STUDY SYNOPSIS

Study Title	A pre-market, multi-center, international, open-label, single-arm study to evaluate the safety and performance of a Class III medical device (GreenBone Implant) for surgical repair of long bone defects
Study Code	GB-02-18
Study Sponsor	GreenBone Ortho S.r.l. Via Albert Einstein, 8 48018 Faenza (RA) – Italy
Clinical Phase	Pre-market clinical investigation of a Class III Investigational Medical Device.
Study Design	Multi-center, international, prospective, open-label, single-arm, first-in-human clinical investigation. The Patients enrolled in this clinical investigation will undergo a scheduled surgery for the treatment of long bone defects up to 6 cm using GreenBone Implant. After the surgery, the Patients will be monitored at pre-scheduled visits up to 12 months. Adverse events, pain, quality of life and functional parameters, as well as X-ray and CT scan, will be evaluated at scheduled follow-up visits. An independent DSMB will review the safety reports at regular intervals and Serious Adverse Events (SAE) as soon as reported, to protect Patients participating in the study. The initial phase of the study contemplates the treatment of bone defects up to 3 cm. An adaptive interim analysis will be performed when the first 7 Patients will have completed the 6-month follow-up visit. The DSMB will review the results of the interim analysis with respect to the primary endpoint (safety), and provide one of the following recommendations to the Sponsor: a) to stop the study for unacceptable frequency and severity of adverse events or b) to continue the study up to 25 Patients recruited and to include at least 5 Patients with a longer bone defect (> 3 cm up to 6 cm).
Investigational Medical Device	GreenBone Implant for bone substitution, a Class III Medical Device. The GreenBone Implant had not yet received the CE mark at the time of the Study first submissions and first patient enrollment. The Device received the CE mark in December 2019.
Investigational Medical Device Use	GreenBone Implant is used in the surgical procedures for repair of bones defects.
Investigational Medical Device Description	GreenBone Implant is a ceramic reabsorbable scaffold with a geometrical structure, engineered to reflect anatomical and physiological bone hierarchical structures. GreenBone Implant scaffold is made of biomimetic substituted calcium phosphate phases (HA and β-TCP and minerals). It is suitable for repair of defects in bones. GreenBone Implant is a EU class III Medical Device.

Clinical Investigation Rationale	Bone defects can result from malformation, high-energy traumatic events, bone resection due to different pathologies such as tumors or infections, or from the treatment of complex non-unions, all very challenging conditions in orthopedic practice. Current treatment of bone defects usually implies the use of bone grafts and/or biocompatible materials, to create a scaffold that bridges the defect, favoring the migration of cells from the neighboring tissues to fill the gap. Autograft and Allograft are the treatments most currently used for large bone loss, but both treatments have significant disadvantages. Different types of synthetic materials have been tested for repair of long bone gaps. However, at the moment, there is not any bone substitute with the ideal/desirable osteoinductive, osteoconductive and mechanical properties. GreenBone Implant is a synthetic, acellular, reabsorbable, new generation bone graft, being suitable for surgical reconstruction of bone defects.
Study Objectives	The primary objective of this study is to assess the safety profile of the GreenBone Implant in Patients undergoing surgery for the treatment of bone defects up to 6 cm, specifically for long bone repair. The initial phase of the study contemplates the treatment of bone defects up to 3 cm. Following DSMB interim assessment, at least 5 Patients, of the 25 patients, with a longer bone defect (> 3 up to 6 cm) will be enrolled. The secondary objectives of this study are: - To evaluate the progression of bone healing and bone regeneration at the level of the intervention site after use of GreenBone Implant throughout a 12-month observation period; - To evaluate the degree of pain throughout a 12-month observation period; - To evaluate the functional recovery of Patient throughout a 12-month observation period. - To evaluate the health related quality of life of Patient throughout a 12-month observation period.
Implant of the Investigational Medical Device	GreenBone Implant is applied to the bone gap by the surgeon during the scheduled intervention, according to standard clinical practice and to the information provided by GreenBone Ortho S.r.l. in the device Instructions For Use (IFU) document.
Study Population	Male and female Patients, aged between 18 and 65 years, undergoing surgery for the treatment of long bone defects up to 6 cm. *Please note:* The initial phase of the study contemplates the treatment of bone defects up to 3 cm. After an adaptive interim analysis review by the DSMB, inclusion criteria will be amended; specifically to include the recruitment of patients suffering from bone defects up to 6 cm.

