seen	0 - 1	0 - 1
Nursing activity – normal		
Number of animals affected	21	20
Number of times recorded	454	435
% of affected animals	100	100
First to last seen	0 - 21	0 - 21
Nesting activity – normal		
Number of animals affected	21	20
Number of times recorded	454	435
% of affected animals	100	100
First to last seen	0 - 21	0 - 21

Table 20: Summary of maternal observations: F0 generation - Cohort 2 – Lactation, sponsor provided (Study no. 20312409).

Litter observations

No test article-related effect on litter observations were reported. The viability and lactation indices (98.28% and 78.22%, respectively) and live male pups/litter (53.57%) were similar when compared with the control group or were within the historical control range of the testing facility.

Preweaning F1 generation pups

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
Group Size - Females		22	22
Females with Liveborn	N+ve	21	20
Viability Index Birth-4 (%) [k]	Mean	97.95	98.28
	SD	3.48	3.06
	N	21	20
	%Diff	-	0.34
Lactation Index (%) [k]	Mean	80.00	78.22
	SD	0.00	5.23
	N	21	20
	%Diff	-	-2.22
Live Male Pups/Litter (%) 21 [G]	Mean	51.79	53.57
	SD	9.91	12.60
	N	21	20
	%Diff	-	3.45

Table 21: Summary of litter observations: F0 generation - Cohort 2, sponsor provided (Study no. 20312409).

Clinical observations

No test article-related effect on the clinical observations in F1 generation male and female pups were reported. All clinical observations, including an absent tail, absent anal opening, thin cover fur, domed head, fur loss, discolored and/or pale skin, and cold to the touch were considered unrelated to test article treatment because: 1) the observations were limited to the control group; and/or 2) the observations were limited to one pup and/or one litter in the group.

Observation Type: Pup Observations From Day 1 to 21 (Litter Date)		0 ug/dose Group 1	96 ug/dose Group 2
LITTERS EXAMINED	N	21	20
Anal Opening Absent			
Number of Times Recorded	N	2	0
Number of Pups Affected	N	1	0
Number of Litters Affected	N	1	0
Tail, Absent (PT)			
Number of Times Recorded	N	1	0
Number of Pups Affected	N	1	0
Number of Litters Affected	N	1	0
Fur, Thin Cover			
Number of Times Recorded	N	22	0
Number of Pups Affected	N	10	0
Number of Litters Affected	N	1	0
Other (Domed Head)			
Number of Times Recorded	N	0	1
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1

Observation Type: Pup Observations From Day 1 to 21 (Litter Date)		0 ug/dose Group 1	96 ug/dose Group 2
LITTERS EXAMINED	N	21	20
Fur, Loss			
Number of Times Recorded	N	0	48
Number of Pups Affected	N	0	8
Number of Litters Affected	N	0	1
Skin, Discolored			
Number of Times Recorded	N	0	1
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1
Skin, Pallor			
Number of Times Recorded	N	0	1
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1
Cold to Touch			
Number of Times Recorded	N	0	1
Number of Pups Affected	N	0	1
Number of Litters Affected	N	0	1

Table 22: Summary of pup clinical observations: F1 generation - Cohort 2, sponsor provided (Study no. 20312409).

Pup body weights

No test article-related effect on mean body weights in preweaning F1 generation male and female pups were reported. Mean combined pup body weights were 102.5% of controls on day 21 postpartum. All other differences, regardless of statistical significance, were considered unrelated to test article treatment because the differences were transient.

Sex: Female			0 ug/dose Group 1	96 ug/dose Group 2
Day(s) Relative to Littering (Litter: A)			Group 1	Group 2
Mean Pup BW all	1 [G]	Mean	7.29	7.48
		SD	0.75	0.75
		N	21	20
		%Diff	-	2.55
	4 [G]	Mean	10.15	10.64
		SD	1.20	1.51
		N	21	20
		%Diff	-	4.90
	7 [G]	Mean	16.43	16.82
		SD	1.46	1.94
		N	21	20
		%Diff	-	2.41
	10 [G]	Mean	23.88	24.20
		SD	2.27	2.22
		N	21	20
		%Diff	-	1.34
	13 [G]	Mean	30.20	31.37

Sex: Female			0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)			Group 1	Group 2
		SD N %Diff	2.41 21 -	2.61 20 3.86
Mean Pup BW all	14 [G]	Mean SD N %Diff	31.46 2.36 21 -	32.75 3.15 20 4.08
	18 [G]	Mean SD N %Diff	39.43 3.92 21 -	42.27* 4.08 20 7.22
	21 [G]	Mean SD N %Diff	51.86 5.20 21 -	53.17 5.04 20 2.53
Mean Pup BW males	1 [G]	Mean SD N %Diff	7.49 0.77 21 -	7.68 0.80 20 2.48
	4 [G]	Mean SD N %Diff	10.42 1.19 21 -	10.86 1.52 20 4.22
Mean Pup BW males	7 [G]	Mean SD N %Diff	16.90 1.41 21 -	17.14 1.95 20 1.46
	10 [G]	Mean SD N %Diff	24.48 2.22 21 -	24.62 2.36 20 0.57
	13 [G]	Mean SD N %Diff	30.89 2.08 21 -	32.13 2.96 20 4.00
	14 [G]	Mean SD N %Diff	32.23 2.50 21 -	33.11 3.06 20 2.72
	18 [G]	Mean SD N %Diff	40.39 4.23 21 -	42.75 3.90 20 5.85
Mean Pup BW males	21 [G]	Mean SD N %Diff	52.96 5.58 21 -	53.86 4.95 20 1.70
Mean Pup BW females	1 [G]	Mean SD N %Diff	7.11 0.71 21 -	7.25 0.68 20 1.97

Sex: Female			0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)			Group 1	Group 2
	4 [G]	Mean	9.82	10.39
		SD	1.19	1.53
		N	21	20
		%Diff	-	5.87
	7 [G]	Mean	15.91	16.52
		SD	1.52	1.96
		N	21	20
		%Diff	-	3.80
	10 [G]	Mean	23.25	23.80
		SD	2.32	2.14
		N	21	20
		%Diff	-	2.35
Mean Pup BW females	13 [G]	Mean	29.51	30.61
		SD	3.00	2.46
		N	21	20
		%Diff	-	3.71
	14 [G]	Mean	30.68	32.07
		SD	2.25	2.99
		N	21	19
		%Diff	-	4.50
	18 [G]	Mean	38.46	41.53*
		SD	3.75	4.28
		N	21	19
		%Diff	-	7.98
	21 [G]	Mean	50.82	52.22
		SD	5.26	5.36
		N	21	19
		%Diff	-	2.75
Mean Pup BW Postcull all	4 [G]	Mean	10.15	10.63
		SD	1.19	1.53
		N	21	20
		%Diff	-	4.80
Mean Pup BW Postcull male	4 [G]	Mean	10.45	10.86
		SD	1.21	1.53
		N	21	20
		%Diff	-	3.88
Mean Pup BW Postcull female	4 [G]	Mean	9.81	10.42
		SD	1.17	1.54
		N	21	20
		%Diff	-	6.14

[G] - Dunnett: * = $p \leq 0.05$

Table 23: Summary of litter mean pup body weights: F1 generation - Cohort 2, sponsor provided (Study no. 20312409).

Reflex and physical development

No test article-related effect on eye opening, surface righting, pinna detachment auditory startle, hair growth or pupil constriction in F1 generation preweaning male and female pups were reported.

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
LMean Eyes Opened [k]	Mean	13.9	14.1
	SD	0.7	0.7
	N	21	20
	%Diff	-	1.6
LMean Surface Righting [k]	Mean	1.7	1.8
	SD	0.6	0.5
	N	21	20
	%Diff	-	6.0
LMean Pinna Detached [k]	Mean	2.7	3.0
	SD	0.7	0.5
	N	21	20
	%Diff	-	10.3
LMean Auditory Startle [k]	Mean	13.0	13.0
	SD	0.0	0.0
	N	21	20
	%Diff	-	0.0
LMean Hair Growth [k]	Mean	7.0	7.0
	SD	0.0	0.0
	N	21	20
	%Diff	-	0.0

Table 24: Summary of litter mean day pup reflex and development: F1 generation - Cohort 2, sponsor provided (Study no. 20312409).

Sex: Female		0 ug/dose	96 ug/dose
Day(s) Relative to Littering (Litter: A)		Group 1	Group 2
Pups Pupil Constriction (%) d21 [k]	Mean	100.00	100.00
	SD	0.00	0.00
	N	20	15
	%Diff	-	0.00

Table 25: Summary of litter percentages pup reflex and development: F1 generation - Cohort 2, sponsor provided (Study no. 20312409).

Pup necropsy

No test article-related effect on the macroscopic observations detected in preweaning F1 generation male and female pups during necropsy examination were reported. At scheduled euthanasia on day 21 postpartum, necropsy examination revealed a misshapen brain and a moderately small renal papilla in group 1. Moderate dilatation of the brain and a hole located between the right cerebral hemisphere and diencephalon were reported in group 2. These observations were not considered test article related because they were limited to a single pup in one litter and/or the observation was limited to the control group.

Litter: A

Exam Type: Pup Necropsy 2		0 ug/dose Group 1	96 ug/dose Group 2
Number of Pups Examined:		170	148
Number of Litters Examined:		21	20
Brain			
Brain, Dilatation, Moderate	Pups N(%)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	1(5.0)
Brain, Hole - Malformation	Pups N(%)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	1(5.0)
Brain, Misshapen - Malformation	Pups N(%)	1(0.6)	0(0.0)
	Litters N(%)	1(4.8)	0(0.0)
Kidney			
Renal papilla, Left, Small, Moderate - Variation	Pups N(%)	1(0.6)	0(0.0)
	Litters N(%)	1(4.8)	0(0.0)
Stomach			
Stomach content, Absent	Pups N(%)	0(0.0)	1(16.7)
	Litters N(%)	0(0.0)	1(20.0)

Table 26: Summary of pup gross pathology: F1 generation - Cohort 2 - Terminal euthanasia, sponsor provided (Study no. 20312409).

