**CELON PHARMA TOXICOLOGY DETAILED STUDIES SCOPE**

**Compound: mRNA – LNP**

**Attachment 2.**

**Detailed scope of work ( for RfQ no. 124/2025/M/RNA )**

1. **General Toxicology**
   - 1.1 Non-clinical studies in Sprague-Dawley Rat
   - 1.1.1 Determination of the Maximum Tolerated Dose (MTD)/Dose Range Finding (DRF) after intravenous administration in the Sprague-Dawley Rat
   - 1.1.2 8-weeks Repeat Dose Toxicology Study after intravenous administration in Sprague-Dawley Rats followed by recovery period
   - 1.2 Non-clinical studies in Cynomolgus Monkey
   - 1.2.1 Determination of the Maximum Tolerated Dose (MTD) after intravenous administration in Cynomolgus Monkey
   - 1.2.2 8-weeks Repeat Dose Toxicology Study after intravenous administration in Cynomolgus Monkey followed by recovery period
2. **Safety Pharmacology**
   - 2.1 Study of the Central Nervous System (CNS) employing a Functional Observational Battery (FOB) after intravenous administration in Sprague-Dawley Rat
   - 2.2 Study of Cardiovascular assessment after intravenous administration in Cynomolgus Monkey
   - 2.3 Effect on Cloned hERG Potassium Channels Expressed in Mammalian Cells
3. **Analytical Support**
   - 3.1 Dose Formulation Analysis
   - 3.1.1 Method Development of dose formulation samples
   - 3.1.2 Method Validation of dose formulation samples
4. **Bioanalysis**
   - 4.1 Bioanalytical Method Development for Test Item determination in Rat plasma
   - 4.2 Bioanalytical Method Validation for Test Item determination in Rat plasma
   - 4.3 Bioanalytical Method Development for Test Item determination in Monkey plasma
   - 4.4 Bioanalytical Method Validation for Test Item determination in Monkey plasma
5. **Genetic Toxicology**
   - 5.1 Bone marrow micronucleus test in the Wistar Han Rat
   - 5.2 In Vitro chromosomal aberration assay (Chinese hamster ovary (CHO)
   - 5.3 Bacterial reverse mutation test in vitro (AMES)

Regulatory test guidelines from the EU, OECD, FDA and ICH are going to be used as the reference material for preparation of the following studies.

1. **General Toxicology**
   - **1.1 Non-clinical studies in Sprague-Dawley Rat**

### 1.1.1 And MTD / DRF Intravenous Infusion Study in Sprague-Dawley Rats

Compliance: non-GLP
Frequency of dosing: MTD Phase: Single dose, 3-Days between dosing groups
DRF Phase: Weekly for 2 weeks
Route of administration: Intravenous Infusion (1 hour)
Species/Strain: Rat (Sprague-Dawley)
Age: 8 weeks at initiation of dosing
Weight: 252 to 309 g (males), 176 to 214 g (females)
No. of Animals on Study: MTD Phase: 20/sex plus 2 spares/sex
DRF Phase: 20/sex plus 2 spares/sex
Acclimation: As required

Study Design:

**MTD Phase:**

| Group No. | Treatment | No. of Main Animals* | |
|---|---|---|---|
| | | Males | Females |
| 1 | Dose 1 | 5 | 5 |
| 2 | Dose 2 | 5 | 5 |
| 3 | Dose 3 | 5 | 5 |
| 4 | Dose 4 | 5 | 5 |

*Animals sacrificed after a 7-day observation period (Day 8).

Cage side observations and mortality: Daily (cage side am and mortality at the end of the working day)
Detailed examinations: pre-study and prior to necropsy
Cutaneous examination: 1 hour and 24 hours post-dose (continue daily until resolution)
Body weights: Pre-treatment and prior to necropsy (not fasted)
Food consumption: Daily, qualitative
Gross pathology: Not required
Organ weights: Not required
Histopathology: Not required

**DRF Phase:**

| Group No. | Treatment | No. of Main Animals* | |
|---|---|---|---|
| | | Males | Females |
| 1 | Control | 5 | 5 |

| | | | |
|---|---|---|---|
| **2** | **Dose 1** | **5** | **5** |
| **3** | **Dose 2** | **5** | **5** |
| **4** | **Dose 3** | **5** | **5** |

*Animals sacrificed after a 7-day observation period (Day 15).

