Clinical Study Protocol

Title page

Study title	A First-In-Human Phase I, Open-Label, Dose-Escalating Trial to Assess the Safety, Tolerability and Immunogenicity/Preliminary Antitumor Activity of ES2B-C001 With or Without [adjuvant] [redacted] in HER2 Expressing Metastatic Breast Cancer.
Lay title	A First-in-Human clinical trial assessing safety of ES2B-C001 with or without [adjuvant] [redacted] in patients with HER2 expressing metastatic breast cancer.
Sponsor	ExpreS2ion Biotechnologies ApS [redacted]
Coordinating investigator	Univ. Prof. Dr. Bernd Jilma
Study code	ES2B-C001-S01
EU CT number	2024-516333-12-00
Phase of development	Phase I
Version and date	V2.0; 30-Oct-2024

This clinical trial, including archiving of essential documents, will be conducted in compliance with Good Clinical Practice (GCP) and with all applicable laws and regulations.

Signature Page

Signature of sponsor

This clinical study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational medicinal product (IMP), as well as with the moral, ethical and scientific principles governing clinical research as set out in the guidelines on GCP applicable to this clinical study.

ExpreS2ion Biotechnologies ApS
[redacted]

Sponsor [redacted] [redacted]

Signature of investigator

The PI agrees to supervise and conduct the study in compliance with the protocol, ICF, sponsor instructions, IRB/EC ICH-GCP, and local regulations.

Univ. Prof. Dr. Bernd Jilma
Medizinische Universität Wien
[redacted]

Principal investigator | Date | Signature

Protocol Version History

Version no.	Date	Summary of changes
V1.0	30-Jul-2024	Initial release
V2.0	30-Oct-2024	- Sequential recruitment of patient populations - Initial three dose cohorts start with adjuvanted ES2B-C001, in case of tolerability issues, treatment can be switched to non-adjuvanted ES2B-C001 - Assessment of injection site reactions according to FDA guidance 2007 (primary endpoint)

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List of Abbreviations

ADC	Antibody-drug conjugates
AE	Adverse event
AESI	Adverse events of special interest
AI	Aromatase inhibitor
BC	Breast cancer
CBR	Clinical benefit rate
ChT	Chemotherapy
COPD	Chronic obstructive pulmonary disease
COV	Coefficient of variation
CTRCD	Cancer therapy-related cardiac dysfunction
CR	Complete response
CRO	Clinical research organisation
CRS	Cytokine release syndrome
CT	Computer tomography
CTL	Cytotoxic T-lymphocytes
cVLP	Capsid virus like particle
DCR	Disease control rate
DFS	Disease-free survival
DLT	Dose limiting toxicity
DSMB	Date Safety Monitoring Board
DRF	Dose rate finding
eGFR	Estimated glomerular filtration rate
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ELISA	enzyme-linked immunosorbent assay
EOS	End of Study
ET	Endocrine therapy
FiH	First in human
FISH	Fluorescent in-situ hybridization
FVB	Friend virus B
GGT	Gamma glutamyl transferase
GLS	Global longitudinal strain
HER2	Human epidermal growth factor receptor 2
HR	Hormone receptor
HRT	Hormone replacement therapy
ICH	Immunohistochemistry
IEC	Independent ethics committees
IMP	Investigational Medicinal Product

IRB	Institutional review boards
LVEF	Left ventricular ejection fraction
mAbs	Monoclonal Antibodies
MAD	Maximum administered dose
MBC	Metastatic breast cancer
MRI	Magnetic resonance imaging
MRSD	Maximum recommended starting dose
MTD	Maximum tolerated dose
NCI-CTCAE	National cancer institute-common terminology criteria for adverse events
NYHA	New York Heart Association
OID	Optimal immunological dose
OBD	Optimal biological dose
OFS	Ovarian function suppression
ORR	Objective response rate
OS	Overall survival
OTC	Over the counter
pAb	Polyclonal antibodies
PBMC	Peripheral blood mononuclear cells
PFS	Progression free survival
PR	Partial response
Q3Q	Once every 3 weeks
RBD	Receptor-binding domain
RECIST	Response evaluation criteria in solid tumors
RP2D	Recommended phase 2 dose
SD	Stable disease
SAE	Serious adverse event
SAP	Statistical analysis plan
SERD	Selective estrogen receptor degrader or downregulator
SERM	Selective estrogen receptor modulators
SoA	Schedule of Activities
SoC	Standard of care
SUSAR	Suspected unexpected serious adverse reaction
TNBC	Triple-negative breast cancer

1 Protocol Summary

1.1 Protocol Synopsis

Protocol Title	A first-in-human phase I, open-label, dose-escalating trial to assess the safety, tolerability and immunogenicity / preliminary antitumor activity of ES2B-C001 with or without [adjuvant] [redacted] in HER2 expressing metastatic breast cancer.
Brief Title	A First-in-Human clinical trial assessing safety of ES2B-C001 with or without [adjuvant] [redacted] in patients with HER2 expressing metastatic breast cancer.
Study Numbers	ES2B-C001-S01
Development Phase	Phase 1 study in breast cancer patients
Sponsor	ExpreS2ion Biotechnologies ApS
Study Centers	Single site (Austria)
Study Objectives	**Primary Objective:** To determine the safety, tolerability, maximum tolerated dose (MTD) for ES2B-C001 alone or in combination with the adjuvant [redacted]. **Secondary Objectives:** To investigate the immunogenicity of ES2B-C001 alone or in combination with the adjuvant [redacted]. **Exploratory Objectives:** To determine the preliminary antitumor activity of ES2B-C001 alone or in combination with the adjuvant [redacted].
Study Endpoints	**Primary Endpoint:** • Nature and frequency of dose-limiting toxicities (DLTs). • Incidence, nature and severity of AEs graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0. • Incidence, nature and severity of injection site reactions according to FDA Guidance 2007. **Secondary Endpoints:** Immunogenicity as humoral immune response:

	• Total anti-HER2 Immunoglobulin titers in sera by enzyme-linked immunosorbent assay (ELISA). • Isotyping of anti-HER2 Immunoglobulins in sera (e.g. IgM, IgG (IgG1-4), IgD, IgE, IgA). **Exploratory Endpoints:** • Percentage of patients with measurable disease at baseline achieving Complete Remission (CR) or Partial Response (PR) according to RECIST 1.1 as assessed by the investigator during routine follow up. • Percentage of patients with Disease Control Rate (DCR) according to RECIST 1.1 as assessed by the investigator during routine follow up. • Progression free survival (PFS) in patients with Stable Disease (SD), or PR, at the time of first dosing of ES2B-C001 alone or in combination with the adjuvant [redacted] according to RECIST 1.1. • Disease-free survival (DFS) in patients with CR at the time of first dosing of ES2B-C001 alone or in combination with the adjuvant [redacted] according to RECIST 1.1. • Overall survival (OS).
Study Period	This dose escalation part will be conducted according to an adapted 3+3 design. For each patient enrolled, there will be a: • Screening period between 1 and 28 days prior to the first dose ES2B-C001. • An out-patient treatment period of 12 weeks; [redacted]. • Safety follow-up for ~6 weeks following the last dose of ES2B-C001. • An end-of-study visit.
Study Design	The trial is a first-in-human, phase I, open-label, dose-escalating trial to assess the safety and tolerability of ES2B-C001 combined with [adjuvant] [redacted] or without [adjuvant] [redacted], in patients with human epidermal growth factor receptor 2 (HER2) expressing metastatic breast cancer. [redacted]

[redacted]

Dose Escalation:

Dose escalation will follow an adapted 3+3 design.

The number of subjects may be increased from n=3 to n=6 or n=9 in each of the cohorts.

If one of the 3 patients in a dosing cohort experiences a DLT, 3 additional patients are added to the same dose level cohort. If 2 or more patients among these 6 patients experience DLTs, no further dose escalation can occur, and lower dose levels can be further explored. Dose escalation will continue until not more than 1 out of 6 patients experience DLT in a dose level cohort or the highest dose level group has completed treatment or no trial stopping criterion has been met.

	The decision to escalate the dose to the next dosing cohort or initiate the expansion part will be taken by the sponsor based on the data safety monitoring board (DSMB) recommendations. The DSMB will base their recommendations on an evaluation of accumulated safety data and, if available, on immunogenicity data (antibody titers), whenever 3 patients of a specific cohort have received at least one dose, and the last patient completed the DLT observation period of 8 weeks. Enrollment will be fully staggered in each of the populations, meaning that an observation period of 3 weeks plus 24 hours is foreseen before the second patient is dosed in one cohort. Dosing of the next subject in each cohort should ideally not take place until 1 week after the preceding patient has received the first dose. Expansions of the cohorts are foreseen without further amendment. Expansion of cohorts will be considered in the case of high variability of the emerging immunogenicity data or in case it appears desirable to obtain more safety data from a given cohort. Patients will be considered to have completed the study protocol if they attend an end of study (EOS visit) 6 weeks after the last dose of the primary vaccination series. Booster doses may be offered to patients under a compassionate use program (CUP) based on the investigator's choice for patients with at least stable disease or better who show a good immunologic response and a good tolerability, if they wish to receive such boosters.
Investigational Medicinal Product (IMP)	**Test Product ES2B-C001:** Type: Vaccine Formulation: solution for intramuscular injection Strength: 3.0 mg/mL Route of administration: intramuscular (I.M.) [redacted]: Type: Adjuvant Formulation: 3.0 mL solution; mixed in 1:1 (v/v) ratio with ES2B-C001

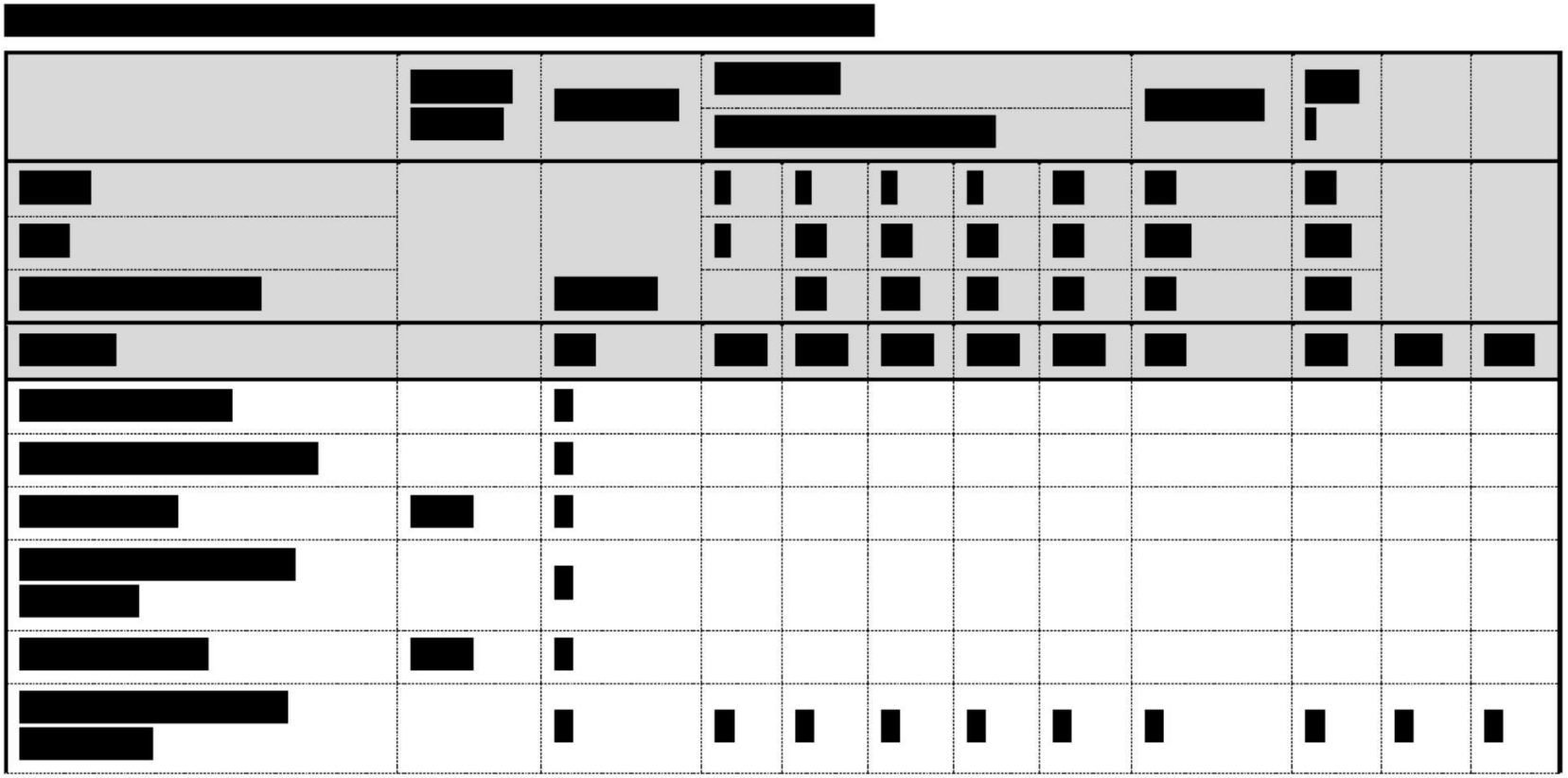
	Strength: N/A Route of administration: intramuscular (I.M.)
Number of Participants	9 patients (3 per cohort) will be enrolled in the dose escalation parts with 3 dose levels each. Up to 3 expansions of cohorts are planned, which may increase cohort sizes to 6-9 patients. In total, up to 27 patients may be dosed (not accounting for replacement of potential dropouts).
Inclusion Criteria	Subjects who meet the following criteria will be eligible to participate in the clinical study: [redacted] 9. [redacted]
Exclusion Criteria	Subjects who meet one or more of the following criteria will not be considered eligible to participate in the clinical study: [redacted]