Antibody evaluation

Serum antibody evaluation

Mean anti-RSV serum antibody concentrations in F0 generation females increased from SD 15 to GD 13. Thereafter, mean anti-RSV antibody concentrations were lower but remained higher than the SD 15 serum concentrations.

Fetal and pup serum antibody evaluation

Mean anti-RSV serum antibody levels in F1 generation male and female fetuses and pups increased from GD 21 to LD 13 and was highest on LD 13 (113,770 AU/mL).

Milk antibody evaluation

Mean anti-RSV milk antibody levels in F0 generation females ranged from 9,295 to 9,993 AU/mL from LD 13 to LD 21.

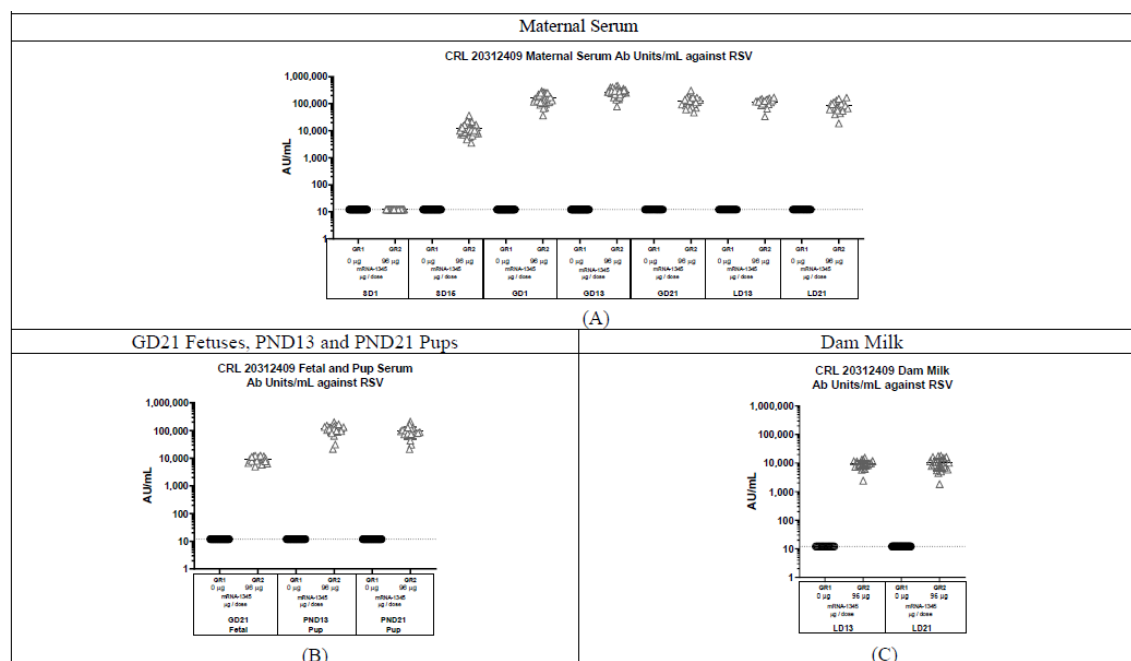


Figure 1: Graphs of antibody titers against RSV pre-fusion protein, sponsor provided (Study no. 20312409).

Conclusion

In conclusion, test article treatment during the premating period (28 and 14 days prior to mating) and on gestation days (GD) 1 and 13 was tolerated in F0 or F1 generation rats. Test article-related effects, including injection site reactions (limited usage of the hindlimbs and swollen hindlimbs) that were considered secondary to the route of administration were reported during the premating and gestation periods, with the onset mimicking the timing of dose administration. Test article-related reductions in body weight gain and food consumption were reported. Following dose administration, anti-RSV antibodies were not detected in the serum samples of the F0 females on SD 1. Anti-RSV antibodies were present on SD15 continuing through LD 21, F1 fetuses on GD 21, F1 pups on LD 13 and 21, and in the milk samples on LD 13 and 21.

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

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes () or no (X).

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

Study number 2: T Cell Immunogenicity of mRNA-1345 in Mice. Study number: 3797-1
 Objective

The objective of this study was to evaluate the immunogenicity of several different candidate RSV mRNA vaccines, including mRNA-1345, in mice. This sub-study summarizes the T cell response induced by mRNA-1345.

Study design

As shown in the table below, 8-week-old female (b) (4) mice (n=10, (b) (4)) were vaccinated with mRNA-1345 via intramuscular (IM) injection in 50 µL volume administered twice, once on day 1 and once on day 22. Control animals (n = 4) were injected IM with PBS. On day 36, spleens were collected from each animal.

Group	n	Test Article ^a	Dose (mRNA)	Dosing Schedule	Endpoint
7	10	mRNA-1345	1 µg	Days 1 & 22	RSV F-specific T cells in day 36 spleens by (b) (4)
13	4	PBS	--		

^a Doses were administered in an injection volume of 0.05 mL via intramuscular injection in the hind leg.

Abbreviations: F = fusion; mRNA = messenger RNA; PBS = phosphate buffered saline; RSV = respiratory syncytial virus

Table 27: Study design, sponsor provided (Study no. 3797-1).

Methods:

Test articles

mRNA-1345 is formulated in a mixture of 4 lipids to form a drug lipid complex (lipid nanoparticle [LNP]). The four lipids are heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6 (undecyloxy) hexyl) amino) octanoate (SM-102); 1,2-dimyristoyl-racn-glycerol, methoxypolyethyleneglycol (PEG2000-DMG); 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and cholesterol.

Test Article	Lot#	Stock Concentration
mRNA-1345	mRNA Drug Substance: (b) (4)	(b) (4)
	Drug product: DP-013188	

Table 28: Test article lot number, sponsor provided (Study no. 3797-1).

Blood was collected via the submandibular vein

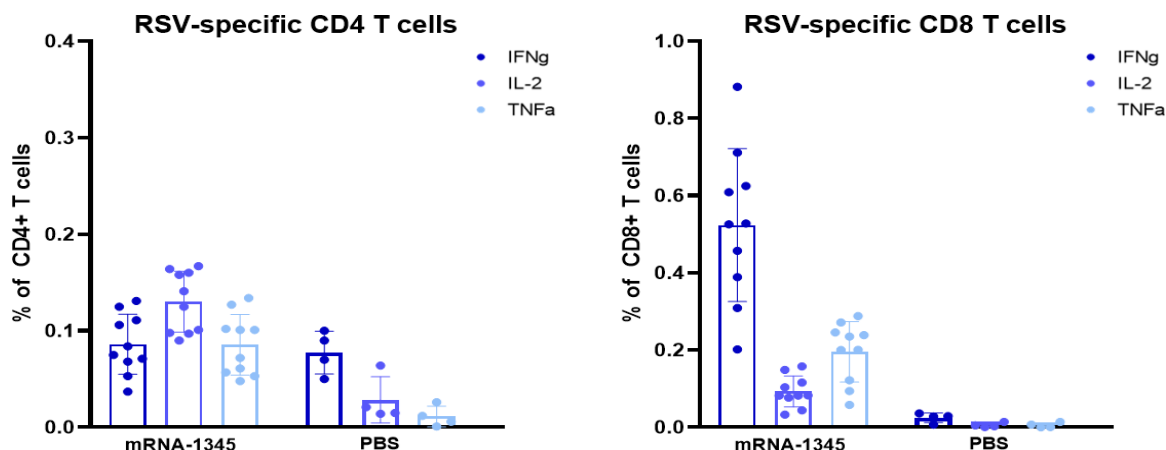
(b) (4)

Whole mouse spleens were collected from (b) (4) mice on day 36 and cells were prepared for this assay.

Results

As shown in the figure below, mouse splenocytes were restimulated with a pool of overlapping peptides covering the F protein (15mers overlapping by 11 amino acids) and intracellular IFN γ ,

IL-2, and TNF α were measured by (b) (4). mRNA-1345 induced RSV-specific CD4+ and CD8+ T cells that produced the type 1 cytokines IFN γ , IL-2 and TNF α . Following RSV infection, CD8+ T cells producing these cytokines are known to contribute to viral clearance. Little to no F-specific cytokine production in the PBS control mice were reported.



Abbreviations: IFN γ = interferon gamma; IL-2 = interleukin 2; mRNA = messenger RNA; PBS = phosphate buffered saline; RSV = respiratory syncytial virus; TNF α = tumor necrosis factor alpha.

Note: Each symbol represents an individual mouse, bars represent mean response and error bars indicate SD.

Figure 2: RSV-F specific T cell responses on day 36, sponsor provided (Study no. 3797-1).

Conclusion

mRNA-1345 elicited RSV-specific type 1 cytokine-producing CD4+ and CD8+ T cell responses in mice.

Study number 3: Immunogenicity of mRNA-1345, (b) (4) in (b) (4) Mice Relative to ERD Controls. Study number: X-107.

Methods:

Test articles

All mRNA drug products were stored at (b) (4). mRNAs drug products were thawed on ice the day of dosing and diluted in PBS to reach the target mRNA dose level.

Test Article	Lot#	Stock concentration
mRNA-1345	mRNA Drug Substance: (b) (4) Drug Procut: FID: 17874	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)
RSV preF protein	(b) (4)	(b) (4)
(b) (4) preF protein	(b) (4)	(b) (4)
Alhydrogel adjuvant	(b) (4)	(b) (4)
(b) (4)	(b) (4)	N/A
(b) (4)	(b) (4)	N/A
Live RSV (b) (4)	(b) (4)	N/A
Live (b) (4)	(b) (4)	N/A

Table 29: Test article lot number, sponsor provided (Study no. X-107).

