PROTOCOL TITLE

2021-PT053: A Phase I Open Label, Placebo-controlled,Randomized Dose Escalation Study to Evaluate Safety and Pharmacokinetics of NATRUNIX via Subcutaneous Injection in Healthy Subjects

NCT NUMBER

NCT05099510

22 December 2021

Trial Protocol

2021-PT053: A Phase I Open Label, Placebo-controlled, Randomized Dose Escalation Study to Evaluate Safety and Pharmacokinetics of NATRUNIX™ via Subcutaneous Injection in Healthy Subjects

SPONSOR

XBiotech USA, Inc.

DATE

22 December 2021

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Basic Information

STUDY TITLE: A Phase I Open Label, Placebo-controlled, Randomized Dose Escalation Study to Evaluate Safety and Pharmacokinetics of Natrunix™ via Subcutaneous Injection in Healthy Subjects

INVESTIGATIONAL PRODUCT: Natrunix™

IND Number: **157422**

PROTOCOL NUMBER: 2021-PT053

PROTOCOL VERSION / DATE: **Version 3.1 / 22 December 2021**

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STUDY TITLE: A Phase I Open Label, Placebo-controlled, Randomized Dose Escalation Study to Evaluate Safety and Pharmacokinetics of Natrunix™ via Subcutaneous Injection in Healthy Subjects

Protocol Version / Date: 3.1 / 22 Dec 2021

Protocol Number: 2021-PT053

STUDY PRINCIPAL INVESTIGATOR SIGNATURE:

I have read the protocol and appendices. I understand the contents and intend to comply fully with all requirements and the applicable current local and international regulations and guidelines. No changes will be made without formal authorization by XBiotech USA, Inc., in the form of a protocol amendment.

INVESTIGATOR SIGNATURE:

_____
Printed name of Investigator

_____
Signature

_____
Date

SPONSOR SIGNATURE:

XBiotech USA, Inc.

_____
Printed name

_____
Signature

_____
Date

Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase (ALT, SGPT)
PT/aPTT	Prothrombin Time/Activated Partial Thromboplastin Time
ALP	Alkaline phosphate
AUC	Area under plasma concentration-time curve
BP	Blood pressure
CBC	Complete blood counts
CI	Confidence interval
CH	Heavy chain constant region
CL	Light chain constant region
CL	Systemic Clearance
eCRF	Electronic Case report form
Cmax	Maximum concentration
CRA	Clinical Research Associate
CT	Computed tomography
DEXA	Dual Energy X-Ray Absorptiometry
EC	Ethics Committee
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assay
EORTC-QLQ	European Organization for Research and Treatment of Cancer – Quality of Life Questionnaire
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
HIV	Human immunodeficiency virus
IgG	Immunoglobulin G
IL-1 a	Interleukin-1 a
IL-1b	Interleukin-1 b
IL-1 RA	Interleukin-1 receptor antagonist
IRB	Institutional review board
LBM	Lean Body Mass
MedDRA	Medical Dictionary for Regulatory Activities
pK	Pharmacokinetics
RPR	Rapid Plasma Reagin
TB	Tuberculosis
TNF	Tumor necrosis factor
ULN	Upper limit of normal
VOD	Volume of distribution
WOCBP	Women of childbearing potential

1. BACKGROUND

1.1. Natrunix™ True Human™ Therapeutic Antibody

XBiotech USA, Inc. has developed a True Human™ monoclonal antibody, Natrunix™ that binds human IL-1α with high affinity and is an effective blocker of its biological activity. IL-1α is a key cytokine that regulates numerous sterile inflammatory responses, including pathological inflammation associated with tumor progression, rheumatoid arthritis, vascular disease, diabetes, and chronic skin conditions. Accordingly, targeting IL-1α may be a safe and effective way to abrogate the inflammatory process that drives these diseases.

Natrunix™ is a True Human™ therapeutic antibody. The antibody was generated by a natural human immune response and was cloned directly from a human peripheral B lymphocyte. No *in vitro* affinity maturation or modifications have been made to improve its natural binding affinity. A true human antibody should be effectively non-immunogenic in humans and exhibit activity and pharmacokinetics indistinguishable from native IgG_4 immunoglobulin.

1.2. Summary of Non-Clinical Findings

Natrunix™ is a recombinant IgG_4 monoclonal antibody that binds to human IL-1α, and prevents its interaction with the IL-1 receptor (IL-1R1), thereby inhibiting IL-1α mediated signaling. IL-1 signaling results in upregulation of inflammatory responses, including: upregulation of vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), chemokines, adhesion molecules (ICAM1, E-selectin, etc) and interleukin 6 (IL-6).

Several *in vitro* studies have been used to confirm the biological activity of Natrunix™. These models have confirmed that Natrunix™ binds specifically to both free and cell membrane associated human IL-1α, but not to IL-1β or the IL-1 receptor antagonist. Inhibition of IL-1α with Natrunix™ has also been shown to block the upregulation of adhesion molecules (ICAM-1 and/or E-selectin) using *in vitro* models of human umbilical vein endothelial cells. Finally, Natrunix™ inhibits secretion of IL-6 in fetal human lung fibroblasts.

1.3 Study Rationale:

There is limited clinical experience with Natrunix™ and it has not been previously administered via subcutaneous route. Natrunix™ is being evaluated at three dose levels here for safety and pharmacokinetics against placebo in healthy subjects after receiving a single subcutaneous injection. the dose levels chosen covers the clinical dose ranges that will be used in subsequent studies. The decision to escalate the dose will be made by a dose escalation committee (DEC) based on an interim analysis of clinical and laboratory data obtained by as early as Day 14.