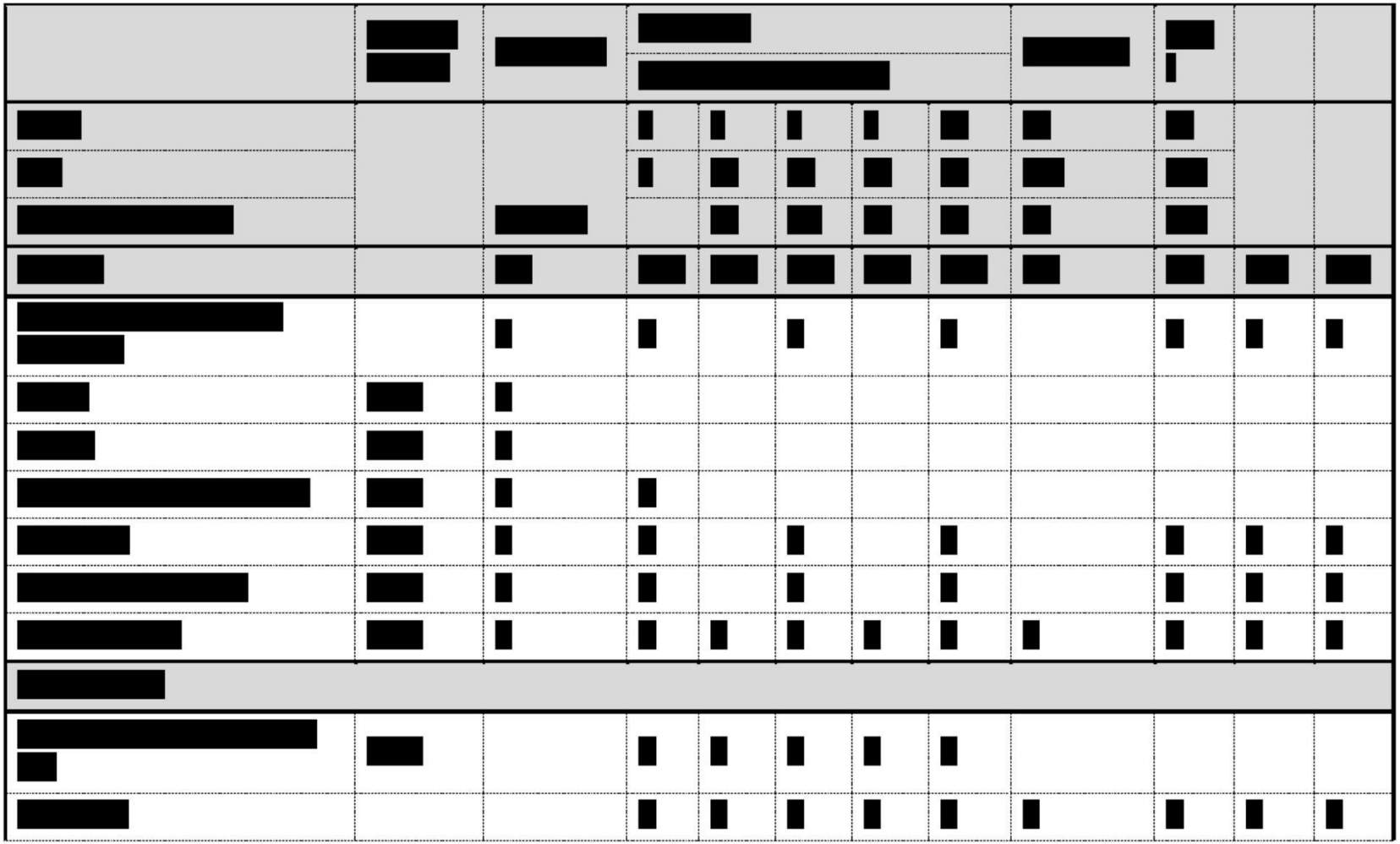
	[redacted] 16. [redacted]
Study Duration	Phase 1 study duration is estimated to be approximately 15 months, including an enrollment period of about 10 months: First subject's first visit: Q4/2024 Last subject's last visit: Q1/2026

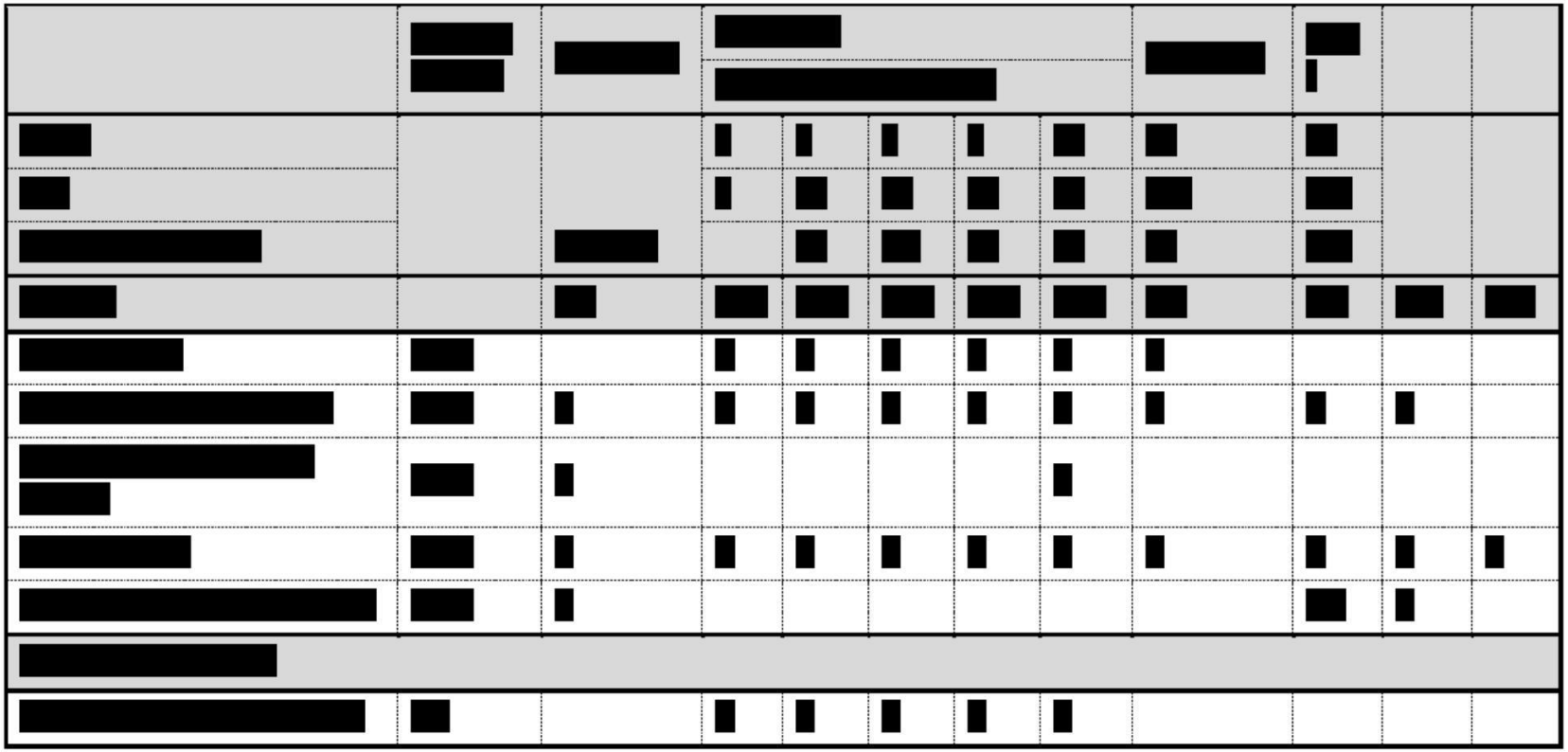
1.2 Study Schema

Figure 1: Schematic overview of the proposed dosing.

1.3 Schedule of Activities (SoA)







2 Introduction

2.1 Purpose of the Trial and Background Information

2.1.1 Breast cancer and HER2

Breast cancer is currently one of the most prevalently diagnosed cancers and the 5th most common cause of cancer-related deaths with an estimated annual number of around 2.3 million new cases worldwide [redacted].

Human epidermal growth factor receptor 2 (HER2) is a receptor tyrosine kinase that is overexpressed in a large subset of breast cancers. Between 15-30% of women with breast cancer are HER2-positive (corresponding to HER2 overexpression detected by immunohistochemistry (IHC) level 3+, and/or HER2 gene amplification detected by fluorescent in-situ hybridization (FISH)) [redacted]. The historically poor prognosis of HER2-positive breast cancer improved dramatically since the introduction of targeted treatment with HER2-directed agents. Until recently, HER2-low expression tumors (IHC level 1+ or 2+ and FISH negative) were considered ineligible for anti-HER2 therapies. But the development of a new generation of anti-HER2 antibody-drug conjugates (ADCs) reshaped this scenario leading to the recognition of HER2-low as a new targetable subgroup of breast cancers (BCs) [redacted].

HER2-directed agents comprise monoclonal antibodies (mAbs), (like trastuzumab and pertuzumab), tyrosine kinase inhibitors (TKIs) (like lapatinib and tucatinib), and ADCs (like ado-trastuzumab emtansine and fam-trastuzumab deruxtecan), of which some are used in the pre-operative (neoadjuvant), post-operative (adjuvant) setting, and others in metastatic settings [redacted].

2.1.2 Therapeutic cancer vaccines

The treatment goal of therapeutic cancer vaccines is to achieve tumor regression, eradicate minimal residual disease, and establish lasting antitumor memory, without inducing adverse reactions (Saxena et al., 2021). FDA, EMA, and national regulatory agencies have authorized therapeutic cancer vaccines before for indications such as advanced melanoma and metastatic prostate cancer, and one containing Bacillus Calmette–Guérin for the treatment of non-muscle-invasive bladder cancer [redacted].

2.1.3 HER2 and HER2 vaccines

HER2 is a tumor-associated antigen, which is overexpressed on some tumor cells and to a lesser extent expressed on normal cells, which makes HER2 an obvious target for therapeutic cancer vaccines. Thus, HER2 is a self-protein. Most self-proteins in the body, such as HER2, are not antigenic because of established self-tolerance, a process in which self-reacting cytotoxic T-lymphocytes and autoantibody-producing B-lymphocytes are deleted or inactivated "centrally" in the thymus and "peripherally" in the spleen and lymph nodes when exposed to self-antigens in a non-danger signal context. Conversely, any self-protein, that for some reason has not been

exposed to the immune system in the thymus, may later trigger an immune response, since central self-tolerance has not been developed. This may include normal proteins that are well sequestered from the immune system, proteins that are normally produced in extremely small quantities, or proteins whose structure is modified due to mutation.

