Appendix No. 1 to the Request for Proposal No. Z01/11/2023/INKAROS

Detailed description of the subject of the contract

Preclinical pharmacokinetic evaluation of a drug candidate substance in mice animal model

Details not covered in the text below will be described in the detailed study protocol developed upon contract preparation.

1.1. Objective

The study aims to perform the PK-half life, body weight and clinical abnormalities of 10 tested articles + 1 control after e.g. 10 mg/kg (or e.g. 20 mg/kg) dose via intravenously (IV) administration to subject mice group.

1.2. Animals

6- 9 week old B6.Cg-*Fcgrttm1Dcr Prkdcscid* Tg(FCGRT)32DcrJ mice (preferably females) that will be **homozygous** for the human FcRn transgene Tg(CAG-FCGRT)276Dcr, *Fcgrttm1Dc*, and *Prkdcscid* will be used for experiments. Mice age should be matched and at least n=6 mice per experimental group should be maintained. The offers without proper certifications will be excluded.

1.3. Test articles and administration

The biologically active test articles 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10 and the control will be provided by the sponsor along with formulation instructions. All the compounds will be prepared freshly before administration via IV. Overview of the experimental details are shown in Table 1.

1.4. Dosage

IV single dose.

1.5. Procedure

The test articles 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and the control will be administrated at a concentration of e.g. 10 or 20 mg/kg. The study period after IV administration can be between 2-3 weeks. The study plan includes blood sampling in 10 different time points, body weight (BW) and clinical abnormalities recording. Pilot ELISAs will be used to determine the optimum plasma sample dilution with different dilutions for both the early time after IV injection and 1 day. So that these test articles plasma concentrations hit near the top of the standard curve within its linear range. All the plasma samples will be assessed in triplicate.

1.6. General study plan

Minimum number of mice per study group should be n=6. PK analysis - half life ($t_{1/2}$), body weight, clinical abnormalities must be measured and recorded daily as outlined in Table 2. All these data should be analyzed via scientifically proven software.

1.7. Evaluated parameters

Cytokine level analysis will be performed on the blood samples (TNF alpha, IFN-gamma, IL-4, IL-10, IL-9, IL-17) before and after the study. Moreover, % of T-cells including gamma delta T-cells and NK cells will be recorded before and after the study. Clinical signs and individual body weight (BW) will be measured before administration of the compound, and parameters will be collected daily until termination of the study. The Contractor should inform about the raw data availability during the study course and experimental updates frequency. The Contractor should inform the Sponsor about the schedule of test article administration and blood sample collection timepoints, as well as the plasma sample storage maps.

1.8. Report

Non-GLP study and report should be prepared. All experimental data will be provided to sponsor as raw data, and as a PowerPoint summary including plasma concentration time curves, PK parameters, half-life ($t_{1/2}$), body weight, clinical observations, cytokines levels and % of T-cells including gamma delta T-cells and NK cells. A comprehensive interpretive study report must be delivered to the sponsor.

Table 1: Overview of the project

Group no.	Treatment	Dose [mg/kg bw]	Route of administration, frequency	Mouse strain, no of mice	Blood sampling times
1	control	TBD	IV, 1x	*scid* FcRn-/- hFcRn (32) Tg, 6	10 time points
2	Substance 1 to 10	TBD	IV, 1x	*scid* FcRn-/- hFcRn (32) Tg, 60	10 time points
total				66 mice	

TBD: To be determined, IV: intravenous

Table 2: Time schedule of blood sampling, cytokines analysis, percentage of cells analysis, body weight and clinical observations

Day	Hours	Process plasma, store	Body weight [mg]	Cytokine analysis	% of immune cells analysis	Clinical symptoms
TBD	TBD	Injection of test articles	YES	Day TBD backwards	DayTBD backwards	Yes
TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES

TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES
TBD	TBD	YES	YES	-	-	YES
Day TBD onwards	-	ELISA and PK analysis	Analysis	Day TBD onwards	Day TBD onwards	Analysis

TBD: To be determined, PK: Pharmacokinetics, ELISA: Enzyme-linked immunosorbent assay