FINAL REPORT

Study Title

NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Test Article

NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles

Author

PPD , PhD

Study Completion Date

30 June 2020

Testing Facility

BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AF87FU.125012NGLPICH.BTL

Sponsor

Moderna Therapeutics
200 Technology Square
3rd Floor
Cambridge, MA 02139

1. REGULATORY REQUIREMENTS

Study No. AF87FU.125012NGLPICH.BTL was conducted in accordance with Standard Operating Procedures and as an exploratory study; it did not fall within the scope of the FDA/EPA Good Laboratory Practice (GLP) regulations. I, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

PPD

PPD

2. TABLE OF CONTENTS

3. STUDY INFORMATION

Study Conduct

Sponsor:	Moderna Therapeutics 200 Technology Square 3rd Floor Cambridge, MA 02139
Study Monitor:	PPD , MS, MBA, DABT
Testing Facility:	BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850
BioReliance Study No.:	AF87FU.125012NGLPICH.BTL

Test Article

ID:	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles
Purity:	90.5% (per Summary of Analysis) A correction factor of 1.105 was used for dose formulations.
Storage Conditions:	-65 to -90°C, protected from light
Concentration:	Lipid: 24.84 mg/mL mRNA: 1.30 mg/mL
Receipt Date:	20 November 2019

Negative/Vehicle Control

ID:	25 mM Tris/sucrose 1mM DTPA pH 7.5
BioReliance TAID:	AF99YN
Lot No.:	MTDS18021
Storage Conditions:	-65 to -90°C, protected from light
Receipt Date:	20 November 2019

Study Initiation Date:	06 December 2019
Experimental Starting Date (First day of Data Collection):	02 December 2019
Experimental Start Date (First Day of Dosing):	09 December 2019
Experimental Completion Date:	22 December 2019

Key Personnel

Study Director:	PPD , PhD
Test Facility Management:	PPD , MSc, Ph.D. Director, Genetic Toxicology Study Management
Laboratory Supervisor:	PPD , BS
Laboratory Supervisor (Dose Formulation Preparation):	PPD , BS, RLATG, CMAR
Report Writer:	PPD , BS
Principal Investigator (Bioanalytical– 2 hour samples):	PPD , BS
Analytical Test Site (Bioanalytical– 2 hour samples):	Moderna Therapeutics 200 technology Square, 3rd Floor Cambridge, MA 02139)
Principal Investigator (Cytokine analysis – 6 hour samples):	PPD
Analytical Test Site (Cytokine analysis – 6 hour samples):	[redacted]

4. SUMMARY

The test article, NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles, was evaluated for its clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocytes (PCEs) cell in rat bone marrow.

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 at 5 mL/kg once via intravenous injection. 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.

There was no Test Article-related mortality or clinical observations.

Test article-related increases in body temperature at 3.21 or 60 mg/kg (mRNA or SM-102, respectively) were observed males and females from 1-2 hours postdose to 8 hours postdose and met the protocol-specified parameters for hyperthermia (≥1°C increase for at least 4.5 hours).

Test article-related increases in IL-6, MCP-1, MIP-1α, and/or IP-10 were noted 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). Fold increases observed were up to 3.68x for IL-6, up to 4.66x for MCP-1, up to 2.62x for MIP-1 α, and up to 30.47x for IP-10.

There was no significant increase in the incidence of micronuclei in the test article dosed animals at either time point (24 or 48 hours). A slight, but statistically significant, decrease in %PCEs was observed in the low dose males at the 48 hour time point.

In conclusion, the test article, NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles, was determined to be negative (non-clastogenic) under the conditions of this study at doses up to 3.21 or 60 mg/kg (mRNA or SM-102, respectively)

5. PURPOSE

The objective of this study was to evaluate a 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. This assay design is based on OECD Guideline 474 (OECD, 2016), the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (2011), and ISO/IEC 17025:2005 (ISO/IEC, 2005).

Historical control data are found in Appendix I. A copy of the study protocol and amendments is found in Appendix II.

6. CHARACTERIZATION AND PREPARATION OF TEST AND CONTROL ARTICLES

A copy of the Summary of Analysis for characterization of the test article is included in Appendix V.

The vehicle, 25 mM Tris/sucrose 1 mM DTPA pH 7.5, was provided by the Sponsor and assigned the BioReliance TAID AF99YN. Any vehicle samples were used during testing, and there is none remaining to return.

The vehicle used to prepare the test article formulations is characterized by the Certificates of Analysis provided. A copy of the Certificate of Analysis is kept on file at BioReliance.

Scoring positive control slides (fixed and unstained), generated from BioReliance Study No. AF65AY.125M012.BTL, were included to verify scoring. These slides were generated from male rats treated once with cyclophosphamide monohydrate (CP) at 40 mg/kg, and the bone marrow harvested 24 hours after treatment.

The positive control articles have been characterized as per the Certificates of Analysis on file with the testing facility. The stability of the positive control article is demonstrated by acceptable results that met the criteria for a valid test.

Preparation of Dose Formulations

The bulk test item was thawed, mixed gently by swirling, and diluted with 25 mM Tris/sucrose 1mM DTPA pH 7.5 to achieve target concentrations. Final mixtures were inverted/swirled for one minute until uniform. Material was mixed using aseptic techniques in a biological safety cabinet. Dose formulations were stored at room temperature prior to delivery to the dosing lab and were stored refrigerated (2 to 8°C) prior to dosing. Refrigerated dose formulations were equilibrated at room temperature for at least 30 minutes prior to the start of dosing and were used within 3 hours after being equilibrated at room temperature.

Residual dose formulations were discarded.

7. MATERIALS AND METHODS

The assay was conducted according to established procedures (Heddle, 1973; Mavournin et al., 1990; Hayashi et al., 1994; OECD, 2016).

Test System

Sprague-Dawley (Hsd:SD) rats were received from Envigo RMS, Inc., Frederick, MD on 02 December 2019.

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		

Justification for the Test System

This species has been routinely used as an animal model of choice for the mammalian bone marrow erythrocyte micronucleus assay. This strain is an outbred strain that maximizes genetic heterogeneity and therefore tends to eliminate strain-specific response to the test article.

Animal Welfare Provisions

This study is not duplicative or unnecessary. The number of animals, procedures, and design used for this study, has been reviewed and were approved by the BioReliance Institutional Animal Care and Use Committee. Procedures involving animals performed at BioReliance follow the specifications recommended in the most current version of *The Guide for the Care and Use of Laboratory Animals* adopted by BioReliance (National Academy Press, Washington, D.C., 2011).

Animal Receipt and Acclimation

Virus antibody-free (VAF) animals were acclimated as noted above and were judged to be healthy prior to utilization in the study.

Housing

Animals were housed in a controlled environment at 72 ± 3°F and 50 ± 20% relative humidity with a 12-hour light/dark cycle. The light cycle was interrupted for study related

activities. The animal rooms were supplied with at least 10 changes of fresh HEPA-filtered air per hour. Animals of the same sex were housed three per Micro-Barrier cage. Cages were placed on racks equipped with an automatic watering system and Micro-VENT full ventilation, HEPA filtered system.

Environmental Enrichment

Animals were provided with Nylabones as environmental enrichment.

Bedding, Food and Water

Heat treated hardwood chips were used for bedding to absorb liquids. A certified laboratory rodent chow (Envigo 2018C Teklad Global 18% Protein Rodent Diet) was provided *ad libitum*. The food was analyzed by the manufacturer for the concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates and specified nutrients. Animals had free access to tap water, which met U.S. EPA drinking water standards [Washington Suburban Sanitary Commission (WSSC) Potomac Plant]. Drinking water was monitored at least annually for levels of specified microorganisms, pesticides, heavy metals, alkalinity and halogens. The results of bedding, food and water analyses are on file at BioReliance. There were no contaminants in the bedding, feed and water that were expected to interfere with the study.

Randomization and Identification

Animals were assigned to groups using a randomization procedure within Provantis™. At the time of randomization, the weight variation of animals did not exceed ±20% of the mean weight. Following randomization, animals were identified by sequentially numbered ear tags. The cage card contained, at least, the animal number(s), sex, study number, treatment group number, dose level, test article ID and route of administration. Cage cards were color coded by treatment group. Raw data records and specimens were also identified by the unique animal number.

Body Weights and Animal Observation

Body weights were recorded prior to the first dose for the purpose of dose volume calculations. Body weights were also recorded on the day of sacrifice excluding animals used for bioanalysis. Animals were observed once daily for signs of illness and poor health during the acclimation period. Once dosing was initiated, animals were observed twice daily for signs of illness or poor health. Animals were observed prior to dose administration, approximately one and two hours after dose administration and at least once daily on non-dosing days, excluding animals used for bioanalysis for clinical signs of toxicity.

Dose Administration

All dose formulations were administered once at a volume of 5 mL/kg by intravenous injection (slow push over 1.5 to 2.5 minutes) using appropriately sized disposable

polypropylene syringes. The route has been routinely used and is widely-accepted for use in the mammalian bone marrow erythrocyte micronucleus assay.

Body Temperatures

Body temperatures were monitored in animals in Groups 1-4 using implantable programmable temperature transponders. Temperatures were taken at approximately 48 and 24 hours prior to dose, prior to dose on the day of dosing, 0.5, 1, 2, 4, 5, 6, 8, and approximately 24 and 48 hours post dose. The 48 hour body temperature was recorded only for animals not sacrificed at the 24 hour bone marrow collection time point.

Group mean body temperatures (and standard deviation) were calculated for each dose level and time point, by sex. The test article was considered to cause hyperthermia at a particular dose level if group body temperature increased by ≥1.5°C for more than one hour, or by ≥1°C for more than 4.5 hours. If the mean group body temperature decreased by ≥ 3°C for more than 4.5 hours, the test article was considered to cause hypothermia at that dose. Body temperature changes exceeding those above have been reported to induce micronucleus formation (Guzman et al, 2004; King and Wild, 1983; Asanami and Shimono, 1997; Asanami and Shimono, 1999).

Micronucleus Assay

The assay design as follows:

Euthanasia Time (hours postdose)[B]					24	48
Group No.	Test Article	Dose Level of Test Article (mg/kg [mRNA/SM102 lipid])	Concentration (mg/mL [mRNA/SM102 lipid])	Dose Volume[A] (mL/kg)	Number of Animals/Sex	
1	Vehicle/ Negative Control	0/0	0/0	5	5	5
2	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	0.32/6.0	0.064/1.2	5	5	5
3	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	1.07/20	0.22/4	5	5	5
4	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	3.21/60	0.64/12	5	5	5

[A]Based upon individual body weight
[B]Range(s): 24-27 hours and 45-48 hours, respectively

The high dose for the micronucleus assay was 60 mg/kg of SM102 Lipid based upon information provided by the Sponsor.

Blood (Plasma) Collection and Sample Handling for TK and Cytokine Analysis

Group No.	Test Article	Dose Level of Test Article (mg/kg [mRNA/SM102 lipid])	Concentration (mg/mL [mRNA/SM102 lipid])	TK (Bioanalysis)/ Cytokine Animals/Sex	Sample Collection Timepoint (hours postdose)
6	Vehicle/ Negative Control	0/0	0/0	3	2 and 6
7	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	0.32/6.0	0.064/1.2	3	2 and 6
8	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	1.07/20	0.22/4	3	2 and 6
9	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	3.21/60	0.64/12	3	2 and 6

Frequency 1st day of dosing

Collection Site Retro-orbital Sinus

Target Volume 0.5mL of whole blood.

