*Appendix No. 1*

Preclinical studies of WNV VLP/mRNA/Alhydrogel/MPLA Combination Vaccine candidates – Main Study protocol.

# MAIN STUDY PROTOCOL:

Title: Preclinical Evaluation of a WNV VLP/mRNA/Alhydrogel/MPLA Combination Vaccine candidates: Immunogenicity and Protective Efficacy in Mouse Models

1. Objective:

To assess the immunogenicity and protective efficacy of a novel West Nile Virus vaccine candidates combining Virus-Like Particles/ Virus-Like Partticles- mRNA, and Alhydrogel/MPLA adjuvant in mouse model in challenge study.

Object of the Contract

The purpose of this Agreement is to conduct the study to proved the effective immunoprotection of experimental vaccine against WNV on mice model.

1. Study Design:

Animal models:

C57BL/6 mice, 6-8 weeks old, equal numbers of males and females according the Table 1

Table 1 Number of mice in each experiments

|  |  |  |
| --- | --- | --- |
| Experimental group | Challenge study: VLP | Challenge study: VLP-mRNA |
| Number of mice | 10+10; 20 in total | 10+10; 20 in total |
| i.m. immunisation | VLP + Alhydrogel/MPLA | VLP-mRNA + Alhydrogel/MPLA |

* Duration:

up to 14 weeks (vaccination to final sample collection)

3. Experiments:

1. Challenge study

Performed under BSL-3 conditions, the scheme and procedure for both group are exactly the same. The challenge study will be divided into 2 separated experiments according the groups in Table 1.

* Immunization Schedule: Challenge study

Day 0: Prime vaccination, i.m.

Day 21: Boost 1, i.m.

Day 42: Boost 2, i.m.

Sample Collection Schedule:

Day 0: Pre-immunization serum

Day 21: Pre-boost serum

Day 42: Post-boost I serum

Day 63 post-boost II serum

Day 70: Pre-challenge serum and splenocytes (n=5 per group)

Days 70 -98 Post-challenge morbidity and survival evaluation (every other day)

Day up to 98 or at euthanasia: Terminal blood and tissue collection

Endpoints and Assessments:

Primary Endpoints:

a) Survival rate

b) Neutralizing antibody titers e.g. Plaque Reduction Neutralization Test (PRNT90) using homologous virus

Secondary Endpoints:

a) Clinical score (0-5 scale: 0=normal, 1=ruffled fur, 2=hunched posture, 3=reduced activity, 4=paralysis, 5=moribund or dead)

b) Weight change ( every day since challenge day)

c) Viremia levels in blood ( every day)

d) Viral load in tissues (brain, spinal cord, spleen, liver) using the RT-qPCR

e) T cell responses (IFN-γ ELISpot and intracellular cytokine staining: TNF-α, and IL-2) (pre- and post-challenge samples)

f) Antibody isotype profile (IgG1, IgG2a/c) (pre- and post-challenge samples)

g) Histopathological changes in CNS tissues

Deliverabiles:

A final report containing a detailed description of the tests performed, the results obtained and the final conclusions. All results must be provided both as raw data and also processed and subjected to the appropriate statistical analysis for the test.

The offer is addressed to bidders who have experience in providing similar services and have a certificate of the necessary quality standards. Experience should be demonstrated by attaching a declaration of meeting these criteria which constitutes **Appendix** :

* the facility must have broad expertise and a track record in performing and completing such projects evidenced by at least 3 years of activity of on the market in terms of this specific service
* Certificate of BSL-3 Laboratory
* Declaration of experienced working with West Nile Virus.
* Declaration of virus availability on-site or the possibility of purchase and cultivation in the laboratory.