Historically, very few HER2-reactive T cell responses in healthy individuals have been identified. This finding is indicative of either a lack of prior exposure to HER2 epitopes or tolerance. The data points to the lack of prior exposure, as HER2 epitope and protein vaccines elicit T-cell and antibody immunity that remain stably elevated for at least two years after last vaccine [redacted].

The definition of HER2-positive breast cancer is based on patients expected to benefit from immunotherapy (e.g. trastuzumab and pertuzumab) per the inclusion criteria used in pivotal trials. Low expressing HER2 (IHC 1+, or 2+ and no gene expression/FISH -) may be a distinct, clinically relevant breast cancer population warranting reassessment of traditional diagnostic and therapeutic paradigms (Prat et al., 2022). Up to 85% of patients have traditionally defined HER2-negative breast cancer and are ineligible for current anti-HER2 directed therapies; however, 50% of patients with HER2-negative BC have low levels of HER2 expression.

HER2-low patients were extensively studied in multiple HER2 vaccine trials over the last two decades, summarized in Table 2.

Table 2: Overview of Vaccine Trials in HER2-low Patients.

First author/year of publication	Vaccine	Population	Safety	Efficacy
[redacted]	E75 vs GM-CSF alone	ICH 0-2+/3+	Grade 1-2 local tox, minimal systemic toxicity	DFS better (p=0.04) in vaccinated patients, immunogenic
[redacted]	E75	IHC 1-2+/3+	no issues	larger immunologic responses in HER2-low, mortality decrease in IHC 1+ (p=0.05)
[redacted]	virosomal formulated Her-2/neu multi-peptide vaccine	IHC 1-2+	Grade 2 tox, no change in LVEF	immunogenic
[redacted]	GP2+GM-CSF	IHC 1-2/3+	well tolerated	immunogenic

First author/year of publication	Vaccine	Population	Safety	Efficacy
[redacted]	GP2+GM-CSF+trastuzumab	IHC 3+	Grade 2 tox, mean LVEF 60% before and after	immunogenic together with trastuzumab
[redacted]	GP2+GM-CSF+/-trastuzumab	IHC 1-3+	1 Grade 3 maculopapular rash	DFS 94% vs 85% in the per treatment population
[redacted]	E75 (nelipepimut) vs GM-CSF alone	IHC 1-3+	rarely Grade 3 tox.	DFS numerically improved by 9 percentage points from 80% to 89%
[redacted]	E75 (nelipepimut) vs GM-CSF alone	IHC 1-2+	safety similar to placebo	DFS similar between groups
[redacted]	E75 (nelipepimut) vs GM-CSF in combi with trastuzumab	IHC 1-2+	combination with trastuzumab was safe	triple negative BC (HER2-lowhave significantly increased DFS
[redacted]	AE37 vs GM-CSF alone	IHC 1-2/3+	safe and tolerable	DFS trendwise better 89% vs 51% (p=0.1)
[redacted]	AE37 vs GM-CSF alone	IHC 1-2/3+	safety not different from control	DFS trendwise better in HER2-low
[redacted]	GP2 vs GM-CSF alone	IHC 1-2/3+	safety not different from control	

Overall, more than 1.000 patients with HER2-low expressing breast cancer were exposed to various HER2 vaccines with and without adjuvant in these trials, and no serious safety concerns were raised with only rare incidence of grade 3 toxicities. Most importantly, a trend for efficacy was observed by improved disease-free survival (DFS) in this patient population. This means that preferentially if not exclusively patients were studied after the first line of therapy. Therefore, the overall risk-benefit ratio of including HER2-low patients in HER2 vaccine trials seems favorable.

The proposed trial design will include [redacted] patients first to assess safety and tolerability, and potential immunogenicity of this novel HER2-directed vaccine. [redacted].

2.1.4 ES2B-C001

ES2B-C001, either alone or adjuvanted with [adjuvant] [redacted], is being developed as a therapeutic cancer vaccine for the treatment of HER2-expressing breast cancer. The vaccine consists of [redacted] HER2 coupled to the Acinetobacter Phage 205 capsid virus-like particle (cVLP). The virus like particle (VLP) technology has been utilized in vaccines like Cervarix®, Gardasil®, Gardasil-9® and HEPLISAV-B®. The novelty of ES2B-C001 is the display of [redacted] HER2 covalently linked to the surface of a VLP. By displaying the [redacted] HER2 antigen on the VLP in a repeated, high-density, and directed way to the immune system, ES2B-C001 may potentially overcome self-tolerance to HER2 and the limitations associated with mAbs (i.e. HER2 mAb immunogenicity leading to treatment resistance) and induce anti-HER2 humoral immunity [redacted].

2.2 Rationale for the clinical study

This protocol pertains to the first-in-human study with adjuvanted and non-adjuvanted ES2B-C001. ES2B-C001 has not previously been tested in clinical studies.

The nonclinical program with ES2B-C001 includes non-clinical *in vitro* and *in vivo* studies and nonclinical *in vivo* studies characterising the systemic and local toxicity profile of ES2B-C001. Proof-of-concept studies have revealed promising results regarding the anti-tumor efficacy of polyclonal anti-HER2 antibodies elicited by adjuvanted ES2B-C001 administrations in FVB mice and Delta16 mice (Delta16 FVB transgenic mice, carry the splice variant of Delta16 human HER2 oncoprotein and spontaneously develop mammary carcinomas). Both demonstrated significant inhibition of tumor growth in both trastuzumab-sensitive and trastuzumab-resistant human breast cancer cell lines expressing varying levels of HER2.

In a therapeutic local tumor model in FVB mice, ES2B-C001 with adjuvant totally blocked tumor development. ES2B-C001 without adjuvant partly blocked tumor development and if tumors developed, growth was significantly inhibited.

Taken together, the primary pharmacodynamic studies conducted clearly show the potential of the adjuvanted as well as non-adjuvanted vaccine ES2B-C001 for the targeted indication of any HER2 expressing breast cancer.

Adjuvanted ES2B-C001 will initially be evaluated in [redacted] breast cancer patients [redacted]. In case of tolerability issues patients might be switched to non-adjuvanted ES2B-C001.

2.3 Benefit/Risk Assessment

Although considerable progress has been achieved in the treatment of HER2 expressing breast cancer, and the development of more effective treatment regimens are ongoing, women with HER2-positive breast cancer continue to have inferior outcomes. Hence, the evaluation of investigational agents in this clinical context is still warranted.

The ES2B-C001 vaccine is expected to generate a polyclonal antibody response targeting the HER2 receptor. Monoclonal HER2 directed antibodies have been shown to be effective in HER2-positive (IHC 3+, and/or, FISH positive) cancers and recently, four novel generations of anti-HER2 ADCs have proven activity in HER2-low BC. [redacted]

Participation in this FIH trial includes the risk of experiencing adverse events related to trial procedures (e.g., related to blood sampling, tumor biopsies), or the administration of adjuvanted and non-adjuvanted ES2B-C001. ES2B-C001 has not been tested in humans before and its clinical safety profile is unknown. Adverse events of ES2B-C001 may be locally related to the injections or systematically related to the immune response expected to be raised by exposure to the vaccine.

As noted above, ES2B-C001 alone was well tolerated in the repeat dose GLP toxicity study. Conversely, ES2B-C001 emulsified in [adjuvant] [redacted], showed a poor local tolerance with persistent induration and oedema noted at injection sites ≥ 0.45 mg/dose, with a higher severity and incidence at 0.75 mg/dose. These findings were considered to be adverse in view of the magnitude and/or grade, and related to [adjuvant] [redacted], although a slight exacerbation by ES2B-C001 cannot be excluded given the slightly higher severity of the findings at 0.75 mg/dose compared to 0.45 mg/dose.

Safety events may therefore be locally related to the injections or systemically related to the immune response expected to be raised by exposure to the vaccine. Although HER2 is expressed at low levels in normal tissue, e.g., heart and lung, autoimmunity involving normal tissue is unlikely to occur. Notwithstanding, baseline and continuous evaluation of cardiac function is implemented throughout the study. However, reassuringly, side effects with approved HER2-targeting mAbs or adverse events seen during clinical development of similar treatment modalities (monoclonal

antibodies directed at epitopes from the HER2 receptor and vaccinations with recombinant HER2 or epitopes) have been mild to moderate and primarily transient with no indications of autoimmunity. Use of approved HER2-targeting mAbs is associated with an established low risk of cardiac and pulmonary side effects. In summary, clinical studies with recombinant HER2 or epitopes have thus shown an acceptable safety profile with minimal toxicity, with no publicly available information on any development programs being stopped for safety reasons [redacted].

This clinical study protocol has been designed to minimize the risks to participants. Enrollment of the patients will be staggered in each of the study populations. Subjects will be monitored to detect DLTs and other adverse events (AEs) including serious adverse events (SAEs) during the study and consequent follow-up periods, to ensure appropriate resolution of any unpredicted effects. The trial is intended to use gradual ascending dose levels that begin at the Maximum Recommended Starting Dose (MRSD) for the adjuvanted vaccine, in order to allow a reasonable rapid attainment of the trial objectives (i.e., assessment of the therapeutic's safety and tolerability, as well as immunogenicity/preliminary antitumor activity). In case of intolerable local reactions, further investigations of the non-adjuvanted vaccine will be initiated.

2.3.1 VLP vaccines

[redacted]

[redacted]

The safety of [adjuvant] [redacted] has been assessed in a systematic review of 91 clinical trials [redacted]. Results from clinical studies with vaccines formulated with [adjuvant] [redacted] (usually <2 mL injection volume) report that the most common side effects are local reactions such as transient mild-to-moderate pain, erythema, swelling and granuloma formation at the injection site. The most intensive local reactions were often linked to the highest antigenic dose, volume or number of injections. No serious adverse advents have been reported and

general/systemic reactions were mainly mild-moderate transient fatigue, chills, fever and headache.

Since the adjuvant [redacted] is reactogenic and has limited local tolerability the volume of the i.m. injections should be kept to a minimum: [redacted]

2.4 Overall Benefit-Risk Conclusion

In conclusion, this trial aims to evaluate the safety profile of the novel HER2-directed vaccine ES2B-C001 with and without adjuvant. The risks for the patients participating in the trial are considered low and it is expected that the polyclonal anti HER2 antibody response will have anticancer effects beneficial to patients [redacted] breast cancers [redacted] participating in the trial. The overall risk-benefit-ratio of participating in this trial is judged to be favorable in patients [redacted].

3 Trial Objectives and Endpoints

Table 3: Description of the objectives and endpoints of this clinical study.

Objectives	Endpoints
Primary	
To determine the safety, tolerability, maximum tolerated dose (MTD) for ES2B-C001 alone or in combination with the adjuvant [redacted].	• Nature and frequency of dose-limiting toxicities (DLTs). • Incidence, nature and severity of AEs graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0. • Incidence, nature and severity of injection site reactions according to FDA Guidance on Toxicity Grading Scales in Vaccine Trials (FDA, 2007).

Objectives	Endpoints
Secondary	
To investigate the immunogenicity of ES2B-C001 alone or in combination with the adjuvant [redacted].	Immunogenicity as humoral immune response: • Total anti-HER2 Immunoglobulin titers in sera by enzyme-linked immunosorbent assay (ELISA). • Isotyping of anti-HER2 Immunoglobulins in sera (e.g. IgM, IgG (IgG1-4), IgD, IgE, IgA), Optional and obligatory in the non-adjuvanted and adjuvanted treatment group, respectively.
Exploratory	
To determine the preliminary antitumor activity of ES2B-C001 alone or in combination with the adjuvant [redacted] observed	• Percentage of patients with measurable disease at baseline achieving Complete Remission (CR) or Partial Response (PR) according to RECIST 1.1 as assessed by the investigator during routine follow up. • Percentage of patients with Disease Control Rate (DCR) according to RECIST 1.1 as assessed by the investigator during routine follow up. • Progression free survival (PFS) in patients with Stable Disease (SD), or PR, at the time of first dosing of ES2B-C001 alone or in combination with the adjuvant [redacted] according to RECIST 1.1. • Disease-free survival (DFS) in patients with CR at the time of first dosing of ES2B-C001 alone or in combination with the adjuvant [redacted] according to RECIST 1.1. • Overall survival (OS)

4 Trial Design

4.1 Description of the Trial Design

The trial is an open label, FiH dose escalation trial to assess the safety and tolerability as well as immunogenicity/preliminary antitumor activity of ES2B-C001 with and without the adjuvant [redacted] in patients with [redacted] breast cancer [redacted]. The trial is planned in a single center in Austria. The IMP ES2B-C001, in combination with [adjuvant] [redacted], is defined as the adjuvanted IMP or the adjuvanted ES2B-C001, or without [adjuvant] [redacted] defined as the non-adjuvanted IMP or the non-adjuvanted ES2B-C001 throughout the protocol.

