Appendix F

CLINICAL PROTOCOL

A PHASE I/IIA SAFETY AND EFFICACY STUDY OF A SYNTHETIC BIOACTIVE BONE GRAFT DEVICE FOR CRANIAL BONE REGENERATION IN ADULTS

Protocol Number:	xxxxx
Phase of Study:	Phase I/IIa
IDE Number:	xxxxx
Investigational Product:	Plasmid DNA (pln.247-pBI, 200 μg/ml) for transient expression of human perlecan domain 1 with 100 μg/ml rhBMP-2 in a gelatin/tricalcium phosphate scaffold for cranial bone regeneration (B-247 Cranial Bone Graft Implant).
Protocol Issue Date:	xx/xx/xxxx

Sponsor: Agenta Biotechnologies, Inc.
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Investigator: Melissa R. Chambers, M.D.
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Birmingham, Alabama 35294

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INVESTIGATOR AGREEMENT

I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol. Deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment. I agree to conduct the study in accordance with generally accepted standards of good clinical practice. I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in Section 10.2 of this protocol. I also agree to handle all clinical supplies provided by the sponsor and collect and handle all clinical specimens in accordance with the protocol. I understand that information that identifies me will be used and disclosed as described in the protocol, and that such information may be transferred to countries that do not have laws protecting such information.

_____ _____

Melissa R. Chambers, M.D. Date
Principle Investigator,

1. STUDY CONTACTS

The Clinical Trial is sponsored by Agenta Biotechnologies, Inc. (Agenta). Clinical monitoring and study management will be performed by representatives from the organizations listed in the table below.

Table 1: Investigators and Study Administrative Structure

Medical Monitor and SAE Contact	Donald Marks, M.D. Cooper Green Mercy Hospital Dept of Medicine 1515 6th Ave South Birmingham, AL 35233	Cell phone: 205-283-1688 FAX: 603-372-4813 e-mail: extant4@hotmail.com
Principal Investigator	Melissa R. Chambers, M.D. Division of Neurological Surgery 1034 Faculty Officer Tower 510 20th Street South Birmingham, Alabama 35294	Phone: (205) 532-4669 e-mail: mrchambers@uabmc.edu
Project Management and Clinical Study Monitoring	Arthur DeCarlo, DDS, Ph.D. CSO, Agenta Inc. 1500 1st Avenue North, Suite L105 Birmingham, AL 35203	Phone: (205) 307-6500, Fax: (205) 307-6501 e-mail: decarlo@agentabio.com
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2. PROTOCOL SYNOPSIS

Protocol Title: A Phase I/IIa safety and efficacy study of a synthetic bioactive bone graft device for cranial bone regeneration in adults

Protocol Number:

Investigational Product: Plasmid DNA (pln.247-pBI, 200 μg/ml) for transient expression of human perlecan domain 1 with 100 μg/ml rhBMP-2 in a gelatin/tricalcium phosphate scaffold for cranial bone regeneration (B-247 Cranial Bone Graft Implant).

Placebo (Vehicle): none

Sponsor: Agenta Biotechnologies, Inc.
1500 1st Avenue North, Suite L105
Birmingham, AL 35203

Study Site: UAB Health System
Birmingham, Alabama

Investigator: Melissa R. Chambers, M.D.
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Name of IRB: Western IRB
3535 Seventh Ave SW
Olympia, WA 98502-5010

Proposed Start Date: Quarter 3, 2013

Study Duration: For each subject, from day of device implant to final clinic visit at 1 year, with follow-up through 2 years.

Study Objectives: The objectives of this study are as follows:

- To evaluate the safety of a B-247 Cranial Bone Graft Implant
- To evaluate bone regeneration associated with placement of the B-247 Cranial Bone Graft Implant.

Study Design: Single center, single dose study unblinded safety and efficacy trial.

Number of Subjects: 12 total subjects, one group

	Number of Subjects	Dose Level
Group 1	12	200 μg/ml pln.247-pBI + 100 μg/ml rhBMP-2

Study Population: Trauma and Surgical Craniotomy patients, male, 19 to 59 years of age

Route of Administration: Localized direct surgical placement of investigational product in cranial defect

Dose and Schedule: Subjects will receive investigational product at time of surgery.

General Study Conduct: Subjects who consent to participate in the trial will be screened for eligibility immediately. Baseline data, including pre-operative CT images, will be made prior to surgery. Subjects and investigators will be unblinded to the treatment.

1) in-hospital surveillance for the first 7 days after administration of investigational product;
2) in-clinic physical assessments on Days 0, 3, 7, 42, 180, and 360;
3) lab testing at Days 0, 7 and 42;
4) solicited adverse events (AEs) recorded on diary cards for 1 years after administration; and during phone contact at 2 yr. follow-up.
5) collection of unsolicited adverse events during active follow up to Day 360 and during phone contact at 2 year follow-up.

Subjects will receive compensation for their time and effort at the completion of each clinic visit beginning with the 6 week follow-up.

Data and Safety Monitoring: The first three subjects will be enrolled at a rate of no more than one subject per week. After review of the 7 day safety data for each of the first 3 subjects, the Safety Review Committee (SRC) will recommend either to open enrollment for the remaining subjects or to discontinue enrollment. The SRC will review the occurrence of AEs weekly, with 24 hour reporting for SAEs. Stopping rules will be defined in the protocol.