2. INVESTIGATIONAL PRODUCT

2.1. Active Ingredient, Pharmacologic Class

XB2001 is the active ingredient in the drug product Natrunix™. XB2001 is an immunoglobulin (IgG4k). The antibody sequence was cloned from a naturally occurring anti-IL-1α immunoglobulin present in a healthy

human volunteer. Naturally occurring anti-IL-1α antibody is present in 5% to 28% of blood samples from healthy subjects[1,2], and is naturally occurring in cord blood, children and adults[3]. XB2001 is indistinguishable from the naturally occurring immunoglobulin.

Antibodies undergo a process in the human body whereby chemical modifications make the antibody uniquely bind its target with extremely high affinity. For NATRUNIX™, affinity modifications had naturally taken place in the human body, and therefore no synthetic alterations were necessary to modify XB2001 for use as a drug product. All marketed antibodies, without exception, are either animal derived and/or have been subjected to synthetic modifications to increase suitability for use as a drug product. Natrunix™ (XB2001) therefore has minimal potential for side effects or injection site/infusion related reactions.

2.2. Drug Product (NATRUNIX™) Description

[redacted]

[redacted] ubjects in 100 mg and 200 mg cohorts will use syringes filled with 1 mL drug product. Syringes will be graduated and only a portion will be used for the 100mg cohort. Subjects in 400 mg will use syringes filled with 2 mL drug product.

Placebo is a sterile liquid solution containing the exact same buffer matrix used for Natrunix drug product. The placebo subjects will receive subcutaneous injection of the same volume of the buffer compared to the drug product subjects in the same cohort.

2.3. Storage

Recommended storage of Natrunix™ drug product is at 2-8° C and protected from light.

2.4. Stability

The drug product is formulated in a buffer in which most of the tonicity comes from [redacted] is an effective stabilizer against oxidation, aggregation, thermal, and mechanical stress. [redacted] was selected as the buffering agent due to its antioxidant properties.

Stability data indicates that the drug product is very stable. Short exposure to room temperature has no negative effect on the drug.

The drug product is initially labeled with a 6 month retest/expiry date from the Manufacturing Date. Stability testing is performed according to a stability test plan following ICH guidelines. With more stability data obtained at later stability time points, drug product shelf life may be extended and re-issued by XBiotech Quality.

2.5. Method of Administration

2.5.1. Materials

1. XB2001 pre-filled syringe(s) for injection, warmed to room temperature or hand warmed.
   NOTE:
   Subjects in 100 mg cohort will be injected with 0.5 mL (graduated mark) from the 1 mL filled syringes.
   Subjects in 200 mg cohort will be injected with the entire content of 1 mL filled syringes.
   Subjects in 400 mg cohort will be injected with the entire content from 2 mL filled syringes.
2. Sterile alcohol wipes.
3. Band-aids, along with 2x2 gauze bandages and paper tape.
4. Latex-free gloves.

2.5.2. Instructions for Subcutaneous Injection

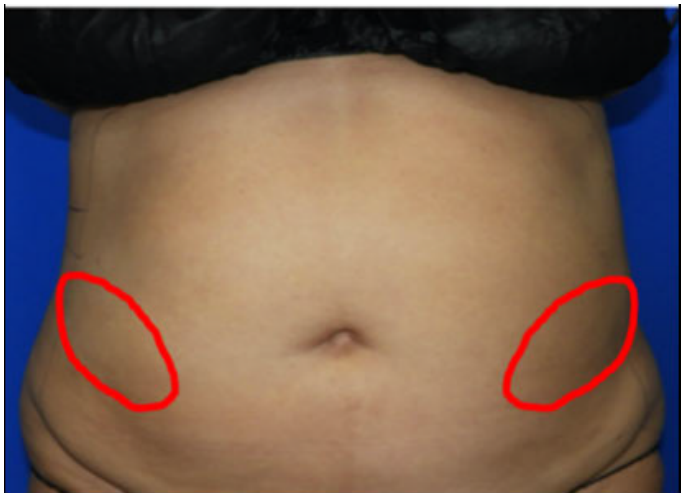
Syringe preparation: Approximately 30 minutes prior to injection, remove the pre-filled syringe(s) from refrigeration and let it naturally warm to room temperature. The drug has been highly concentrated to minimize injection volume. Warming to room temperature will make the formulation flow easier and reduce the amount of pressure needed to be applied to the plunger when injecting. The syringe may also be warmed by holding in palm of hand. *Do not heat the syringe*.

Only For the Cohort 1 for 100mg injection: please follow the instruction below-

Prior to injecting, hold the syringe by the barrel with the tip cap pointing up, remove the needle shield and push the plunger rod to remove the air in the headspace. Next, invert the syringe, remove 0.5ml of drug product into a tube (provided with the syringe) by pushing the stopper plunger until the plunger reaches the red line on the syringe. The volume will now be 0.5 mL containing 100mg of drug product (red-line). Follow the injection procedure below to inject the 0.5 mL (100mg) of drug into subject.