The overall trial design is illustrated in Figure 1.

The study is expected to be completed within 15 months according to the interventional periods described below:

For each patient enrolled, there will be a:

- **Screening period:**
  The screening period starts upon signing the Informed Consent Form (ICF) by a patient and lasts for a maximum of 28 days. During this period a patient will undergo procedures for evaluation against study inclusion and exclusion criteria. Tumor imaging does not need to be repeated if it was performed before the ICF was signed but within the screening period as standard of care.
- **Treatment period:**
  Each patient will be dosed [redacted]
- **Safety follow-up** for ~6 weeks following last dose of ES2B-C001.

The trial design envisions a total of 3 cohorts. The dose escalation will be conducted according to an adapted 3+3 design. Three dose level cohorts are planned [redacted] for the adjuvanted ES2B-C001. The number of patients included in one dose cohort is 3 but may be increased from n=3 to n=6 or n=9 in each of the cohorts. Eligible patients may be added at lower dose levels via "backfilling" allowing patients access to the study and potentially providing a larger sample size for exploratory immunogenicity testing.

Dose cohorts are described in Table 4. The preparation and exact dose volumes of the IMP are detailed in the IMP manual.

Table 4: Description of the ascending-dose cohorts, and respective route of administration (Cohort 1-3)

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

4.2 Rationale for Trial Design

This Phase 1 clinical trial is designed to determine safety, and tolerability of adjuvanted and non-adjuvanted ES2B-C001 to establish a maximum tolerated dose for this novel HER2-directed

vaccine in breast cancer patients. The primary rationale for conducting such phase 1 studies is to obtain sufficient information about safety and preliminary efficacy such as immunogenicity and antitumor activity to permit the design of subsequent well-controlled, scientifically valid phase 2 studies. The number of subjects included in such phase 1 trials is small, just enough to determine the safety, proper tolerated dose, and method of administration of the IMP, with or without adjuvant, for a next phase study. Enrollment in this FiH trial will be fully staggered in each of the populations. Repeated safety evaluations will be conducted during pre-dosing calls between PI(s), CRO medical monitor, and Sponsor, where safety and reactogenicity data from the first patient of each cohort will be reviewed. Additional safety evaluations will be performed at DSMB meetings to ensure a progression according to 3+3 design through a limited number of dose levels guided by safety. After accumulation of safety data across the dose levels, a maximum tolerated dose (MTD) will be established or if no unacceptable side effects or DLTs occur up to the highest dose level cohort, MTD will be determined as the maximum administered dose (MAD) level.

4.3 Dose escalation

A DSMB will be appointed to oversee the trial. The composition and responsibilities of the DSMB is described in a dedicated charter, which will include additional instructions for the dose escalation procedure.

The decision to escalate the dose to the next dosing cohort or initiate the expansion part will be taken by the sponsor based on the DSMB recommendations. For the decision of dose escalation, AEs collected during the DLT period will be assessed by the DSMB. Other available data beyond the DLT evaluation period for all patients in the trial will also be assessed before a decision on escalation to the next dose level can be taken. Dose escalation rules will be based on the number of patients with AEs, specifically DLTs (Section 4.3.2). The DSMB will base recommendations on an evaluation of accumulated safety data and may include other available data, including data on immunogenicity (antibody titers) in their review.

Patients who discontinue for reasons other than toxicity before having DLT evaluable safety data may be replaced at sponsor's discretion.

A schedule of trial procedures is provided in Section 1.3. The administration of adjuvanted and non-adjuvanted ES2B-C001 is described in Section 6 and the trial assessments are described in Section 8.

The dose escalation will include 3 planned dose level cohorts with [redacted] adjuvanted ES2B-C001 (Section 6.2).

Enrollment will be fully staggered in each of the populations, meaning that an observation period of 3 weeks plus 24 hours is foreseen before the second patient is dosed in one cohort. Dosing of the next subject in each cohort should not take place until 1 week after the preceding patient has received the first dose. Prior to dosing the second patient, safety and reactogenicity data from the previous patient will be discussed at a pre-dosing call between PI(s), CRO medical monitor, and

Sponsor. At subsequent administrations, pre-dosing calls will be scheduled if safety concerns warrant it. This will ensure an evaluation of safety prior to administration of the next patients.

Any safety concerns, such as potential DLTs and/or events that may meet a criterion for initiation of rescue medication, will be escalated to the DSMB who will review accumulated safety data and may provide recommendations for trial adjustments e.g., applying additional overnight stays for patients.

4.3.1 Dose escalation rules

Dose escalation to a higher dose will be driven by an evaluation by the DSMB. At the time of DSMB evaluation, the last patient in the current dose level cohort must have received the 2nd dose and must have accumulated safety data for 8 weeks following the first dose. This allows for the DLT evaluation period to include an assessment of a potential delay in dose 2 due to toxicity concerns, as described for DLT number 5 in Section 4.3.2.

If one of the 3 patients in a dosing cohort experiences a DLT, 3 additional patients are added to the same dose level cohort. If 2 or more patients among these 6 patients experience DLTs, no further dose escalation can occur, and lower dose levels can be further explored. Dose escalation will continue until not more than 1 out of 6 patients experience DLT in a dose level cohort or the highest dose level group has completed treatment or no trial stopping criterion has been met. See Figure 2.

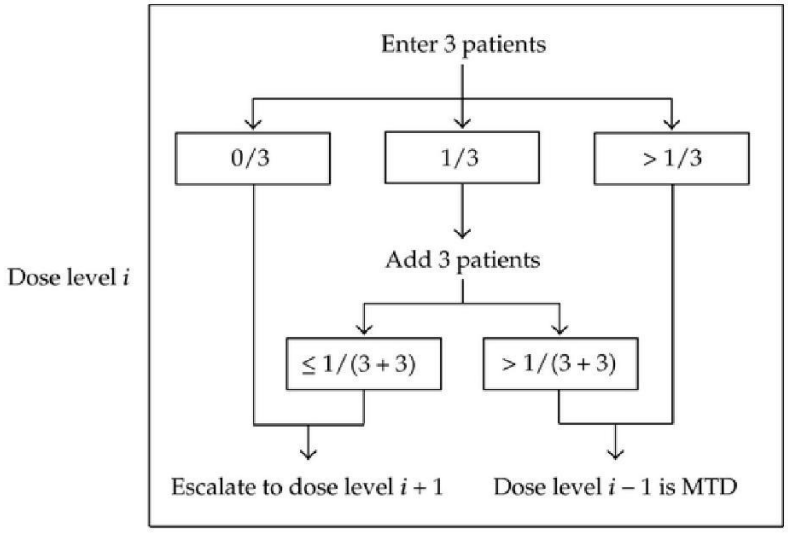


Figure 2: Dose-escalation according to a 3+3 design

Dose escalation will continue until not more than 1 out of 6 patients experiences a DLT in a dose level cohort (see DLT definitions in Section 4.3.2) or the dose level of [redacted] for the adjuvanted

treatment has been completed (maximum administered dose MAD) or a trial stopping criterion has been met. The stopping criteria for dose escalation and trial are provided in Section 7.

Patients experiencing DLT will have their administration of adjuvanted or non-adjuvanted ES2B-C001 discontinued. These patients are allowed to remain in the trial to follow safety, disease progression and immune response. The DSMB will convene and recommend whether a re-start of these patients' treatment is advised and if a dose reduction may be required.

4.3.2 Definition of Dose-limiting Toxicity (DLT)

A DLT is defined as any grade ≥ 3 AE according to the NCI-CTCAE v. 5.0 observed at any time from the first administration of ES2B-C001 (alone or adjuvanted) to the 8 weeks thereafter, which despite standard supportive care and in the opinion of the Investigator, is possibly, probably, or definitely related to adjuvanted or non-adjuvanted ES2B-C001, or other event as described below. Investigators evaluation of relatedness to trial treatment administration should take concomitant medication into account.

DLTs will include:

1. Any grade ≥ 3 adverse events except:
    a. Grade ≥ 3 nausea, vomiting, or diarrhea lasting ≤ 72hours with optimal medical therapy Grade 3 fatigue or anorexia lasting < 7 days.
    b. Fever that occurs within 48 hours of IMP administration and resolves to Grade 2 within 48 hours and is fully resolved within 7 days.
    c. Non-clinically relevant isolated biochemical abnormalities (e.g., isolated increase in gamma-glutamyltransferase [GGT]).
    d. Laboratory values out of normal range without related clinical symptoms, clinically transient isolated in nature.
    e. Laboratory abnormality that is asymptomatic and deemed by the Investigator not to be clinically significant.
2. Grade 3 autoimmune reactions, except Grade 3 autoimmune thyroiditis or other endocrine abnormality that can be managed by endocrine therapy.
3. Intolerable or > grade 3 injection-site reactions according to FDA guidance 2007 (table 16 appendix 2).
4. Grade 2 decrease in left ventricular ejection fraction (LVEF).
5. Treatment delay of >14 days due to any related adverse event(s).

In any case, the reported toxicity should be discussed between the Investigators and both the DSMB and the Sponsor. All toxicities will be graded using NCI-CTCAE Version 5.0 based on investigator assessment.

4.4 Start of Trial and End of Trial

The study will be a single-center trial intended to start in the fourth quarter of 2024 with the recruitment of the participants according to the inclusion/exclusion criteria and initial signing of the

Informed Consent Form (ICF). The end of this trial is expected in the first quarter of 2026. The end of the trial is defined as the date of the last visit of the last patient in the trial or last scheduled procedure shown in the schedule of activities for the last patient in the trial.

A patient is considered to have completed the trial if the patient has completed all periods of the trial including the last scheduled procedure shown in the SoA in Section 1.3.

5 Trial Population

5.1 Trial Population

[redacted]

5.2 Rationale for Trial Population

[redacted]

[redacted]

5.3 Inclusion Criteria

Patients are eligible to be included in the trial only if all of the following criteria apply:

[redacted]

5.4 Exclusion criteria

Patients are excluded from the trial if any of the following criteria apply:

[redacted]

[redacted]

5.5 Contraceptive rules

5.5.1 Women Participants

Females of childbearing potential must use highly effective methods of contraception Table 5, of which one at least is a barrier method, from screening until the end-of-study visit (or minimum 4 weeks after receipt of the IMP).

Table 5: Highly effective methods of contraception.

	Highly effective methods of contraception
1.	Total abstinence (when this is in line with the preferred and usual lifestyle of the participant). Periodic abstinence (e.g., calendar, ovulation, symptom-thermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
2.	Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy, or tubal ligation at least 6 weeks before taking study intervention. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow-up hormone level assessment.

3.	Sterilization of male partner (at least 6 months prior to screening) with post-procedural semen specimen to verify a successful procedure (the report of the male partner will not be collected since the partner is not study participant). For female participants on the study, the vasectomized male partner should be the sole partner for that participant.
4.	Placement of an intrauterine device or intrauterine system, or other forms of non-hormonal contraception that have comparable efficacy (failure rate < 1%).

Female participants as described in Table 6 are considered NOT to have childbearing potential and do not need to receive contraceptive advice. Treating physicians are advised to refer their participant for a gynecological opinion if at all unsure as to whether a woman meets the criteria for being of non-childbearing potential.

Table 6: Women not considered to have childbearing potential.