Cage side observations and mortality: Daily (cage side am and mortality at the end of the working day)
Detailed examinations: 5 occasions (prior to each dosing occasion, X hours post each dose, and prior to termination)
Cutaneous Tolerance Testing: 4 occasions (1 hr and 24 hr post-dose on each dosing occasion)
Body weights: Pre-treatment, twice weekly, and at termination
Food consumption: Daily, qualitative
Clinical Pathology (H, CC, Coag): 3 occasions (Days 3, 10, and at termination)
Urinalysis: At termination
Cytokine analysis: 2 occasions: Days 1 and 8 at 2, 4, 24-hours post dose (IL-1 , IL-6, TNF-alfa by Flow cytometer/ELISA).
Complement factors: 2 occasions: Days 1 and 14 at 2h, 24h, -hours post dose (Bb and C4d, CH50).
Gross pathology: All animals
Tissue collection and Organ weights: All animals, full list (including BM smears)
Histopathology: Main organs and injection sites from control and high-dose; gross lesions all animals.
Draft report: 12 weeks following the end of study
Archiving: 5 years
SEND dataset: To be quoted as an option

### 1.1.2 8-Weeks Repeat Dose Toxicology Study after Intravenous Infusion Administration in Sprague-Dawley Rats followed by a 4-Week Recovery Period

Compliance: GLP
Frequency of dosing: Once weekly
Route of administration: Intravenous Infusion (1 hour)
Species/Strain: Rat (Sprague-Dawley)
Age: 8 weeks at initiation of dosing
Weight: 252 to 309 g (males), 176 to 214 g (females
No. of Animals on Study: Main: 50/sex; TK: 39/sex; Recovery: 15/sex; Spares: 10 /sex
Acclimation: As required

**Study Design:**

| Group No. | Treatment | No. of Main Animals[a] | | No. of Toxicokinetic Animals[b] | | No. of Recovery animals[c] | |
|---|---|---|---|---|---|---|---|
| | | Males | Females | Males | Females | Males | Females |
| 1 | Control | 10 | 10 | 3 | 3 | 5 | 5 |
| 2 | Control-LNP | 10 | 10 | 9 | 9 | - | - |
| 3 | Dose 1 (low) | 10 | 10 | 9 | 9 | - | - |
| 4 | Dose 2 (mid) | 10 | 10 | 9 | 9 | 5 | 5 |
| 5 | Dose 3 (high) | 10 | 10 | 9 | 9 | 5 | 5 |

[a]Main animals sacrificed on Day 51
[b]TK animals sacrifieced without further examination
[c]Recovery animals sacrificed on Day 78

| | |
|---|---|
| Cage side observations and mortality: | Daily (cage side am and mortality at the end of the working day) |
| Detailed examinations: | Main and Recovery, pre-treatment and weekly |
| Cutaneous examination: | After each administration, 1 hr and 24 hr post-dosing (continue daily until resolution) |
| Body weights: | Main and Recovery, pre-treatment and weekly |
| Food consumption: | Main and Recovery, weekly, qualitative |
| Ophthalmoscopy: | Main and recovery at predose and at the end of dosing (End of recovery at additional cost) |
| Respiratory evaluation: | 5 Main animals / sex / group at 4 timepoints on Day 1 |
| FOB (Including CNS): | 5 main per sex per group; 5 timepoints on Day 1. |
| Clinical pathology (H, CC, Coag, Urinalysis): | Main and Recovery animals at termination. |
| Cytokines: | Main animals. Blood samples will be collected on Day 1-2h after dosing and 24h after dosing _cytokine levels IL-1 , IL-6, TNF-alfa. Measured by flow cytometer/ELISA. |
| Complement Factors: | Main animals. Blood samples for complement factor (*Bb and C4d, CH50):* analysis will be collected predosing and on Day 1 and 14 at 2h and 4 h postdose<br>Samples will be analyzed using a qualified method (method provided by the Bidder, eg. ELISA) |
| ADA: | TK animals, 8 occasions (prior to each dosing occasion) |
| Toxicokinetic Sampling: | 2 occasions (Days 1 and 50). 2 Timepoints for Group 1; 8 timepoints (2h, 6h, 24h, 48h, 78h, 96h, 168h and 336h) for Groups 2 to 5 (3/sex/group/timepoint). |
| Bioanalysis (option): | Analysis will be conducted for 3 analytes. Plasma samples (0.5ml) will be analyzed by LC/MS/MS (**LNP**), qPCR (**mRNA**) and ELISA (**protein**). |
| TK non-compartmental Analysis (option): | WinNonlin Table Assembly. Toxicokinetic subreport should be included. |
| Gross pathology: | Main and Recovery animals |