Anesthesia Animals were anesthetized prior to collection by 70% CO_2/30% O_2.

Anticoagulant K_2EDTA

Sample Handling Blood samples were maintained on wet ice until centrifugation.

Centrifugation Blood collection was conducted in group number sequence order from Groups 6 to 9. Blood samples were centrifuged for 5 minutes, 2-8°C, at 2000 g within 1 hour of collection and plasma was harvested into two, approximately equal, aliquots (primary and back up).

Sample Storage One set of plasma samples were stored at ≤ -60°C until packed on dry ice and shipped to the Test Site for analysis. The remaining set was retained at BioReliance at ≤ -60°C as a backup.

Animal Disposition Animals were sacrificed by CO_2 overdose after their last collection timepoint.

Bioanalysis (BioA)

A non-validated method (bDNA) was used to analyze the concentration of mRNA in the plasma samples. Plasma samples (3 samples/sex/group; Groups 6, 7, 8, and 9) collected at 2 hours post-dose were shipped on dry ice by overnight courier to the Principal Investigator for Bioanalysis. Upon receipt, samples were stored at -80°C or below until required for analysis. Unused samples were discarded upon acceptance of the analytical results by the Study Director. Due to technical issues with the assay, results were considered to be unreliable and thus not reported.

For BioA (2 hr post-dose collections) samples were shipped to:

PPD , BS
Moderna, Inc.
200 Technology Square, 3rd Floor
Cambridge, MA 02139
Phone: PPD
E-mail: PPD

Cytokine analysis

A non-validated method (Luminex) was used to analyze the concentrations of cytokines (MIP-1a, MCP-1, IL-6, IL-1B, TNFα, IP-10) in the plasma samples. Plasma samples collected at 6 hours post-dose (3 samples/sex/group; Groups 6, 7, 8, and 9) were shipped on dry ice by overnight courier to the Principal Investigator for Cytokine Analysis. Upon receipt, samples were stored at -80°C or below until required for analysis. Unused samples were discarded upon acceptance of the analytical results by the Study Director in consultation with the Sponsor Representative. The cytokine analysis report is included in Appendix IV.

For cytokine analysis (6 hr post-dose) samples were shipped to:

PPD
[redacted]
Email: PPD

Bone Marrow Collection and Slide Preparation

Femoral bone marrow was collected at approximately 24 and 48 hours after dose administration, as indicated above. Animals were euthanized by carbon dioxide inhalation. Immediately following euthanasia, the femurs were exposed, cut just above the knee, and the bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow was transferred to a centrifuge tube containing 2 mL fetal bovine serum, the cells were

pelleted by centrifugation, and the supernatant was drawn off leaving a small amount of fetal bovine serum with the pellet. Cells were re-suspended and a small drop of the bone marrow suspension was spread onto a clean glass slide. Four slides were prepared from each animal, air dried and fixed by dipping in methanol. One set of two slides (including at least five positive control slides) was stained with acridine orange for microscopic evaluation. The other set of slides was kept as backup and were archived at report finalization. Stained slides were discarded prior to report finalization. Each slide was identified by the harvest date, study number, and animal number. Slides were coded using a random number table by an individual not involved with the scoring process.

Scoring

Bone marrow was evaluated by fluorescent microscopy. The staining procedure permitted the differentiation by color of polychromatic and normochromatic erythrocytes (bright orange PCEs and ghost-like, dark green NCEs, respectively).

The criteria for the identification of micronuclei are those of Schmid (1975). Micronuclei are brightly stained bodies that generally are round and that generally are between 1/20 and 1/5 the size of the PCE. Scoring was based upon the micronucleated cell, not the micronucleus; thus, occasional cells with more than one micronucleus were counted as one micronucleated PCE (MnPCE), not two (or more) micronuclei.

4000 PCEs/animal were scored for the presence of micronuclei (MnPCEs), whenever possible. In addition, at least 500 total erythrocytes (PCEs + NCEs) were scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity.

Stained slides were discarded prior to report finalization.

Statistical Analysis

Statistical analysis was performed on the micronucleus frequency (%MnPCE) and %PCE using the animal as the unit. The mean and standard deviation of %MnPCE and %PCE were presented for each treatment group.

The use of parametric or non-parametric statistical methods in the evaluation of data was based on the variation between groups. The group variances for micronucleus frequency for the vehicle and test article groups at the respective sampling time were compared using Levene's test (significance level of $p \leq 0.05$). Since the variation between groups was found not to be significant, a parametric one-way ANOVA was performed followed by a Dunnett's post-hoc analysis to compare each dose group to the concurrent vehicle control.

A linear regression analysis was conducted to assess dose responsiveness in the test article treated groups ($p \leq 0.01$).

A pair-wise comparison (Student's T-test) was used to compare the positive control group to the concurrent vehicle control group.

Criteria for Determination of a Valid Test

The group mean frequency of MnPCEs for the vehicle control group should ideally be within the 95% control limits of the distribution of the historical negative control database. If the concurrent negative control data fall outside the 95% control limits, they may be acceptable as long as these data are not extreme outliers (indicative of experimental or human error).

The frequency of MnPCEs for the scoring positive controls must be significantly greater than the concurrent vehicle control ($p \leq 0.05$) and should be compatible with those observed in the historical positive control data base.

At least three doses were tested for at least one sampling time. Five animals/sex/group were available for analysis.

The maximum dose evaluated for micronucleus induction was the MTD or MFD.

Evaluation of Test Results

A test article was considered to have induced a positive response if:

a) at least one of the test article doses exhibited a statistically significant increase when compared with the concurrent negative control ($p \leq 0.05$), and
b) when multiple doses were examined at a particular sampling time, the increase was dose-related ($p \leq 0.01$ $R^2 \geq 70\%$), and
c) results of the group mean or of the individual animals in at least one group were outside the 95% control limit of the historical negative control data.

A test article was considered to have induced a clear negative response if none of the criteria for a positive response were met and there was evidence that the bone marrow was exposed to the test article (unless intravenous administration was used).

Electronic Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis included, but were not limited to, the following:

System	Purpose
CCI	Test Article Tracking Captures in-life toxicology, animal randomization and management data Calculations/Randomization Environmental Monitoring Generates in-life toxicology tables

Records and Archives

Upon issue of the final report, all raw data for procedures performed at BioReliance will be returned to the Sponsor.

The raw data, Reports, and other documents generated at locations other than BioReliance will be archived by the Test Site.

8. RESULTS AND DISCUSSION

Micronucleus Assay

Clinical signs are presented in Table 1 (Hands-On) and Table 2 (Cage side and Mortality). Mean group body weight data are found in Table 3.

There was no Test article-related mortality or clinical observations.

Increased temperatures in both males and females were observed at the high dose level (3.21/60 mg/kg) from 1-2 hours postdose to 8 hours postdose and met the protocol-specified parameters for hyperthermia (≥1°C increase for at least 4.5 hours). Body temperature results are included in Appendix III.

Bone Marrow Analysis

The incidence of MnPCEs per 40,000 PCEs scored (4000 PCEs/animal) and the proportion of polychromatic erythrocytes per total erythrocytes are summarized and presented for each treatment group by sacrifice time in Table 4. Individual animal data are presented in Table 5.

The scoring results and a statistical analysis of data indicated the following:

- A statistically significant reduction in the PCEs/EC ratio was observed 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 in the vehicle and test article groups were compared using Levene's test. The test indicated that there was no significant difference in the group variance ($p > 0.05$); 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 observed 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 (Appendix I).

Based upon this, all criteria for a valid test were met as specified in the protocol. NPI Luciferase 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

A copy of the report is included in Appendix IV.

Administration of NPI Luciferase mRNA in SM102-containing lipid nanoparticles to rats when given by slow intravenous injection elicited cytokine changes including increases in IL-6, MCP-1, MIP-1α, 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).

9. CONCLUSION

Under the conditions of the assay described in this report, NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles was concluded to be negative for the induction of micronucleated polychromatic erythrocytes.

10. REFERENCES

Asanami, S. and Shimono, K. Hypothermia induces micronuclei in mouse bone marrow cells. Mutation Res 393 91-98, 1997.

Asanami, S. and Shimono, K. The effect of hyperthermia on micronucleus induction by mutagens in mice. Mutation Res 446: 149-154, 1999.

Guzman, A., C. Garcia, A.P. Marin, M.T. Ruiz, A. Tortajada, A.R. Fernandez de Henestrosa. Induction of micronuclei in mouse bone-marrow erythrocytes in association with hypothermia after administration of the sigma receptor ligand E-5842. Mutation Res. 565: 11-22, 2004.

Heddle J.A. A rapid in vivo test for chromosomal damage. Mutation Res. 18:187 190, 1973.

Hayashi M., Tice R.R., MacGregor J.T., Anderson D., Blakey D.H., Kirsch-Volders M., Oleson Jr. F.B., Pacchierotti F., Romagna F., Shimada H., Sutou S. and Vannier B. In vivo rodent erythrocyte micronucleus assay. Mutation Res. 312: 293-304, 1994.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. S2(R1) document recommended for adoption at step 4 of the ICH process on 9 November 2011. Adopted at Step 5 in Europe by CHMP December 2011 (issued as EMA/CHMP/ICH/126642/2008). Adopted at Step 5 in US by FDA on June 7, 2012 (issued as 77 FR 33748 pages 33748-33749). Adopted in Japan at Step 5 September 20, 2012 (issued as PFSB/ELD Notification No. 0920-2).

ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories.

King, M.T. and Wild, D. The mutagenic potential of hyperthermia and fever in rats. Mutation Res.; 111:219-226, 1983

Mavournin K.H., Blakey D.H., Cimino M.C., Salamone M.F. and Heddle J.A. The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene Tox Program. Mutation Res. 239:29-80, 1990.

National Research Council, Institute of Laboratory Animal Resources Commission on Life Sciences. Guide for the Care and Use of Laboratory Animals. National Academy Press. Washington, D.C., 2011.

OECD Guidelines for Testing of Chemicals, Guideline 474 (Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test), Updated guidance adopted 29 July 2016.

Schmid W. The micronucleus test. Mutation Res. 31:9-15, 1975.