	Women not considered to have childbearing potential
1.	Age equal to or more than 50 years (≥ 50 years) and naturally (spontaneously) amenorrhoeic (postmenopausal) for ≥12 months*.
2.	Premature ovarian failure confirmed by a specialist gynecologist.
3.	Previous surgically sterilized (have documented sterilization) as one of the following: bilateral oophorectomy or bilateral salpingectomy at least 90 days before the start of the trial and/or hysterectomy. NOTE: Documentation as per review of the participant's medical records, medical examination, or medical history.
4.	Turner syndrome, uterine agenesis.

* A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

5.5.2 Male Participants

Male participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the criteria defined in Table 7 for the duration of the study until 90 days after the last dose of study intervention.

Table 7: Contraception rules for male participants – at least ONE needs to apply

	Contraception rules for male participants

1.	Agree to use a male condom (not made of natural [animal] membrane [e.g., latex, or polyurethane condoms]) plus partner use of an acceptable contraceptive method. Plus, female partners of childbearing potential must agree to use one of the acceptable contraceptive methods.
2.	Males with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.
3.	Must agree to abstain from donating semen or sperm for the duration of the study and for 90 days after the dose(s) of study intervention.

Female partners of childbearing potential (even if they have amenorrhea) must agree to use one of the contraception methods described in Table 8 during the following time periods related to this study: 1) for at least 28 days before the dose(s) of study intervention in the male participant; 2) during the study; and 3) until 90 days after the dose(s) of study intervention. The adequacy of other methods of contraception will be assessed on a case-by-case basis by the PI.

Table 8: Contraception methods for female partners of childbearing potential – at least ONE needs to apply

	Contraception methods for female partners of male participants
1.	Intrauterine device.
2.	Diaphragm combined with spermicidal foam/gel/film/cream/suppository.
3.	To practice complete (absolute and continuous) abstinence from heterosexual contact.

5.6 Screen Failures

A screen failure occurs when a patient who has consented to participate in the clinical trial is not subsequently assigned to trial treatment.

A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the CONSORT publishing requirements and to respond to queries from regulatory authorities. As a minimum, the following data will be collected in the eCRF for screening failures: demography, screen failure details and eligibility criteria.

Re-screening of screening failures is not allowed. However, if the reason for screening failure is not due to the patient failing to meet the eligibility criteria but is administrative or operational (e.g. reserve patient or screened patients who were not dosed within the screening window due to time constraints), re-screening may be permitted.

6 Trial Intervention and Concomitant Therapy

ES2B-C001 is a therapeutic cancer vaccine comprised of a capsid virus-like particle (VLP) decorated with the [redacted] human HER2 receptor.

ES2B-C001 is formulated as a solution for intramuscular (I.M.) injection and will be administered with or without the adjuvant [redacted] mixed at a v:v ratio of 1:1.

Table 9 describes the intervention to be administered during the trial period, including route/mode of administration, dose, dosage regimen, duration of intervention. [redacted]

[redacted]

All trial products are supplied to sites by the sponsor.

6.1 Description of Trial Intervention

Table 9: Trial Intervention(s) Administered

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

6.2 Rationale for Dose

In the GLP repeat-dose toxicity study in cynomolgus monkeys, 8 intramuscular injections of ES2B-C001 alone were locally and systemically well tolerated with no evidence of systemic toxicity at 750 µg/dose. However, ES2B-C001 emulsified in [adjuvant] [redacted] showed a poor local tolerance at injection sites at dose levels [redacted]. Based on these results, the no observed adverse effect level (NOAEL) was considered to be [redacted] for ES2B-C001 alone and [redacted] for ES2B-C001 together with [adjuvant] [redacted] . [redacted] Although the WHO guideline does not specifically apply to therapeutic vaccines for non-infectious diseases, our starting dose considerations are aligned with its principles. Hence, the doses planned to be used in humans are in the range of therapeutically active doses in animals on a dose per subject basis.

The dose ranges chosen for this trial are anticipated to be immunogenic, with the expectation for an immune response being fairly conservative even at the lowest dose. This assumption is based on immunogenicity results across three species: mouse, rat and cynomolgus monkey. For instance, the sera of Delta 16 mice vaccinated twice 2 weeks apart with 5-40 µg adjuvanted ES2B-C001 strongly inhibited tumor growth *in vitro*. An increase in the anti-HER2 antibody titers was seen when increasing the dose from 5 to 10 µg, but titers plateaued at higher doses, with no substantial increase seen from 20 to 40 µg. Furthermore, administration of adjuvanted ES2B-C001 to Delta16 FVB mice completely blocked tumor development, whereas all control mice had lung nodules. Non-adjuvanted ES2B-C001 prevented tumor development in 6/9 mice (67%), while the remaining mice had 1-2 lung nodules only. On a body weight basis, the starting dose has a safety margin of 2000 (mice of 20 g body weight versus women of 40 kg body weight). Taken together, the planned starting doses in this study in BC patients are considered to be safe, but still sufficient to induce an immune response and thereby inhibit tumor growth.

The pre-planned dose levels and dose increments are shown in Table 10. [redacted]

The dose escalation steps may be smaller, but never larger, dependent on the safety evaluation by the DSMB and the Sponsor's safety committee.

In case the first dose level is found to be dose limiting, a lower dose level may be investigated at a level recommended by the DSMB and agreed upon in Sponsor´s safety committee.

Table 10: Pre-planned dose levels of ES2B-C001

[redacted]	[redacted]	[redacted]
[redacted]		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	
[redacted]	[redacted]	

*If the DSMB suggests investigating additional dose levels, any additional cohort for adjuvanted or non-adjuvanted treatment will be named A- or NA-cohort-X.

6.3 ES2B-C001 Dosing and Administration

In this trial ES2B-C001 should only be administered in a hospital or clinic with immediate access to equipment, medications, and appropriately qualified staff for resuscitating and stabilizing individuals in an acute emergency, along with ready availability of an intensive care unit and other hospital facilities.

Each administration of adjuvanted or non-adjuvanted ES2B-C001 will be given in the shoulder, thigh, or buttocks (alternating) by an authorized site staff proficient in English. If axillary lymph nodes have been resected on one side, the shoulder on that side should not be used for vaccination, if only 1 or 2 sentinel nodes have been removed administration may be done in the corresponding shoulder. If a region planned for administration of adjuvanted or non-adjuvanted ES2B-C001 has any clinically significant findings on day of dosing (e.g., swelling or ulceration) an alternative administration site must be chosen. Selection of region for injection should be done considering SoC treatments and allow for preferred regions for SoC to be used for that purpose (and not the vaccine).

All administrations of adjuvanted or non-adjuvanted ES2B-C001 in the ambulatory setting must be undertaken allowing for in-person monitoring and observation period of one hour following the injections.

The IMP Manual contains specific instructions for the preparation of the solutions and administration, including how to prepare the adjuvanted formulation of the final product, mixing [adjuvant] [redacted] with ES2B-C001 solution.

6.4 Preparation, Handling, Storage, and Accountability

The investigator or designee must confirm appropriate conditions (e.g., temperature) have been maintained during transit for all IMP received, and any discrepancies are reported and resolved before use of the trial intervention. Only patients enrolled in the trial may receive adjuvanted or

non-adjuvanted ES2B-C001, and only authorized site staff may supply, prepare, or administer adjuvanted or non-adjuvanted ES2B-C001.

All trial supplies must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, or authorized site staff is responsible for accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records) throughout the clinical study. The drug accountability log includes information such as, subject number, randomization number, amount dispensed and remaining amount. The remaining IMP volume per vial will be stored until monitor reconciliation.

The used/opened vials should be marked as 'used' and kept separate from the products not yet dispensed. All dispensing and accountability records will be available for Sponsor review. When the Study Monitor visits the study site, he/she will reconcile the drug accountability log with the products stored at the study site IMP storage.

Packaging and labelling of ES2B-C001 will be outsourced to a clinical packaging contract manufacturing organization. All products are labelled according to Annex 13, EudraLex, volume 4 and local requirements. Each unit of product is uniquely numbered.

After receiving Sponsor approval in writing, study site is responsible for returning all unused or partially used study medications to the Sponsor or designated third party or for preparing the study medications for destruction via incineration. Further guidance and information for the final disposition of unused IMP are provided in the IMP Manual.

The trial interventions supply, packaging, labeling, and storage are outlined in Table 11.

Table 11: Trial intervention packaging, storage, and handling.

Trial intervention	Supplier	Packaging	Labeling	Storage	Storage after preparation
ES2B-C001	ExpreS2ion Biotechnologies ApS	10 vials per box. The primary packaging will be glass 2R vials which will be closed with rubber stoppers.	according to applicable local and regulatory requirements	-25°C to -15°C	15°C to 25°C

Trial intervention	Supplier	Packaging	Labeling	Storage	Storage after preparation
[adjuvant] [redacted]	ExpreS2ion Biotechnologies ApS	10 vials per box. The primary packaging will be amber glass 2R vials which will be closed with rubber stoppers.	according to applicable local and regulatory requirements	15°C to 25°C	15°C to 25°C

6.5 Trial allocation

After the patient has signed the consent form, the site will assign a unique patient number through the EDC system. Once assigned, it becomes the permanent trial identifier for that patient. A patient number cannot be re-used for any reason. If the patient is re-screened a new unique patient number will be assigned.

Trial participation begins once written informed consent is obtained. Refer to Section 10.3 for details on the informed consent process. Once informed consent is obtained, the screening evaluations to assess eligibility criteria may begin.

Patients that fulfill all inclusion and none of the exclusion criteria will be offered to be enrolled in the trial.

6.6 Trial Intervention Compliance

When the individual dose for a patient is prepared from a bulk supply, the preparation of the dose should be confirmed by a second member of the trial site staff.

Patients are dosed at the hospital site or clinic, hence they will receive trial intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered will be recorded in the source documents and eCRF. The dose of trial intervention and trial patient identification will be confirmed at the time of dosing by a member of the trial site staff other than the person administering the trial intervention.

A record of the quantity of IMP administered to each patient must be maintained and reconciled with trial intervention and compliance records. Intervention start and stop dates, including dates and reason for intervention delays and/or dose reductions will also be recorded.

6.7 Medication Errors

Medication errors and uses outside what is foreseen in the protocol may include:

- Administration of wrong drug
- Wrong route of administration, i.e. not IM
- Accidental administration of a lower or higher dose than intended. An overdose is defined as a patient receiving a dose of adjuvanted or non-adjuvanted ES2B-C001 exceeding 10% as compared to the protocol-specified dose. An underdose is defined as a patient receiving a dose of adjuvanted or non-adjuvanted ES2B-C001 less than 10% as compared to the protocol-specified dose.

If a medication error results in an AE, the AE must also be reported in the eCRF. If the event qualifies as a SAE, it must be reported as described in Section 12.4.

6.8 Continued Access to Trial Intervention after the End of the Trial

Patients will be considered to have completed the study protocol if they attend an end of study (EOS) visit 6 weeks after the last dose of the primary vaccination series.

Booster doses may be offered to patients under a compassionate use program (CUP) based on the investigator's choice to patients with at least stable disease or better who show a good immunologic response and a good tolerability, if they wish to receive such boosters.

6.9 Prior and Concomitant Therapy

6.9.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record in the eCRF prior medication taken by the patient during the past 2 months.

All previous lines of anti-cancer therapy must be documented in the eCRF at baseline, including previous CHT regimens and neoadjuvant or adjuvant treatment given at time of primary diagnosis of breast cancer, if applicable.

6.9.2 Concomitant Therapy

Concomitant therapy consists of any medications (including prescription drugs, OTC drugs, nutritional supplements, herbal or homeopathic remedies) used by the patient between Day -28 and End of Study (EOS) in addition to protocol mandated treatment.

Medications or vaccinations prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from ES2B-C001 may be required and will be evaluated by the medical monitor and agreed with Sponsor on a case-by-case basis.

The investigator is to discuss prohibited medication/vaccination with the Sponsor. The final decision on any supportive therapy or vaccination rests with the investigator and/or the patient's

primary physician. However, the decision to continue the patient on trial treatment requires the mutual agreement of the investigator, the Sponsor, and the patient.

Listed below are specific restrictions for concomitant therapy or vaccination during the course of the trial:

[redacted]

All concomitant medication should be recorded in the eCRF.

6.9.3 Rescue Medicine

Due to the potential of autoimmunity as response to the vaccine with self-antigen (HER2), start of rescue treatment according to institutional standard, must be evaluated and started without delay by the treating physician (or a medically qualified delegate on-site).