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| | |
|---|---|
| Tissue collection and Organ weights: | Main and Recovery animals, full list (including BM smears and injection sites) |
| Histopathology: | Full list of Main and Recovery animals found dead or euthanized pre-terminally; Control (Groups 1) and high dose (Group 5); target organs for lower dose levels<br>All gross lesions from all Main and Recovery animals |
| Draft report: | 12 weeks following the end of study |
| Final report: | 6 weeks following approval of Draft report |
| Archiving: | 5 years |
| SEND dataset: | To be quoted as an option |
| Archiving: | 5 years |

### 1.2 Non-clinical studies in Cynomolgus Monkey

### 1.2.1 A DRF Intravenous Infusion Study in Cynomolgus Monkey

| | |
|---|---|
| Compliance: | non-GLP |
| Frequency of dosing: | Single Incremental doses, two weeks apart. (Up to 4 escalating dose levels will be given one week apart to alternating groups) |
| Route of administration: | Intravenous Infusion (1 hour) |
| Species: | Cynomolgus monkey |
| Age: | 2.5 – 3 years |
| Weight: | 2.3 to 3.1 kg (males), 2.1 to 2.8 kg (females) |
| Number of animals: | 4 / sex; spares: 1/sex |
| Acclimation: | As required |

| Group No. | Treatment | No. of Main Animals* | |
|---|---|---|---|
| | | Males | Females |
| 1 | Control, Dose 2, Dose 4 | 2 | 2 |
| 2 | Dose 1, Dose 3 | 2 | 2 |

*Animals sacrificed 15 Days after the last dose administration

| | |
|---|---|
| Cage side observations and mortality: | Daily (cage side am and mortality at the end of the working day) |
| Detailed examinations: | Pre-treatment and weekly |
| Cutaneous Tolerance Testing: | After each administration, 1 hr and 24 hr post-dosing |
| Body weights: | Pre-treatment and twice weekly |
| Food consumption: | Daily, qualitative |
| Clinical pathology (H, CC, Coag): | Pre-dose and before each dosing occasion. |

| | |
|---|---|
| Cytokines: | Blood samples will be collected on each dosing occasion at 2 timepoints. Cytokine levels IL-1, IL-6, IL -8, TNF-alfa, IFN-alfa, and IFN-gamma. Measured by flow cytometer/ELISA. |
| Complement Factors: | Blood samples for complement factor (*Bb and C4d, CH50):* analysis will be collected on each dosing occasion at 2 timepoints.<br>Samples will be analyzed using a qualified method (method provided by the Bidder, eg. ELISA) |
| ADA: | TK animals, 8 occasions (prior to each dosing occasion) |
| Toxicokinetic Sampling: | On each dosing occasion at 8 timepoints (2h, 6h, 24h, 48h, 78h, 96h, 168h and 336h). |
| Bioanalysis (option): | Analysis will be conducted for 3 analytes. Plasma samples (0.5ml) will be analyzed by LC/MS/MS (**LNP**), qPCR (**mRNA**) and ELISA (**protein**). |
| TK non-compartmental Analysis (option): | WinNonlin Table Assembly. Toxicokinetic subreport should be included. |
| Gross pathology: | Not required |
| Organ weights: | Not required |
| Tissue preservation: | Not required |
| Histopathology: | Not required |
| Draft report: | 12 weeks following the end of study |
| Archiving: | 5 years |
| SEND dataset: | To be quoted as an option |