11. DATA TABLES

Table 1: – Clinical Signs (Hands On)

RTA001-02/01 Provantis (v.9.4.6.3) Date: 01/28/2020 14:42

Clinical Observations - Clinical Signs by Animal

AF87FU125DEF - AF87FU.125012NGLPICH.BTL NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	1
1	m	295	No Abnormalities Detected	X
		296	No Abnormalities Detected	X
		297	No Abnormalities Detected	X
		298	No Abnormalities Detected	X
		299	No Abnormalities Detected	X
		300	No Abnormalities Detected	X
		301	No Abnormalities Detected	X
		302	No Abnormalities Detected	X
		303	No Abnormalities Detected	X
		304	No Abnormalities Detected	X
2	m	305	No Abnormalities Detected	X
		306	No Abnormalities Detected	X
		307	No Abnormalities Detected	X
		308	No Abnormalities Detected	X
		309	No Abnormalities Detected	X
		310	No Abnormalities Detected	X
		311	No Abnormalities Detected	X
		312	No Abnormalities Detected	X
		313	No Abnormalities Detected	X
		314	No Abnormalities Detected	X
3	m	315	No Abnormalities Detected	X
		316	No Abnormalities Detected	X
		317	No Abnormalities Detected	X
		318	No Abnormalities Detected	X
		319	No Abnormalities Detected	X
		320	No Abnormalities Detected	X
		321	No Abnormalities Detected	X
		322	No Abnormalities Detected	X
		323	No Abnormalities Detected	X
		324	No Abnormalities Detected	X
4	m	325	No Abnormalities Detected	X
		326	No Abnormalities Detected	X
		327	No Abnormalities Detected	X
		328	No Abnormalities Detected	X
		329	No Abnormalities Detected	X
		330	No Abnormalities Detected	X
		331	No Abnormalities Detected	X
		332	No Abnormalities Detected	X
		333	No Abnormalities Detected	X
		334	No Abnormalities Detected	X

Severity Codes: X = Present

Group 1 - 0/0 mg/kg/day Group 2 - 0.32/6.0 mg/kg/day
Group 3 - 1.07/20 mg/kg/day Group 4 - 3.21/60 mg/kg/day

Table 1 Cont.: – Clinical Signs (Hands On)

RTA001-02/01 Provantis (v.9.4.6.3) Date: 01/28/2020 14:42

Clinical Observations - Clinical Signs by Animal

AF87FU125DEF - AF87FU.125012NGLPICH.BTL NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	1
1	f	335	No Abnormalities Detected	X
		336	No Abnormalities Detected	X
		337	No Abnormalities Detected	X
		338	No Abnormalities Detected	X
		339	No Abnormalities Detected	X
		340	No Abnormalities Detected	X
		341	No Abnormalities Detected	X
		342	No Abnormalities Detected	X
		343	No Abnormalities Detected	X
		344	No Abnormalities Detected	X
2	f	345	No Abnormalities Detected	X
		346	No Abnormalities Detected	X
		347	No Abnormalities Detected	X
		348	No Abnormalities Detected	X
		349	No Abnormalities Detected	X
		350	No Abnormalities Detected	X
		351	No Abnormalities Detected	X
		352	No Abnormalities Detected	X
		353	No Abnormalities Detected	X
		354	No Abnormalities Detected	X
3	f	355	No Abnormalities Detected	X
		356	No Abnormalities Detected	X
		357	No Abnormalities Detected	X
		358	No Abnormalities Detected	X
		359	No Abnormalities Detected	X
		360	No Abnormalities Detected	X
		361	No Abnormalities Detected	X
		362	No Abnormalities Detected	X
		363	No Abnormalities Detected	X
		364	No Abnormalities Detected	X
4	f	365	No Abnormalities Detected	X
		366	No Abnormalities Detected	X
		367	No Abnormalities Detected	X
		368	No Abnormalities Detected	X
		369	No Abnormalities Detected	X
		370	No Abnormalities Detected	X
		371	No Abnormalities Detected	X
		372	No Abnormalities Detected	X
		373	No Abnormalities Detected	X
		374	No Abnormalities Detected	X

Severity Codes: X = Present

Group 1 - 0/0 mg/kg/day
Group 2 - 0.32/6.0 mg/kg/day
Group 3 - 1.07/20 mg/kg/day
Group 4 - 3.21/60 mg/kg/day

Table 2: Clinical Signs (Cage side and Mortality)

RTA001-02/01 Provantis (v.9.4.6.3) Date: 12/12/2019 19:19

Clinical Observations - Clinical Signs by Animal

AF87FU125DEF - AF87FU.125012NGLPICH.BTL NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	1	2	3
1	m	295	No Abnormalities Detected	X	X	.
		296	No Abnormalities Detected	X	X	.
		297	No Abnormalities Detected	X	X	.
		298	No Abnormalities Detected	X	X	.
		299	No Abnormalities Detected	X	X	.
		300	No Abnormalities Detected	X	X	X
		301	No Abnormalities Detected	X	X	X
		302	No Abnormalities Detected	X	X	X
		303	No Abnormalities Detected	X	X	X
		304	No Abnormalities Detected	X	X	X
2	m	305	No Abnormalities Detected	X	X	.
		306	No Abnormalities Detected	X	X	.
		307	No Abnormalities Detected	X	X	.
		308	No Abnormalities Detected	X	X	.
		309	No Abnormalities Detected	X	X	.
		310	No Abnormalities Detected	X	X	X
		311	No Abnormalities Detected	X	X	X
		312	No Abnormalities Detected	X	X	X
		313	No Abnormalities Detected	X	X	X
		314	No Abnormalities Detected	X	X	X
3	m	315	No Abnormalities Detected	X	X	.
		316	No Abnormalities Detected	X	X	.
		317	No Abnormalities Detected	X	X	.
		318	No Abnormalities Detected	X	X	.
		319	No Abnormalities Detected	X	X	.
		320	No Abnormalities Detected	X	X	X
		321	No Abnormalities Detected	X	X	X
		322	No Abnormalities Detected	X	X	X
		323	No Abnormalities Detected	X	X	X
		324	No Abnormalities Detected	X	X	X
4	m	325	No Abnormalities Detected	X	X	.
		326	No Abnormalities Detected	X	X	.
		327	No Abnormalities Detected	X	X	.
		328	No Abnormalities Detected	X	X	.
		329	No Abnormalities Detected	X	X	.
		330	No Abnormalities Detected	X	X	X
		331	No Abnormalities Detected	X	X	X
		332	No Abnormalities Detected	X	X	X
		333	No Abnormalities Detected	X	X	X
		334	No Abnormalities Detected	X	X	X

Severity Codes: X = Present

Group 1 - 0/0 mg/kg/day
Group 2 - 0.32/6.0 mg/kg/day
Group 3 - 1.07/20 mg/kg/day
Group 4 - 3.21/60 mg/kg/day

Table 2 Cont.: Clinical Signs (Cage side and Mortality)

RTA001-02/01 Provantis (v.9.4.6.3) Date: 12/12/2019 19:19

Clinical Observations - Clinical Signs by Animal

AF87FU125DEF - AF87FU.125012NGLPICH.BTL NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	1	2	3
1	f	335	No Abnormalities Detected	X	X	.
		336	No Abnormalities Detected	X	X	.
		337	No Abnormalities Detected	X	X	.
		338	No Abnormalities Detected	X	X	.
		339	No Abnormalities Detected	X	X	.
		340	No Abnormalities Detected	X	X	X
		341	No Abnormalities Detected	X	X	X
		342	No Abnormalities Detected	X	X	X
		343	No Abnormalities Detected	X	X	X
		344	No Abnormalities Detected	X	X	X
2	f	345	No Abnormalities Detected	X	X	.
		346	No Abnormalities Detected	X	X	.
		347	No Abnormalities Detected	X	X	.
		348	No Abnormalities Detected	X	X	.
		349	No Abnormalities Detected	X	X	.
		350	No Abnormalities Detected	X	X	X
		351	No Abnormalities Detected	X	X	X
		352	No Abnormalities Detected	X	X	X
		353	No Abnormalities Detected	X	X	X
		354	No Abnormalities Detected	X	X	X
3	f	355	No Abnormalities Detected	X	X	.
		356	No Abnormalities Detected	X	X	.
		357	No Abnormalities Detected	X	X	.
		358	No Abnormalities Detected	X	X	.
		359	No Abnormalities Detected	X	X	.
		360	No Abnormalities Detected	X	X	X
		361	No Abnormalities Detected	X	X	X
		362	No Abnormalities Detected	X	X	X
		363	No Abnormalities Detected	X	X	X
		364	No Abnormalities Detected	X	X	X
4	f	365	No Abnormalities Detected	X	X	.
		366	No Abnormalities Detected	X	X	.
		367	No Abnormalities Detected	X	X	.
		368	No Abnormalities Detected	X	X	.
		369	No Abnormalities Detected	X	X	.
		370	No Abnormalities Detected	X	X	X
		371	No Abnormalities Detected	X	X	X
		372	No Abnormalities Detected	X	X	X
		373	No Abnormalities Detected	X	X	X
		374	No Abnormalities Detected	X	X	X

Severity Codes: X = Present

Group 1 - 0/0 mg/kg/day
Group 2 - 0.32/6.0 mg/kg/day
Group 3 - 1.07/20 mg/kg/day
Group 4 - 3.21/60 mg/kg/day

Table 3: Group Mean Body Weights

RTA023-05/00 Provantis (v.9.4.6.3) Date: 12/12/2019 19:20

Bodyweights - Intergroup Comparison of Bodyweight Gains

AF87FU125DEF - AF87FU.125012NGLPICH.BTL NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay

Body Weight Gain (Grams)

Day numbers relative to Start Date

Group	Sex	Base Weight Day 1	From: To:	1 2	1 3	Abs Gain 1 3	% Gain 1 3
1	m	206.89	Mean	-1.76	10.84	10.84	5.15
		4.95	S.D.	2.02	5.46	5.46	2.43
2	m	205.95	Mean	-1.42	8.34	8.34	4.09
		6.07	S.D.	3.00	2.19	2.19	1.06
3	m	208.67	Mean	-6.90	1.08	1.08	0.51
		5.14	S.D.	3.12	5.30	5.30	2.58
4	m	209.02	Mean	-10.82	-6.04	-6.04	-2.88
		6.57	S.D.	2.75	7.78	7.78	3.68
1	f	163.27	Mean	-7.14	3.00	3.00	1.84
		3.69	S.D.	2.74	1.17	1.17	0.70
2	f	160.46	Mean	-5.54	0.66	0.66	0.43
		2.68	S.D.	1.66	3.39	3.39	2.12
3	f	162.64	Mean	-3.84	2.58	2.58	1.60
		3.17	S.D.	2.32	5.11	5.11	3.10
4	f	162.23	Mean	-4.80	0.50	0.50	0.28
		4.17	S.D.	2.25	2.56	2.56	1.58

Statistical analysis not performed - Arithmetic mean values presented

Abs Gain = absolute bodyweight gain between base period and end of the analysis period
% Gain = percentage bodyweight gain between base period and end of the analysis period

Group 1 - 0/0 mg/kg/day
Group 2 - 0.32/6.0 mg/kg/day
Group 3 - 1.07/20 mg/kg/day
Group 4 - 3.21/60 mg/kg/day

Table 4: Summary of Bone Marrow Micronucleus Analysis

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
NPI Luciferase 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
C							
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
NPI Luciferase 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 5: Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow Collected 24 Hours Following Dose Administration