Local and systemic AEs related to the adjuvanted ES2B-C001 injection should be treated symptomatically to relieve the patients as deemed necessary by the treating physician (or a medically qualified delegate on-site).

In case of suspected immune system disorders, such as hypersensitivity, allergic reaction/anaphylaxis or autoimmune disease symptomatic supportive treatment as needed (care guided by organ specific symptoms) should be provided as well as and rescue treatment to brake immune response, these may include antihistamines, steroids, and any standard acute treatment regimens for anaphylactic shock as per local practice.

Symptomatic suspected cancer therapy-related cardiac dysfunction (CTRCD) should be handled according to the local best practice, including referral to cardiologist as needed. Symptomatic heart failure should be treated according to current international guidelines, e.g. "2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure" [redacted].

The use of rescue medications is allowable at any time during the trial. The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded and linked to relevant AEs in the eCRF.

7 Discontinuation and delaying of Trial Intervention and Patient Discontinuation/ Withdrawal

7.1 Discontinuation of Trial Treatment

Patients may stop treatment at any time during the trial at their discretion. The treating physician may, for safety reasons or the well-being of the patient, refrain from dosing at the discretion of the treating physician.

If adjuvanted or non-adjuvanted ES2B-C001 is permanently discontinued, the patient should, if at all possible, have an early termination visit and remain in the trial for follow-up visit 7 and 8. See the SoA (Section 1.3) for data to be collected at the End of Trial and during follow-up.

Discontinuation of trial treatment does not represent withdrawal from the trial.

Patients who discontinue for reasons other than toxicity before having DLT evaluable safety data may be replaced at sponsor's discretion.

A patient should be considered for discontinuation from trial treatment but continue to be monitored in the trial for any of the following reasons:

- Unacceptable toxicity.
- Intercurrent illness of sufficient magnitude to preclude safe continuation of the trial.
- Investigator's judgement on optimal benefit/risk not supporting continuation of the trial for the individual patient.
- Non-compliance with trial requirements (e.g. treatment delay of 28 days or more).
- Patient's decision.
- Pregnancy during the trial.
- Patient is lost to follow-up.
- Death.

7.2 Temporary Discontinuation of Trial Treatment

Patients experiencing a symptomatic decline in organ function suspected to be related to (adjuvanted) ES2B-C001 must be evaluated by the investigator and submitted to treatment pause as needed.

Declines in organ function that a are not symptomatic, not clinically significant and/or discovered only through routine testing, without associated clinical symptoms should not lead to treatment pauses.

7.2.1 Cancer therapy-related cardiac dysfunction

Symptomatic suspected cancer therapy-related cardiac dysfunction (CTRCD) should be handled according to the local best practice and include referral to cardiologist as needed.

Moderate and severe asymptomatic suspected CTRCD, as defined in the 2022 ESC cardio-oncology guidelines [redacted], see Table 12, should lead to pause of trial treatment and referral to cardiologist.

Table 12: Definition of Asymptomatic cancer therapy-related cardiovascular toxicity

Severe	New LVEF reduction to <40% or reduction by ≥20 percentage points from baseline
Moderate	New LVEF reduction by ≥10 percentage points to an LVEF of 40–49% OR New LVEF reduction by <10 percentage points to an LVEF of 40–49% AND either new relative decline in GLS by >15% from baseline OR new rise in cardiac biomarkers
Mild	LVEF ≥ 50% AND new relative decline in GLS by >15% from baseline AND/OR new rise in cardiac biomarkers

7.2.2 Immune system disorders

Immune system disorders judged to be driven by autoimmunity by the investigator and grade 3 or above for suspected CRS, if related to adjuvanted or non-adjuvanted ES2B-C001 as judged by the treating physician should lead to pause in trial treatment and follow-up and following action should be taken by the investigator:

- Initiate appropriate symptomatic treatment.
- Fill in designated eCRF page + SAE page, if applicable.
- Notify sponsors, and CROs medical monitor immediately and plan for further evaluation.
- Evaluate with sponsor, and CROs medical monitor ~2 weeks post event for continuation in trial on treatment, or early treatment discontinuation, to the farthest extent possible continued participation for safety follow up should be pursued.

7.3 Patient Withdrawal from the Trial

- A patient may withdraw from the trial at any time at the patient's own request for any reason (or without providing any reason).
- A patient may be withdrawn at any time at the discretion of the investigator for safety or compliance reasons.

- At the time of discontinuing from the trial, if possible, an early termination visit should be conducted, as shown in the SoA (Section 1.3).
- The patient will be permanently discontinued from the trial intervention and the trial at that time.
- If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a patient withdraws from the trial, the patient may request destruction of any samples taken and not tested, and the investigator must document this in the site trial records.

7.4 Lost to Follow-up

A patient will be considered lost to follow-up if the patient repeatedly fails to return for scheduled visits and is unable to be contacted by the trial site.

The following actions must be taken if a patient fails to return to the clinic for a required trial visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible, counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether the patient wishes to and/or should continue in the trial.
- Before a patient is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the patient (where possible via 3 telephone calls, and if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's medical record.
- Should the patient continue to be unreachable, the patient will be considered to have withdrawn from the trial.

7.5 Criteria for Temporarily Delaying Enrollment in the Trial and Stopping Rules

Enrollment of new patients in the trial will be temporarily halted if 2 or more out of 6 patients experience DLTs in the same dosing group (as per Section 4.3.2).

In these cases, DLT level has been reached and enrollment at that dose level cannot continue if DLTs are confirmed. The DSMB will review all available, accumulated safety data and recommend further course of action and potentially identify intermediate lower doses for further testing.

The trial includes following trial stopping criterion:

- DSMB recommendation based on patients' medical data and intolerable toxicities.
- Decision from the sponsor to terminate the trial.

8 Trial Assessments and Procedures

For timing of assessments and procedures, refer to the SoA presented in Table 1 (Section 1.3).

In the event of a significant trial-continuity issue (e.g., caused by a pandemic), alternate strategies for patient visits, assessments, medication distribution and monitoring may be implemented by the sponsor or the investigator, as per international, national or local health authority/ethics requirements.

8.1 Screening/Baseline Assessments

Patients will be informed about all aspects of the study including the purpose and risks, having the possibility to clarify the questions they may have. Any study-specific assessment may only be done after the patient has signed the ICF. An eligibility screening can take place within 28 days prior to the first dose administration.

All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

8.1.1 Demography

The following demographic data will be recorded:

- Year of birth and age.
- Sex: female, male.
- Ethnicity (if allowed by local legislation).

8.1.2 Smoking status

Never, current, previous smoker and duration of smoking.

8.1.3 Medical and Surgical History

Relevant general medical and surgical history including concurrent diagnoses within the previous 12 months, or longer if considered clinically significant, must be reported.

For each condition, diagnosis, or surgical procedure, the start date and stop date will be recorded; it will also be recorded if the condition or diagnosis is ongoing.

Relevant medical history also includes diseases which are specifically listed as exclusion criteria and diseases for which specific treatments are listed as exclusion criteria.

8.1.4 History of Breast Cancer

First onset of breast cancer must be documented, along with any primary surgery, and first diagnosis of metastatic breast cancer (if this differs from first onset of breast cancer), cancer stage including TNM classification, histological grade, HER2 as well as hormone receptor status All previous neo-adjuvant and adjuvant treatment regimens must be noted (type of compounds, number of cycles, overall duration), as well as all previous lines of therapy in the metastatic setting (type of compounds, number of cycles, overall duration) (see Section 6.9.1).

A history prior of axillary lymph node dissection must be taken, and documented in the eCRF, it must also be documented if axillary lymph node dissection has not been performed. If a limited number of sentinel nodes have been removed, this must be documented.

8.1.5 Height and Weight

The patients' height (cm) and weight (kg) as well as BMI (kg/m^2) will be measured or calculated, respectively, and will be recorded in the eCRF.

8.1.6 Viral Serology

Viral serology to HBsAg, HCV antibody, and/or HIV types 1 and 2 antibodies will be assessed during screening period.

8.2 Efficacy and Immunogenicity Assessments

Planned timepoints for all efficacy and/or immunogenicity assessments are provided in the SoA (Section 1.3).

Objective response rate (ORR), DCR, PFS, DFS, and OS, following RECIST 1.1 will be assessed by the investigator according to routine follow up.

8.2.1 Immunogenicity Assessments

Samples will be collected from all patients according to the SoA. Additionally, serum samples should be collected at the final visit from patients who discontinued trial intervention or were withdrawn from the trial.

Antibodies to ES2B-C001 will be evaluated in serum samples collected from all patients. Samples will be screened for antibodies binding to HER2 and the titer of confirmed positive samples will be reported.

Anti-HER2 antibodies and titers will be evaluated by enzyme-linked immunosorbent assay (ELISA) after last administration for all patients (up to Day 127 sample), or as applicable.

Isotyping of anti-HER2 Immunoglobulins in serum will be performed by ELISA or immunocapture LC/MS for determination of e.g. IgM, IgG (IgG1-4), IgD, IgE, IgA.

Other analyses may be performed to verify the stability of antibodies to HER2 and/or further characterize the immunogenicity of ES2B-C001.

The detection and characterization of antibodies to HER2 will be performed by central or special laboratory using a validated assay method by or under the supervision of the sponsor. Details regarding sample collection and processing will be provided in the Laboratory Manual.

8.2.2 Tumor Imaging and Assessments of Disease

Throughout this section, the term 'scan' refers to any medical imaging data used to assess tumor burden and may include cross-sectional imaging (such as CT or MRI) medical photography, or other methods as specified in this protocol.

8.2.2.1 Initial Tumor Scans

At screening, patients require a baseline scan evaluated per RECIST 1.1. Tumor scans performed as part of routine clinical management are acceptable for screening if they are of acceptable diagnostic quality and performed within the allowed screening period, up to 28 days prior to start of ES2B-C001 administration.

Availability of CNS imaging in medical records, are required to evaluate radiographically detectable CNS metastases if applicable.

8.2.2.2 Tumor Scans During the Trial

After enrollment, tumor scans should be performed at routine follow up every 12 weeks (± 2-week window) for MBC or more frequently if clinically indicated. If a patient discontinues trial treatment, tumor scans should be performed at the time of discontinuation (± 2-week window) unless previous scans were obtained within 2 weeks of discontinuation.

8.3 Safety Assessments

Planned timepoints for all safety assessments are provided in the SoAs.

8.3.1 Assessment of the injection site

In the eCRF the injection site (shoulder, thigh, or buttocks) and side (right or left), must be documented. It must be documented that the region of choice has no clinically significant findings prior to administration of adjuvanted ES2B-C001 (see Section 6.3 for details).

8.3.2 Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems.
- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).
- Investigators should pay special attention to clinical signs related to previous serious illnesses and previous reaction to vaccines.

8.3.3 Vital Signs

- Body temperature (°C), pulse rate (bpm), respiratory rate (breaths/minute), and supine blood pressure [mmHg] will be recorded (before blood collection for laboratory tests).

- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Vital signs will be measured after 5 minutes rest and will include tympanic body temperature, systolic and diastolic blood pressure, and pulse and respiratory rate.

8.3.4 ECOG

The ECOG assessment is to be performed according to the seminal paper Toxicity and Response Criteria of the Eastern Cooperative Oncology Group published in the American Journal of Clinical Oncology in 1982 [redacted].

8.3.5 Cardiac evaluations

8.3.5.1 Electrocardiograms

At screening a triplicate 12-lead ECG(s) will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Holter ECG monitoring is acceptable to replace 12-lead ECG, provided the heart rate and measures PR, QRS, QT, and QTc intervals can be produced and entered in the eCRF.

During treatment, one 12-lead ECG(s) will be obtained at all dosing visits.

A triplicate 12-lead ECG is obtained at the End of Study visit at week 18 (+/- 14 days).

Triplicate ECGs include 3 individual ECG tracings obtained as closely as possible in succession, but no more than 2 minutes apart.

8.3.5.2 Echocardiographic evaluation of left ventricular function

At baseline: Echocardiography (LVEF and GLS) will be measured, unless an assessment has been performed within 2 weeks of screening.

