PRE-IND MEETING BRIEFING DOCUMENT

Type B Meeting with FDA/CBER/OVRR/DVRPA

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
BLA	Biologics License Application
CBER	Center for Biologics Evaluation and Research
CMC	Chemistry, Manufacturing, and Controls
CSP	Circumsporozoite Protein
DVRPA	Division of Vaccines and Related Products Applications
ELISA	Enzyme Linked Immunosorbent Assay
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
IND	Investigational New Drug
LNP	Lipid Nanoparticle
NANP	Asparagine Alanine Asparagine Proline (repeat sequence)
NHP	Nonhuman Primate
NOAEL	No Observed Adverse Effect Level
PfCSP	Plasmodium falciparum Circumsporozoite Protein

1. EXECUTIVE SUMMARY

Aethon Biologics Inc. is developing MalariVax-CSP (ABX-4521), a recombinant protein subunit vaccine for the prevention of *Plasmodium falciparum* malaria. The vaccine targets the circumsporozoite protein (CSP), the predominant surface antigen on sporozoites, which has been validated as a protective antigen through the development of RTS,S/AS01 (Mosquirix™) and R21/Matrix-M vaccines. MalariVax-CSP incorporates design improvements intended to enhance immunogenicity and durability of protective responses compared to first generation CSP vaccines. Key differentiating features include: (1) inclusion of the full length NANP repeat region and C-terminal domain containing T-cell epitopes; (2) conjugation to a modified carrier protein to enhance T-helper responses; and (3) formulation with a novel lipid nanoparticle adjuvant system designed to promote both humoral and cellular immunity. Preclinical studies in mice and nonhuman primates have demonstrated robust anti-CSP IgG responses exceeding titers associated with protection in RTS,S clinical trials. The proposed Phase 1 study will evaluate safety, tolerability, and immunogenicity of three dose levels in healthy malaria naive adults. The purpose of this Pre-IND meeting is to obtain FDA feedback on: (1) the adequacy of the proposed nonclinical toxicology program; (2) CMC considerations for IND submission; and (3) the proposed Phase 1 clinical trial design.

2. INTRODUCTION AND BACKGROUND

2.1 Malaria Disease Burden

Malaria remains one of the most significant global infectious disease threats, with the World Health Organization reporting approximately 249 million cases and 608,000 deaths in 2022. The burden falls disproportionately on sub-Saharan Africa, where 95% of cases and 96% of deaths occur, predominantly in children under five years of age. *Plasmodium falciparum* is responsible for the vast majority of severe disease and mortality. Despite decades of vector control efforts and antimalarial drug development, malaria elimination remains elusive. The emergence of insecticide resistant mosquitoes and drug resistant parasites underscores the critical need for effective vaccines as a complementary prevention strategy.

2.2 Rationale for CSP as a Vaccine Target

The circumsporozoite protein (CSP) is the most abundant surface protein on *P. falciparum* sporozoites and plays essential roles in sporozoite motility and hepatocyte invasion. Antibodies targeting the central NANP repeat region can neutralize sporozoites and prevent liver infection, providing sterilizing immunity. Additionally, T-cell responses to epitopes in the C-terminal region contribute to protection through elimination of infected hepatocytes. The clinical validation of CSP as a protective antigen has been established through: **RTS,S/AS01 (Mosquirix™):** WHO recommended vaccine demonstrating approximately 30% efficacy against severe malaria in pediatric populations; first malaria vaccine to receive regulatory approval. **R21/Matrix-M:** Next generation CSP vaccine showing 77% efficacy in Phase 2b trials, achieving the WHO's 75% efficacy target. These successes validate CSP as a target while highlighting opportunities for further improvement in vaccine design to enhance efficacy and durability of protection.