Microneutralization assays

An RSV-A microneutralization assay was performed to detect RSV-specific neutralizing antibodies.

(b) (4)

(b) (4)

T cell stimulation and (b) (4)

Whole mouse spleens are collected from (b) (4) mice on day 36. A (b) (4) tissue dissociator ((b) (4)) is used to generate mononuclear single-cell suspensions from (b) (4) mouse whole spleens.

Animals were immunized (i.m.) in groups 1 thru 8, 11, and 12 as indicated in the table below at a volume of 50 µl per animal. Animals in group 9 were infected (i.n.) with (b) (4) per animal. Animals in group 10 were infected (i.n.) with (b) (4) per animal. Back titrations were performed to confirm priming.

Group	n	Treatment	Route	Dosing Schedule
1	5	PBS	IM	Day 0, 21, 49
2	8	5 µg mRNA-1345	IM	Day 0, 21, 49
3	8	10 µg (b) (4)	IM	Day 0, 21, 49
4	8	10 µg (b) (4)	IM	Day 0, 21, 49
5	8	5 µg (b) (4)	IM	Day 0, 21, 49
6	8	5 µg mRNA/LNP control	IM	Day 0, 21, 49
7	8	10 µg (b) (4)	IM	Day 0, 21, 49
8	8	10 µg (b) (4)	IM	Day 0, 21, 49
9	8	(b) (4)	IN	Day 0
10	8	(b) (4)	IN	Day 0
11	8	(b) (4)	IM	Day 0, 21, 49
12	8	(b) (4)	IM	Day 0, 21, 49

Table 30: Study design, sponsor provided (Study no. X-107).

Results

Neutralizing and binding (IgG) antibody

RSV, (b) (4) serum neutralizing (NT) and binding (IgG) antibody titers are shown in figures below. (b) (4)

(b) (4). mRNA-1345 (RSV) induced RSV neutralizing and binding (preF and postF IgG) antibodies but not (b) (4) antibodies. (b) (4)

The RSV neutralizing and binding antibody titers induced by (b) (4) were similar in magnitude to those induced by mRNA-1345 at a matched RSV mRNA dose level of 5 µg (figure 1). Similarly, the (b) (4) neutralizing and binding antibody titers induced by mRNA-1365 were similar in magnitude to those induced (b) (4)

3 pages have been determined to be not releasable: (b)(4)

(b) (4)

In conclusion, these results demonstrate that mRNA-1345, (b) (4) induce a robust CD8⁺ and Th1-biased CD4⁺ T cell response to the encoded antigen(s), whereas (b) (4) induces a Th2-biased response.

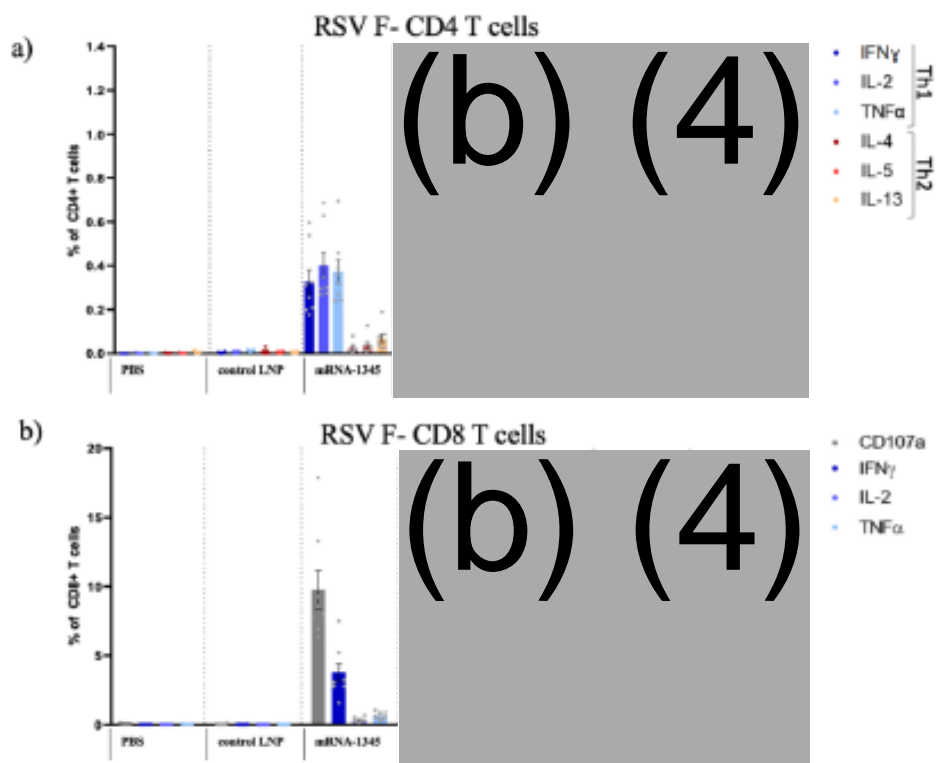


Figure 7: RSV, (b) (4) -specific T cell response (day 56), sponsor provided (Study no. X-107).

(b) (4)

Conclusions:

mRNA-1345, (b) (4) induced a robust RSV (mRNA-1345 and (b) (4)) and/or (b) (4)) neutralizing and binding antibody response.

The RSV (b) (4) antibody response induced by mRNA-1345, (b) (4) was largely functional (neutralizing). In contrast, the antibody response induced by (b) (4) was primarily nonfunctional (non-neutralizing) and directed to the postF conformation. As indicated by type 1 cytokine-producing CD4⁺ and CD8⁺ T cell responses and a balanced IgG2a: IgG1 antibody response, mRNA-1345, (b) (4) induced a Th1 response. In contrast, (b) (4) induced Th2 responses, as indicated by type 2 cytokine producing CD4⁺ T cells, no CD8⁺ T cells, and a IgG1-biased antibody response.

In conclusion, mRNA-1345, (b) (4) induced an immunologic profile characteristic of protection that was distinct from the disease-enhancing phenotype induced by (b) (4).

Study number 4: A GLP Repeat Dose Toxicity Study of mRNA-1345 by Intramuscular Administration in (b) (4) Rats. Study number: 1021-9921.

Performing laboratory: (b) (4)

Study initiation date: March 08, 2022

Final report date: Draft report on July 14, 2022, and January 09, 2023, is the expected date of study director signature of report.

Test article batch/lot:

Test article identification

	Test Item
Identification:	mRNA-1345
Alternate Identification:	MRNA-1345 RSV (b) (4) DP
Batch/Lot No.:	DH-10855.1
Expiration/Retest Date:	19 Nov 2022
Physical Description:	White to off-white dispersion
Concentration:	0.49 mg/mL
Density:	N/A
Storage Conditions (temperature set to maintain):	-20°C
Provided by:	Sponsor

N/A= Not Applicable

Table 31: Test article identification, sponsor provided (Study no. 1021-9921).

Reference item identification

	Vehicle
Identification:	20 mM Tris, 87 g/L Sucrose, (b) (4) mM Acetate, (b) (4)
Alternate Identification:	Diluent buffer
Batch/Lot No.:	DH-10900.2
Expiration/Retest Date:	19 Nov 2022
Density	N/A
Storage Conditions (temperature set to maintain):	(b) (4)
Provided by:	Sponsor

N/A= Not Applicable

Table 32: Reference item identification, sponsor provided (Study no. 1021-9921).

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

Breeder/supplier: (b) (4)

Number of animal per group and sex: 10/sex/group

Age: 7-8 weeks

Body weight range: 159 and 258 grams

Route and site of administration: Once on day 1 (left thigh)¹ and once on day 22 (right thigh)
Volume of injection: 0.2 mL

Frequency of administration and study duration: Days 1 and 22

Dose: 0 or 98 µg/animal

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.

All samples had mean concentrations within or equal to the acceptance criteria of (b) (4) (individual values within or equal to (b) (4)) of their theoretical concentrations. For homogeneity, the RSD of concentrations for all samples in each group tested was within the acceptance criteria of (b) (4).

The mean measured concentration was (b) (4) mg/mL, the (b) (4) and the main peak purity was (b) (4). The obtained concentration, purity and (b) (4) results were consistent with the concentration value (b) (4) and the main peak purity (b) (4) value reported in the initial summary of analysis provided by the sponsor. This demonstrate that the test article was stable.

Means of administration: Intramuscular (IM)

Report status: Draft report

Experimental design:

Animals were randomized and assigned to 2 different groups. Animals, 10/sex/group, were dosed by IM on study days 1 and 22. The dose levels were 0 and 98 µg/animal and administered at a dose volume of 0.2 mL/dose. The details of the study design are listed in the following table:

Group No.	Test Material	Dose Level (µg/animal)	Dose Volume (mL/animal)	Dose Concentration (µg /mL)	Animal ID	
					Males	Females
1	Reference Item	0	0.2	0	1001-1010	1501-1510
2	mRNA-1345	98		490	2001-2010, 2101	2501, 2502, 2504-2510, 2603

Prior to the start of dosing, animal No. 2001 and animal No. 2503 were rejected from the study due to ophthalmology findings and were replaced by spare animals which became animal No. 2101 and animal No. 2603 respectively. All animals remaining unassigned to groups after day 6 were released from the study and their disposition documented.

Table 33: Experimental design, sponsor provided (Study no. 1021-9921).

Methods:

Randomization procedure: Yes

Statistical analysis plan: Yes.

¹ On day 1, the test item was suspected to have been administered to the left gastrocnemius muscle instead of the left thigh (quadriceps).