Injection Site: See Figure 1 below. Either left or right side of the belly can be used.
Note: avoid areas where the skin is burned, scarred, hardened, inflamed, swollen or damaged

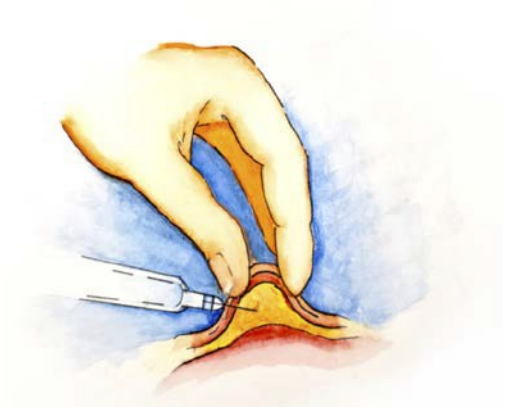
Figure 1: Injection Site Illustration



Injection Procedure:

- Put on gloves.
- Assemble syringe with finger grip wing attachment.
- Wipe injection site clean with alcohol pads.
- Pinch and gently raise a fold of skin to create a skin flap (see Figure 2 below) approximately 3 to 6 inches to the left or right of the belly button.
- Place index and middle fingers on either side of the grip attachment and thumb on the plunger. Insert needle fully into the base of the pinched skin where a pocket has been formed until it cannot go in any further. Creating this pocket allows space for the injection fluid to enter without causing tissue disruption or pain. See Figure 2 below. Press plunger and inject drug as rapidly as is comfortable for the subject until the appropriate volume of the syringe has been injected (refer to 2.5.1).
- Withdraw needle. No adhesive bandage is necessary. However, if desired, an adhesive bandage (ie. band-aid) may be placed over injection site (*Note: If bleeding occurs, hold pressure on the injection site until bleeding stops, and then apply gauze bandage secured by tape*).

Figure 2: Picture of Injecting into Skin Flap



2.6. Agent Ordering

The Responsible Investigational Pharmacy will order drug product from XBiotech as needed.

2.7. Potential Drug Interactions

- Live vaccines should not be given concurrently with Natrunix™.
- Combinations of Natrunix™ with tumor necrosis factor alpha (TNFα) inhibitors or other IL-1 inhibitors (IL-1 receptor antagonist, alpha or beta) may increase infection risk. Use in combination with these agents is prohibited.

2.8. Prohibited and Restricted Therapies

The following concomitant therapies are prohibited while on trial:

- Therapeutic agents and biologics that specifically target the cytokines IL-1 (IL-1 RA, alpha, or beta) or TNF-alpha
- Live virus vaccines
- Investigational agents

3. STUDY DESIGN AND OBJECTIVES

3.1 Endpoints

Primary Endpoint:

1. Number of subjects with treatment emergent adverse events assessed according to "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials"

Participants will be monitored for acute reactions, blood chemistry and hematology immediately after dosing and at multiple time points up to 28 days. All adverse events will be documented at multiple time points and assessed in terms those possibly, probably and definitely related to test article. Anti-drug antibodies (ADA) will also be evaluated.

Secondary Endpoints:

1. Maximum plasma concentration of test article

Blood draws will be taken prior to injection and at multiple time points after injection. The maximum plasma concentration will be assessed using a proprietary immunoassay to detect circulating Natrunix™.

2. Terminal plasma concentration

Plasma levels will be determined at various time points from blood draws. Terminal plasma concentration will be assessed at 28 days.

3. Half-life

Plasma levels will be determined at various time points from blood draws. Peak and terminal plasma levels will be used in a two compartmental model to assess plasma half-life.

4. Total exposure

Area under the curve will be determined based on measured plasma levels over 28 days.

3.2 Design

Number of Planned Subjects: Eight healthy subjects per cohort (including six for Natrunix™ and two for placebo). Three cohorts for a total of 24 healthy volunteers.

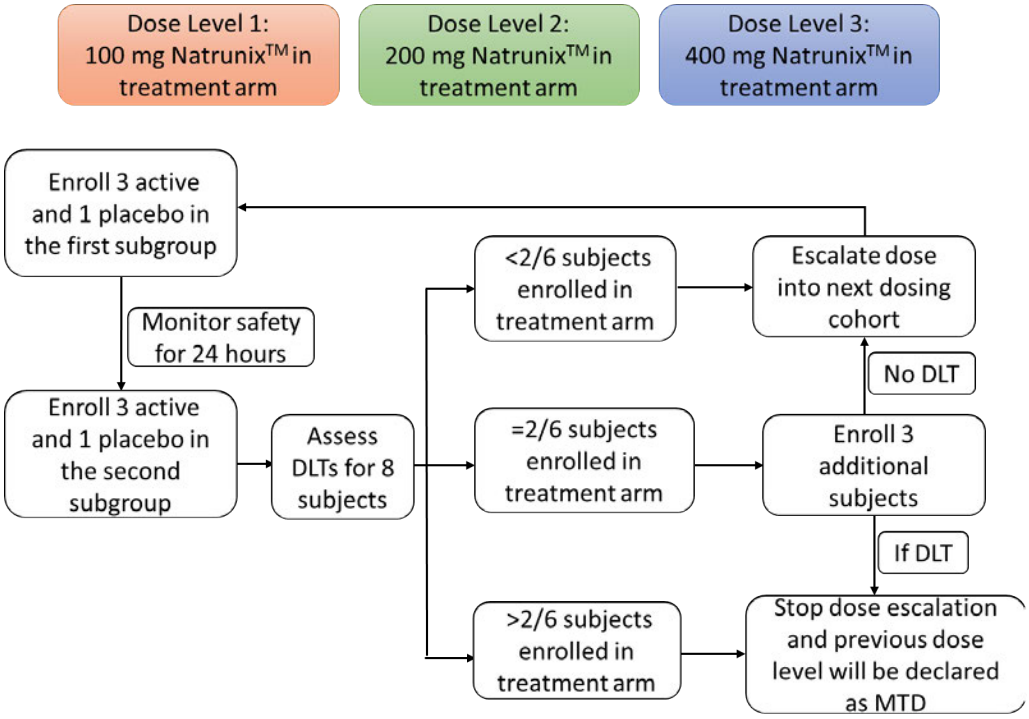
Approximate Duration:

Approximately 38 days for each subject which includes a screening period of up to 10 days followed by one subcutaneous dose of Natrunix™, and then evaluation over 28 days. Blood will be sampled at various time points for blood chemistry, hematological analysis and Natrunix™ serum/plasma concentrations.