3. PRODUCT DESCRIPTION

3.1 Vaccine Composition

MalariVax-CSP (ABX-4521) is a recombinant protein subunit vaccine comprising the following components:

Component	Description	Function
ABX-4521 Antigen	Recombinant PfCSP fusion protein (aa 91-387) conjugated to modified diphtheria toxoid (CRM197)	Immunogen; elicits anti-CSP antibodies and T-cell responses
ABX-ADJ01 Adjuvant	Lipid nanoparticle containing synthetic TLR4 agonist (GLA) and saponin (QS-21 analog)	Enhances immunogenicity; promotes Th1 responses
Buffer System	Phosphate buffered saline, pH 7.2, with trehalose stabilizer	Maintains antigen stability and isotonicity

3.2 Antigen Design

The ABX-4521 antigen was designed to optimize immunogenicity while maintaining manufacturability. The construct includes: **N-terminal Region (aa 91-110):** Contains the Region I cleavage site and junction domain, recently identified as a target of potent neutralizing antibodies. **Central Repeat Region (aa 111-300):** Comprises 27 NANP repeats interspersed with 4 NVDP variants, representing the immunodominant B-cell epitope region. **C-terminal Region (aa 301-387):** Contains the Th2R and Th3R T-cell epitopes critical for CD4+ T-helper responses and the thrombospondin like type I repeat (TSR) domain. The fusion protein is chemically conjugated to CRM197 (a nontoxic mutant of diphtheria toxin) using a heterobifunctional crosslinker. This carrier protein provides additional T-helper epitopes and leverages existing immunological memory from DTP vaccination to enhance responses.

3.3 Adjuvant System

The ABX-ADJ01 adjuvant system was developed to promote both robust antibody responses and Th1 polarized cellular immunity. The lipid nanoparticle formulation contains: **GLA (Glucopyranosyl Lipid A):** A synthetic TLR4 agonist that activates dendritic cells and promotes Th1 cytokine production (IFN-γ, IL-2). **QS-21 Analog:** A purified saponin fraction that enhances antigen uptake and cross presentation, promoting CD8+ T-cell responses. The LNP delivery system provides sustained antigen presentation at the injection site and facilitates lymph node trafficking. This adjuvant platform has demonstrated acceptable safety in previous clinical studies with unrelated antigens.

3.4 Mechanism of Action

MalariVax-CSP is designed to prevent malaria infection by inducing immune responses that target sporozoites before they can establish liver infection: **1. Humoral Immunity:** Anti-NANP repeat antibodies bind to sporozoites in the skin and bloodstream, forming a circumsporozoite precipitate that immobilizes parasites and prevents hepatocyte invasion. High titer, durable antibody responses are essential for protection. **2. Cellular Immunity:** CD4+ T-cells recognizing C-terminal epitopes provide help for B-cell responses and antibody class switching. CD8+ T-cells may contribute to elimination of infected hepatocytes expressing CSP derived peptides. **3. Memory Responses:** Generation of long lived plasma cells and memory B-cells is critical for sustained protection in endemic settings with ongoing exposure.

4. NONCLINICAL DEVELOPMENT PROGRAM

4.1 Completed Studies Summary

Proof of concept studies have been completed in mice and nonhuman primates to evaluate immunogenicity and inform dose selection. A summary of completed studies is provided below:

Study	Species	Key Findings
ABX-001 Immunogenicity	BALB/c Mice (n=10/group)	Dose dependent anti-CSP IgG responses; Peak titers >10^6 at 25 μg dose; Th1 biased cytokine profile (IFN-γ, IL-2)
ABX-002 Dose Optimization	C57BL/6 Mice (n=10/group)	Three dose regimen (0, 28, 56 days) optimal; ABX-ADJ01 superior to alum comparator; Durable responses at 6 months postvaccination
ABX-003 NHP Immunogenicity	Rhesus Macaques (n=5/group)	Anti-NANP IgG titers comparable to RTS,S; Functional antibody activity confirmed; No adverse clinical observations

4.2 Proposed GLP Toxicology Program

The following GLP compliant toxicology studies are proposed to support the initial IND submission:

Study Type	Species	Design	Key Endpoints
Repeat Dose Toxicity with TK	Sprague Dawley Rat (15/sex/group)	3 doses (Days 1, 29, 57) High dose: 5X clinical 4 week recovery	Clinical observations, body weight, clinical pathology, histopathology, anti-CSP antibody, TK
Repeat Dose Toxicity with TK	Cynomolgus Monkey (3/sex/group)	3 doses (Days 1, 29, 57) High dose: 5X clinical 4 week recovery	Clinical observations, body weight, clinical pathology, immunophenotyping, histopathology, TK
Local Tolerance	NZW Rabbit (3/sex/group)	Single IM injection High dose	Injection site evaluation (Draize scoring), histopathology

4.3 Toxicology Study Rationale

Species Selection: Rats and cynomolgus monkeys were selected based on demonstration of immunological responses to ABX-4521 and relevance to human immune function. Both species mounted anti-CSP antibody responses following vaccination in pilot studies. **Dose Selection:** The high dose in toxicology studies (5X the proposed maximum clinical dose) provides adequate safety margins while remaining within the range demonstrating immunogenicity. Dose selection accounts for body surface area scaling between species. **Dosing Regimen:** The three dose regimen mirrors the proposed clinical schedule, allowing evaluation of effects from repeated immunization including potential hypersensitivity responses. **Study Duration:** A four week recovery period is included to assess reversibility of any treatment related findings and persistence of immune responses.

4.4 Developmental and Reproductive Toxicology

Consistent with FDA guidance for preventive vaccines, developmental and reproductive toxicology (DART) studies are not planned prior to IND submission. Women of childbearing potential will be excluded from the Phase 1 study unless using adequate contraception, and pregnancy testing will be performed prior to each vaccination. DART studies will be conducted to support the BLA submission in accordance with ICH S5(R3) guidelines.

5. CHEMISTRY, MANUFACTURING, AND CONTROLS (CMC)

5.1 Drug Substance: ABX-4521 Antigen

Expression System: The ABX-4521 antigen is produced in *Escherichia coli* BL21(DE3) using an IPTG inducible T7 expression system. The expression plasmid contains the PfCSP sequence (NF54 reference strain) codon optimized for *E. coli* expression. **Manufacturing Process:** The manufacturing process consists of fermentation, cell harvest, inclusion body solubilization, refolding, and multistep chromatographic purification (ion exchange, hydrophobic interaction, and size exclusion chromatography). The purified protein is chemically conjugated to CRM197 using a SMCC crosslinker, followed by ultrafiltration/diafiltration into formulation buffer. **Characterization:** The drug substance is characterized by identity (SDS-PAGE, Western blot, peptide mapping), purity (SEC-HPLC ≥95%, residual HCP <100 ng/mg, endotoxin <10 EU/mg), potency (anti-CSP ELISA binding assay), and conjugation ratio (MALDI-TOF MS, target 4-8 CSP per CRM197).

5.2 Drug Substance: ABX-ADJ01 Adjuvant

Manufacturing Process: The ABX-ADJ01 lipid nanoparticle adjuvant is manufactured using a microfluidic mixing process. Lipid components (DSPC, cholesterol, synthetic GLA, QS-21 analog) are dissolved in ethanol and rapidly mixed with aqueous buffer to form uniform nanoparticles. The resulting dispersion is processed through tangential flow filtration to remove ethanol and concentrate to target lipid concentration. **Characterization:** The adjuvant is characterized by particle size (DLS, target 80-120 nm, PDI <0.2), lipid content (HPLC quantification), GLA activity (TLR4 reporter cell assay), and sterility/endotoxin testing.

5.3 Drug Product

Formulation: The final drug product is a liquid formulation supplied as two vials for bedside mixing: Vial A contains the ABX-4521 antigen in PBS/trehalose buffer; Vial B contains the ABX-ADJ01 adjuvant dispersion. Contents are combined immediately prior to injection. **Presentation:** Single dose vials (0.5 mL per vial) **Dose Strengths:** 10 μg, 25 μg, and 50 μg antigen per 0.5 mL dose (fixed adjuvant quantity) **Storage:** 2-8°C; do not freeze **Shelf Life:** Provisional 12 month shelf life supported by ongoing stability studies