The following parameters were evaluated:

Parameter	Frequency (minimum required)	Comments
Mortality	At least twice daily (morning and afternoon) beginning upon arrival through termination/release.	Animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Cage-side Observations	At least once daily, on non-dosing days; from at least week -1, excluding the day of necropsy.	Animals were observed within their cage unless necessary for identification or confirmation of possible findings. ^a For deviation, see appendix 1.
Detailed Clinical Observations	Weekly; from at least week -1 and throughout the study. Days 1 and 22 at 6 hours post-dose.	Animals are normally removed from the cage. A detailed clinical examination was not performed during week -1. For deviation, see appendix 1.
Post-dose Observations	Days 1 and 22 at 24 hours post dose	The animals were observed within their cage unless necessary for identification or confirmation of possible findings. Each animal was evaluated for new observations or increases in the severity of existing observations following dose administration. Pre-existing conditions, considered to be unaffected by dosing, were recorded only in the cage side observations.
Individual Body Weights	Weekly; from at least week -1 and throughout the study, including day 1 and day 22.	Body weight was not recorded during week -1. Last body weight was performed prior to terminal fasting. Fasted body weight was not performed as required. For deviation, see appendix 1.
Food Consumption (Cage Measurement)	Weekly; from at least week -1 and throughout the study.	Quantitatively measured ^b
Injection Site Monitoring (Modified Draize)	Days 1 and 22 at 6- and 24-hours post-dose. Monitoring continued every 24 hours until resolution of reaction or scheduled termination.	The presence (present/not present) of erythema and/or edema were recorded for individual animals at the injection site. Animals were removed from their cages for these observations.
Ophthalmic Examinations	Prior to initiation of dosing and day 23 (prior to necropsy).	All animals were subjected to funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations. All examinations were conducted by a board-certified veterinary ophthalmologist.

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

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

Table 34: General in-life assessments, sponsor provided (Study no. 1021-9921).

Group Numbers	Time Point	Hematology	Coagulation	Clinical Chemistry
All animals	Day 23	X	X	X
Overnight Fasting:		-	-	Yes
Method/Comments:		Abdominal aorta		

Group Numbers	Time Point	Hematology	Coagulation	Clinical Chemistry
Target Volume (mL):		1.3	1.3	1.1
Anticoagulant:		K ₃ EDTA	Sodium citrate	None ^a
Special Requirements:		-	-	-
Processing:		None	Plasma	Serum

X = Sample to collect; - = Not applicable

^a = Samples were collected in serum separator tubes (SST)

Table 35: Samples for clinical pathology evaluation, sponsor provided (Study no. 1021-9921).

Table of antibody sample collection

	Study Days	
	Week -1	Day 23
All Animals	X	X
Method/Comments:	Jugular venipuncture or other appropriate veins; abdominal aorta ^a	
Target Volume (mL):	1.0	
Anticoagulant:	None ^b	
Special Requirements:	None	
Processing:	Serum	

X = Sample collected.

^a For day 23 where the sample was taken prior to necropsy.

^b Samples were collected in serum separator tubes (SST)

Table 36: Antibody sample collection, sponsor provided (Study no. 1021-9921).

Postmortem procedures:

Group No.	No. of Animals		Scheduled Euthanasia Day	Necropsy Procedures			Histology Processing	Microscopic Evaluation
	M	F		Necropsy	Tissue Collection	Organ Weights		
1	10	10	23	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a
2	10	10	23	X	Full List ^a	Full List ^a	Full List ^a	Full List ^a

X = Procedure to be conducted. M= Male, F= Female.

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

^a See tables below for the list of tissues applicable to each procedure.

Table 37: Terminal procedure, sponsor provided (Study no. 1021-9921).

Brain Epididymis ^a Gland, adrenal ^a Gland, pituitary Gland, prostate Gland, thyroid ^{a,b} Heart Kidney ^a	Liver Ovary ^a Spleen Testis ^a Thymus Uterus Cervix
-------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------

^a Paired organ weight.

^b Weighed with parathyroid glands.

Table 38: Organs weighed at necropsy; sponsor provided (Study no. 1021-9921).

Administration site (Muscle, quadriceps)	Large intestine, cecum
Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Body cavity, nasal	Liver
Bone marrow smear	Lung
Bone marrow	Lymph node, iliac
Bone, femur	Lymph node, inguinal
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Epididymis	Muscle, skeletal (gastrocnemius)
Esophagus	Nerve, optic
Eye	Nerve, sciatic
Gland, adrenal	Ovary
Gland, harderian	Pancreas
Gland, mammary	Skin
Gland, parathyroid	Small intestine, duodenum
Gland, pituitary	Small intestine, ileum
Gland, prostate	Small intestine, jejunum
Gland, salivary, mandibular	Spinal cord (cervical, thoracic, lumbar)
Gland, seminal vesicle	Spleen
Gland, thyroid	Stomach
Gross lesions/masses	Testis
Gut-associated lymphoid tissue	Thymus
Heart	Tongue
Joint, femorotibial (knee)	Trachea
Joint, humeroradial (elbow)	Urinary bladder
Kidney	Uterus/Cervix
	Vagina

Note: Tissues that were supposed to be microscopically evaluated per study plan but were not available on the slide (and therefore not evaluated) are listed in the individual animal data of the pathology report as not present. These missing tissues did/not affect the outcome or interpretation of the pathology portion of the study because the number of tissues examined from each treatment group was sufficient for interpretation.

Table 39: Tissue collection and preservation, sponsor provided (Study no. 1021-9921).

Results:

No test article-related mortality was reported.

Clinical chemistry and hematology:

Test article-related changes consisted of increases in GLOB (range: 1.39x-1.45x mean control) and decreases in ALB (0.84x mean control) and A/G (range: 0.57x-0.61x mean control) in groups 2 males and females. Increases in TRIG (range: 1.46x-1.54x mean control) were also reported.

Other test article-related increases in CREAT (range: 1.14x-1.33x mean control) in males and females and an increase in UREA (1.26x mean control) in females only, on day 23 were also reported.

There was test article-related decrease in GLUC (0.89x mean control) and a minimal increase in CA (1.04x mean control) in males only.

Remaining differences in clinical chemistry parameters, regardless of statistical significance, were not considered test article-related because they were of small magnitude, absence of a dose response, general overlap of individual values with the range of control values, and/or were of a magnitude of change commonly reported in rats under similar study conditions.

	Group Dose (µg/animal) Sex	1 0* M	0* F	2 98 M	98 F
UREA (mmol/L)					
Day 23		4.36	3.78	—	1.26x
CREAT (umol/L)					
Day 23		30.9	30.3	1.14x	1.33x
ALB (g/L)					
Day 23		41.61	46.67	0.84x	0.84x
GLOB (g/L)					
Day 23		17.12	14.97	1.45x	1.39x
A/G ratio					
Day 23		2.49	3.16	0.57x	0.61x
GLUC (mmol/L)					
Day 23		9.174	7.785	0.89x	—
TRIG (mmol/L)					
Day 23		0.700	0.367	1.46x	1.54x
CA (mmol/L)					
Day 23		2.768	2.782	1.04x	—

M = Males F = Females

A dash (—) indicates absence of change. Numerical values indicate fold changes of treated group value relative to control group mean value. Bolded values indicate mean values were statistically different from controls at $P \leq 0.05$. * Control group values are reported for reference.

Table 40: mRNA-1345-related clinical chemistry changes, sponsor provided (Study no. 1021-9921).

Test article-related increases in NEUT (range: 4.62x-6.12x mean control) and EOS (range: 2.48x-3.27x mean control) and decreases in LYMPH (range: 0.51x-0.62x mean control) and MONO (range: 0.35x-0.50x mean control) in males and females were reported on day 23.

Test article-related decreases in RETIC (range: 0.59x-0.84x mean control) and increases in RDW (range: 1.07x-1.09x mean control), in absence of any changes in red blood cell counts were also reported. There were decreases in PLT (range: 0.68x-0.74x mean control) levels in males and females.

These changes might be indication of inflammation.

Other differences in hematology parameters, regardless of statistical significance, were not considered test article-related because they were of small magnitude, absence of a dose response, general overlap of individual values with the range of control values, and/or were of a magnitude of change commonly reported in rats under similar study conditions.

	Group Dose (µg/animal) Sex	1 0* M	0* F	2 98 M	98 F
NEUT 10⁹/L					
Day 23		0.930	0.586	6.12x	4.62x
LYMPH 10⁹/L					
Day 23		7.027	5.054	0.51x	0.62x
MONO 10⁹/L					
Day 23		0.207	0.142	0.50x	0.35x

	Group Dose (µg/animal) Sex	1		2	
		0* M	0* F	98 M	98 F
EOS 10⁹/L Day 23		0.063	0.071	2.48x	3.27x
RDW % Day 23		12.53	10.98	1.07x	1.09x
RETIC 10⁹/L Day 23		246.79	155.42	0.59x	0.84x
PLT 10⁹/L Day 23		1291.4	1310.6	0.74x	0.68x

M = Males F = Females

Numerical values indicate fold changes of treated group value relative to control group mean value. Bolded values indicate mean values were statistically different from controls at $P \leq 0.05$. * Control group values are reported for reference.

Table 41: mRNA-1345-related hematology changes, sponsor provided (Study no. 1021-9921).

Test article-related increases in FIB (range: 3.29x-3.35x mean control) and APTT (range: 1.10x-1.33x mean control) in males and females, and an increase in PT (1.06x mean control) in females only, on day 23 were reported. These changes might be an indication of inflammation.

Remaining differences in coagulation parameters, regardless of statistical significance, were not considered test article-related based on their small magnitude, absence of a dose response, general overlap of individual values with the range of control values, and/or were of a magnitude of change commonly reported in rats under similar study conditions.