During the trial, echocardiography (LVEF and GLS, if available) will be measured prior to administration of the 5th dose at week 12 (+/- 7 days).

8.3.6 Clinical Safety Laboratory Tests

Clinical laboratory tests will be performed by the laboratories mentioned in the study-specific Laboratory Manual. Samples will be collected in appropriate tubes and handled according to standard procedures of the applicable laboratory and according to the study-specific laboratory manual. Clinical laboratory variables will be determined as outlined in Table 13.

Table 13: Safety laboratory assessments

Clinical laboratory assessments		
Hematology	White blood cell (WBC) count Red blood cell (RBC) count Hemoglobin (Hb) Hematocrit (HCT) Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Platelet count	Lymphocytes (% and absolute) Neutrophils (% and absolute) Monocytes (% and absolute) Eosinophils (% and absolute) Basophils (% and absolute)
Coagulation	Prothrombin time D-dimer Activated partial thromboplastin time	
Clinical chemistry	Alanine aminotransferase (ALT) Albumin Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) Blood urea nitrogen (BUN) Calcium Creatinine Creatinine Kinase (CK) Troponin (T or I) Natriuretic peptides (NTproBNP)	Glucose Lactate dehydrogenase (LDH) Phosphorus Potassium Sodium Total bilirubin Uric acid C-reactive protein (CRP) Total protein
Urinalysis	Bilirubin Blood Glucose Ketones Leukocytes Creatinine	pH Protein Specific weight Urobilinogen Nitrite Urine microscopic analysis (cells, bacteria, casts, crystals)

- The investigator must review the laboratory results, document this review, and record any clinically significant changes occurring during the trial as an AE. The laboratory results must be retained with source documents.
- Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the investigator to be more severe than expected for the patient's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the trial should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.
    - If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.
    - All protocol-required laboratory tests must be conducted in accordance with the laboratory manual and the SoA (Section 1.3).
    - If laboratory values from non-protocol-specified laboratory tests performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the investigator, then the results must be recorded as AEs.

8.3.7 Pregnancy Testing

Pregnancy testing is to be conducted with a highly sensitive serum or urine human chorionic gonadotropin (hCG) pregnancy test at screening and the days of vaccine administration as well as follow up visits (see Table 1 SoA). If pregnancy is suspected, additional pregnancy tests should be performed, and the result recorded in the patient's medical records. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IEC.

8.4 Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Safety Reporting

Progression of the breast cancer under trial is not considered an adverse event (AE) or serious adverse event (SAE) unless it results in hospitalization or death.

The definitions of AEs, AESIs and SAEs can be found in Appendix 1, Section 12.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all reportable safety events.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 1, Section 12.

8.4.1 Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from start of first dose administration on day 1 (visit 2) until the follow-up visit at the timepoints specified in the SoA (Section 1.3). All reportable safety events that occur

after the consent form is signed but before the first administration of adjuvanted or non-adjuvanted ES2B-C001 will be recorded as medical history/current medical conditions, not as AEs.

All AEs occurring from the first administration of adjuvanted or non-adjuvanted ES2B-C001 must be reported by the investigator until the follow-up visit.

All SAEs and AESIs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 1, Section 12. The investigator will submit any updated SAE and AESI data to the sponsor within 24 hours of it being available.

All pregnancies from the time of first administration of adjuvanted or non-adjuvanted ES2B-C001 until the follow-up visit must be reported by the investigator.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the trial participation. However, if the investigator learns of any SAE, including a death, at any time after a patient has been discharged from the trial, and the investigator considers the event to be reasonably related to the trial intervention or trial participation, the investigator must promptly notify the sponsor.

8.4.2 Method of Detecting AEs and SAEs

AEs will be collected at each trial visit. In addition, there will be telephone calls at 2 days +/- 1 day after each dose administration, and a paper patient diary that will be handed out to the patient at each dose administration and reviewed before the next dosing (see Table 1 SoA) as well as spontaneous reports at any time and patients should be instructed to promptly report any symptoms and signs of potential drug reactions to allow timely intervention.

Care will be taken not to introduce bias when detecting AEs. Open-ended and non-leading verbal questioning of the patient is the preferred method to inquire about AE occurrences.

8.4.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs and AESIs will be followed until resolution, stabilization or is considered chronic, the event is otherwise explained, or the patient is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is provided in Appendix 1, Section 12.

8.4.4 Regulatory Reporting Requirements for SAEs

The PI will review each SAE and evaluate the severity according to the CTCAE criteria V5.0 and the causal relationship of the event to the IMP. All SAEs will be recorded from day 1 (after dosing) until the end-of-study visit. Serious AEs occurring after the end-of-study visit and coming to the attention of the PI must be reported only if there is, in the opinion of the PI, reasonable causal relationship with the IMP. The PI is responsible for providing notification to the Sponsor of any SAE, whether deemed IMP-related or not, that a subject experiences during their participation in the study within 24 hours of becoming aware of the event. As a minimum requirement, the initial

report should provide details of the study, site, subject, details of the SAE and associated treatment with study drug, seriousness criteria and causality assessment. Further relevant information, including concomitant medication, medical history, relevant laboratory evaluation and outcome, should be provided as soon as possible. The Sponsor will request clarification of omitted or discrepant information from the initial notification. The PI or an authorized delegate is responsible for delivering the requested information to the Sponsor within 24 hours of the Sponsor's request or as soon as the requested information is available. Initial reports of SAEs must be followed later with detailed descriptions, including clear photocopies of other documents as necessary (e.g., hospital reports, consultant reports, autopsy reports), with the subject's personal identifiers removed. All relevant information obtained by the PI through review of these documents will be recorded and reported to the Sponsor within 24 hours of receipt of the information. Refer to Section 7.5 for stopping rules in case of a SAE related to the IMP.

8.4.5 Suspected Unexpected Serious Adverse Reaction (SUSAR) Reporting

Information on SUSARs will be collected and reported to the regulatory authority and the IEC in accordance with European Commission Guidance 2011/C 172/01 ("CT3"), or as per national regulatory requirements in the participating country. This process will be described in detail in the Safety Monitoring Plan (SMP). If the SUSAR is fatal or life-threatening, associated with the use of the IMP, and unexpected, the regulatory authority and the IEC will be notified within 7 calendar days after being made aware of the case. Additional follow-up (cause of death, autopsy report, hospital report) information should be reported within an additional 8 days (15 days in total). SUSARs which are not fatal and nor life-threatening are to be notified within 15 days. Follow-up information may be submitted if necessary. The Sponsor will also provide annual safety reports for submission to the regulatory authority and the IEC responsible for the clinical study. These updates will include information on SUSARs and other relevant safety findings.

SERIOUS ADVERSE EVENT REPORTING INSTRUCTIONS

Send the SAE Report Form and any supporting documentation via email or fax within 24 hours of becoming aware of the event.

Contact person for SAE and SUSAR reporting:

[redacted]

8.4.6 Pregnancy and Postpartum Information

Any female participant who becomes pregnant while participating in the study will discontinue study intervention or will be withdrawn from the study. The PI must follow-up and document the course and the outcome of all pregnancies, including those of female partners of male participants, even if the subject was withdrawn from the clinical study or if the clinical study has finished.

The Investigator will collect pregnancy information on any female participant, or female partner of a male participant, who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a participant's or female partners pregnancy and the pregnant woman will be referred to a physician specialized or experienced in teratology for evaluation and advice. The participant will be followed to determine the outcome of any pregnancy. All outcomes of pregnancy must be reported by the PI to the Sponsor on the pregnancy outcome report form within 24 hours after he/she has gained knowledge of the delivery or elective abortion.

The Investigator will collect any follow-up information on the participant/female partner and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of the pregnancy will be reported, regardless of fetal state (presence or absence of anomalies) or indication for the procedure.

Pregnancy alone is not regarded as an AE unless there is a suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication. Elective abortions without complications should not be regarded as AEs, unless they were therapeutic abortions (see below). While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy-related SAE considered reasonably related to study intervention by the Investigator will be reported to the Sponsor. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of any pregnancy related information through spontaneous reporting.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

Hospitalization for normal delivery of a healthy newborn should not be considered a SAE.

9 Statistical Considerations

The Statistical Analysis Slan (SAP) will be finalized prior to database lock, and it will include a more technical and detailed description of the statistical analyses described in this section.

9.1 Sample Size Determination

This is a phase I study following a traditional 3+3 design, and no formal statistical hypothesis will be tested. The sample size was estimated to be 9 evaluable patients assuming 3 possible dose levels. However, due to the cohort expansion design, a maximum of 27 evaluable patients in total could be dosed.

9.1.1 Operating characteristics of the 3+3 design

Since true probabilities of DLTs under specific dose levels are unknown, four theoretical scenarios are presented to illustrate operating characteristics of the 3+3 design in different locations of the MTD. The calculation was performed considering all possible trial pathways for the standard 3+3 design (Wheeler et al., 2016).

Two extreme scenarios are considered, where the true MTD is lower or higher than dose levels tested in the study. Then, two scenarios where the MTD is within the interval of dose levels that are tested in the study.

Operating characteristics of 3+3 design with 3 dose levels under 4 different scenarios are in Table 14.

Table 14: Operating characteristics of 3+3 design with 3 dose levels under 4 different scenarios.

9.2 Analysis Sets

Safety Set: will include all patients who received at least 1 dose of the trial drug. All safety/tolerability and DLT analyses will be performed using this analysis set and will be descriptive, with no statistical inference to be drawn from the data.

Immunogenicity Set: will include all patients who received at least 1 dose of the trial drug and have provided immunogenicity data. All immunogenicity analyses will be performed using this population.

Efficacy-evaluable Set: will include all patients receiving at least 1 dose of the trial drug and who have provided at least 1 tumor evaluation at baseline and 1 tumor evaluation following treatment administration.

9.3 Statistical Analyses

In general, continuous data will be summarized using number of available data, mean, standard deviation, median, minimum, and maximum by dose level. Categorical and ordinal data will be summarized using number and percentage of patients by dose level.

The detailed statistical analysis will be described in the SAP.

9.3.1 Primary endpoint analysis

Total number and incidence of dose limiting toxicities (DLTs), number of patients with DTLs and percentage of patients with DLTs in each dose level and patient population will be reported.

Additionally, the number of DLTs, affected patients and percentage of affected patients will also be reported by preferred term and severity according to CTCAE v5.0 in each dose level and patient population.

The maximum tolerated dose will be determined for each patient population separately as lowest dose level at which ≥33% of patients have record of DLT.

The primary endpoint will be evaluated on the Safety Set.

9.3.2 Secondary endpoint analysis

All immunogenicity variables will be analyzed descriptively following general rules defined at the beginning of this section. In addition to the standard set of descriptive statistics, geometric mean will be reported by study visit for each population separately. For each visit after first study drug administration, the change from baseline will be analyzed descriptively.

Secondary endpoint analysis will be done on Immunogenicity Set.

9.3.3 Exploratory endpoint analysis

Number and percentage of patients with Complete Remission (CR) or Partial Response (PR) (according to RECIST 1.1) at the latest assessment during the study period out of patients with measurable disease at baseline will be reported by study visit, dose level and patient population.

The number and percentage of patients with Disease Control Rate (DCR) according to RECIST 1.1 will be reported by dose level and patient population.

In addition, best percent change from baseline in sum of target tumor measurements will be summarized and presented graphically.

Progression free survival (PFS) in patients with SD, PR, or CR at the time of first dosing according to RECIST 1.1. will be evaluated using Kaplan-Meier estimate by dose level and patient population.

Overall survival (OS) will be evaluated using Kaplan-Meier estimate by dose level and patient population.

Exploratory endpoint analysis will be done on Efficacy-evaluable Set.

9.3.4 Safety analysis

Safety analysis will be evaluated on the Safety Set.

9.3.4.1 Adverse events

All recorded adverse events (AEs) will be summarized descriptively by preferred term and system organ class. Total number of AEs, total number of subjects with AE and percentage will be reported by dose level and patient population.

Additionally, AEs will be summarized by seriousness, severity (according to CTCAE v5.0), relationship to treatment and outcome.

9.3.4.2 Physical examination, Vital signs, ECOG, Cardiac evaluations, Clinical laboratory tests

Safety variables will be analyzed descriptively according to general rules defined at the beginning of this section by study visit and patient population.