		Animal		Micronucleus Frequency		
Treatment	Sex	No.	%PCE	MnPCE	PCE	%
Vehicle	M	295	64.0	4	4000	0.10
0 mg/kg/day		296	63.4	4	4000	0.10
		297	60.6	1	4000	0.03
		298	53.4	6	4000	0.15
		299	50.2	4	4000	0.10
Vehicle	F	335	62.6	6	4000	0.15
0 mg/kg/day		336	74.8	4	4000	0.10
		337	69.6	5	4000	0.13
		338	63.4	2	4000	0.05
		339	63.0	6	4000	0.15
NPI Luciferase mRNA in SIV	M	305	69.2	5	4000	0.13
0.32 mg/kg/day		306	61.8	4	4000	0.10
		307	64.6	4	4000	0.10
		308	62.6	5	4000	0.13
		309	73.0	4	4000	0.10
NPI Luciferase mRNA in SIV	F	345	80.0	5	4000	0.13
0.32 mg/kg/day		346	67.0	5	4000	0.13
		347	61.0	2	4000	0.05
		348	70.4	5	4000	0.13
		349	65.0	3	4000	0.08
NPI Luciferase mRNA in SIV	M	315	61.2	4	4000	0.10
1.07 mg/kg/day		316	62.8	2	4000	0.05
		317	68.6	6	4000	0.15
		318	60.0	4	4000	0.10
		319	56.0	4	4000	0.10
NPI Luciferase mRNA in SIV	F	355	58.0	5	4000	0.13
1.07 mg/kg/day		356	64.2	3	4000	0.08
		357	60.8	3	4000	0.08
		358	64.2	3	4000	0.08
		359	73.2	5	4000	0.13
NPI Luciferase mRNA in SIV	M	325	70.0	2	4000	0.05
3.21 mg/kg/day		326	62.4	3	4000	0.08
		327	66.8	4	4000	0.10
		328	64.2	4	4000	0.10
		329	68.2	5	4000	0.13
NPI Luciferase mRNA in SIV	F	365	63.6	3	4000	0.08
3.21 mg/kg/day		366	66.6	5	4000	0.13
		367	48.8	5	4000	0.13
		368	65.2	4	4000	0.10
		369	61.0	4	4000	0.10
CP	M	CP 348	33.0	131	4000	3.28
40 mg/kg/day		CP 349	22.8	157	4000	3.93
		CP 350	24.0	177	4000	4.43
		CP 351	30.2	136	4000	3.40
		CP 352	28.4	139	4000	3.48

PCE – Polychromatic Erythrocytes; MnPCE – Micronucleated Polychromatic Erythrocytes

Table 5 (Cont): Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow Collected 48 Hours Following Dose Administration

Treatment	Sex	Animal No.	%PCE	Micronucleus Frequency		
				MnPCE	PCE	%
Vehicle	M	300	67.6	3	4000	0.08
0 mg/kg/day		301	72.8	2	4000	0.05
		302	72.6	3	4000	0.08
		303	73.6	4	4000	0.10
		304	63.4	3	4000	0.08
Vehicle	F	340	45.6	5	4000	0.13
0 mg/kg/day		341	62.0	3	4000	0.08
		342	60.2	5	4000	0.13
		343	63.6	2	4000	0.05
		344	77.4	5	4000	0.13
NPI Luciferase mRNA in SIV	M	310	64.9	4	4000	0.10
0.32 mg/kg/day		311	43.8	3	4000	0.08
		312	62.2	2	4000	0.05
		313	53.6	4	4000	0.10
		314	65.2	4	4000	0.10
NPI Luciferase mRNA in SIV	F	350	49.2	4	4000	0.10
0.32 mg/kg/day		351	65.6	6	4000	0.15
		352	61.8	3	4000	0.08
		353	65.6	5	4000	0.13
		354	68.2	5	4000	0.13
NPI Luciferase mRNA in SIV	M	320	60.6	4	4000	0.10
1.07 mg/kg/day		321	68.6	2	4000	0.05
		322	63.2	4	4000	0.10
		323	61.4	5	4000	0.13
		324	65.0	2	4000	0.05
NPI Luciferase mRNA in SIV	F	360	61.6	6	4000	0.15
1.07 mg/kg/day		361	64.6	4	4000	0.10
		362	64.0	4	4000	0.10
		363	63.2	6	4000	0.15
		364	68.4	5	4000	0.13
NPI Luciferase mRNA in SIV	M	330	68.2	2	4000	0.05
3.21 mg/kg/day		331	60.6	2	4000	0.05
		332	66.2	3	4000	0.08
		333	70.8	5	4000	0.13
		334	72.8	3	4000	0.08
NPI Luciferase mRNA in SIV	F	370	56.8	4	4000	0.10
3.21 mg/kg/day		371	57.2	4	4000	0.10
		372	72.8	6	4000	0.15
		373	74.4	6	4000	0.15
		374	73.2	1	4000	0.03

PCE – Polychromatic Erythrocytes; MnPCE – Micronucleated Polychromatic Erythrocytes

12. APPENDIX I: Historical Control

Rat Micronucleus Test Historical Control Data
2016-2018

Historical Vehicle Control in Male Rats[1]

	Individual Animals		Studies	
	PCE%	MN%	PCE%	MN%
N	816	816	156	156
Mean[3]	53.3	0.08	53.3	0.08
SD	5.8	0.04	4.5	0.03
95% UCL	64.9	0.16	62.4	0.13
95% LCL	41.7	0.00	44.2	0.02
Max[4]	84.8	0.30	77.0	0.18
Min[4]	35.2	0.00	39.8	0.01

Historical Positive Control in Male Rats[2]

	Individual Animals		Studies	
	PCE%	MN%	PCE%	MN%
N	588	588	118	118
Mean[3]	43.6	2.77	43.6	2.76
SD	6.0	0.99	4.5	0.90
95% UCL	55.7	4.74	52.5	4.57
95% LCL	31.5	0.79	34.7	0.96
Max[4]	71.8	6.78	55.6	5.41
Min[4]	17.6	0.18	24.7	0.53

[1]Since no appreciable differences in the induction of MnPCEs by different vehicles and solvents (test article carriers) and different routes of administration were observed, this table contains data from carriers and routes of administration widely used during the conduct of contract studies at BioReliance.

Vehicles: water, water soluble vehicles (methylcellulose, carboxymethylcellulose, dextrose), saline, corn oil, and other vehicles.

Routes of administration: intraperitoneal (IP), intravenous (IV), oral gavage (PO), subcutaneous (SC).

Bone marrow collection time: approximately 24 and/or 48 hours post-final dose for Micronucleus studies; 3-4 hours post-final dose for the Micronucleus portion of combined Micronucleus/Comet studies.

[2]Positive control article: Cyclophosphamide monohydrate (CP); Doses: 20 or 40 mg/kg; Route of administration: PO.

[3]Average of the PCE ratio observed out of 500 or 1000 erythrocytes scored per animal for the total number of animals used; average of the number of MnPCE per 2000 or 4000 PCE for the total number of animals used; average of number of MnPCE/per group (containing 5-6 animals per group) for total number of groups used.

[4]Minimum and maximum range of PCE ratio observed out of 500 or 1000 erythrocytes scored per animal, the minimum and maximum range of MnPCE observed out of 2000 or 4000 PCE for the total number of animals used and the minimum and maximum range of MnPCE observed out of 10000 to 24000 PCE for the total number of groups used.

Formula: 95% control limit ranges = mean ± 2 x standard deviation

Note: This historical control data includes data from non-GLP studies.

Historical Vehicle Control in Female Rats[1]

	Individual Animals		Studies	
	PCE%	**MN%**	**PCE%**	**MN%**
N	413	413	79	79
Mean[3]	52.6	0.09	52.5	0.08
SD	5.8	0.04	4.4	0.03
95% UCL	64.1	0.17	61.4	0.14
95% LCL	41.0	0.01	43.6	0.03
Max[4]	74.2	0.25	63.5	0.16
Min[4]	27.0	0.00	42.8	0.04

Historical Positive Control in Female Rats[2]

	Individual Animals		Studies	
	PCE%	**MN%**	**PCE%**	**MN%**
N	23	23	5	5
Mean[3]	40.6	1.98	40.6	1.95
SD	5.3	0.70	4.8	0.65
95% UCL	51.1	3.37	50.1	3.25
95% LCL	30.1	0.59	31.0	0.65
Max[4]	50.2	3.03	48.5	2.84
Min[4]	32.6	0.98	36.4	1.34

[1]Since no appreciable differences in the induction of MnPCEs by different vehicles and solvents (test article carriers) and different routes of administration were observed, this table contains data from carriers and routes of administration widely used during the conduct of contract studies at BioReliance.

Vehicles: water, water soluble vehicles (methylcellulose, carboxymethylcellulose, dextrose), saline, corn oil, and other vehicles.

Routes of administration: intraperitoneal (IP), intravenous (IV), oral gavage (PO), subcutaneous (SC).

Bone marrow collection time: approximately 24 and/or 48 hours post-final dose for Micronucleus studies; 3-4 hours post-final dose for the Micronucleus portion of combined Micronucleus/Comet studies.

[2]Positive control article: Cyclophosphamide monohydrate (CP); Doses: 20 or 40 mg/kg; Route of administration: PO.

[3]Average of the PCE ratio observed out of 500 or 1000 erythrocytes scored per animal for the total number of animals used; average of the number of MnPCE per 2000 or 4000 PCE for the total number of animals used; average of number of MnPCE/per group (containing 5-6 animals per group) for total number of groups used.

[4]Minimum and maximum range of PCE ratio observed out of 500 or 1000 erythrocytes scored per animal, the minimum and maximum range of MnPCE observed out of 2000 or 4000 PCE for the total number of animals used and the minimum and maximum range of MnPCE observed out of 10000 to 24000 PCE for the total number of groups used.

Formula: 95% control limit ranges = mean ± 2 x standard deviation

Note: This historical control data includes data from non-GLP studies.

13. APPENDIX II: Study Protocol and Amendments

PROTOCOL AMENDMENT 1

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

1. Page 2, Section 1, Key Personnel – Test Site(s) Information, Principal Investigator (Cytokine analysis – 6 hour samples)

   Effective: Date of Study Director signature on this amendment.

   Original:
   Principal Investigator (Cytokine analysis – 6 hour samples) — To be added by Amendment

   Change To:
   Principal Investigator (Cytokine analysis – 6 hour samples)
   PPD
   Charles River Laboratories
   54943 N Main St
   Mattawan, MI 49071
   Phone PPD
   Email: PPD

   Reason: To include the information for the PI for cytokine analysis.

2. Page 12, Section 8, Experimental Design and Methodology – Cytokine analysis, Last two lines in the section

   Effective: Date of Study Director signature on this amendment.

   Original:
   For cytokine analysis (**6 hr post-dose**) ship samples to:

   Contact and address To be added by Amendment

   Change To:
   For cytokine analysis (**6 hr post-dose**) ship samples to the PI for Cytokine analysis listed in Section 1.

   Reason: To include the shipping contact and address.

PROTOCOL AMENDMENT 1

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Sponsor Approval:

PPD

PPD

Misc.

Sponsor Representative

PPD

Date

PROTOCOL AMENDMENT 1

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Study Director Approval:

PPD

BioReliance Study Director

PPD

Date

PROTOCOL AMENDMENT 2

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

1. Page 2, Section I, Key Personnel

Effective: Date of Study Director's signature on this amendment.