	Group Dose (µg/animal) Sex	1		2	
		0* M	0* F	98 M	98 F
FIB (g/L) Day 23		3.083	2.300	3.35x	3.29x
PT (sec) Day 23		16.59	16.38	—	1.06x
APTT (sec) Day 23		19.66	16.36	1.10x	1.33x

M = Males F = Females

A dash (—) indicates absence of change. Numerical values indicate fold changes of treated group value relative to control group mean value. Bolded values indicate mean values were statistically different from controls at $P \leq 0.05$. * Control group values are reported for reference.

Table 42: mRNA-1345-related coagulation changes, sponsor provided (Study no. 1021-9921).

Systemic toxicity:

No treatment-related mortality or ophthalmologic changes were reported.

Test article clinical signs, including limited use of the hindlimb and/or hindpaw, skin discoloration and swelling, were attributed to the inflammatory reaction.

Test article-related edema and/or erythema associated with the injection site, were reported approximately 24 hours postdose, following the day 1 and day 22 dosing. This finding was resolved within 6 days of the dose administration.

Other clinical observations were transient, not dose-responsive, occurred sporadically, were reported in animals in the control group, and/or are commonly reported within this strain, age, and species.

During the week following the day 1 dosing (day 1 to 8), the group 2 males were noted to have a lower mean body weight gain of almost 20g (29.6%), compared to the control group. This is correlated with decreased food consumption reported over the same period. For the remaining study days (day 8 to 22), the body weight gains of the group 2 males, were comparable to the control group. This resulted in a lower body weight of the treated males, throughout the study, compared to the control group.

Group 2 males had a 14.4% lower average food consumption from day 1 to 8 compared to control, which is consistent with the lower body weights reported at that occasion.

Organ Weight:

Increases in the spleen weight of group 2 of both sexes were reported. The mean spleen weight (absolute and relative to brain weight) of group 2 males and females was increased by approximately 21-26%, when compared to concurrent controls. The increase in spleen weights were not correlated with microscopic findings.

	Males		Females	
Group	1	2	1	2
Dose (µg/animal)	0	98	0	98
No. of Animals per Group	10	10	10	10
Terminal body weight^a	-	-	-	-
Spleen (No. Weighed)^a	10	10	10	10
Absolute value	+0.74	+20.96	+0.53	+24.21
% of body weight*	-	-	-	-
% of brain weight	+35.98	+21.99	+28.32	+25.77

^a All values expressed in dosed groups are expressed as percent difference of control group means.

Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

*Not available due to lack of data for terminal body weights.

Table 43: Summary of organ weight data – Terminal euthanasia (day 23), sponsor provided (Study no. 1021-9921).

Gross pathology:

In all treated animals, macroscopic findings at the injection site (right quadriceps muscle) were reported and consisted of swollen muscle with or without adherence of pale/clear, firm/gelatinous material to muscle and/or subcutaneous tissues. These lesions were microscopically correlated with acute subcutaneous inflammation with occasional myofiber degeneration/necrosis of quadriceps muscle at the injection site.

Occasionally, group 2 had swollen (enlarged) or abnormal consistency of the regional draining iliac and popliteal lymph nodes with microscopic correlation of perinodal inflammation. Isolated

group 2 animals had abnormal consistency of sciatic nerves that were microscopically correlated with epineurial and fascial inflammation in the sciatic nerves.

	Males		Females	
	1	2	1	2
Group	0	98	0	98
Dose (µg/animal)	10	10	10	10
No. of Animals per Group				
Injection site, quadricep muscle (No. Examined)	(10)	(10)	(10)	(10)
Material accumulation; pale	0	9	0	8
Material accumulation	0	0	0	1
Swelling	0	6	0	5
Sciatic nerve (No. Examined)	(10)	(10)	(10)	(10)
Abnormal consistency	0	2	0	0
Lymph node, iliac (No. Examined)	(10)	(10)	(10)	(10)
Enlargement	0	1	0	2
Lymph node, popliteal (No. Examined)	(0)	(3)	(0)	(4)
Abnormal consistency	0	3	0	4

Table 44: Summary of gross pathology findings –Terminal euthanasia (day 23), sponsor provided (Study no. 1021-9921).

Microscopic findings:

In all group 2 males and females, microscopic findings were reported at the injection sites (right quadricep muscle). These findings were characterized by one or more of the following: mild to moderate acute inflammation with infiltration of large numbers of neutrophils, few macrophages that have vacuolated cytoplasm, occasional mast cells, and lesser lymphocytes and plasma cells, variable subcutaneous edema, and increased incidence of minimal myofiber degeneration/necrosis with minimal to mild inflammatory infiltrates.

In group 2 males and females, minimal to mild mixed inflammation was also reported in the right gastrocnemius muscle. Muscle swelling and degeneration/necrosis that typically associated with injection were not reported in the right gastrocnemius muscle. No inflammatory lesions were reported in the left gastrocnemius muscle at the terminal euthanasia.

In the right regional lymph nodes (popliteal, iliac, and inguinal) of group 2 of both sexes, minimal to moderate neutrophilic to mixed cell inflammatory infiltrates in the perinodal adipose tissues with increased macrophages in the lymphoid sinuses of occasional lymph nodes were reported. In the white pulp of spleen of most group 2 males, there was minimal decrease in lymphoid cellularity of marginal zone characterized by depletion of lymphocytes. Additionally, in the white pulp of spleen of group 2 of both sexes, there was decreased lymphoid cellularity with minimal loss of small lymphocytes of periarteriolar lymphoid sheaths (PALS) and minimal apoptosis/necrosis of lymphocytes predominantly in the germinal centers.

Infiltration with neutrophils admixed with few macrophages, lymphocytes, and mast cells were reported in the fascial and epineurial tissues of the right sciatic nerves of group 2 of both sexes. These findings were considered likely a local extension of the inflammatory changes at the injection site. No changes were reported in the nervous tissue itself.

Group Dose (µg/animal) No. Animals per Group	Males		Females	
	1	2	1	2
	0	98	0	98
	10	10	10	10
Injection Site, Quadricep Muscle (No. Examined)	10	10	10	10
Inflammation, neutrophilic; subcutaneous tissue; right	(0) ^a	(10)	(0)	(10)
Mild	0	0	0	1
Moderate	0	10	0	9
Inflammation, neutrophilic; myofiber; right	(0)	(10)	(0)	(10)
Minimal	0	0	0	2
Mild	0	10	0	8
Degeneration/necrosis; myofiber; right	(1)	(9)	(0)	(7)
Minimal	1	9	0	7
Skeletal Muscle, Gastrocnemius (No. Examined)	10	10	10	10
Mixed cell inflammation; right	(0)	(8)	(0)	(7)
Minimal	0	6	0	6
Mild	0	2	0	1
Sciatic Nerve (No. Examined)	10	10	10	10
Inflammation, neutrophilic; epineurial; fascia; right	(0)	(9)	(0)	(10)
Minimal	0	5	0	4
Mild	0	4	0	6
Lymph Node, Iliac (No. Examined)	10	10	10	10
Increased macrophage cellularity, sinusoid; right	(0)	(7)	(0)	(7)
Minimal	0	5	0	6
Mild	0	2	0	1
Adipose tissue inflammation, mixed cell, perivascular, perivascular/focal; right	(0)	(0)	(0)	(3)
Minimal	0	0	0	2
Mild	0	0	0	1
Adipose tissue infiltration, neutrophilic; right	(0)	(3)	(0)	(6)
Minimal	0	3	0	6
Lymph Node, Inguinal (No. Examined)	10	10	10	10
Increased macrophage cellularity, sinusoid; right	(0)	(1)	(0)	(1)
Minimal	0	1	0	1
Mixed cell infiltration, adipose tissue; right	(0)	(1)	(0)	(1)
Minimal	0	1	0	1
Lymph Node, Popliteal (No. Examined)	0	3	0	4
Adipose tissue inflammation, neutrophilic; right	(0)	(3)	(0)	(4)
Minimal	0	0	0	1
Mild	0	3	0	2
Moderate	0	0	0	1

Group Dose (µg/animal) No. Animals per Group	Males		Females	
	1	2	1	2
	0	98	0	98
	10	10	10	10
Spleen (No. Examined)	10	10	10	10
Decreased cellularity; lymphoid; marginal zone	(0)	(7)	(0)	(0)
Minimal	0	7	0	0
Apoptosis/necrosis; lymphoid; white pulp	(0)	(3)	(0)	(2)
Minimal	0	3	0	2
Decreased cellularity; lymphoid; periarteriolar lymphoid sheath; white pulp	(0)	(10)	(0)	(10)
Minimal	0	10	0	10

^a Numbers in parentheses represent the number of animals with the finding.

Table 45: Summary of microscopic findings – Terminal euthanasia (day 23), sponsor provided (Study no. 1021-9921).

Body temperature:

Not collected.

Serology:

To assess IgG antibodies against RSV pre-fusion protein, in rat serum samples immunized with mRNA-1345 collected under (b) (4) study No. 1021-9921, (b) (4) assay against RSV pre-fusion protein were used.

A total of 81 serum samples (42 samples from the week-1 timepoint and 39 samples from day 23 timepoint) were successfully tested to detect antibodies against RSV pre-fusion protein.

No detectable IgG titers were reported in groups 1 and 2 (mRNA-1345) samples at week-1 timepoint. Following two immunizations of mRNA-1345 vaccine at 98 µg/animal, administered on days 1 and 22, robust IgG titers were reported in group 2 (mRNA-1345) samples for day 23 timepoint.