5.4 Manufacturing Facility and Analytical Methods

Drug substance and drug product for Phase 1 clinical studies will be manufactured at Aethon Biologics' GMP facility in Cambridge, MA. The facility has been inspected by FDA within the past 2 years with no significant findings. CRM197 carrier protein is sourced from a qualified GMP supplier with an established Drug Master File. Validated analytical methods have been developed for release and stability testing per ICH Q2(R2). Key release specifications include: Identity (SDS-PAGE, Western Blot conforming to reference), Purity (SEC-HPLC ≥95%), Potency (Anti-CSP ELISA 80-120% of reference), Conjugation Ratio (MALDI-TOF MS 4-8 CSP/CRM197), Endotoxin (LAL <10 EU/mL), Sterility (USP <71> No growth), and Particle Size for adjuvant (DLS 80-120 nm, PDI <0.2).

6. PROPOSED PHASE 1 CLINICAL STUDY

6.1 Study Synopsis

Parameter	Description
Study Title	A Phase 1, Randomized, Observer Blind, Placebo Controlled Study to Evaluate the Safety, Tolerability, and Immunogenicity of MalariVax-CSP in Healthy Adults
Study Design	First in human, dose escalation study with sentinel dosing
Study Population	Healthy adults aged 18-50 years, malaria naive, residing in nonendemic area
Sample Size	Approximately 60 subjects (15 per dose group including placebo)
Dose Groups	Cohort 1: 10 μg	Cohort 2: 25 μg	Cohort 3: 50 μg	Cohort 4: Placebo
Dosing Regimen	3 doses administered IM at Days 0, 28, and 56
Route	Intramuscular (deltoid)
Study Duration	12 months (6 months post third vaccination)
Primary Endpoints	Safety: AEs, SAEs, solicited local/systemic reactions, laboratory parameters
Secondary Endpoints	Immunogenicity: Anti-CSP IgG titers, seroconversion rates, T-cell responses

6.2 Study Design Rationale

Dose Selection: The proposed dose range (10-50 μg) is based on NHP immunogenicity data demonstrating robust anti-CSP responses at 25 μg, with the 10 μg and 50 μg doses exploring the lower and upper bounds of the expected efficacious range. **Dosing Schedule:** The three dose regimen at 0, 28, and 56 days is consistent with successful malaria vaccine schedules and allows evaluation of prime boost kinetics. **Sentinel Dosing:** Within each cohort, two sentinel subjects (1 active, 1 placebo) will be dosed first, with a 72 hour safety observation period before enrolling the remaining subjects. **Observer Blind Design:** An observer blind design is employed to minimize bias in safety assessments while maintaining practical considerations for the two vial formulation.

6.3 Safety Monitoring

Solicited Adverse Events: Subjects will record local (pain, erythema, swelling, induration) and systemic (fever, headache, fatigue, myalgia, arthralgia) reactions in a diary card for 7 days following each vaccination. **Unsolicited Adverse Events:** All unsolicited AEs will be collected for 28 days following each vaccination. SAEs and AESIs will be collected throughout the study duration. **Laboratory Monitoring:** Hematology, serum chemistry, and urinalysis will be performed at baseline and Days 7, 35, 63, and 84. **Safety Monitoring Committee:** An independent SMC will review safety data prior to each dose escalation and may recommend protocol modifications based on predefined stopping rules.

6.4 Immunogenicity Assessments

Humoral Immunity: Anti-CSP (NANP repeat) IgG by ELISA at Days 0, 28, 56, 70, 84, 180, 365; Anti-CSP IgG subclass distribution (IgG1, IgG2, IgG3, IgG4); Functional antibody activity (sporozoite inhibition assay as exploratory endpoint). **Cellular Immunity:** Antigen specific T-cell responses by IFN-γ ELISpot at Days 0, 70, 180; Intracellular cytokine staining (ICS) for polyfunctional T-cell analysis (exploratory). **Correlate Analysis:**

Immunogenicity data will be analyzed to identify potential correlates of protection for comparison with published thresholds from RTS,S clinical trials.