The trial will be conducted in compliance with the protocol, good clinical practice and applicable regulatory guidance. Each participant will receive one single subcutaneous injection of either placebo or 100 mg (Cohort 1), 200 mg (Cohort 2), or 400 mg (Cohort 3), of Natrunix™. Using a standard 6+2 design, doses will be explored in cohorts of 8 subjects. Each cohort is comprised of two sub-groups, each containing three subjects receiving active drug and one subject receiving placebo. A 24-hour observation window will be given to the participants in the first sub-group for safety observation before enrolling the subjects in the second sub-group. Safety assessment for the initial 24 hours will be based on the clinical safety (vitals, adverse events) and safety laboratory data after start of injection for the first sub-group in each cohort.

The dose levels, schedule and dose escalation scheme are summarized in Figure 3 below.

Figure 3: Dose Escalation Design



The decision to escalate the dose will be made by a dose escalation committee (DEC) based on an interim analysis of clinical safety and safety laboratory data obtained by as early as Day 14.

The dose limiting toxicities (DLTs) are assessed in the subjects that are enrolled into the treatment arm at Day 14. DLT is defined as any grade 3 adverse event that is deemed possibly related to the study drug. Adverse events (AEs) will be defined by "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials".

1. Dose escalation will continue if there are less than two of six treatment subjects experience a dose-limiting toxicity (DLT).
2. A dose cohort is expanded to 3 additional subjects if two of the six subjects experience an apparent DLT during the 14 days. If there are no other DLTS observed in the 3 additional subjects, the dose escalation will continue. However, if there is at least one DLT, then the dose escalation will be stopped, and the previous dose level will be declared as the Maximum Tolerated Dose (MTD). MTD of Natrunix™ is defined as the highest dose level at which less than one third of subjects experience a DLT.
3. If more than two out of six subjects experience a DLT, then the dose escalation will be stopped, and the previous dose level will be declared as the MTD.

Subjects who do not finish until Day 14 due to AEs other than DLTs will be replaced.

Study drug will be administered under close observation in a facility equipped to treat anaphylaxis or injection reactions. Subjects will be closely monitored until at least 1 hour following the administration of the antibody or 1 hour after their vital signs have stabilized. Due to the frequent pK sampling regimen, subjects may remain in the Phase I facility for the first 24 hours.

The expected duration of participation for each subject is up to 10 days of screening and 28 days following Natrunix™ administration.

3.3 Dose Escalation Committee (DEC)

A Dose Escalation Committee (DEC) will provide recommendations about stopping, modifying or continuing the trial. The DEC will convene for cohort safety review after completion of each cohort—and intermittently if necessary—to provide safety oversight and dose escalation recommendations.

The committee will be composed of:

- Sponsor's medical expert
- Principal Investigator
- Sponsor's designate

4. ELIGIBILITY CRITERIA

4.1. Inclusion Criteria

Subjects may be included in the study if they meet all of the following criteria:

1. Age**:** ≥18
2. Adequate bone marrow function defined as:
   - absolute neutrophil count (neutrophil and bands) of ≥1,500/mm^3 (≥ 1.5 x 10^9/L)
   - platelet count > 150,000/mm^3
   - hemoglobin of ≥ 10 g/dL
3. Adequate renal function, defined by serum creatinine ≤ 1.5 x lab ULN.
4. Adequate hepatic function defined as:
   - serum albumin ≥ 3.0 g/dL
   - total bilirubin ≤ 1.5 times lab ULN.
   - alanine aminotransferase (ALT) ≤ 2.0 times lab ULN.
   - aspartate aminotransferase (AST) ≤ 2.0 times lab ULN
5. For WOCBP, a negative pregnancy test at screening. For subjects with reproductive potential, willingness to use one method of contraception of high efficacy during the entire study period. These methods can include but not limited to hormonal contraceptives, intrauterine devices, condoms, diaphragms etc. Women of non-childbearing potential include those considered to have a medical history that indicates that pregnancy is not a reasonable risk, including post-menopausal women and those with a history of hysterectomy or surgically sterilized.
6. If you are a male participating in this clinical research study, you should not get a sexual partner pregnant during your participation in this research study as the effect of the study drug on sperm is not known. The male contraception methods can include but not limited to mechanical methods (abstinence, withdrawal, non-vaginal intercourse) or contemporary methods comprising condoms and vasectomy.
7. Signed and dated Institutional Review Board (IRB) approved informed consent before any protocol-specific screening procedures are performed.

4.2. Exclusion Criteria

Subjects will be excluded from the study if they meet any of the following criteria:

1. Treatment with any biologicals (including intravenous immunoglobulin) or investigational agents within the last 4 weeks (or 5 half-lives, whichever is longer).
2. Uncontrolled or significant cardiovascular disease, including:
   - A myocardial infarction within the past 6 months.
   - Uncontrolled angina within the past 3 months.
   - Congestive heart failure within the past 3 months, defined as New York Heart Association (NYHA) Class II or higher.
   - Uncontrolled hypertension (blood pressure >160 mm Hg systolic or >100 mm Hg diastolic).
3. Dementia or altered mental status that would prohibit the understanding or rendering of informed consent.
4. Treatment with immunosuppressant agents, including corticosteroids or cyclosporine within the last 4 weeks.
5. Serious uncontrolled medical disorders, such as uncontrolled diabetes, active peptic ulcer disease, cerebrovascular accident within three months, ongoing congestive heart failure, and any other condition, which in the opinion of the investigator, would put the subject at risk by participating in the trial.
6. History of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies.
7. Abnormal ECG with any clinically significant findings or with QTc > 470 ms.
8. Infection requiring treatment with antibiotics within 3 weeks prior to screening.
9. Infectious disease:
   - Positive HIV, RPR, Hepatitis B or C, TB (QuantiFERON-TB Gold (QFT)/ IGRA)
10. History of immunodeficiency.
11. Female subjects who are pregnant, planning to become pregnant during the course of the study, or breast-feeding.
12. Major surgery within 28 days prior to Day 0.
13. History of progressive multifocal leukoencephalopathy (PML) or other demyelinating disease.