	Animal	Week -1	Day 23		Animal	Week -1	Day 23
Group 1 Reference Item	1001A	<12	<12	Group 2 mRNA-1345 Dose 98 µg/animal	2001A	<12	No Sample
	1002A	<12	<12		2101A	<12	3526
	1003A	<12	<12		2002A	<12	7983
	1004A	<12	<12		2003A	<12	3691
	1005A	<12	<12		2004A	<12	5705
	1006A	<12	<12		2005A	<12	7062
	1007A	<12	<12		2006A	<12	3006
	1008A	<12	<12		2007A	<12	3979
	1009A	<12	No Sample		2008A	<12	1362
	1010A	<12	<12		2009A	<12	2123
	1501A	<12	<12		2010A	<12	5033
	1502A	<12	<12		2501A	<12	12259
	1503A	<12	<12		2502A	<12	25248
	1504A	<12	<12		2503A	<12	No Sample
	1505A	<12	<12		2603A	<12	9491
	1506A	<12	<12		2504A	<12	33575
	1507A	<12	<12		2505A	<12	19178
	1508A	<12	<12		2506A	<12	24527
	1509A	<12	<12		2507A	<12	13491
	1510A	<12	<12		2508A	<12	20223
		Antibody Units/mL against RSV pre-fusion Protein					Antibody Units/mL against RSV pre- fusion protein

Figure of “antibody units/mL” titers of group 1 (reference item) and group 2 (mRNA-1345)

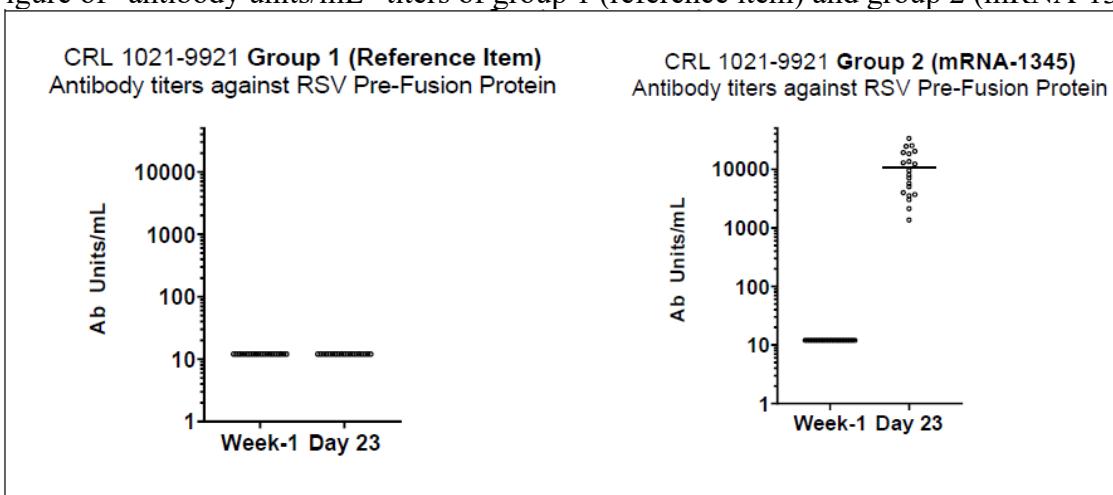


Figure 9: Antibody (units/mL) titers of group 1 (reference item) and group 2 (mRNA-1345), sponsor provided (Study no. 1021-9921).

Test article related effects are listed in the table below:

Test article related effects
<ul style="list-style-type: none"> ↓ A/G ratio ↑ Neutrophils ↓ Lymphocytes ↓ Monocytes ↑ Eosinophils ↑ Fibrinogen ↑ Spleen weight Lymph nodes (popliteal, iliac and inguinal) microscopic findings Injection site findings Immune responses

Table 47: Test article related effects (Study no. 1021-9921).

Assessment:

No treatment-related mortality or ophthalmologic changes were reported.

A low A/G ratio could indicate your albumin levels are too low (hypoalbuminemia), or your globulin levels are too high. High globulin indicates inflammation and immune system activity.

Overall, a low A/G ratio result is associated with: kidney disease (nephrotic syndrome), liver disease and indicator of overall liver function, chronic infections (including HIV, tuberculosis, and hepatitis), malnutrition, pancreatitis, autoimmune diseases (such as rheumatoid arthritis) certain cancers (liver cancer, multiple myeloma and other blood (hematologic) cancers, colorectal cancer, pancreatic cancer, and lung cancer), and type 2 diabetes (low albumin can indicate insulin deficiency)².

² <https://www.healthline.com/health/a-g-ratio-high#low-a-g-ratio>

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.

A lymphocyte is any of 3 types of white blood cell (all 3 are agranulocytes) in a vertebrate's immune system. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). Thus, any decrease, in one or all of these cell types, might affect the immune responses.

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair.

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

The increase in fibrinogen levels were considered to be related to treatment and is considered as an expected (inflammatory) response following treatment with immunogenic substances.

In the spleen, the weight increases might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system³. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal⁴. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

Minimal to moderate neutrophilic to mixed cell inflammatory infiltrates in the perinodal adipose tissues with increased macrophages in the lymphoid sinuses of occasional lymph nodes were reported in the right regional lymph nodes (popliteal, iliac, and inguinal) of group 2 of both sexes. Minimal decrease in lymphoid cellularity of marginal zone characterized by depletion of lymphocytes were reported in the white pulp of spleen of the majority of group 2 males. Additionally, in the white pulp of spleen of group 2 of both sexes, there was decreased lymphoid cellularity with minimal loss of small lymphocytes of periarteriolar lymphoid sheaths (PALS) and minimal apoptosis/necrosis of lymphocytes predominantly in the germinal centers.

Test article-related injection site findings (edema and/or erythema) were reported. Inflammation is a relatively common occurrence as part of the acute phase response following administration of some vaccines.

Test article-related immune responses in group 2 were reported.

³ Spleen, Internet Encyclopedia of Science.

⁴ Mebius RE, Kraal G. (2005). Structure and function of the spleen. *Nat Rev Immunol*. 5(8):606-16.

Based on the overall findings in this study, it can be concluded that in rats, repeat dose of mRNA 1345 on study days 1 and 22 had no adverse effects in terms of systemic toxicity at the dose level of 98 µg/animal.

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

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes () or no (X).

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

Study number 5: A Non-GLP Repeat Dose Immunogenicity and Toxicity Study of mRNA-1345 by Intramuscular Injection in (b) (4) Rats. Study number: 2308-121.

Performing laboratory: (b) (4)

Study initiation date: February 18, 2020

Final report date: June 29, 2020

Test article batch/lot:

Test Article Identification

Identification	DHM-45266
Alternate Identification	520 µg/vial mRNA-1345 (RSV)
Batch (Lot) No.	DHM-45266
Expiration/Retest Date	04 Aug 2020
Purity	(b) (4) (From RNA purity test)
Storage Conditions	Frozen at (b) (4) °C to -30°C, protected from light
Provided by	Moderna

Table 48: Test article identification, sponsor provided (Study no. 2308-121).

Test Article Reconstitution Buffer Identification

Identification	0.9% Sodium Chloride for Injection, USP
Batch (Lot) No.	Y315119
Expiration/Retest Date	Feb 2021
Storage Conditions	At controlled room temperature
Provided by	(b) (4)

Table 49: Test article reconstitution buffer identification, sponsor provided (Study no. 2308-121).

Control Article/Formulation Buffer Identification

Identification	Diluent SD-0724
Alternate Identification	(b) (4)
Batch (Lot) No.	6005219001
Expiration/Retest Date	10 Jul 2020

Identification	Diluent SD-0724
Storage Conditions	Refrigerated at (b) (4), protected from light
Provided by	Moderna

Table 50: Control article/Formulation buffer identification, sponsor provided (Study no. 2308-121).

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

Breeder/supplier: (b) (4)

Number of animal per group and sex: 5/sex/group

Age: 7 weeks

Body weight range: 149 and 266 g

Route and site of administration: Bolus intramuscular injection into one of the quadriceps (hind leg, thigh). A unique site was used for each injection (left quadricep on day 1; right quadricep on day 22)

Volume of injection: 0.2 mL

Frequency of administration and study duration: Days 1 and 22

Dose: 15, 50, or 100 µg/dose

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. Date of manufacturing was reported as (b) (4) and the expiration date was reported as (b) (4). Storage temperature is -20°C.

Means of administration: Intramuscular (IM)

Report status: Final report

Experimental design:

Animals were randomized and assigned to 4 different groups. Animals, 5/sex/group, were dosed by IM on study days 1 and 22. The dose levels were 15, 50, and 100 µg/dose and administered at a dose volume of 0.2 mL/dose. The details of the study design are listed in the following table:

Group No.	Test Material	Dose Level (µg/dose)	Dose Volume (mL/dose)	Dose Concentration (µg/mL)	Animal Numbers	
					Male	Female
1	Control Article	0	0.2	0	1001-1005	1501-1505
2	mRNA-1345	15	0.2	75	2001-2005	2501-2505
3	mRNA-1345	50	0.2	250	3001-3005	3501-3505
4	mRNA-1345	100	0.2	500	4001-4005	4501-4505

Table 51: Experimental design, sponsor provided (Study no. 2308-121).

Methods:

Randomization procedure: Yes

Statistical analysis plan: Yes.

The following parameters were evaluated:

Table of general in-life assessments

Parameter	Population(s)	Frequency (minimum required)	Comments
Mortality/Cageside Observations	All Animals	At least twice daily ^{a,b} (morning and afternoon) beginning upon arrival through termination/release.	Animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Injection Site Observations	All Animals	Immediately, 6 hours and 24 hours post dose.	Animals were observed for erythema, edema, and any other associated observations at the injection site.
Detailed Clinical Observations	All Animals	Once daily; from at day -7 and throughout the study.	Animals were removed from the cage. Observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.
Individual Body Weights	All Animals	At receipt, day -1, and once weekly during the study.	The body weights recorded at receipt are not reported but are maintained in the study file. Body weight changes were calculated for animals between each weighing interval.