Study Sample Size	In the absence of published guidance on first-in-human trials on long bone surgery, the sample size (n=25 including a 20% drop-out rate) has been chosen to be consistent with first-in-human studies of pharmacological agents which are usually conducted in 10-15 patients (*Rubinstein LV et al 2010*).
Inclusion Criteria	1. Male or female Patients. 2. Patients aged ≥ 18 and ≤ 65 years old. 3. Patients requiring long bone defect reconstructions up to 3 cm, resulting from high-energy traumatic events, or from the treatment of complex bone non-unions in the extremities (arms and legs), or patients undergoing the second stage of the Masquelet technique. 4. Patients understanding the nature of the study and providing their informed consent to participation. 5. Patients willing and able to attend the follow-up visits and procedures foreseen by study protocol. *Please note*: after interim analysis review by the DSMB, inclusion criteria n. 3 will be modified as follows (all Investigators will be notified by the Sponsor, and adequate training by CRA will be conducted): *Change of inclusion criteria n. 3:* Patients requiring long bone defect reconstructions up to 6 cm, resulting from high-energy traumatic events, or from the treatment of complex bone non-unions in the extremities (arms and legs), or patients undergoing the second stage of the Masquelet technique.
Exclusion Criteria	For the specific characteristics of this study, the final decision on Patient enrolment will be made by the Investigator at time of surgery (during surgery, before implantation). 1. Patients with bone infection at the time of enrolment. 2. Patients with bone malignant tumor(s) at the time of enrolment. 3. Patients who have been treated with chemotherapy or radiotherapy within 12 months before the enrolment. 4. Patients with concomitant infectious systemic diseases at the time of enrolment. 5. Patients with known inflammatory systemic diseases at the time of enrolment. 6. Patients with concomitant myeloproliferative disorders at the time of enrolment. 7. Patients currently treated with systemic immunosuppressive agents, excluding steroids. 8. Patients with active autoimmune disease. 9. Patients with a pre-existing calcium metabolism disorder (i.e. hypercalcemia). 10. Known hyperthyroidism or autonomous thyroid adenoma. 11. Patients with coagulopathy or bleeding disorders.

<table>
<tr><td></td><td colspan="3">12. Patients who are not allowed to undergo the study procedures involving imaging (X-rays, CT scan) based on Investigator's judgement.
13. Patients with known or suspected allergy or hypersensitivity to the GreenBone Implant components.
14. Patients who are participating or have participated in other clinical studies within the 30 days before the study enrolment.
15. Patients identified by the Investigator to have intra-operative findings that may preclude conduct of study procedure.
16. Patients with occurrence of major intra-operative complications that require resuscitation or deviation from the planned surgical procedure.
17. Women who are pregnant or breast-feeding or who wish to become pregnant during the period of the clinical investigation and for three months later.
18. Female Patients of childbearing age (less than 24 months after the last menstrual cycle) who do not use adequate contraception *.

* Methods at low risk of contraceptive failure (less than 1% per year) when used consistently, including: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), some intra-uterine devices.

Please note: exclusion criteria after interim analysis will remain the same.</td></tr>
<tr><td>Not Allowed Concomitant Medications</td><td colspan="3">Systemic immunosuppressive agents, excluding steroids.</td></tr>
<tr><td rowspan="2">Study Flow Chart</td><td>Visit</td><td>Procedures</td><td>Day</td></tr>
<tr><td>Visit 1
(V1 – Screening)</td><td>• Eligibility criteria evaluation
• Demography / medical history
• Informed consent collection
• Patient enrolment
• Standard preoperative lab assessment
• Vital signs
• Physical examination
• ECG
• X-Ray examination
• CT scan examination (if necessary)
• Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire)
• VAS score (0-10 point scale)
• Functional assessment (36-Item Short Form Survey – SF-36)
• Concomitant medications
• Pregnancy test (for female Patients of childbearing age)</td><td>Up to 7 days before V2</td></tr>
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	Visit 2 (V2 – Surgery)	Before surgery: • Informed Consent confirmation by the Patient During and after surgery: • Device implantation: application of the GreenBone Implant at the target site • Evaluation of technical success • Concomitant medications • Adverse events • Provide Implant Card to each patient • Return of discarded devices (if any) and completion of the report of Investigational Medical Device Deficiency	D 0
	Visit 3 (V3 – Control 1)	• Physical examination of the operated limb • X-Ray examination • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +30 ±3 (1M)
	Visit 4 (V4 – Control 2)	• Physical examination of the operated limb • X-Ray examination • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +60 ±5 (2M)