Remove:

Sponsor's Authorized Representative	PPD MSc. Moderna, Inc. 200 Technology Square, 3rd Floor Cambridge, MA 02139 Phone: PPD Email: PPD

Reason: Sponsor Request

PROTOCOL AMENDMENT 2

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Sponsor Approval:

PPD

PPD

PPD

Study Monitor

PROTOCOL AMENDMENT 2

Sponsor: Moderna, Inc.

BioReliance Study No.: AF87FU.125012NGLPICH.BTL

Title: NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Study Director Approval:

PPD

PPD

Date

BioReliance.

Protocol

Study Title	*NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo* Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat
Study Director	PPD PhD
Testing Facility	BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850 USA
BioReliance Study Number	AF87FU.125012NGLPICH.BTL

1. KEY PERSONNEL

Sponsor Information:

Sponsor	Moderna, Inc. 200 Technology Square, 3rd Floor Cambridge, MA 02139 USA
Sponsor's Authorized Representative	PPD MSc. Moderna, Inc. 200 Technology Square, 3rd Floor Cambridge, MA 02139 Phone PPD Email: PPD
Study Monitor	PPD MS, MBA, DABT Moderna, Inc. 200 Technology Square 3rd Floor Cambridge, MA 02139 Phone PPD Email: PPD

Test Facility Information:

Study Director	PPD PhD BioReliance Corporation Phone: PPD Email PPD

Test Site(s) Information:

Principal Investigator (BioAnalysis – 2 hour samples)	PPD BS Moderna,Inc. 200 Technology Square, 3rd Floor Cambridge, MA 02139 Phone PPD E-mail PPD
Principal Investigator (Cytokine analysis – 6 hour samples)	To be added by Amendment

2. TEST SCHEDULE

Proposed Experimental Initiation Date	09 DEC 2019
Proposed Experimental Completion Date	22 JAN 2020

Proposed Report Date 05 FEB 2020

3. REGULATORY REQUIREMENTS

This study will be performed using the Good Laboratory Practice (GLP) Regulations for nonclinical laboratory studies as a guideline; however, this study will not meet GLP requirements.

4. PURPOSE

The objective of this study is to evaluate the Test Article, NPI Luciferase mRNA in SM102-containing lipid nanoparticles, when administered by slow intravenous injection, for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in rat bone marrow. This assay design is based on OECD Guideline 474 (OECD, 2016), the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (2011), and ISO/IEC 17025:2005 (ISO/IEC, 2005).

5. TEST ARTICLE AND CONTROL ARTICLE INFORMATION

Test Article

Identification	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles
Storage Conditions	-60°C or below Protected from light
Purity	90.5% A correction factor of 1.105 will be used for dose formulations
Concentration	Lipid: 24.31 mg/mL mRNA: 1.3 mg/mL

Negative/Vehicle Control

Identification	25 mM Tris/sucrose 1mM DTPA pH 7.5
BioReliance TAID	AF99YN
Storage Conditions	-60°C or below Protected from light

Positive Control

Identification	Cyclophosphamide monohydrate (CP)

Characterization of Test Article

Characterization of the Test Article is the responsibility of the Sponsor.

Test Article Reserve Sample
Since the in-life portion of this study is less than four weeks in duration, a reserve sample will not be retained.

Characterization of Vehicle
The vehicle used to prepare the Test Article formulations will be characterized by the Certificate of Analysis provided by the Supplier. Copies of the Certificate of Analysis will be kept on file at BioReliance.

Characterization of Dose Formulations
Dose formulations will not be analyzed.

Stability of Test Article in Vehicle
Stability of the Test Article in Vehicle, under the conditions of use, is the responsibility of the Sponsor.

Preparation of Negative/Vehicle Control
The Negative/Vehicle control, consisting of tris/sucrose, will be removed from the freezer and allowed to thaw at 2 to 8°C overnight. The storage of the Negative/Vehicle control at room temperature should not exceed 4 hours. The Negative/Vehicle control may be stored refrigerated (2 to 8) for up to 8 hours if not used for dose administration or formulation preparation within 3 hours of removal from the freezer. Refrigerated Negative/Vehicle control will be equilibrated at room temperature for at least 30 minutes prior to the start of dosing.

Preparation of Test Article Formulations
Dose formulation preparations will be performed under a Biological safety cabinet using aseptic procedures.

The bulk Test Article stock will be removed from the freezer and allowed to thaw at room temperature for no more than 1 hour before dose formulation preparation. The dosing formulations will be prepared by diluting the bulk Test Article with the Negative/Vehicle Control as necessary to the target concentration for administration and should not be filtered. The storage of dose formulations at room temperature should not exceed 4 hours from the time of preparation to the dose administration. The dosing formulations may be stored refrigerated (2 to 8°C) for up to 8 hours if not used for dose administration within 3 hours of preparation. Refrigerated dose formulations will be equilibrated at room temperature for at least 30 minutes prior to the start of dosing. Formulations will be used within 3 hours after being equilibrated at room temperature. The formulation will NOT be vortexed or sonicated but may be gently swirled to ensure even mixing during formulation. Stock solution vials will be used only on the day of dose formulation preparation once thawed and will not be used on subsequent dosing days.

Positive Control
Scoring control slides (fixed and unstained), generated from a BioReliance study, will be included to verify scoring, and the study number will be documented in the raw data. These slides were generated from male rats treated once with cyclophosphamide monohydrate (CP) at 40 mg/kg and the bone marrow harvested 24 hours after the treatment.

Disposition of Test Article, Negative/Vehicle Control, and Dose Formulations
All unused Test Article and Negative/Vehicle Control will be returned to the Sponsor at the address listed below after the last day of dosing on a Monday through Thursday. Any thawed, unused Test Article will be returned on cold packs, any frozen and unused Test Article will be shipped on dry ice, and any unused Negative/Vehicle Control will be shipped on dry ice to:

TBD
Moderna, Inc.
200 Technology Square
2nd Floor
Cambridge, MA 02139
Phone: TBD
Email: TBD

Residual dose formulations will be stored at 2 to 8°C and discarded upon report finalization.

6. **TEST SYSTEM**

Species	Rat
Strain	Sprague-Dawley (Hsd:SD)
Justification for Selection of Species and Strain	The rat has been routinely used as an animal model of choice for the mammalian bone marrow erythrocyte micronucleus assay. This strain is an outbred strain that maximizes genetic heterogeneity and therefore tends to eliminate strain-specific response to Test Article.
Source	Envigo RMS, Inc. (Frederick, MD or alternate Envigo location)

Animal Welfare Provisions

This study is not duplicative or unnecessary. The number of animals, procedures, and design used for this study, has been reviewed and were approved by the BioReliance Institutional Animal Care and Use Committee Protocol Number 10. All procedures involving animals performed at BioReliance follow the specifications recommended in the most current version of The Guide for the Care and Use of Laboratory Animals adopted by BioReliance.

Number and Sex of Animals

	Definitive (DEF*)
Males (Main/TK)	40/12
Females (Main/TK)	40/12

*Additional animals may be added to the top dose as possible replacements in the case of mortality

Body Weight at Randomization and Age of Animals on First Day of Dosing

	Age	Weight
Males	6-8 weeks	150-350 g
Females	6-8 weeks	120-250 g

Animals outside these body weight ranges may be used with written approval from the Study Director. The body weight ranges will be reported in the final study Report.

Acclimation

The animals will be acclimated for at least five days. All animals will be judged to be healthy prior to use in the study. Prior to the first day of dose administration, animals will be observed at least once daily for signs of illness and poor health.

7. HUSBANDRY

Housing

Animals will be housed in a controlled environment at 72 ± 3°F and 50 ± 20% relative humidity with a 12-hour light/dark cycle. The light cycle may be temporarily interrupted for study related activities. The animal rooms will be supplied with at least 10 changes of fresh HEPA-filtered air per hour. Animals of the same sex may be housed up to five per Micro-Barrier cage. Cages will be placed on the racks equipped with an automatic watering system and Micro-VENT full ventilation, HEPA filtered system. If needed, an alternative housing system may be used.

Environmental Enrichment

Animals will be provided with environmental enrichment; the type of enrichment will be recorded in the raw data.

Food

A certified laboratory rodent chow (Teklad 2018C Global 18% Protein Rodent Diet) will be provided ad libitum. The food is

	analyzed by the manufacturer for the concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates and specified nutrients.
Water	Animals will have free access to tap water, which meets U.S. EPA drinking water standards [Washington Suburban Sanitary Commission (WSSC) Potomac Plant]. Drinking water is monitored at least annually for levels of specified microorganisms, pesticides, heavy metals, alkalinity and halogens. If needed, animals may be given supplemental water, as tap water in a petri dish or Napa Nectar™ (Systems Engineering; Napa, CA) or an equivalent hydration gel.
Bedding	Heat treated hardwood chips will be used for bedding to absorb liquids.
Bedding, Food and Water Analysis	The results of bedding, food and water analyses are on file at BioReliance. Based on historical test results, there are no contaminants in the bedding, feed and water that are expected to interfere with the study.

8. EXPERIMENTAL DESIGN AND METHODOLOGY

The assay will be conducted according to established procedures (Heddle, 1973; Mavournin et al., 1990; Hayashi et al., 1994, and OECD, 2016).

Randomization

The weight variation per sex of all animals assigned to study will not exceed ±20% of the mean weight without Study Director approval. Animals will be randomized to achieve random placement of animals throughout all groups.

Animal Identification

Following randomization, animals will be identified by sequentially numbered ear tags and/or programmed microchip. The cage card will contain, at least, the animal number(s), sex, study number, treatment group number, dose level and route of administration. Cage cards will be color coded by treatment group. Raw data records and specimens also will be identified by the unique animal number.

Body Temperatures

Body temperatures will be monitored approximately 48 and 24 hours prior to dose, prior to dose on the day of dosing, 0.5, 1, 2, 4, 5, 6, 8, and approximately 24 and 48 hours post dose. The 48 hour body temperature will only be recorded for animals not sacrificed at the 24 hour bone marrow collection time point) in animals in Groups 1-4 using implantable programmable temperature transponders.

Group mean body temperatures (and standard deviation) will be calculated for each dose level and time point, by sex. The Test Article will be considered to cause hyperthermia at a particular dose level if group body temperature increases by ≥1.5°C for more than one hour, or by ≥1°C for more than 4.5 hours. If the mean group body temperature decreases by ≥ 3°C for more than 4.5 hours, the Test Article will be considered to cause hypothermia at that dose. Body temperature changes exceeding those above have been reported to induce micronucleus formation (Guzman et al, 2004; King and Wild, 1983; Asanami and Shimono, 1997; Asanami and Shimono, 1999).

Micronucleus Assay
Animals will be dosed with the Test Article or Negative/vehicle control and euthanized at the appropriate time.

The high dose for the micronucleus assay will be the Sponsor-indicated dose level of 60 mg/kg of SM102 Lipid. This high dose level was chosen to match the previous SM102 lipid dose level used on *in vivo* bone marrow micronucleus study 9800399. Two additional doses will be evaluated.