5. TREATMENT PLAN

5.1. Dose Modifications

The study design consists of a single dose of Natrunix™ with no modifications.

5.2. Discontinuation of Therapy

The study design consists of only one dose of Natrunix™, therefore discontinuation of therapy does not apply. However, if a participant withdraws consent for any reason, they will no longer be followed for pK or safety data.

5.3. Study Procedures

Screening (maximum 10 days): The screening period begins once the informed consent is signed.

- Informed Consent, inclusion/exclusion
- Demographics
- Medical History
- Concomitant Medications
- Physical Examination
- Vital Signs (blood pressure, heart rate, respiratory rate and body temperature)
- Electrocardiogram (ECG/EKG)
- Height and weight
- Chemistry panel: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, ALT, AST, total bilirubin, alkaline phosphatase, total protein, albumin
- Hematology Panel: Complete Blood Count (CBC) with differential, hemoglobin and platelets
- Infectious disease screening: HIV, Hepatitis B and Hepatitis C panel (HBsAg, anti-HBc, anti-HBs), TB, RPR
- Serum pregnancy test for WOCBP

DAY 0:

1) Pre injection:
    - Weight
    - Physical exam & Vital signs
    - Electrocardiogram
    - Urine Pregnancy Test
    - Blood draw for baseline pK/ADA assessment: one 6mL EDTA plasma tube.
    - Blood draw for Chemistry and Hematology
    - Concomitant medications/treatments
2) Subcutaneous administration of Placebo or Natrunix™: 100 mg (0.5 mL) for Cohort 1; or 200 mg (1 mL) for Cohort 2; or 400 mg (2 mL) for Cohort 3.
3) Post- injection:
    - Vital Signs
        - 30 minutes post injection (+/- 5 minutes)
        - 60 minutes post injection (+/- 5 minutes)
    - PK sampling schedule:
        - 3 hours post injection (+/- 15 minutes): one 6mL EDTA plasma tube
        - 6 hours post injection (+/- 30 minutes): one 6mL EDTA plasma tube
        - 10 hours post injection (+/- 45 minutes): one 6mL EDTA plasma tube
    - Adverse Event Monitoring
        - 60 minutes post injection (+/- 5 minutes)

DAY 1:

- Physical exam & Vital signs
- Electrocardiogram
- PK sampling schedule:
    - 24 hours post injection (+/- 60 minutes): one 6mL EDTA plasma tube
- Labs: Chemistry and Hematology
- Adverse event monitoring

- Concomitant medications/treatments

DAY 2:

- Vital signs
- PK sampling schedule:
  - 48 hours post injection (+/- 2 hours): one 6mL EDTA plasma tube
- Adverse event monitoring
- Concomitant medications/treatments

DAY 3:

- Vital signs
- PK sampling schedule:
  - 72 hours post injection (+/- 3 hours): one 6mL EDTA plasma tube
- Adverse event monitoring
- Concomitant medications/treatments

DAY 4:

- Vital signs
- PK sampling schedule:
  - 96 hours post injection (+/- 4 hours): one 6mL EDTA plasma tube
- Labs: Chemistry and Hematology
- Adverse event monitoring
- Concomitant medications/treatments

DAY 14:

- Urine Pregnancy Test
- Vital signs
- Electrocardiogram
- PK/ADA sampling schedule:
  - Day 14 post injection (+/- 1 day): one 6mL EDTA plasma tube
- Labs: Chemistry and Hematology
- Adverse event monitoring
- Concomitant medications/treatments
- Confirm any Dose Limiting Toxicities (DLTs)

DAY 28:

- Urine Pregnancy Test & Weight
- Physical exam & Vital signs
- Electrocardiogram
- PK/ADA sampling schedule:
  - Day 28 post injection (+/- 1 day): one 6mL EDTA plasma tube
- Labs: Chemistry and Hematology
- Adverse event monitoring
- Concomitant medications/treatments
- Confirm any Dose Limiting Toxicities (DLTs)

5.4. Study Calendar

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 14	Day 28
Visit (V)	**Screening (Max 10 days)**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**
Screening/ Baseline								
Informed Consent	X							
Inclusion/ Exclusion	X							
Medical History/ Demographics	X							
Height	X							
Treatment								
Administer Placebo or Natrunix™		X						
Safety								
Weight	X	X						X
ECG	X	X	X				X	X
Physical Examination	X	X	X					X
Vital Signs	X	Pre-injection and 30 min and 60 min post-injection	X	X	X	X	X	X
AEs/ SAEs		60 min post-injection	X	X	X	X	X	X
Con Meds/ Treatments	X	X	X	X	X	X	X	X
DLTs							X	X
Laboratory Testing								
Infectious Disease	X							
Pregnancy Test (WOCBP only)	Serum	Urine					Urine	Urine
Chemistry and Hematology	X	X	X			X	X	X
PK		Pre-injection and 3 hrs, 6 hrs, 10 hrs post-injection	X[1]	X[1]	X	X	X	X

[1] PK for Day 1 occurs at 24 hours and Day 2 at 48 hours. Please check section 5.3 on the allowable sampling window.

