

LANDRY INDUSTRIES INC.

PhytoIntelligence 3.2: Experimental Protocol Design

In Vivo Malaria Model Study: Efficacy, Pharmacokinetics & Safety

OSF Pre-Registration Compatible Protocol

Author: Marie-Soleil Seshat Landry (ORCID: 0009-0008-5027-3337)

Date: 2026-04-27 | Version: 1.0 (PRE-REGISTRATION READY)

1. STUDY OVERVIEW & OBJECTIVES

1.1 Primary Objective

To evaluate the in vivo antimalarial efficacy of a synergistic botanical pill (artemisinin + quercetin + piperine) vs. artemisinin monotherapy in a rodent malaria model (*Plasmodium berghei* ANKA), measured by:

- **Parasitemia reduction (%)**, measured via blood smear microscopy at days 3, 5, 7, 10, 14, 21.
- **Parasite clearance time (T_{clear})**, defined as first day parasitemia $<0.01\%$.
- **Survival extension and clinical signs** (neurological, cerebral malaria phenotype).

1.2 Secondary Objectives

- **Plasma pharmacokinetics (PK):** C_{max} , AUC, $t_{1/2}$ of artemisinin and its metabolite dihydroartemisinin (DHA).
- **Hepatotoxicity markers:** ALT, AST, total bilirubin at study end.
- **Inflammatory cytokines:** $\text{TNF}\alpha$, $\text{IL-1}\beta$ (serum, day 10 & 21).
- **Organ pathology:** Histology (brain, liver, spleen) for drug-induced vs. malaria-induced lesions.

2. STUDY DESIGN

2.1 Design Type

Randomized, controlled, blinded (animals & outcome assessor), parallel-group in vivo efficacy study.

2.2 Study Schema

4 groups, 10 animals per group (N=40 total, female C57BL/6J mice, 6-8 weeks old):

Group	Treatment	Dose (mg/kg)	Route
Group 1 (Control)	Vehicle (0.5% CMC-Na)	—	Oral, 200 µL
Group 2 (Monotherapy)	Artemisinin only	20	Oral, 200 µL
Group 3 (Combination)	Artemisinin + Quercetin + Piperine	20+15+4	Oral, 200 µL
Group 4 (Positive Control)	Chloroquine (CQ)	10	Intraperitoneal (IP)

Note: Group 4 (chloroquine) serves as baseline efficacy control. Doses scaled from human equivalents using allometric scaling (mouse ~10x human/kg). Oral administration chosen to mimic intended human use.

2.3 Infection Model

Parasite Strain: P. berghei ANKA (lethal, fast-progressing, mimics severe malaria)

Treatment Initiation: Day 3 post-infection (when parasitemia ≈ 5%), 1x daily for 7 days

3. METHODS & MEASUREMENTS

3.1 Primary Outcome: Parasitemia

Blood smears collected from tail vein on Days 3, 5, 7, 10, 14, 21. Giemsa-stained, 5,000 RBCs counted per mouse, % parasitemia calculated.

Analysis: Two-way ANOVA (group × day), post-hoc Bonferroni. Primary outcome: AUC parasitemia (integral of % parasitemia over 21-day period).

3.2 Survival & Phenotype

- **Daily mortality monitoring through Day 28 (or moribund endpoint).**
- **Clinical scoring (0-4):** ruffled fur, hunched posture, seizures, paralysis, death.
- **Kaplan-Meier survival curves; log-rank test for group comparison.**

3.3 Plasma Pharmacokinetics

Terminal (non-infected) mice: 8 per group. Collect plasma at t = 0.5, 1, 2, 4, 8 h post-dose (Day 3 of treatment). Measure artemisinin & DHA via HPLC-MS/MS.

Pharmacokinetic parameters: C_{max}, T_{max}, AUC_{0-∞}, t_{1/2}, CL/F calculated using standard methods.

3.4 Blood Chemistry & Cytokines

Serum collected on Days 10 & 21 post-infection (or at death). Measured:

- AST, ALT, total bilirubin (Roche Cobas analyzer)
- TNF α , IL-1 β (Luminex multiplex ELISA)
- Hemoglobin, RBC, WBC (IDEXX hematology)

3.5 Organ Pathology

Terminal necropsy (Day 21 or endpoint): Brain, liver, spleen collected. Fixed in 10% buffered formalin, paraffin-embedded, H&E-stained. Blinded pathologist reviews for drug-induced necrosis, sequestration, hemorrhage.

4. STATISTICAL ANALYSIS PLAN

Primary analysis: Intent-to-treat (ITT). Power calculation: N=10/group achieves 80% power to detect 30% AUC reduction (artemisinin vs. combination) at $\alpha=0.05$ (two-tailed).

Pre-specified comparisons: Combination vs. Monotherapy; Combination vs. Vehicle. Bonferroni correction ($\alpha=0.025$) for multiple comparisons.

5. ETHICAL COMPLIANCE & REGULATORY

- IACUC approval (Institutional Animal Care & Use Committee) prior to initiation
- Humane endpoints: Severe parasitemia (>40%), neurological signs (seizure), weight loss >15%
- Housing: SPF (specific pathogen-free) facility; water & food ad libitum

6. OSF PRE-REGISTRATION CHECKLIST

This protocol is formatted for submission to Open Science Framework (OSF Registries, <https://osf.io/registries>) and complies with the PRE-REG format.

- **Study Title:** In Vivo Efficacy of Artemisinin + Quercetin +

Piperine vs. Monotherapy in *P. berghei* Malaria

- **Primary Hypothesis:** Combination treatment reduces parasitemia AUC by $\geq 30\%$ vs. artemisinin monotherapy
- **Secondary Hypothesis:** Combination increases artemisinin $t_{1/2}$ & AUC by $\geq 40\%$; reduces $TNF\alpha$ by $\geq 25\%$
- **Pre-Specified Exclusions:** Animals failing to reach 3% parasitemia by Day 3 post-infection

7. STUDY TIMELINE

Phase	Timeline
IACUC Submission	Weeks 1–4
Animal Acclimation	Weeks 5–8
Infection & Treatment	Weeks 9–13 (Day 0–28 post-infection)
Data Analysis	Weeks 14–16
Manuscript Preparation	Weeks 17–20

Total study duration: ~20 weeks from IACUC approval.

8. CONCLUSION

This protocol is designed to rigorously test the efficacy and safety of the PI 3.2 botanical combination in a validated malaria model. Pre-registration on OSF establishes research integrity and enables transparent reporting of results regardless of outcome.