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

[redacted] 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.

[redacted] Date

Study Director

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3. STUDY INFORMATION

Study Conduct

Sponsor:	Moderna Therapeutics 200 Technology Square 3rd Floor Cambridge, MA 02139
Study Monitor:	[redacted] 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:	[redacted] PhD
Test Facility Management:	[redacted] MSc, Ph.D. Director, Genetic Toxicology Study Management
Laboratory Supervisor:	[redacted] BS
Laboratory Supervisor (Dose Formulation Preparation):	[redacted] BS, RLATG, CMAR
Report Writer:	[redacted] BS
Principal Investigator (Bioanalytical– 2 hour samples):	[redacted] 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):	[redacted]
Analytical Test Site (Cytokine analysis – 6 hour samples):	Charles River Laboratories 54943 N Main St Mattawan, MI 49071

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 ≤ -60ºC until packed on dry ice and shipped to the Test Site for analysis. The remaining set was retained at BioReliance at ≤ -60ºC 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:

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

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:

[redacted]
Charles River Laboratories
54943 N Main St
Mattawan, MI 49071
Phone: [redacted]
Email: [redacted]

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
LIMS Labware System	Test Article Tracking
Provantis™ (Instem)	Captures in-life toxicology, animal randomization and management data
Excel (Microsoft Corporation)	Calculations/Randomization
Kaye Lab Watch Monitoring system (Kaye GE)	Environmental Monitoring
Provantis™ Tables and Stats (Instem)	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

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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 Proventis (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 Luciferaae mRNA in SM102-Containing Lipid Nanoparticlea: In Vivo Mammalian Bone Marrow Erythrocyte Micronucleua Assay

Body Weight Gain (Grama)

Group	Sex	Base Weight Day 1		Day numbers relative to Start Date From: 1 To: 2	From: 1 To: 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
CP							
40 mg/kg/day	M	24	5	27.7 ± 4.3**	-53	3.70 ± 0.47**	740 /20000
Vehicle							
0 mg/kg/day	M	48	5	70.0 ± 4.4	---	0.08 ± 0.02	15 /20000
0 mg/kg/day	F	48	5	61.8 ± 11.3	---	0.10 ± 0.04	20 /20000
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

Treatment	Sex	Animal No.	%PCE	Micronucleus Frequency 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 5M	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 5M	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 5M	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 5M	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 5M	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 5M	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 SN	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 SN	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 SN	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 SN	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 SN	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 SN	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