6.5 Inclusion/Exclusion Criteria

Key Inclusion Criteria: Healthy adults aged 18-50 years; BMI 18-32 kg/m²; No prior malaria infection or residence in malaria endemic region; Willing to comply with study procedures and contraception requirements; Able to provide informed consent. **Key Exclusion Criteria:** History of malaria infection or receipt of investigational malaria vaccine; Travel to malaria endemic area within 6 months prior to enrollment or planned during study; Receipt of licensed vaccine within 14 days or investigational product within 30 days; Immunocompromised status or receipt of immunosuppressive therapy; History of severe allergic reaction to vaccine components; Pregnant or breastfeeding women; women of childbearing potential not using adequate contraception; Clinically significant laboratory abnormalities at screening.

6.6 Clinical Study Sites

The Phase 1 study will be conducted at 2-3 clinical research sites in the United States with experience in vaccine clinical trials. Sites will be selected based on access to healthy volunteer populations, clinical research infrastructure, and malaria naive demographics. All sites will have IRB approval prior to enrollment.

7. QUESTIONS FOR FDA

Aethon Biologics Inc. respectfully requests FDA feedback on the following questions regarding the planned IND submission for MalariVax-CSP (ABX-4521):

Nonclinical Development

Question 1: Does FDA concur that the proposed GLP toxicology program (repeat dose toxicity studies in rats and cynomolgus monkeys with a three dose regimen mirroring the clinical schedule) is adequate to support the initiation of Phase 1 clinical studies? *Sponsor Position:* The proposed program is consistent with FDA guidance for preventive vaccines and provides evaluation in both rodent and nonrodent species with relevant immune responses.

Question 2: Given that the ABX-ADJ01 adjuvant system has been evaluated in previous clinical studies with a different antigen, does FDA agree that separate adjuvant only toxicology studies are not required, provided the formulated vaccine (antigen + adjuvant) is tested in the GLP toxicology program? *Sponsor Position:* The adjuvant safety profile has been established in prior clinical experience. Testing the final formulated product is most relevant to clinical safety assessment.

Question 3: Does FDA agree that developmental and reproductive toxicology (DART) studies are not required prior to IND submission, given that women of childbearing potential will be excluded from the Phase 1 study unless using adequate contraception? *Sponsor Position:* This approach is consistent with FDA guidance for preventive vaccines and standard practice for early phase vaccine development.

Chemistry, Manufacturing, and Controls

Question 4: Does FDA have any concerns with the proposed two vial presentation (antigen and adjuvant supplied separately for bedside mixing) for the Phase 1 study? Are there specific stability or compatibility studies FDA would recommend? *Sponsor Position:* The two vial presentation ensures optimal stability of both components and allows dose flexibility. In use stability studies following reconstitution will be provided.

Question 5: The potency assay for ABX-4521 is an anti-CSP binding ELISA. Does FDA concur that this assay is appropriate for release testing at the IND stage, with development of a functional potency assay (sporozoite inhibition) planned for later development? *Sponsor Position:* The binding ELISA is well characterized and correlates with functional activity in preclinical studies. A functional assay will be developed for BLA submission.

Clinical Development

Question 6: Does FDA concur with the proposed Phase 1 study design, including the dose range (10-50 μg), three dose regimen, and sentinel dosing approach for dose escalation? *Sponsor Position:* The design is consistent with standard first in human vaccine study approaches and is informed by NHP immunogenicity data and published literature on CSP vaccines.

Question 7: Does FDA have recommendations regarding the immunogenicity assay panel, particularly regarding the assessment of functional antibody responses (sporozoite inhibition)? *Sponsor Position:* The proposed panel includes both binding antibody (ELISA) and T-cell (ELISpot) assessments. Sporozoite inhibition assays are included as exploratory endpoints.

Question 8: Are there specific safety monitoring requirements or adverse events of special interest (AESIs) that FDA would recommend tracking for this CSP based malaria vaccine? *Sponsor Position:* We propose standard vaccine AESI monitoring and are aware of the meningitis signal observed in RTS,S trials. We welcome FDA guidance on specific monitoring.

8. REFERENCES

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