### 1.2.2 An 8-Week Repeat Dose Toxicology Study after intravenous administration in Cynomolgus Monkey followed by a 4-Week Recovery Period

| | |
|---|---|
| Compliance: | GLP |
| Frequency of dosing: | Weekly for 8 weeks |
| Route of administration: | Intravenous Infusion (1 hour) |
| Species: | Cynomolgus monkey |
| Age: | 2.5 – 3 years |
| Weight: | 2.3 to 3.1 kg (males), 2.1 to 2.8 kg (females) |
| Number of animals: | 21/sex (Main animals 15/sex; recovery 6/sex) + 2 spares/sex |
| Acclimation: | As required |

**Dosing scheme:**

| Group No. | Treatment | No. of Main Animals[a] | | No. of Recovery animals[b] | |
|---|---|---|---|---|---|
| | | Males | Females | Males | Females |
| 1 | Control | 3 | 3 | 2 | 2 |
| 2 | Control- LNP | 3 | 3 | - | - |
| 3 | Dose 1 (low) | 3 | 3 | - | - |
| 4 | Dose 2 (mid) | 3 | 3 | 2 | 2 |
| 5 | Dose 3 (high) | 3 | 3 | 2 | 2 |

[a]Main animals euthanized on Day 51.
[b]Recovery animals euthanized on Day 78.

| | |
|---|---|
| Cage side observations and mortality: | Daily (cage side am and mortality at the end of the working day) |
| Detailed examinations: | All NHPs, pre-treatment and weekly |
| Cutaneous Tolerance Testing: | After each administration, 1 hr and 24 hr post-dosing |
| Body weights: | Pre-treatment and weekly |
| Food consumption: | Daily, qualitative |
| Clinical pathology (H, CC, Coag): | All animals on Weeks 1, 4, 8 and prior to necropsy |
| Urinalysis: | At termination |
| Cytokines: | All animals on each dosing occasion (2 time points). Cytokine levels IL-1, IL-6, TNF-alfa will be measured by flow cytometer/ELISA. |
| Complement Factors: | Blood samples for complement factor (*Bb and C4d, CH50):* All animals on each dosing occasion (2 timepoints). Samples will be analysed using a qualified method (method provided by the Bidder, eg. ELISA) |
| ADA: | 8 occasions (prior to each dosing occasion) Samples will be analysed using a qualified ADA method (method provided by the Bidder, eg. ELISA) |
| Toxicokinetic Sampling | TK analysis will be conducted on 2 occasions (Day 1 and on the last day of dosing. Plasma samples (0.5ml) will be collected at 8 timepoints (2h, 6h, 24h, 48h, 78h, 96h, 168h and 336h) |
| Bioanalysis (option): | Analysis will be conducted for 3 analytes. and analyzed by LC/MS/MS (**LNP**), qPCR (**mRNA**) and ELISA (**protein**). |
| TK non-compartmental Analysis: | WinNonlin Table Assembly. Toxicokinetic subreport should be included. |
| Electrocardiograms (option): | All animals. 1 occasion prior to dosing as well as 4 timepoints (up to 48 hours) following each treatment. Evaluated quantitatively (RR, PR, QRS, QT, QTc; 12 ECG/animal/dose). |
| Cardiovascular Safety Pharm (Telemetry by jacket; option): | All animals on two occasions, commencing 2-hour monitoring period prior to dosing followed by at least a 6-hour monitoring period post-dose. Quantitative evaluation by software |
| Ophthalmoscopy: | Predose and at the end of dosing (end of recovery at additional cost) |
| Gross pathology: | All animals |
| Tissue collection and Organ weights: | All animals, full list (include BM smears and injection sites) |
| Histopathology: | Full list including injection sites, Study plan tissues from all animals found dead or euthanized pre-terminally; Study plan tissues from all control and high dose animals; gross lesions from all Main and Recovery animals; target organs for lower dose levels. |

| | |
|---|---|
| Draft reporting: | 12 weeks following ending the Study |
| Final reporting: | 6 weeks following approval of Draft Report |
| SEND dataset: | To be quoted as an option |
| Archiving: | 5 years |