6. PHARMACOKINETIC AND SAFETY MEASUREMENTS

6.1. Pharmacokinetics (PK) Sample Collection

An enzyme-linked immunosorbent assay (ELISA) has been developed to specifically measure XB2001 levels in human plasma. Six milliliters of blood will be drawn into a single blood collection (plasma) tube at each PK collection time point. These samples will be transferred/shipped to XBiotech in batches for PK analysis.

6.2. Assessment of Safety

Safety will be assessed by monitoring pre-injection and post injection vital signs, as well as by monitoring the occurrence of treatment emergent adverse events from Day 0 post- injection to Day 28. If any serious adverse events occur, these will be followed until resolution. See next section.

Study drug will be administered under close observation in a facility equipped to treat anaphylaxis or injection site reactions. Subjects should be closely monitored until at least 1 hour following the administration of the antibody or 1 hour after their vital signs have stabilized. Due to the frequent pK sampling regimen, patients may remain in the Phase I facility for the first 24 hours.

Treatment-emergent ADA will be evaluated using the pK samples collected. A screening assay has been developed to detect the anti-drug antibodies.

7. ADVERSE EVENTS

7.1. Definition of Adverse Event (AE)

An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including laboratory findings), symptom, or disease temporally associated with the use of drug, whether or not it is apparently related to drug;
- A concurrent illness;
- An exacerbation, or an unexpected increase in frequency or intensity of a preexisting condition, including intermittent or episodic conditions;
- A significant or unexpected worsening of the condition/indication under investigation;
- A suspected interaction between the investigational drug and concomitant medications;
- An abnormal laboratory finding (including radiological interpretations, histopathological findings, etc.).

7.2. Definition of Serious Adverse Event (SAE)

An adverse event is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening adverse event;

- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/ birth defect;
- An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Note that seriousness and severity should not be confused. A subject could experience a severe headache that would not qualify as an SAE, while another might experience a mild stroke that, while not severe, would be considered serious.

7.3. Recording of Adverse Events

All untoward events occurring between visit 1 (Day 0 post- injection) and the end of study (28 days after the last injection of the treatment regimen or if the subject withdraws from the study early) should be recorded on the eCRF, regardless of whether they are considered related to the study drug.

All AEs should be recorded in a standard medical terminology, as concisely as possible. The AE recorded should not be a procedure or a clinical/laboratory measurement but should reflect the event leading to the procedure or the cause of the clinical/laboratory abnormality, if known. Whenever possible, AEs should be evaluated and recorded as a diagnosis, rather than individual signs and symptoms. However, if a definitive diagnosis is not possible, the individual signs and symptoms should be recorded. Any AE that worsens in intensity, or becomes serious, should be recorded as a new event.

7.4. Evaluating Adverse Events

All AEs will be graded according to the “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials”. If the reported adverse event term is not present in the above grading scale, then it should be graded as mild, moderate, severe, or life threatening based upon the investigator’s clinical judgment.

7.5. Assessment of Causality

Investigators are required to assess the relationship, if any, of each AE or SAE to the investigational drug using clinical judgment to determine the degree of certainty with which an AE can be attributed to the investigational drug. Alternative causes, such as the natural history relating to any suspected underlying disease, other risk factors, and the temporal relationship of the event to the administration of the study medication must be considered.

Relationship to study drug is summarized as follows:

- **Not Related:** There is another obvious cause of the AE
- **Unlikely to be related:** There is another more likely cause of the AE
- **Possibly related:** The AE could have been due to the investigational drug
- **Probably related:** The AE is probably attributable to the investigational drug
- **Definitely related:** The AE is most likely attributable to the investigational drug

7.6. Reporting Requirements

Any grade 3 or greater adverse event and injection site reaction must be reported to the sponsor within 24 hours of learning of the event.

All serious adverse events (SAEs) should be reported to the Sponsor on the initial SAE report forms within 24 hours of knowledge of the event. These immediate reports should be followed promptly by detailed, written reports (follow-up SAE reports). The subject should be followed up until stabilization of the reported SAE, either with full satisfactory resolution or resolution with sequelae, or until death of the subject. Before declaring the subject is lost to follow-up, three unsuccessful attempts at contact should be made and recorded on the SAE form. The immediate and follow-up reports should identify subjects by unique code numbers (generated by the safety database) assigned to the trial subjects, rather than by the subjects' names, personal identification numbers, and/or addresses. The Investigator should also submit SAEs to the IRB/EC according to their IRB/EC guidelines [ICH-GCP E6]. Drug-related Serious Adverse Events will be reported to the FDA by XBiotech's Medical Safety Officer according to 21 CFR 312.32.

7.7. Reference Safety Information: Potential Adverse Reactions

More than 1,100 subjects have been treated using a previous anti-IL-1α antibody in subjects with advanced solid tumors, advanced hematologic malignancies, metastatic colorectal cancer, peripheral vascular disease, type II diabetes, acne vulgaris, plaque psoriasis, pyoderma gangrenosum, atopic dermatitis and hidradenitis suppurativa. Over 2,000 doses (N=309) of previous anti-IL-1α antibody were administered at 7.5 mg/kg to refractory, metastatic colorectal cancer (CRC) subjects with cancer associated symptoms at baseline (ECOG performance status 1 and 2). In this study, the most common AEs reported (>10%) were abdominal pain, peripheral edema, fatigue, anemia, constipation, decrease in weight, asthenia, decreased appetite, and nausea. The majority of these events were grade 1 or 2 and appeared to be related to the underlying CRC. The prevalence of these events was similar in the treatment and placebo groups.