The assay design will be as follows:

Euthanasia Time (hours postdose)[B]					24	48
Group No.	Test Article	Dose Level of Test Article (mg/kg [mRNA/SM102 lipid])	Concentration (mg/mL [mRNA/SM102 lipid])	Dose Volume[A] (mL/kg)	Number of Animals/Sex	
1	Vehicle/ Negative Control	0/0	0/0	5	5	5
2	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	0.32/6.0	0.064/1.2	5	5	5
3	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	1.07/20	0.22/4	5	5	5
4	NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles	3.21/60	0.64/12	5	5	5

[A]Based upon individual body weight
[B]Range(s): 24-27 hours and 45-48 hours, respectively

Procedure	Micronucleus Assay Animals
Body Weight*	Prior to the first dose (for the purpose of all dose volume calculations) Once on the day of euthanasia, excluding animals used for bioanalysis
Moribundity and Mortality Check**	At least twice daily, beginning on the first day of dose administration
Detailed Hands-On Clinical Observations	Pre-dose on Day 1, excluding animals used for bioanalysis
Cage Side Observations*	1 to 2 hours post-dose and at least once daily on non-dosing days, excluding animals used for bioanalysis
Dose Frequency	Once

Route of Administration***	Intravenous injection (slow push over **1.5 to 2.5 minutes**)

*Additional observations may be performed as needed.
**Moribund animals will be sacrificed immediately by CO_2 overdose. No additional data points or sample collection will be taken from moribund sacrificed or found dead animals. Animals found dead or sacrificed moribund will be discarded without necropsy.
***This route has been routinely used and is widely-accepted for use in the mammalian bone marrow erythrocyte micronucleus assay.

Blood (Plasma) Collection and Sample Handling for TK and Cytokine Analysis

Group No.	Test Article	Dose Level of Test Article (mg/kg [mRNA/SM102 lipid])	Concentration (mg/mL [mRNA/SM102 lipid])	TK (Bioanalysis)/ Cytokine Animals/Sex	Sample Collection Timepoint (hours postdose)
6	Vehicle/ Negative Control	0/0	0/0	3	2 and 6
7	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	0.32/6.0	0.064/1.2	3	2 and 6
8	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	1.07/20	0.22/4	3	2 and 6
9	Luciferase mRNA in SM102-Containing Lipid Nanoparticles	3.21/60	0.64/12	3	2 and 6

Frequency	1st day of dosing
Collection Site	Retro-orbital Sinus
Target Volume	0.5mL of whole blood. Any volume less than this will be documented in the raw data.
Anesthesia	Animals will be anesthetized prior to collection by 70% CO_2/30% O_2.

Anticoagulant	K_2EDTA
Sample Handling	Blood samples will be maintained on wet ice until centrifugation.
Centrifugation	Blood collection will be conducted in a group number sequence order from Groups 6 to 9. Blood samples will be centrifuged for 5 minutes, 2-8°C, at 2000 g within 1 hour of collection and plasma will be harvested into two, approximately equal, aliquots (primary and back up).
Sample Storage	Plasma samples will be stored at ≤ -60°C until packed on dry ice and shipped to the Test Site for analysis.
Animal Disposition	Animals will be sacrificed by CO_2 overdose after their last collection timepoint.

Bioanalysis (BioA)
A non-validated method (bDNA) will be used to analyze the concentration of mRNA in the plasma samples. Plasma samples (3 samples/sex/group; Groups 6, 7, 8, and 9) collected at **2 hours** postdose will be shipped on dry ice by overnight courier to the Principal Investigator for BioAnalysis. They will be sent on a non-holiday Monday, Tuesday, or Wednesday that does not immediately precede a holiday. Upon receipt, samples will be stored at -80°C or below until required for analysis. Unused samples will be discarded upon acceptance of the analytical results by the Study Director. A BioA Contribution Report, signed by the Principal Investigator from the Test Site, will be provided and included in the main *in vivo* Micronucleus Report.

For BioA (**2 hr post-dose collections**) ship samples to:
PPD BS
Moderna, Inc.
200 Technology Square, 3rd Floor
Cambridge, MA 02139
Phon PPD
E-mail PPD

Cytokine analysis
A non-validated method (Luminex) will be used to analyze the concentrations of cytokines (MIP-1a, MCP-1, IL-6, IL-1B, TNFα, IP-10) in the plasma samples. Plasma samples collected at **6-hour** postdose (3 samples/sex/group; Groups 6, 7, 8, and 9) will be shipped on dry ice by overnight courier to the Principal Investigator for Cytokine Analysis. They will be sent on a non-holiday Monday, Tuesday, or Wednesday that does not immediately precede a holiday. Upon receipt, samples will be stored at -80°C or below until required for analysis. Unused samples will be discarded upon acceptance of the analytical results by the Study Director in consultation with the Sponsor Representative. A Cytokine Analysis Contribution Report, signed by the Principal Investigator from the Test Site, will be provided and included in the main *in vivo* Micronucleus Report.

For cytokine analysis (**6 hr post-dose**) ship samples to:

Contact and address To be added by Amendment

Bone Marrow Collection
Femoral bone marrow will be collected at approximately 24 or 48 hours after dose administration, as indicated. Animals will be euthanized by carbon dioxide inhalation. Immediately following euthanasia, the femurs will be exposed, cut just above the knee, and the bone marrow will be aspirated into a syringe containing fetal bovine serum.

Preparation of Micronucleus Slides
The bone marrow will be transferred to a centrifuge tube containing 1-3 mL fetal bovine serum, the cells will be pelleted by centrifugation, and the supernatant drawn off leaving a small amount of fetal bovine serum with the pellet. Cells will be re-suspended and a small drop of the bone marrow suspension will be spread onto a clean glass slide. At least four slides will be prepared from each animal, air dried and fixed by dipping in methanol. One set of two slides (including at least 5 Positive Control slides) will be stained with acridine orange for microscopic evaluation. The other set of slides will be kept as backup. Each slide will be identified by the harvest date, study number, and animal number (or slide number for Positive Control slides). Slides will be coded using a random number table by an individual not involved with the scoring process.

Scoring of Micronucleus Slides
Slides will be evaluated by fluorescent microscopy. The staining procedure permits the differentiation by color of polychromatic and normochromatic erythrocytes (bright orange PCEs and ghost-like, dark green NCEs, respectively).

The criteria for the identification of micronuclei are those of Schmid (1975). Micronuclei are brightly stained bodies that generally are round and that generally are between 1/20 and 1/5 the size of the PCE. The frequency of micronucleated cells will be recorded with cells containing one or more micronuclei counted as one micronucleated PCE (MnPCE).

At least 4000 PCEs/animal will be scored for the presence of micronuclei (MnPCEs) whenever possible. In addition, at least 500 total erythrocytes (PCEs + NCEs) will be scored per animal to determine bone marrow cytotoxicity.

Stained slides will be discarded prior to report finalization.

Statistical Analysis
Statistical analysis will be performed on the micronucleus frequency (%MnPCE) and %PCE using the animal as the unit. The mean and standard deviation of %MnPCE and %PCE will be presented for each treatment group.

The use of parametric or non-parametric statistical methods in evaluation of data will be based on the variation between groups. The group variances for micronucleus frequency for the Vehicle and Test Article groups at the respective sampling time will be compared using Levene's test (significance level of $p \leq 0.05$). If the variation between groups is found not to be significant, a parametric one-way ANOVA will be performed followed by a Dunnett's post-hoc analysis to compare each dose group to the concurrent vehicle control. If Levene's test indicates heterogeneous group variances (significance level of $p \leq 0.05$), the suitability of a transformation of the original data will be evaluated (e.g. using logarithm transformed values of the original data) in an attempt to meet the normality criteria. Afterwards, statistical analysis will be performed using the parametric tests described above. If parametric tests are not acceptable, non-parametric statistical methods (Kruskal Wallis and/or Mann Whitney test) may be used in evaluation of data.

A linear regression analysis will be conducted to assess dose responsiveness in the Test Article treated groups ($p \leq 0.01$ and $R^2 \geq 70\%$). Alternative statistical methods (e.g., Jonckheere's Test) may be used in the evaluation of data ($p \leq 0.01$).

A pair-wise comparison (Student's T-test; $p \leq 0.05$) will be used to compare the Positive Control group to the concurrent Vehicle Control group. If parametric tests are not acceptable, non-parametric statistical methods (Kruskal Wallis and/or Mann Whitney test) may be used in evaluation of data.

9. CRITERIA FOR DETERMINATION OF A VALID ASSAY

Vehicle Controls

The group mean frequency of MnPCEs should ideally be within the 95% control limits of the distribution of the historical Negative Control database. If the concurrent Negative Control data fall outside the 95% control limits, they may be acceptable as long as these data are not extreme outliers (indicative of experimental or human error).

Positive Controls

The frequency of MnPCEs for the scoring controls must be significantly greater than the concurrent Vehicle Control ($p \leq 0.05$) and should be compatible with those observed in the historical Positive Control data base.

Test Conditions

At least three doses will be tested for at least one sampling time. Five animals/sex/group should be available for analysis. In the event of mortality, sufficient replacement animals will be added to the study to meet this criterion. If fewer than five animals/sex/group are available for analysis, bone marrow will be collected and slides will be prepared from all surviving animals, but a decision about evaluation of the slides from the surviving animals will be made by the Study Director in consultation with the Sponsor.

Cell Analysis

At least 4000 PCEs/animal will be scored for the presence of micronuclei (MnPCEs) whenever possible. In addition, at least 500 total erythrocytes (PCEs + NCEs) will be scored per animal to determine the proportion of PCEs as an index of bone marrow cytotoxicity. A reduction in the PCE proportions to less than 20% of vehicle control value will be considered excessively cytotoxic and the animal data will be excluded from evaluation.

Maximum Dose Evaluated
The maximum dose evaluated for micronucleus induction must

a) be the MTD or MFD, or
b) demonstrate cytotoxicity in the bone marrow (reduction in the PCE/NCE ratio of more than 50% but not less than 20% of the control value), or
c) in the absence of cytotoxicity or MFD, a dose of 2000 mg/kg/day (limit dose) is used.

10. EVALUATION OF TEST RESULTS

A Test Article will be considered to have induced a positive response if

a) at least one of the Test Article doses exhibits a statistically significant increase when compared with the concurrent Negative Control ($p \leq 0.05$), and
b) when multiple doses are examined at a particular sampling time, the increase is dose-related ($p \leq 0.01$ and $R^2 \geq 70\%$), and
c) results of the group mean or of the individual animals in at least one group are outside the 95% control limit of the historical negative control data.

A Test Article will be considered to have induced a clear negative response if none of the criteria for a positive response were met and there is evidence that the bone marrow was exposed to the Test Article (unless intravenous administration was used).

If the response is neither clearly positive nor clearly negative, or in order to assist in establishing the biological relevance of a result, the data will be evaluated by expert judgment and/or further investigations. Possible additional work may include scoring additional cells (where appropriate) or performing an additional experiment that could employ the use of modified experimental conditions. Such additional work will only be carried out following consultation with, and at the request of, the Sponsor Representative.

In some cases, even after further investigations, the data set will preclude making a conclusion of positive or negative, at which time the response will be concluded to be equivocal. In such cases, the Study Director will use sound scientific judgment and report and describe all considerations.