^a Included alternate animals until released from study.^b Except on days of receipt and necropsy where frequency was at least once daily.

Table 52: General in-life assessments, sponsor provided (Study no. 2308-121).

Parameters	Frequency of Testing
Clinical chemistry*	Day 23 (24 hours post the last dose)
Hematology*	Day 23 (24 hours post the last dose)
Coagulation*	Day 23 (24 hours post the last dose)
Virus neutralization assay*	Pretest and on day 35
Postmortem study evaluations	Study day 35 (terminal groups)

* Site collection of blood samples were sublingual vein.

Table 53: Clinical pathology testing, sponsor provided (Study no. 2308-121).

Postmortem procedures:

Not examined.

Results:

No test article-related mortality was reported.

Clinical chemistry and hematology:

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≤ 1.5)	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Aspartate aminotransferase (AST or SGOT) SD23 F \uparrow = 2.1 G4	
B) HEPATOBILIARY	Alanine aminotransferase (ALT or SGPT) SD23 F \uparrow = 3.8 G4	
	Total bilirubin SD23 F \uparrow = 1.7 G4	Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Fasting triglycerides SD23 F \uparrow = 1.6 G3 SD23 F \uparrow = 1.9 G4	Albumin (A) Total protein Globulin A/G ratio Total Cholesterol Gamma-GT CK

Table 54: Clinical chemistry results (Study no. 2308-121).

Clinical chemistry results showed an increase in AST levels in group 4 females at study day 23. Clinical chemistry results showed an increase in ALT levels in group 4 females at study day 23. An increase in bilirubin levels in group 4 females at study day 23 was reported. An increase in triglyceride levels in groups 3 and 4 females at study day 23 was reported.

Group Dose Level ($\mu\text{g}/\text{dose}$) Sex	1* 0		2		3		4 100	
	M	F	M	F	M	F	M	F
Albumin (g/dL) Day 23 (24 hr post)	3.50	3.70	0.93x	–	0.89x	0.91x	0.84x	0.85x
Globulin (g/dL) Day 23 (24 hr post)	3.44	3.16	–	1.13x	–	1.13x	–	1.06x
Albumin/Globulin Ratio Day 23 (24 hr post)	1.02	1.17	0.87x	0.84x	0.83x	0.81x	0.80x	0.80x
AST (U/L) Day 23 (24 hr post)	122.2	116.6	–	–	–	–	–	2.07x

Group Dose Level (µg/dose) Sex	1* 0		2		3		4 100	
	M	F	M	F	M	F	M	F
ALT (U/L) Day 23 (24 hr post)	42.8	39.4	—	—	—	—	—	3.82x
Total Bilirubin (mg/dL) Day 23 (24 hr post)	0.12	0.14	—	—	—	—	—	2.00x
Triglycerides (mg/dL) Day 23 (24 hr post)	55.6	33.6	—	—	1.54x	1.62x	1.47x	1.88x
Calcium (mg/dL) Day 23 (24 hr post)	10.50	10.10	—	—	0.95x	—	0.93x	—
Chloride (mEq/L) Day 23 (24 hr post)	99.4	102.0	—	—	0.97x	0.95x	0.98x	0.96x

M = Males; F = Females; hr = hour/hours; post = postdose.
 ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.
 A dash (—) indicates absence of a mRNA-1345-related change. Numerical values indicate fold change of the treated group mean value relative to the control group mean value.
Bolded values indicate the mean value was statistically different from controls ($p < 0.05$ or $p < 0.01$).
 * Control group values are reported for comparison.

Table 55: mRNA-1345-related clinical chemistry changes, sponsor provided (Study no. 2308-121).

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.55, ie, ≥ 1.6 or ≤ 1.6)	Not of NOTE
Red blood cells	Reticulocytes SD23 M ↓ = 0.6 G4	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
White blood cells	Neutrophil count SD23 M ↑ = 3.1 G2 SD23 M ↑ = 5.5 G3 SD23 M ↑ = 4.7 G4 SD23 F ↑ = 6.7 G2 SD23 F ↑ = 9.9 G3 SD23 F ↑ = 7.9 G4 Lymphocyte count SD23 M ↓ = 0.6 G2 SD23 M ↓ = 0.6 G3 SD23 M ↓ = 0.4 G4 SD23 F ↓ = 0.6 G4	Macrophage White Blood Cells (WBC) Large Unstained Cells (LUC)

⁵ With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.55, ie, ≥ 1.6 or ≤ 1.6)	Not of NOTE
	Leukocytes SD23 F \uparrow = 1.8 G3 Monocyte count SD23 F \uparrow = 2.0 G2 SD23 F \uparrow = 2.0 G3 Eosinophils count SD23 M \uparrow = 1.7 G3 SD23 F \uparrow = 1.9 G2 SD23 F \uparrow = 4.0 G3 SD23 F \uparrow = 2.8 G4 Basophils SD23 F \uparrow = 1.6 G2 SD23 F \uparrow = 2.6 G3 Other cells SD23 M \uparrow = 2.0 G2 SD23 M \uparrow = 2.1 G3 SD23 F \uparrow = 2.2 G2 SD23 F \uparrow = 1.8 G3 SD23 F \uparrow = 1.7 G4	
Clotting potential		Activated partial-thromboplastin time Prothrombin time Platelet count Fibrinogen
Others		Bone marrow cytology

Table 56: Hematology results (Study no. 2308-121).

Group Dose Level ($\mu\text{g}/\text{dose}$) Sex	1* 0		2		3		4 100	
	M	F	M	F	M	F	M	F
Platelets ($10^3 \times \text{cells}/\mu\text{L}$) Day 23 (24 hr post)	1111.6	1083.4	0.82x	—	0.86x	—	0.79x	0.81x
Reticulocytes ($10^3 \times \text{cells}/\mu\text{L}$) Day 23 (24 hr post)	232.86	189.22	0.78x	—	0.79x	0.71x	0.65x	0.75x
Neutrophils ($10^3 \times \text{cells}/\mu\text{L}$) Day 23 (24 hr post)	1.938	0.82	3.10x	6.74x	5.45x	9.89x	4.74x	7.90x
Lymphocytes ($10^3 \times \text{cells}/\mu\text{L}$) Day 23 (24 hr post)	11.40	7.46	0.57x	0.74x	0.58x	—	0.41x	0.63x
Monocytes ($10^3 \times \text{cells}/\mu\text{L}$) Day 23 (24 hr post)	0.23	0.94	—	2.02x	—	1.98x	—	—

Group Dose Level (µg/dose) Sex	1* 0		2		3		4 100	
	M	F	M	F	M	F	M	F
Eosinophils (10³x cells/µL) Day 23 (24 hr post)	0.14	0.10	—	—	—	3.96x	—	2.80x
RDW (%) Day 23 (24 hr post)	12.26	10.90	—	—	1.08x	1.04x	1.04x	1.09x
MCH (pg) Day 23 (24 hr post)	19.16	19.52	—	—	1.05x	—	1.05x	—
MCHC (g/dL) Day 23 (24 hr post)	31.22	32.84	—	—	1.03x	—	1.02x	—
M = Males; F = Females; hr = hour/hours; post = postdose. MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration. RDW = Red blood cell distribution width. A dash (—) indicates absence of a mRNA-1345-related change. Numerical values indicate fold change of the treated group mean value relative to the control group mean value. Bolded values indicate the mean value was statistically different from controls (p < 0.05 or p < 0.01). * Control group values are reported for comparison.								

Table 57: mRNA-1345-related hematology changes, sponsor provided (Study no. 2308-121).

Neutrophil levels were increased in groups 2, 3, and 4 males and females at study day 23. Lymphocyte levels were decreased in groups 2, 3, and 4 males at study day 23. Lymphocyte levels were decreased in group 4 males at study day 23. Leukocyte levels were increased in group 3 females at study day 23. Monocyte levels were increased in groups 2 and 3 females at study day 23. Eosinophils levels were increased in group 3 males at study day 23. Eosinophils levels were increased in groups 2, 3, and 4 females at study day 23. Basophil levels were increased in groups 2 and 3 females at study day 23. Other cells (not specified) levels were increased in groups 2 and 3 males and groups 2, 3, and 4 females at study day 23.

Systemic toxicity:

No treatment-related mortality or body weight changes were reported.

In group 3 males and females, mRNA-1345-related effects of edema and impaired limb function at the injection site were reported at 24 hours post-test article administration. In group 3, edema was reported on day 2, with decreased incidence by day 3, and was resolved by day 4. However, impaired limb function was reported on day 2 and was resolved by day 3. In group 4, edema was reported on day 2 to day 3 with similar incidence and was resolved by day 4, while impaired limb function was only reported on day 2.

Edema was reported in group 2 males and females following the second dose on day 22. Edema was reported on days 23 and 24 in group 3, with decreased incidence by day 25 and resolved by day 26. In group 4, edema was reported on day 22 to day 25 at similar incidence, with decreasing incidence from day 26 to day 27, and was resolved by day 28. Limb function impairment was reported in a single female in group 3 and the majority of animals in group 4 on day 22. On day 23, limb function impairment was reported in group 3 animals and for one group 2 female. Limb function impairment was decreased in group 3 on day 24 and were all resolved by day 25. After the second dose on day 22, the severity of edema and impaired limb function was greater than

the first dose on day 1. This is based on the time when the clinical signs were first reported, the dose levels with clinical signs, and the time taken for the signs to resolve.

In a single group 4 female, carriage high was reported on days 23 and 24, which was likely a secondary effect to the mRNA-1345-related edema and impaired limb function reported for this animal.