## 2. Safety Pharmacology

### 2.1 Study of Cardiovascular assessment after intravenous administration in Cynomolgus Monkey

Frequency of dosing: Single dose
Route of administration: IV infusion (1h)
Species/Strain: Cynomolgus monkey
Age: 2.5 to 3 years
Weight: 2.3 to 3.1 kg

Compliance: GLP

Animals:
Species: Cynomolgus Monkey (*Macaca fascicularis*)
Total No. of Animals on Study: 12 males
Target Body Weight Range: 2 to 4 kg at onset of treatment
Target Age Range at Start: Young adult at onset of treatment
Acclimation: minimum 4 weeks prior the Study initiation

Treatment:

**Dosing scheme:**

| Group No. | Treatment | No. of Animals |
|---|---|---|
| 1 | Control | 3 |
| 2 | Dose 1 (low) | 3 |
| 3 | Dose 2 (mid) | 3 |
| 4 | Dose 3 (high) | 3 |

Clinical observations: Twice daily
Detailed examinations: Pretreatment and following dosing
Body weight: Pretreatment and prior to each dose
Clinical pathology: All animals pretreatment: full hematology, coagulation and clinical chemistry (standard parameters)
Cardiovascular Assessment (BP & ECG), Body Temp and Activity: all NHPs, Quantitative evaluation by software.

ECG: Evaluated quantitatively (RR, PR, QRS, QT, QTc). Review of all cardiovascular data by a certified veterinary cardiologist.

Draft reporting:
no later than 10 weeks following ending the Study
(at least two rounds of comments between the Purchaser and the Bidder should be included)
Final reporting: no later than 6 weeks following finishing the review of the Draft Study Reports

Archives: at least for 5 year after finishing the Study

### 2.2 Effect on Cloned hERG Potassium Channels Expressed in Mammalian Cells

In vitro cardiovascular assessment will be conducted by whole-cell patch clamp in mammalian cell line (HEK-293) stably expressing hERG channel.
Dose formulation analysis (GLP): Conducted on all dosing formulations

Draft reporting:
no later than 10 weeks following ending the Study
(at least two rounds of comments between the Purchaser and the Bidder should be included)
Final reporting: no later than 6 weeks following finishing the review of the Draft Study Reports

Archives:
at least for 5 year after finishing the Study

## 3. Analytical Support
## 3.1 Dose Formulation Analysis
### 3.1.1 Method Development of dose formulation samples

Compliance: non GLP
Test Item: LNP - mRNA
Analyte: mRNA (1 analyte)
Vehicle: TBD
Method of Analysis: fluorescence measurement or other suitable method for quantitative mRNA determination provided by the Bidder
Study Documentation: The method will be detailed in an Analytical Procedure that will be generated under the validation study

### 3.1.2 Method Validation of dose formulation samples

Compliance: GLP
Test Item: LNP – mRNA
Analyte: mRNA (1 analyte)
Vehicle: TBD

Working range: TBD
Experimental procedure and parameters: Full method validation under this project TBD – based on the validated analytical methods proposed by the Bidder
Reporting for Method Validation: Full audited draft(s) and final report

## 4. Bioanalysis

### 4.1 Bioanalytical Method Development for Test Item determination in Rat plasma

Method Feasibility/Development of an LC-MS/MS , qPCR, ELISA Method for the Determination of Test Item in rat plasma
Analyte: mRNA, LNP component (3 analytes: 1 analyte-LNP, 1 analyte- mRNA, 1 analyte- protein)
Compliance: non GLP
Test Item: LNP - mRNA
Vehicle: TBD
Method of Analysis: LNP- LC/MS/MS; mRNA- qPCR; protein- ELISA
Study Documentation: The method will be detailed in an Analytical Procedure that will be generated under the validation study

### 4.2 Bioanalytical Method Validation for Test Item determination in Rat plasma

Validation of an Analytical Method for the Determination of Test Item in rat Plasma by LC-MS/MS, qPCR, ELISA
Compliance: GLP
Test Item: LNP - mRNA
Vehicle: TBD
Method of Analysis: LC/MS/MS, qPCR, ELISA
Study Documentation: Full audited draft(s) and final report