11. ELECTRONIC DATA COLLECTION SYSTEMS

Electronic systems used for the collection or analysis of data may include but not be limited to the following (version numbers are maintained in the system documentation):

System	Purpose
CCI [redacted]	Test Article Tracking Captures in-life toxicology, animal randomization and management data Calculations/Randomization Environmental Monitoring Deviation and audit reporting Generates in-life toxicology tables

12. REPORT

An abbreviated summary Report will be prepared by BioReliance and include:

- results in tabular form
- interpretation of results
- conclusions

13. RECORDS AND ARCHIVES

Upon issue of the final report, all raw data for procedures performed at BioReliance will be returned to the Sponsor.

The raw data, Reports, and other documents generated at locations other than BioReliance will be archived by the Test Site.

REFERENCES

Asanami, S. and Shimono, K. Hypothermia induces micronuclei in mouse bone marrow cells. *Mutation Res* 393 91-98, 1997.

Asanami, S. and Shimono, K. The effect of hyperthermia on micronucleus induction by mutagens in mice. *Mutation Res* 446: 149-154, 1999.

Guzman, A., C. Garcia, A.P. Marin, M.T. Ruiz, A. Tortajada, A.R. Fernandez de Henestrosa. Induction of micronuclei in mouse bone-marrow erythrocytes in association with hypothermia after administration of the sigma receptor ligand E-5842. *Mutation Res.* 565: 11-22, 2004.

Heddle J.A. A rapid *in vivo* test for chromosomal damage. Mutation Res. 18:187-190, 1973.

Hayashi M., Tice R.R., MacGregor J.T., Anderson D., Blakey D.H., Kirsch-Volders M., Oleson Jr. F.B., Pacchierotti F., Romagna F., Shimada H., Sutou S. and Vannier B. *In vivo* rodent erythrocyte micronucleus assay. Mutation Res. 312: 293-304, 1994.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. S2(R1) document recommended for adoption at step 4 of the ICH process on 9 November 2011. Adopted at Step 5 in Europe by CHMP December 2011 (issued as EMA/CHMP/ICH/126642/2008). Adopted at Step 5 in US by FDA on June 7, 2012 (issued as 77 FR 33748 pages 33748-33749). Adopted in Japan at Step 5 September 20, 2012 (issued as PFSB/ELD Notification No. 0920-2).

ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories.

King, M.T. and Wild, D. The mutagenic potential of hyperthermia and fever in rats. Mutation Res.; 111:219-226, 1983

Mavournin K.H., Blakey D.H., Cimino M.C., Salamone M.F. and Heddle J.A. The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 239:29-80, 1990.

National Research Council, Institute of Laboratory Animal Resources Commission on Life Sciences. Guide for the Care and Use of Laboratory Animals. National Academy Press. Washington, D.C., 2011.

OECD Guidelines for Testing of Chemicals, Guideline 474 (Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test), Updated guidance adopted 29 July 2016.

Schmid W. The micronucleus test. Mutation Res. 31:9-15, 1975.

APPROVALS

PPD

PPD

Sponsor Representative

Date

Study Director Approval

PPD _____ PPD

Date

14. APPENDIX III: Body Temperatures

Male Body Temperature

Treatment	Sex		Pretreatment -48hr Pre-Dose	Pretreatment -24hr Pre-Dose	Pretreatment 0hr Pre-Dose	Pretreatment Average Pre-Dose	Group Mean Body Temperatures (°C) 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															
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**
NPI Luciferase 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

°C Change = Post-treatment temperature - Pretreatment temperature

ND = No data due to mortality

[3]SD = Standard deviation not available due to single surviving animal

[4]SD = No Standard deviation available due to single value reported

Female Body Temperature

Treatment	Sex		Pretreatment -48hr Pre-Dose	Pretreatment -24hr Pre-Dose	Pretreatment 0hr Pre-Dose	Pretreatment Average Pre-Dose	Group Mean Body Temperatures (°C) 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															
24 Hour	F	**Mean**	36 9	36 9	37 2	37 0	37 9	38 5	37 6	37 0	37 9	37 9	38 1	38 1	N/A
		+/-SD	0 1	0 2	0 3		±0 3	±0 2	±0 4	±0 2	±0 4	±0 3	±0 4	±0 4	N/A
		°C Change				--	**0.9**	**1.5**	**0.6**	**0.0**	**0.9**	**0.9**	**1.1**	**1.1**	**N/A**
48 Hour	F	**Mean**	34 8	34 6	35 1	34 8	36 6	36 6	36 4	34 9	38 0	36 3	35 6	34 9	35 8
		+/-SD	4 3	4 1	4 5		±4 2	±4 3	±4 3	±4 2	±0 4	±4 3	±4 1	±4 3	±4 5
		°C Change					**1.8**	**1.8**	**1.6**	**0.1**	**3.2**	**1.5**	**0.8**	**0.1**	**1.0**
NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles 0 32/6 0 mg/kg/day						--									
24 Hour	F	**Mean**	36 4	36 2	36 6	36 4	37 9	38 0	37 7	37 7	38 1	37 8	37 9	37 3	N/A
		+/-SD	0 5	0 7	1 0		±0 9	±0 7	±0 8	±0 9	±0 8	±0 8	±0 5	±0 9	N/A
		°C Change				--	**1.5**	**1.6**	**1.3**	**1.3**	**1.7**	**1.4**	**1.5**	**0.9**	**N/A**
48 Hour	F	**Mean**	35 0	36 5	35 0	35 5	36 6	37 9	36 7	35 9	36 2	35 8	35 7	35 5	35 9
		+/-SD	3 0	1 9	3 3		±2 5	±1 8	±2 7	±2 9	±2 7	±2 8	±2 9	±2 5	±2 8
		°C Change				--	**1.1**	**2.4**	**1.2**	**0.4**	**0.7**	**0.3**	**0.2**	**0.0**	**0.4**
1 07/20 mg/kg/day															
24 Hour	F	**Mean**	37 6	38 3	38 1	38 0	39 2	39 4	38 8	38 2	39 3	38 8	39 0	38 4	N/A
		+/-SD	1 9	1 8	1 7		±1 7	±1 9	±2 0	±2 0	±1 7	±2 0	±1 9	±1 4	N/A
		°C Change				--	**1.2**	**1.4**	**0.8**	**0.2**	**1.3**	**0.8**	**1.0**	**0.4**	**N/A**
48 Hour	F	**Mean**	37 0	36 8	37 7	37 2	38 5	38 6	38 0	37 9	38 0	38 1	38 1	37 0	37 1
		+/-SD	0 7	0 5	0 9		±0 8	±0 8	±1 2	±1 0	±0 8	±0 9	±0 8	±0 8	±0 9
		°C Change				--	**1.3**	**1.4**	**0.8**	**0.7**	**0.8**	**0.9**	**0.9**	**-0.2**	**-0.1**
3 21/60 mg/kg/day															
24 Hour	F	**Mean**	41 4	41 8	41 7	41 6	42 5	42 8	42 9	42 7	42 9	43 0	42 6	41 6	N/A
		+/-SD	1 9	2 0	1 8		±2 1	±2 3	±2 2	±2 1	±2 1	±2 1	±2 3	±2 5	N/A
		°C Change				--	**0.9**	**1.2**	**1.3**	**1.1**	**1.3**	**1.4**	**1.0**	**0.0**	**N/A**
48 Hour	F	**Mean**	39 1	39 3	39 4	39 3	40 5	40 5	40 6	40 7	40 9	40 7	40 5	39 6	39 3
		+/-SD	1 8	1 8	1 5		±1 9	±2 2	±2 1	±2 1	±2 2	±2 2	±2 1	±2 2	±2 1
		°C Change					**1.2**	**1.2**	**1.3**	**1.4**	**1.6**	**1.4**	**1.2**	**0.3**	**0.0**

SD = Standard deviation

N/A - Not Applicable due to study design

°C Change = Post-treatment temperature - Pretreatment temperature

ND = No data due to mortality

[3]SD = Standard deviation not available due to single surviving animal

[4]SD = No Standard deviation available due to single value reported

15. APPENDIX IV: Cytokine Analysis

charles river

FINAL REPORT

Study Phase: Cytokine Analysis

Test Site Reference No. 2308-115

Testing Facility Study No. AF87FU.125012NGLPICH.BTL

NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

TESTING FACILITY:
BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850
USA

TEST SITE:
Charles River Laboratories, Inc.
54943 North Main Street
Mattawan, MI 49071
USA

TABLE OF CONTENTS

REPORT APPROVAL

DocuSigned by:
PPD
Signer Name: PPD
Signing Reason: I approve this document
Signing Time: 18-Feb-2020 | 10:11:47 EST
3D3739B119314D0B839A938410FEA543

PPD BSc
Principal Investigator

1. RESPONSIBLE PERSONNEL

1.1. Test Site

Site Head / General Manager	PPD	PhD, DSP
Senior Director, Safety Evaluation	PPD	BS
Senior Director, Laboratory Sciences	PPD	BA
Principal Investigator	PPD	BSc
Report Coordinator	PPD	BS

2. SUMMARY

This phase of the study was conducted for Moderna, Inc., to evaluate plasma from (Hsd:SD) Sprague-Dawley rats for cytokine analysis.

Administration of NPI Luciferase mRNA in SM102-containing lipid nanoparticles to rats when given by slow intravenous injection at SM102 lipid dose levels of 0, 6, 20, and 60 mg/kg elicited cytokine changes at dose levels of 20 and 60 mg/kg which included increases in IL-6 (3.60x – 3.68x), MCP-1 (2.65x – 4.66x), MIP-1α (1.94x – 2.62x), and/or IP-10 (4.58x – 30.47x) at 6 hours post-dose in both sexes.

3. MATERIALS AND METHODS

3.1. Sample Receipt and Analysis

Plasma samples were received on dry ice from BioReliance Corporation, Rockville, Maryland for the evaluation of cytokine concentrations (MIP-1α, MCP-1, IL-6, IL-1β, TNFα, IP-10) using validated methods (Method No. CP-CLP-042-A). Samples were stored at -80°C or below upon receipt until analyzed.

4. STATISTICS

Statistical analysis was not performed; however, means and standard deviations were calculated.

5. COMPUTER SYSTEMS

Critical computerized systems used in the study are listed below (Text Table 1). All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 1
Critical Computerized Systems

System Name	Version No.	Description of Data Collected and/or Analyzed
CCI		Collection of Part 11 compliant signature
		A comprehensive laboratory information management system used to manage data, including but not limited to: instrumentation, test articles, standards, and samples.
		Client-server, Oracle-based system used for electronic documentation and data management from compound receipt through reporting.
		The CCI System is an integrated system of software products that enables a user to perform data entry, retrieval, data management, reporting, graphics, statistical analysis, and applications development.
		Software used for statistical procedures.
		Environmental monitoring, alarming, and reporting applications.
		The CCI system is a flow cytometer-based dual-laser system for simultaneously identifying and quantitating up to 100 different analytes in a single biomolecular assay (xMAP technology).

6. RETENTION OF RECORDS AND SAMPLES

All study-specific raw data, documentation, protocol, and samples, and final reports from this study were archived at a Charles River archival facility unless otherwise specified in the protocol. At least one year after issue of the draft report, the Sponsor will be contacted. Unused samples will be discarded upon acceptance of the analytical results by the Study Director in consultation with the Sponsor Representative.

7. RESULTS AND DISCUSSION

Appropriate samples were analyzed as directed by the protocol. No deviations occurred during this portion of the study. Cytokine data are presented in Table 1 and Appendix 1.