Other clinical signs reported were not considered to be test article-related due either to low incidence, considered to be procedure-related, or commonly reported in group-housed laboratory animals.

Organ Weight:

Not collected.

Gross pathology:

Not collected.

Microscopic findings:

Not collected.

Injection Site Reactogenicity:

Except for a single group 4 female, there was no mRNA-(b) (4)-related erythema reported following the IM administration on day 1. No mRNA-(b) (4)-related erythema was reported following the second dose on day 22.

Immediately post-dose and at 6 hours post-dose on day 1, there was no mRNA-(b) (4)-related edema. At 24 hours post-dose, mRNA-(b) (4)-related edema was reported in group 3 females, 3 out of 5 males, and all group 4 males and females.

Immediately post day 22 dose, there was no mRNA-(b) (4)-related edema. At 6 hours post-dose, mRNA-(b) (4)-related edema was reported in one group 3 female, and in all group 4 males and females. At 24 hours post-dose, mRNA-(b) (4)-related edema was reported in all group 2 animals. When compared to the first administration on day 1, the severity of edema was greater after the second dose administration on day 22. This is based on the time when edema was first reported, the number of incidences recorded, and the dose levels with edema reported.

Body temperature:

Not collected.

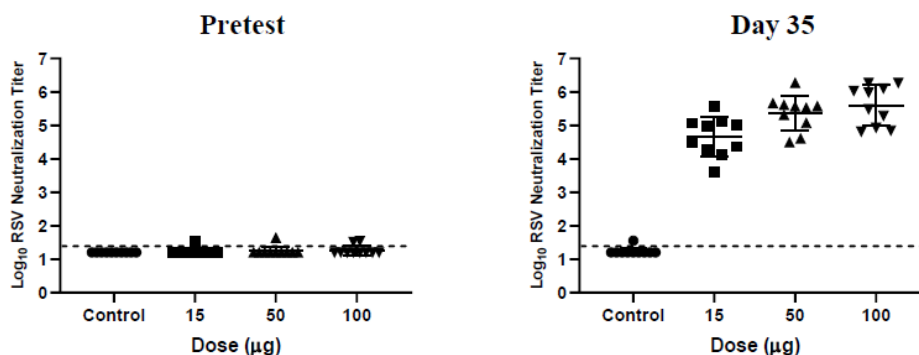
Serology:

To detect RSV-specific neutralization antibodies, an RSV ((b) (4)) ((b) (4)) assay was used. As shown in the table and the figure below, RSV neutralizing antibodies were detected in the serum of all rats administered mRNA-1345 with some evidence of a dose-dependent response at ≥ 15 $\mu\text{g}/\text{dose}$. In serum of animals from pretest and in day 35 samples from the control group, neutralizing antibodies were close to or below the limit of detection.

Group	Test Material	Dose level (ug/dose)	Sample ID	Pretest	Day 35	Group	Test Material	Dose level (ug/dose)	Sample ID	Pretest	Day 35
1	Reference Item	0	1001	16	16	3	mRNA-1345	50	3001	44	31314
			1002	16	36				3002	16	374435
			1003	16	16				3003	16	40584
			1004	16	16				3004	16	334614
			1005	16	16				3005	16	117932
			1501	16	16				3501	16	412903
			1502	16	16				3502	16	370496
			1503	16	16				3503	16	206890
			1504	16	16				3504	16	455771
			1505	16	16				3505	16	250478
2	mRNA-1345	15	2001	16	120182	4	mRNA-1345	100	4001	16	70051
			2002	35	102989				4002	16	85964
			2003	16	29632				4003	16	64941
			2004	16	23568				4004	33	193644
			2005	16	96522				4005	16	306166
			2501	16	374954				4501	16	477406
			2502	16	127010				4502	16	1275247
			2503	16	13584				4503	35	167376
			2504	16	18555				4504	16	977915
			2505	16	3977				4505	16	1084631

* Neutralizing antibody titers are expressed as highest reciprocal serum dilution capable of causing a 50% reduction in RSV plaques, as in the described assay method.

Table 58: RSV neutralizing antibody titers, sponsor provided (Study no. 2308-121).



* Neutralizing antibody titers are expressed as highest reciprocal serum dilution capable of causing a 50% reduction in RSV plaques, as in the described assay method.

Figure 10: RSV neutralizing antibody titers, sponsor provided (Study no. 2308-121).

Test article related effects are listed in the table below:

Test article related effects
<ul style="list-style-type: none"> ↑ Neutrophils ↑ Eosinophils ↓ Lymphocytes Injection site findings Immune responses

Table 59: Test article related effects (Study no. 2308-121).

Assessment:

No treatment-related mortality or body weight changes were reported.

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 hematopoiesis in the bone marrow before migrating into blood.

A lymphocyte is any of 3 types of white blood cell (all 3 are agranulocytes) in a vertebrate's immune system. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). Thus, any decrease, in one or all of these cell types, might affect the immune responses.

Test article-related injection site findings (edema) were reported. Inflammation is a relatively common occurrence as part of the acute phase response following administration of some vaccines.

Test article-related immune responses in groups 2, 3, and 4 were reported.

Based on the overall findings in this study, it can be concluded that in rats, repeat dose on study days 1 and 22 had no adverse effects in terms of systemic toxicity at the dose levels of 15, 50, and 100 µg/dose.

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

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes (X) or no ().

Conclusions:

Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

(b) (4)

(b) (4)

86 pages have been determined to be not releasable: (b)(4)

(b) (4)

Study number 12: (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 135: Experimental design (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 micronucleus 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 (°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
		°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
		°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
		°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
		°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
		°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
		°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
		°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
		°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 Pre-Dose	-24hr Pre-Dose	0hr Pre-Dose	Average Pre-Dose	0.5 Hr Post-Dose	1 Hr Post-Dose	2 Hr Post-Dose	4 Hr Post-Dose	5 Hr Post-Dose	6 Hr Post-Dose	8 Hr Post-Dose	24 Hr Post-Dose	48 Hr Post-Dose		
Vehicle	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	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	36.3	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	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	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

N/A - Not Applicable due to study design

°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.

Table 136: 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, CP, 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

Treatment	Gender	Time (Hrs)	Animals	%PCE (Mean +/- SD)	Toxicity (%)	% MnPCE (Mean +/- SD)	Number of MnPCE/PCE Scored
(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
CP							
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 137: Summary of bone marrow micronucleus 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 138: Summary of cytokine values in males (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 139: Summary of cytokine values in females (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.

Study number 13: (b) (4) **Vaccine:**

Study title: SM-102 Bacterial Reverse Mutation Test in (b) (4)
 . **Reviewed by Claudia. Study number:** 9601567.

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

Study No.: 9601567

Date of dose preparation: 22 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)

Positive control (b) (4)

(b) (4)

Table 140: 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 141: 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 $\pm 10\%$ of the theoretical concentration for the stock solution and $\pm 15\%$ 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) mix.

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

Study no.: 9601568

Conducting laboratory and location: (b) (4)

Date of study initiation (dosing): 15 Sep 2016

Date of study completion: 12 Jan 2012

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) (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 (0S9)	4 Hours (+S9)	24 Hours (0S9)
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	-	-
(b) (4)	1000	10	-	2	-
	1500	15	-	2	-
(b) (4)	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 142: 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 143: SM-102 - summary results and statistical analysis, table provided by the sponsor, sponsor provided (Study no. 9601568).

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

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.

9 pages have been determined to be not releasable: (b)(4)

(b) (4)

Study number 17:

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

Study design:

(b) (4)

(b) (4) were treated with the test items at a range of concentrations up to 5000 µg/plate (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 (µL) ^{(b) (4)}	Final Conc. (µg) ^{(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 152: Study design - (b) (4) assay (per test item) (Study no. 9601035).

Test and reference items**Test items**

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

⁴¹ WHO guidelines on the nonclinical evaluation of vaccines. In: *WHO Expert Committee on Biological Standardization. Fifty-fourth report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 1.

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: Purified sterile water

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)

(b) (4)

Results

Bacterial mutation test

The mean revertant colony counts for the vehicle controls were close to or within the laboratory historical control range. Positive controls (with (b) (4) mix 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 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.

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	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 revertants compared to concurrent vehicle control. SD = Sample standard deviation.

Table 153: (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 revertants compared to concurrent vehicle control. SD = Sample standard deviation.

Table 154: (b) (4) (PEG 2K-DMG) - (b) (4) assay in the presence of (b) (4), sponsor provided (Study no. 9601035).

Strain	Conc.	No. of Revertants						(b) (4) Observations *			Fold
	(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	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 revertants compared to concurrent vehicle control. SD = Sample standard deviation.

Table 155: (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
			x_1	x_2	x_3	mean	SD		x_1	x_2	x_3	Response †
(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 revertants compared to concurrent vehicle control.

SD = Sample 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 156: (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 revertants compared to concurrent vehicle control (b) (4).

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

(b) (4). SD = Sample standard deviation.

Table 157: 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 18:

Title and study number: (b) (4) (PEG 2K-DMG) and (b) (4); (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 $\mu\text{g}/\text{mL}$ for 4 hours in the absence and presence of a supplemented (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. ($\mu\text{g}/\text{mL}$)	Final Conc. ($\mu\text{g}/\text{mL}$)	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 158: 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: (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)

4 (b) (4)

* = Substantial increase compared to concurrent vehicle control.

Table 159: (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) ¹	-	2.0	0.0	2000	0.35
(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)				
⁴	(b) (4)				

* = Substantial increase compared to concurrent vehicle control.

Table 160: (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

It is concluded that (b) (4) (PEG 2K-DMG) and (b) (4) did not (b) (4) in human peripheral blood lymphocytes, with or without an (b) (4).

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