### 4.3 Bioanalytical Method Development for Test Item determination in Monkey plasma

Method Feasibility/Development of an LC-MS/MS, qPCR, ELISA Method for the Determination of Test Item in Monkey plasma
Analyte: mRNA, LNP component (3 analytes: 1 analyte-LNP, 1 analyte- mRNA, 1 analyte- protein)
Compliance: non GLP
Test Item: LNP - mRNA
Vehicle: TBD
Method of Analysis: LC/MS/MS, qPCR, ELISA
Study Documentation: The method will be detailed in an Analytical Procedure that will be generated under the validation study

### 4.4 Bioanalytical Method Validation for Test Item determination in Monkey plasma

Validation of an Analytical Method for the Determination of Test Item in Monkey Plasma by LC-MS/MS, qPCR, ELISA
Compliance: GLP
Test Item: LNP - mRNA
Vehicle: TBD
Method of Analysis: LC/MS/MS, qPCR, ELISA
Study Documentation: Full audited draft(s) and final report

**5. Genetic Toxicology**

**5.1 Bone marrow micronucleus test in the Wistar Han Rat**

Compliance: GLP
Species/Strain Rat/ Wistar Han
Test item: TBD
Test system: Human peripheral blood lymphocytes
Dose formulation preparation: Up to 3 days before administration
Dose formulation analysis As required
Route of administration: Oral gavage
Dosing regimen: Groups 1-5 - Two doses (one at 0h and one at 24h)
Group "positive control" – one dose (at 24h)
Pretreatment period Min. 5 days

| Group No. | Treatment | No. of Main Animals | |
|---|---|---|---|
| | | Males | Females |
| 1 | Control | 5 | 5 |
| 2 | Dose 1 (low) | 5 | 5 |
| 3 | Dose 2 (mid) | 5 | 5 |
| 4 | Dose 3 (high) | 5 | 5 |
| 5 | Positive control | 3 | 3 |

Mortality/Morbidity: Twice daily
Body weight: At randomization and Day -1
Bone marrow collection 48h post first dose

Bone marrow smear preparations - stained using Acridine Orange.
Immature erythrocytes - examined by fluorescence microscopy for the presence of micronuclei.

Draft reporting:
no later than 8 weeks following ending the Study

(at least two rounds of comments between the Purchaser and the Bidder should be included)
Final reporting: no later than 6 weeks following finishing the review of the Draft Study Reports

Archives:
at least for 5 year after finishing the Study

### 5.2 In Vitro chromosomal aberration assay (Chinese hamster ovary (CHO)

Compliance: GLP
Cell line: Chinese hamster ovary (CHO) or TK6 cells
Test item: TBD
Dose formulation preparation: Up to 3 days before administration
Dose formulation analysis As required

In Vitro Micronucleus Test Procedure: according to OECD 487 guideline:
Cultures should be incubated with several concentrations of the test compound for three to four hours in the presence and absence of metabolic activation (S9) and for 21 to 24 hours in the absence of S9
A positive outcome is characterized by a statistically significant, dose-dependent increase in micronucleated cells that exceeds historical negative control limits

Draft reporting:
no later than 8 weeks following ending the Study
(at least two rounds of comments between the Purchaser and the Bidder should be included)
Final reporting: no later than 6 weeks following finishing the review of the Draft Study Reports

Archives:
at least for 5 year after finishing the Study

### 5.3 Bacterial reverse mutation test in vitro (AMES)

Compliance: GLP
Test system: Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2 uvrA
Dose formulation preparation: Up to 3 days before administration
Dose formulation analysis As required

Bacterial reverse mutation test in vitro Procedure: according to OECD 471 guideline:
Cultures should be incubated with several concentrations of the test compound in the presence and absence of metabolic activation (S9).
A positive outcome is characterized by a statistically significant, dose-dependent increase in mutant frequency that exceeds historical negative control limits.

Draft reporting:

no later than 8 weeks following ending the Study
(at least two rounds of comments between the Purchaser and the Bidder should be included)
Final reporting: no later than 6 weeks following finishing the review of the Draft Study Reports

Archives:
at least for 5 year after finishing the Study

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