7.1. Cytokines

For this report, treated animals' values were compared to control (vehicle) animals' values. Fold change (x) in cytokine parameters were determined by comparing the Group 7, 8, and 9 mean to the control (Group 6) mean.

Text Table 2
NPI Luciferase mRNA in SM102-containing lipid nanoparticles -Related Cytokine Changes

Group	6		7		8		9	
SM102 Lipid Dose (mg/kg)	0 (Vehicle)		6		20		60	
Sex	M	F	M	F	M	F	M	F
IL-1β (pg/mL)								
6HPD	103.917	112.927	--	--	--	--	--	--
IL-6 (pg/mL)								
6HPD	1881.760	962.830	--	--	--	3.68x	3.60x	3.63x
MCP-1 (pg/mL)								
6HPD	1263.717	1092.177	--	--	3.05x	--	2.65x	4.66x
TNF-α (pg/mL)								
6HPD	47.440	47.770	--	--	--	--	--	--
MIP-1α (pg/mL)								
6HPD	34.410	48.147	--	--	2.42x	--	1.94x	2.62x
IP-10 (pg/mL)								
6HPD	500.587	405.460	--	--	4.58x	7.24x	7.48x	30.47x

M = Males, F = Females, HPD = Hours Post Dose
Dashes (--) indicate absence of change. Numerical values indicate fold change of the treated group mean value relative to the control group mean value.

Intravenous administration of NPI Luciferase mRNA in SM102-containing lipid nanoparticles resulted in test article-related increases in IL-6, MIP-1α, MCP-1, and IP-10 concentrations 6 hours post-dose at the 60 mg/kg dose level in both males and females. Test article-related increases at the 20 mg/kg dose level were observed 6 hours post dose in IL-6 in females, MIP-1α and MCP-1 in males and IP-10 in both males and females.

Peak IL-6 concentrations fold increase over the baseline ranged from 3.68 and 3.63 in females at 20 and 60 mg/kg, respectively, and 3.60 in males at 60/mg/kg.

Peak MCP-1 concentrations fold increase over the baseline ranged from 3.05 and 2.65 in males at 20 and 60 mg/kg, respectively, and 4.66 in females at 60 mg/kg.

Peak MIP-1α concentrations fold increase over the baseline ranged from 2.42 and 1.94 in males at 20 and 60 mg/kg, respectively, and 2.62 in females at 60 mg/kg.

Peak IP-10 concentrations fold increase over the baseline ranged from 4.58 and 7.48 in males and 7.24 and 30.47 in females at 20 and 60 mg/kg, respectively.

No test article-related cytokine changes were noted in the 6 mg/kg dose.

There was no test article related effects observed on IL-1β or TNF-α in either sex at any dose level.

8. CONCLUSION

Administration of NPI Luciferase mRNA in SM102-containing lipid nanoparticles to rats when given by slow intravenous injection elicited cytokine changes including increases in IL-6, MCP-1, MIP-1α, and IP-10 at 6 hours post-dose in one or both sexes at 20 mg/kg and in both sexes at 60 mg/kg of SM102 lipid.

Table 1
Summary of Cytokine Values

Abbreviation for Cytokine Parameters

6HPD - 6 hour postdose

Summary of Cytokine Values

Sex: Male	Day(s) Relative to Start Date		Group 6	Group 7	Group 8	Group 9
IL-1beta (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-alpha (pg/mL)	1 (6HPD)	Mean	47.440	51.283	104.193	46.857
		SD	2.6710	17.9581	47.9196	2.8656
		N	3	3	3	3
MIP-1alpha (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

Summary of Cytokine Values

Sex: Female	Day(s) Relative to Start Date		Group 6	Group 7	Group 8	Group 9
IL-1beta (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-alpha (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-1alpha (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

Appendix 1
Individual Cytokine Values

Code for Individual Cytokine Values

6HPD - 6 hour postdose

Individual Cytokine Values

Sex: Male

Group 6							
Day(s)	Relative to Start Date	IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
375	1 (6HPD)	94.81	<293.00	1420.53	48.04	34.41	466.99
376	1 (6HPD)	131.05	2302.49	950.09	44.52	34.41	461.06
377	1 (6HPD)	85.89	3049.79	1420.53	49.78	34.41	573.71

2308-115

NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Individual Cytokine Values

Sex: Male

Group 7							
Day(s) Relative to Start Date		IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
378	1 (6HPD)	137.17	4418.06	1703.16	66.07	43.31	380.88
379	1 (6HPD)	108.79	3049.79	1703.16	31.30	34.41	597.76
380	1 (6HPD)	177.40	<293.00	1703.16	56.48	48.01	604.19

Individual Cytokine Values

Sex: Male

Group 8 Day(s) Relative to Start Date		IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
381	1 (6HPD)	67.83	1904.21	5251.66	148.23	105.64	2741.31
382	1 (6HPD)	301.83	1462.13	2583.30	53.18	53.77	3082.60
383	1 (6HPD)	91.18	7159.18	3721.39	111.19	90.88	1046.71

2308-115

NPI Luciferase mRNA in SM102-Containing Lipid Nanoparticles: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleus Assay in the Rat

Individual Cytokine Values

Sex: Male

Group 9							
Day(s) Relative to Start Date		IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
384	1 (6HPD)	103.79	6870.35	2519.46	46.29	50.95	2384.39
385	1 (6HPD)	108.79	7444.94	2943.22	44.52	50.95	1348.17
386	1 (6HPD)	112.82	5983.27	4596.73	49.76	97.96	7506.40

Individual Cytokine Values

Sex: Female

Group 6							
Day(s)	Relative to Start Date	IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
387	1 (6HPD)	88.86	<293.00	877.68	16.14	39.95	429.04
388	1 (6HPD)	118.87	<293.00	1083.03	51.47	48.01	423.47
389	1 (6HPD)	131.05	2302.49	1315.82	73.70	56.48	383.87

Individual Cytokine Values

Sex: Female

Group 7		IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
Day(s)	Relative to Start Date						
390	1 (6HPD)	137.17	1482.13	1260.86	59.73	44.92	541.82
391	1 (6HPD)	124.95	3049.79	1083.03	40.90	49.49	553.13
392	1 (6HPD)	227.86	3049.79	1613.37	93.79	77.18	908.28

Individual Cytokine Values

Sex: Female

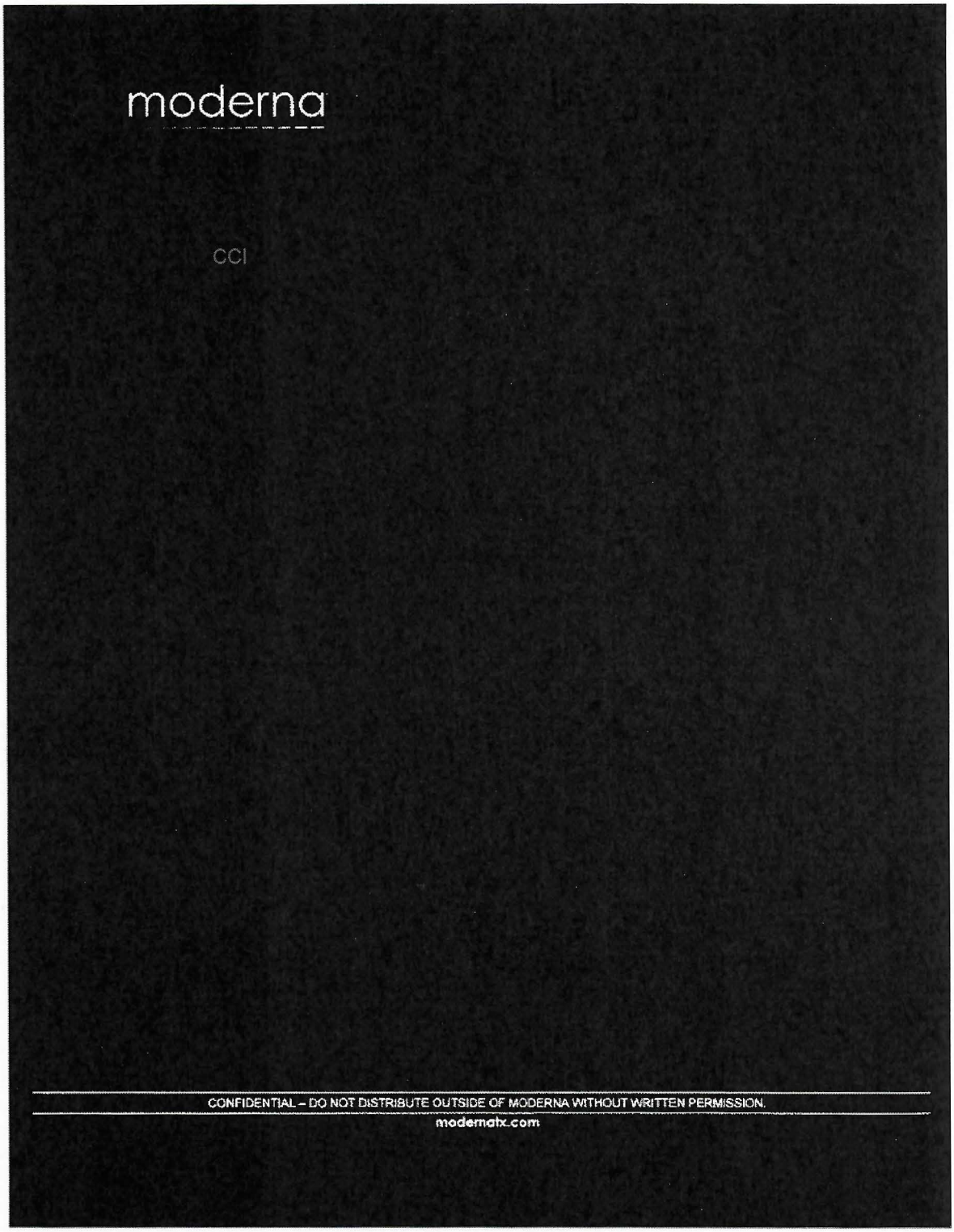
Group 8							
Day(s)	Relative to Start Date	IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
393	1 (6HPD)	189.93	5059.47	1315.82	49.76	56.48	3167.37
394	1 (6HPD)	321.37	4088.00	1566.94	58.11	44.12	3034.81
395	1 (6HPD)	146.38	1482.13	1280.88	40.90	50.95	2601.12

Individual Cytokine Values

Sex: Female

Group 9							
Day(s) Relative to Start Date		IL-1beta (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	TNF-alpha (pg/mL)	MIP-1alpha (pg/mL)	IP-10 (pg/mL)
396	1 (6HPD)	82.94	3750.68	3792.31	46.29	152.26	14963.69
397	1 (6HPD)	131.05	2302.49	2487.05	27.17	132.97	9325.88
398	1 (6HPD)	140.23	4418.06	9001.63	78.15	92.80	12775.66

16. APPENDIX V: Summary of Analysis


moderna
CCI
CONFIDENTIAL – DO NOT DISTRIBUTE OUTSIDE OF MODERNA WITHOUT WRITTEN PERMISSION.
modernatx.com