	Visit 5 (V5 – Control 3)	• Physical examination of the operated limb • X-Ray examination • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +90 ±7 (3M)
	Visit 6 (V6 – Control 4)	• X-Ray examination • CT scan examination (if healing activity inconclusive on radiographs) • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +180 ±14 (6M)
	Visit 7 (V7 – Control 5)	• X-Ray examination • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +270 ±14 (9M)
	Visit 8 (V8 – Control 6)	• X-Ray examination • CT scan examination (if healing activity inconclusive on radiographs) • Evaluation of global tolerability of Patient (by the Investigator) • Quality of Life (EuroQoL EQ-5D-5L Validated Questionnaire) • VAS score (0-10 point scale) • Functional assessment (36-Item Short Form Survey – SF-36) • Concomitant medications • Adverse events	D +360 ±14 (12M)

	Should any of the Patients request at any time an additional visit (i.e. in case of discomfort in the area of intervention), an additional visit should be organized to allow examination by the Investigator.
Study Endpoints	**Primary Endpoint:** Evaluation of adverse events rate during the study period. All adverse occurrences (serious/non-serious or device-related/non device-related) will be recorded prospectively, categorized and evaluated for causality using defined criteria. The study will be monitored by an independent Data Safety Monitoring Board (DSMB).
Study Endpoints	**Secondary Endpoints:** 1. Evaluation of technical success, defined as successful delivery of the GreenBone Implant in the target defect bone evaluated at V2 (during surgical procedure). 2. Evaluation of the bone regeneration in the area of intervention in comparison to baseline (V1), by means of X-ray examination, on visits V3, V4, V5, V6, V7 and V8. X-ray scoring systems have been implemented according to the criteria proposed by Lane and Sandhu (bone formation, union and remodeling) (*Lane 1987*) (See Appendix 1). 3. Evaluation of the bone regeneration in the area of intervention in comparison to baseline (V1), by means of CT scan examination on visits V6 and V8 if indicated. 4. Functional Assessment: evaluation of functional recovery of Patient, by means of a standardized questionnaire (36-Item Short Form Survey – SF-36), on visits V1, V3, V4, V5, V6, V7 and V8 (See Appendix 2). 5. Evaluation of degree of pain perceived by the Patient, by means of a VAS (0-10 point scale), on visits V1, V3, V4, V5, V6, V7 and V8 (*Price 1983*) (See Appendix 3). 6. Evaluation of Quality-of-Life by means of EuroQoL EQ-5D-5L Validated Questionnaire, on visits V1, V3, V4, V5, V6, V7 and V8 (See Appendix 4). 7. Evaluation of global tolerability to treatment of Patient (by the Investigator), through a standardized questionnaire, on visits V3, V4, V5, V6, V7 and V8 (See Appendix 5). 8. Evaluation of the number of re-interventions occurring for the Patient throughout the 12-month observation period.

Statistical Analysis	A descriptive analysis of safety and performance variables will be performed. The descriptive analyses will be performed in order to describe the nature of adverse events, their distribution and frequency. Moreover, all factors which may appear predictive for the rate of adverse events will be analyzed (age, sex, dimension of the implanted device, system used, diabetes history, smoking habits, alcohol consumption, concomitant treatments/diseases, other). If the percentage of Patients with device-related AE would be sufficiently large in respect to the overall sample (≥ 70%) to allow an evaluation of the time interval between the date of surgery and the date of the first adverse event occurence, a median of the time interval will be estimated, with 95% Confidence Interval, through the Kaplan-Meier survival analysis. An adaptive interim analysis will be performed when the first 7 Patients will have completed the 6-month follow-up visit. An independent Data Safety Monitoring Board (DSMB) will review the results of the interim analysis with respect to the primary endpoint (safety), and provide one of the following recommendations to the Sponsor: a) to stop the study for unacceptable frequency and severity of adverse events or b) to continue the study up to a minimum of 25 Patients recruited and to include at least 5 Patients with a longer bone defect (> 3 up to 6 cm). The independent DSMB will receive all the safety data at regular intervals and the data about Serious Adverse Events (SAE) as soon as reported, to protect Patients participating in the study.
Investigational Sites	The study will involve 8 investigational sites in Bosnia & Herzegovina, Israel, Italy, Serbia, Slovenia, Switzerland and UK.