XB2001 is a new recombinant human IgG4 monoclonal antibody specific for human interleukin-1a (IL-1a). As such, it is an immunomodulator that has anti-inflammatory and anti-neoplastic properties. Other agents that could be considered in the same pharmacologic class include biologic agents such as IL-1 receptor antagonist and IL-1β specific antibodies. Potential risks for agents in this class include infusion or injection site reactions, and risk of infection.

XB2001 is considered to be a True Human monoclonal antibody. Unlike previous generations of humanized or fully human antibodies, the entire XB2001 heavy and light chain sequences are identical to those found in naturally-occurring human IgG4k, with the light and heavy chain variable regions being identical to those originally expressed by a peripheral blood B lymphocyte that was obtained from a healthy individual. No *in vitro* affinity maturation or modifications have been made to improve its natural binding affinity (~50 pM). We believe that a true human antibody should be effectively non-immunogenic in humans and thus exhibit optimal activity and pharmacokinetics.

The mechanism behind infusion or injection site reactions associated with monoclonal biologics is not clear in all cases. In most cases, it is a reaction against the antibody products themselves, which are derived from murine sequences or hosts. Reactions may in some cases involve minor residual components from the manufacturing process (i.e. antibody is produced from cell culture, and there is extremely small but inevitable amount of residual cellular material that is not completely eliminated during purification of the product). A previous anti-human IL-1α antibody developed and manufactured by the current Sponsor, was administered to over 1100 and 13,000 doses without a single infusion or injection site-related SAE. Nevertheless, in order to mitigate risk associated historically with monoclonal antibody dosing, monitoring is encouraged for at least 1 hour after the end of the initial Natrunix™ injection. Availability of resuscitation equipment must be present. Pre-medication with antihistamines or corticosteroids is not required.

For the purposes of expedited safety reporting in clinical trials, the following should be considered expected events:

- Infusion Related Reactions
- Injection Site Reactions

8. STUDY MANAGEMENT AND ADMINISTRATION

8.1. Ethical Conduct of Study (GCP)

The guidelines of the World Medical Association Declaration of Helsinki in its revised edition (64th WMA General Assembly, Fortaleza, Brazil, October 2013), the guidelines of ICH GCP E6 (R2), as well as the demands of national drug and data protection laws and other applicable regulatory requirements, will be strictly followed. Approval will be obtained from the appropriate regulatory authorities before sites are initiated.

8.2. IRB and Ethics committee Approval

Prior to initiation of the study, the protocol, the informed consent form, the subject information sheet(s), details of the subject recruitment procedures and any other relevant study documentation will be submitted to the responsible IRB or Ethics Committee (EC). The Investigator will report promptly to the IRB/EC any new information that may adversely affect the safety of subjects or the conduct of the study. Similarly, the Investigator will submit written summaries of the study status to the IRB/EC annually, or more frequently if requested by the IRB/EC. Upon completion of the study, the Investigator will provide the IRB/EC with a brief report of the outcome of the study, if required.

8.3. Protocol Modifications

Modifications of the signed protocol are only possible by approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The IRB/EC must be informed of all protocol amendments and should be asked for its opinion as to whether a full re-evaluation of the ethical aspects of the study is necessary by the committee. This should be fully documented. The Investigator must not implement any deviation from or change to the protocol, without discussion with an agreement by the study Sponsor and prior review and documented approval/favorable opinion of the amendment from the relevant IRB/EC, except where it is necessary to eliminate an immediate hazard to study subjects, or where the change(s) involves only logistical or administrative aspects of the study (e.g., change in CRA(s), change of telephone number(s)). Protocol

amendments will be submitted to the appropriate authority(ies) as required by the applicable regulatory requirement(s).

8.4. Subject Information and Consent

The Investigator is responsible for ensuring that no subject will receive any study-related examination or activity before that subject has given an IRB/EC approved informed consent. The subject must give written consent after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided for the subject. The Investigator will inform the subject of the aims, methods, anticipated benefits and potential hazards of the study, including any discomfort it may entail. The subject must be given every opportunity to clarify any points he/she does not understand and if necessary, ask for more information. At the end of the interview, the subject may be given time to reflect if needed, or if the subject requests more time. Subjects and/or legal guardian forms will be kept and archived by the Investigator in the Investigator's study file. It should be emphasized that the subject is at liberty to withdraw their consent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give, or withdraw, written informed consent may not be included or continued in this study, but this will not impact their subsequent care. The Investigator will notify in writing each subject's primary care physician (or equivalent) of the subject's intent to participate in the study.

8.5. Data Protection and Confidentiality

By signing the final protocol, every participating Investigator agrees to keep all information and results concerning the study and the investigational product confidential. The confidentiality obligation applies to all personnel involved at the investigational site. The Investigator must ensure that each participant's anonymity will be maintained in accordance with applicable laws. On eCRFs or other documents submitted to the Sponsor, subjects should not be identified by their name, but by subject ID number. The Investigator should keep a separate log of ID numbers, names and addresses. Documents that contain the names associated with these ID numbers (e.g., written consent/assent forms), are not for submission to the Sponsor and should be maintained by the Investigator in strict confidence except to the extent necessary to allow auditing by regulatory authorities, auditing or monitoring by the Institutional Review Board/EC, the Sponsor personnel or their affiliates and designees (such as CRAs).

Copies of radiological scans and autopsy reports (and other documents), if applicable, that may be requested by the Sponsor should be de-identified. The Investigator shall obtain all such permissions and authorizations as may be necessary or desirable to allow the collection and use of information protected under federal privacy laws and state privacy laws, including permission/authorization for monitoring and analysis (including re-analysis in combination with results of other studies), for regulatory submission purposes and for applicable reporting (if any) required to be made by Sponsor, its affiliates and their designee.