9.4 Protocol deviations

Protocol deviations will be listed per subject. Classification of protocol deviation will be specified in the respective plan.

10 General Considerations: Regulatory, Ethical and Trial Oversight

10.1 Regulatory and Ethical Considerations

- This trial will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) international ethical guidelines.
  - Applicable ICH Good Clinical Practice (GCP) guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IEC by the investigator and reviewed and approved by the IEC before the trial is initiated.
- Any amendments to the protocol will require IEC approval before implementation of changes made to the trial design, except for changes necessary to eliminate an immediate hazard to trial patients.
- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to trial patients.
- The investigator will be responsible for the following, as applicable:
  - Providing written summaries of the status of the trial to the IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IEC.
  - Notifying the IEC of SAEs or other significant safety findings as required by IEC procedures.
  - Providing oversight of the conduct of the trial at the site and adherence to requirements of 21 CFR, ICH guidelines, the IEC, European regulation 536/2014 for clinical studies, and all other applicable local regulations.

10.2 Financial Disclosure

The financing and insurance arrangements are documented in separate agreements.

10.3 Informed Consent Process

The principles of informed consent in the Declaration of Helsinki and GCP guidelines will be implemented before any protocol-specific procedures or interventions are carried out.

All subjects will be informed that participation is voluntary and that they can cease participation at any time without necessarily giving a reason and without any penalty or loss of benefits to which they are entitled.

- The investigator or the investigator's representative will explain the nature of the trial, including the risks and benefits, to the potential patient and answer all questions regarding the trial.
- The subject must give consent to participate prior to enrollment in the trial. This consent must be given in writing.
- The Investigator who conducts the informed consent discussion must also sign. The Investigator may delegate this responsibility to a suitably qualified member of the study team (e.g., Sub Investigator) if permitted by local regulations. This delegation of responsibility must be recorded in the Study File. By giving signed consent, the subject will confirm that his or her participation is voluntary and that he or she will follow the instructions of the Investigator and answer the questions asked. Signatures must be personally dated.
- The ICF must include all elements required by law, local regulations, GCP guidelines, and ICH guidelines, including consent to allow the Sponsor, Sponsor representative, or external regulatory auditor to review the subject's medical records. This gives permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of the trial.
- The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the trial and the date the written consent was obtained.
- The signed and dated consent form will be kept by the Investigator. A copy of the ICF(s) must be provided to the patient.
- Patients must be reconsented to the most current version of the ICF(s) during their participation in the trial.

10.4 Recruitment strategy

- Patients will be recruited from the existing patient pool at the Department of Gynecology and the Department of Oncology, Medical University Vienna, Austria.

10.5 Data Protection and Data Retention

- Patients will be assigned a unique identifier by the sponsor. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.
- The patient must be informed that their personal trial-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient who will be required to give consent for their data to be used as described in the informed consent.
- The patient must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.
- The contract between sponsor and trial sites specifies responsibilities of the parties related data protection, including handling of data security breaches and respective communication and cooperation of the parties.

- Information technology systems used to collect, process, and store trial-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorized disclosure or access. For eCRF, validated software Clinspire® will be used. The data will be stored on servers at Medical University of Vienna. The trained and designated study site staff as well as persons designated by the sponsor will have access to the data.
- The investigator shall retain all study records during the clinical investigation and for the period required by the applicable regulatory requirements or for at least 25 years after the premature termination or completion of the clinical investigation, whichever period is the longest. However, the investigator should contact the sponsor prior to destruction of any records or reports pertaining to the clinical investigation in order to ensure they no longer need to be retained. In addition, the sponsor should be contacted if the investigator plans to leave the site so that arrangements can be made for transfer of records.

10.6 Serious Deviation/Breach

If a deviation that is likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated in the clinical trial (serious deviation or breach) has occurred, the investigator should inform the sponsor and CRO within 24 hours.

10.7 Data safety monitoring board

The DSMB will constitute representatives from sponsor, CRO and the site (minimally represented by the treating principal investigator or designee). Additionally, up to 2 independent subject experts will be included. A dedicated charter will be established for the DSMB which will include responsibilities and instructions for the dose escalation procedure.

During the dose-escalation the DSMB will review accumulated safety data and available data on immunogenicity and make a recommendation to sponsor which dose may be used in the following dosing cohort.

Any safety concerns throughout the trial such as potential DLTs and/or events that may meet a criterion for initiation of rescue medication, will be escalated to the DSMB who will review accumulated safety data and may provide recommendations for trial adjustments, e.g. to:

- Recommend dose schedule optimizations to the treatment regimen during the trial.
- Recommend intermediate-level doses, provided the safety and immune response data indicate the need for intermediate doses.
- Evaluate if AEs reported as DLTs meet the DLT criteria.
- Recommend additional dose cohorts based on observed safety and tolerability data.
- Apply additional overnight stays for patients for safety precautions.
- Recommend additional patients to a cohort.
- Explore higher doses in the dose escalation part than recommended in the non-clinical toxicity package, if safety and immunogenicity data suggest a higher dose may be more optimal.

Written statements and conclusions of the DSMB decisions will be in place before implementing any adjustments to the trial.

11 Risk Management and Quality Assurance

11.1 Data Quality Assurance

- All patient data relating to the trial will be recorded on electronic CRFs unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- Guidance on completion of CRFs will be provided.
- The investigator must permit trial-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source documents.
- Monitoring details describing strategy, including definition of trial critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the monitoring plan.
- The sponsor or designee is responsible for the data management of this trial, including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., CROs).
- Records and documents, including signed ICFs, pertaining to the conduct of this trial must be retained by the investigator for 25 years after trial completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

11.2 Source Documents

- Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the trial. Also, current medical records must be available.
- Definition of what constitutes source data and its origin can be found in the monitoring plan.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The sponsor or designee will perform monitoring to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source

documents; that the safety and rights of patients are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

11.3 Trial and Site Start and Closure

11.3.1 First Act of Recruitment

The trial start date is the date on which the clinical trial will be open for recruitment of patients. The first act of recruitment is the first site open and will be the trial start date.

11.3.2 Trial/Site Closure and Termination

The sponsor or designee reserves the right to close the trial site or terminate the trial at any time for any reason at the sole discretion of the sponsor. Trial sites will be closed upon trial completion. A trial site is considered closed when all required documents and trial supplies have been collected and a trial-site closure visit has been performed.

The investigator may initiate trial-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a trial site by the sponsor or investigator may include but are not limited to:

For trial termination:

- Discontinuation of further trial intervention development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IEC or local health authorities, the sponsor's procedures, or GCP guidelines.
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of patients by the investigator.
- Total number of patients included earlier than expected.

If the trial is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs, the regulatory authorities, and any CRO(s) used in the trial of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the patient and should assure appropriate patient therapy and/or follow-up.

11.4 Publication Policy

- The results of this trial may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

- The sponsor will comply with the requirements for publication of trial results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

12 Appendix 1: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

12.1 Definition of AE

AE Definition

- An AE is any untoward medical occurrence in a clinical trial patient, temporally associated with the use of trial intervention, whether or not considered related to the trial intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of trial intervention.

Definition of Unsolicited AE

- An unsolicited AE is an AE that was not found using specific questions and that is communicated by a patient who has signed the informed consent. Unsolicited AEs include serious and nonserious AEs.
- Potential unsolicited AEs may be medically significant (i.e., symptoms or illnesses requiring a hospitalization, emergency room visit, or visit to/by a healthcare provider). The patients will be instructed to contact the site as soon as possible to report medically attended event(s), as well as any events that, though not medically attended, are of patient's concern. Detailed information about reported unsolicited AEs will be collected by qualified site personnel and documented in the patient's records.
- Unsolicited AEs that are not medically significant nor perceived as a concern by the patient will be collected during an interview with the patient and by review of available medical records at the next visit.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease, or more severe than expected for the patient's condition).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or severity of the condition.
- New condition detected or diagnosed after trial intervention administration even though it may have been present before the start of the trial.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either trial intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events not Meeting the AE Definition

- Any abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the patient's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen.

12.2 Definition of SAE

An SAE is defined as any untoward medical occurrence that, at any dose, meets one or more of the criteria listed:

a) Results in death

b) Is life threatening

The term *life threatening* in the definition of *serious* refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the patient has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental

trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect

f) Other important medical events

- Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations such as important medical events that that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, convulsions not resulting in hospitalization, or development of intervention dependency or intervention abuse.

12.3 Adverse Event of Special Interest (AESI)

An AESI is a pre-specified medically significant event that has the potential to be causally associated with a vaccine product that needs to be carefully monitored and confirmed by further special studies. In this study the following events are considered AESIs:

- DLT
- CTRCD
- Immune system disorder

Assessment of Severity

The PI will assess all AEs for severity in accordance with the following standard ratings. The severity of each AE (i.e., non-serious and SAEs) is to be assessed by the Investigator using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, Version 5.0) as indicated in Table 15. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the event should be noted for that day. Any change in severity of signs and symptoms over a number of days will be captured by recording a new AE, with the amended severity grade, and the date (and time, if known) of the change.

Table 15: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) reporting (V5.0).

Grade	CTCAE
Grade 1	An AE that is asymptomatic; or involves mild or minor symptoms; or is of marginal clinical relevance; or consists of clinical or diagnostic observations alone; or for which intervention is not indicated; or for which only non-prescription intervention is indicated.
Grade 2	An AE for which only minimal, local, or non-invasive intervention (e.g., packing, cautery) is indicated; or that limits instrumental activities of daily living (e.g., shopping, laundry, transportation or ability to conduct finances).
Grade 3	An AE that is medically significant but not life-threatening; or for which in-patient care or prolongation of hospitalization are indicated; or that is an important medical event that does not result in hospitalization, but may jeopardize the subject or may require intervention either to prevent hospitalization, to prevent the AE from becoming life-threatening or causing death; or that is disabling; or that results in persistent or significant disability, incapacity, or limitation of self-care activities of daily living (e.g., getting in and out of bed, dressing, eating, getting around inside, bathing, or using the toilet).
Grade 4	An AE that has life-threatening consequences; for which urgent intervention is indicated; that puts the subject at risk of death at the time of the event if immediate intervention is not undertaken; or that causes blindness or deafness.
Grade 5	The termination of life as a result of an AE.

Assessment of Causality

- The PI will assess the causality/relationship between the study drug and the AE based on medical judgment and considering the definitions of the "Assessment of Relationship of Adverse Events to Investigational Medicinal Product" described in Table 16, and all contributing factors. The investigator is obligated to assess the relationship between trial intervention and each occurrence of each AE/SAE.
- For causality assessment, the investigator will also consult the IB for ES2B-C001 and/or [adjuvant] [redacted] . The investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Table 16: Assessment of relationship of Adverse Events (AEs) to investigational medicinal product (IMP).

	Assessment of Relationship of Adverse Events to IMP
Definitely Related	A clinical event, including laboratory test abnormality, occurs in a plausible time relationship to treatment administration, and which concurrent disease or other drugs or chemicals cannot explain. The response to withdrawal of the treatment (de-challenge*) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge† procedure if necessary.
Probably Related	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Rechallenge information is not required to fulfill this definition.
Possibly Related	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment, but which could also be explained by concurrent disease or other drugs or chemicals. Information on treatment withdrawal may be lacking or unclear.
Unlikely To Be Related	A clinical event, including laboratory test abnormality, with a temporal relationship to treatment administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Unrelated	A clinical event, including laboratory test abnormality, with little or no temporal relationship with treatment administration. May have negative de-challenge and rechallenge information. Typically explained by extraneous factors (e.g., concomitant disease, environmental factors or other drugs or chemicals).

12.4 Recording, reporting and follow-up of AEs, AESIs and SAEs

Detailed information on recording, reporting and follow-up of AEs, AESIs and SAEs will be provided in a separate safety management plan.

13 Appendix 2: Grading of Injection Site Reactions

Table 17: Grading of Injection Site Reactions.

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[12]
Pain	Does not interfere with activity	Repeated use of non narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness[13]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling[14]	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

[12] Any Grade 4 injection site reaction will be reported as SAE.
[13] In addition to grading the measured local reactions at the greatest single diameter.
[14] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.