8.6. Study Report and Publications

A final integrated clinical/statistical report will be prepared that is compliant with the ICH Harmonized Tripartite Guideline: Structure and Content of Clinical Study Reports (E3). The results of this study will be published and/or presented at scientific meetings in a timely manner. The publication policy is described in the contract between the Sponsor and Investigator.

8.7. Study Files and Retention of Records

Copies of all study documents should be retained by the Investigator until at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product, in accordance with 21 CFR 312.62. These documents should be retained for a longer period however, if required by regulatory requirements or by agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained. The final database will be archived according to the regulatory requirements.

8.8. Case Report Forms

Data for this protocol will be captured electronically in an Electronic Data Capture (EDC) system. Designated study personnel will be provided unique usernames and passwords. Each study personnel will have specific access within the electronic data collection system based on their role. The EDC system contains an audit trail associated with each individual's unique password that will document date and time of data entry and revisions. All protocol-specified data is to be entered into the EDC system in a timely manner for review and audit by XBiotech. All data is to be entered such that it will allow accurate interpretation and tabulation. It is the Investigator's responsibility to ensure that all discontinued orders or changes in study or other medications entered into the database correspond to entries in the subject's medical records (i.e. source documents) and to acknowledge accurate completion of the eCRF.

8.9. Drug Accountability

IMP accountability and chain of custody logs at every site for each randomized subject (per visit) must be kept current and should contain the following information:

- Subject identification number
- PI name and site information
- Study protocol details
- IMP preparation, unit type, date(s) and time(s) of administration
- Quantity of used and unused IMP with lot number(s) and/or with unique identifiers
- IMP disposition related to subject treatment
- IMP destroyed per site’s destruction process
- IMP returned/prepared for return to sponsor/depot

The Investigator (or pharmacist, as appropriate) must maintain records of the delivery of the study medication to the study site, the inventory at the site, the usage for each subject, and destruction. The inventory must be available for monitoring, auditing or inspection.

8.10. Inspections

Investigator sites, the study database and study documentation may be subject to quality assurance audits during the course of the study either by the Sponsor or their appointed representatives. In addition, regulatory bodies at their discretion may conduct inspections. The Investigator shall permit the authorized Sponsor, agents of the Sponsor, and regulatory agency employees to enter and inspect any site where the drug or records pertaining to the drug are held, and to inspect all records relating to an investigation, including subject records. The Sponsor will not, however, copy any source data from the patient’s dossier. Completed eCRFs

must be made available by the Investigator for review by the Sponsor, agents of the Sponsor, the CRA and the regulatory agencies. To ensure the accuracy of data submitted, it is mandatory that representatives of the Sponsor and of the regulatory agencies have direct access to source documents (e.g., subject medical records, charts, laboratory reports, etc.). Subject confidentiality will be protected at all times.

8.11. Access to Information for Monitoring

CRAs will establish and maintain regular contact between the Investigator and the Sponsor. CRAs will evaluate the competence the study center, informing the Sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, CRAs will check that written informed consent has been obtained from all subjects correctly and that data are recorded correctly and completely. CRAs are also entitled to compare entries in eCRFs with corresponding source data and to inform the Investigator of any errors or omissions. CRAs will also monitor adherence to the protocol at the Investigator site. They will arrange for the supply of investigational product and ensure appropriate storage conditions are maintained. The CRA will make written reports to the Sponsor on each occasion when contact with the Investigator is made, regardless of whether it is by phone or in person. During monitoring visits, entries in the eCRFs will be compared with the original source documents. The Investigator must agree to meet with the CRA at regular intervals and to cooperate in resolving any queries or findings made during the monitoring process.

9. STATISTICAL ANALYSIS PLAN

Missing Data: Every effort will be made to identify and recover the missing data from the study centers. If the data remained missing following such efforts, the maximum likelihood method will be implemented while characterizing the pK parameters.

Outliers: No formal outlier tests are planned. Normally distributed values that are outside 3 standard deviations from the mean will be queried and verified with source prior to database lock.

Evaluation of Pharmacokinetic Data: All patients receiving the drug will be included in the analysis. PK analyses will be conducted to evaluate XB2001 concentration-time data to determine PK parameters. The parameter estimates will include, but may not be limited to, Cmax, Tmax, AUC, and half-life ($t_{1/2}$).

Analysis of Safety: All subjects who receive study drug will be included in safety analysis. Safety analysis will be based on adverse events, vital signs, physical examinations, and clinical laboratory measurements. Adverse events will be grouped by System Organ Class (SOC) and Preferred Term (PT) within SOC according to the current version of MedDRA coding dictionary.

Analysis Method: Study data will be presented in subject level listings. Statistical analyses will be descriptive only. There will be no hypothesis testing. Analyses will be performed using SAS for Windows SAS 9.4 (SAS Institute Inc., Cary, NC) and other pk analysis (curve fitting) software (e.g., Thermofisher Kinetica). Descriptive statistics will be presented for continuous variables, and frequency and percentage will be presented for categorical and ordinal variables.

10. REFERENCES

[1] Miossec. Anti-interleukin 1α autoantibodies. Ann Rheum Dis 2002; 61: 577-579.

[2] Gallay et al. Characterization and detection of naturally occurring antibodies against IL-1α and IL-1β in normal human plasma. Eur Cytokine Network 1991; 2: 329-338.

[3] Müller et al. Autoantibodies to IL-1α in sera from umbilical cords, children, and adults, and from subjects with juvenile chronic arthritis. Scand J Rheumatol. 1996; 25 (3): 164-7.