Monitored Parameters:

Safety:

- Hematology, blood chemistry, urinalysis
- Vital signs and physical assessments
- Unsolicited and solicited adverse events (clinic visits, diary cards and telephone contacts)
- Follow-up safety surveillance through 2 years

Bone Regeneration:

- CT analysis

Endpoints:

Safety:

- Incidence of local adverse events including ectopic bone formation if any
- Incidence of systemic adverse events
- Incidence of changes to laboratory parameters relative to baseline values

Bone Regeneration:

- The proportion of subjects at Day 42 and 180 and 360 with evidence of mineralized bone regeneration at the site of the original cranial defect relative to baseline (Day 0)
- The percentage of bone regeneration in the original cranial defect at Day 42 and 180 and 360 relative to 0% at baseline (Day 0)

3. SCHEDULE OF EVALUATIONS

Visit Number	**Screen**	**Visit 1**	**Contacts 1 and 2**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Contact 3**
Trial Timeline	**Day -21 to 0**	**Day 0**	**Days 1 and 2[1]**	**Day 3**	**Day 7**	**Day 42**	**Day 180[1]**	**Day 360[1]**	**Days 720[1]**
Visit/Contact Window (Days)	**N/A**	**N/A**	**+/- 0**	**+/- 1**	**+/- 1**	**+/- 4**	**+/- 28**	**+/- 28**	**+/- 28**
Informed Consent	X								
Inclusion/Exclusion	X	X							
Medical History	X								
Interim Medical History		X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	
Physical Examination	X								
HIV and Illicit DrugTest	X								
Physical Assessment: Vital Signs[2]/Node Check		X		X	X	X	X	X	
Safety Labs (CBC, Blood Chemistry, PT, PTT, Urinalysis)[2,3]	X	X			X	X			
Surgical Treatment		X							
Surveillance (30 minutes)		X							
CT	X	X				X	X	X	
Diary Cards Distributed/ Collected		X			X	X	X	X	X
Unsolicited Adverse Events		X	X	X	X	X	X	X	X

[1] Subjects will be contacted at Days 1 and 2, Days 42 and at 6 months, 1 year, and 2 years with a scripted telephone contact for safety follow-up and scheduling.

[2] Vital signs and laboratory values will be evaluated using the appropriate FDA draft guidance.

[3] Any abnormal laboratory result arising post-administration, and of moderate or greater severity, will be followed until results return to baseline value or for the duration of safety follow-up.

4. LIST OF ABBREVIATIONS

°C	Degrees Celsius
°F	Degrees Fahrenheit
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	According to protocol
BUN	Blood urea nitrogen
CBC	Complete blood count
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CI	Confidence interval
CPE	Cytopathic effects
CPK	Creatine phosphokinase
CRF	Case Report Form
DIC	Disseminated intravascular coagulation
dL	Deciliter
DNA	Deoxyribonucleic acid
ER	Emergency room
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
ICH	International Committee on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intention to treat
IV	Intravenously
MedDRA	Medical Dictionary for Regulatory Activities
mEq	Milliequivalents
mL	Milliliter
mm	Millimeter
mM	Millimolar
N/A	Not applicable
ND	Not determined
NP	Nasopharyngeal
OTC	Over the counter
PCR	Polymerase chain reaction
PIN	Participant identification number
PRBC	Packed red blood cells
PT	Prothrombin time
PTT	Partial thromboplastin time
rbc/hpf	Red blood cells per high power field
SAE	Serious adverse event
SOP	Standard operating procedure
SRC	Safety Review Committee
ULN	Upper limit of the normal range
v/v	Volume by volume
WBC	White blood cell
WHO	World Health Organization
w/v	Weight by volume
mg	microgram
mL	microliter

5. INTRODUCTION

5.1 Investigational Product Description

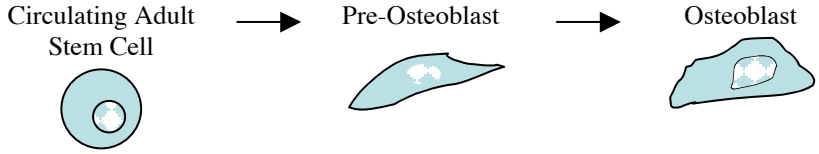
The B-247 Cranial Bone Graft Implant is an experimental device which consists of plasmid DNA (pln.247-pBI, 200 μg/ml of implant volume) for transient expression of human perlecan domain 1 with 100 μg/ml rhBMP-2 in a gelatin/tricalcium phosphate scaffold for cranial bone regeneration. The product is provided as a pliable solid single-phase unit consisting of 80% bioactivated tricalcium phosphate (TCP) crystals 200-600 μm particle size, evenly distributed within a 20% cross-linked gelatin polymer. The implant is bioactivated by incorporating plasmid DNA lipoplexes and rhBMP-2 in 1% sucrose excipient, then lyophilizing the loaded implant to a final concentration of 200 μg/ml of implant of pln.247-pBI plasmid and 100 μg/ml of implant of rhBMP-2. Plasmid lipoplexes are derived from incubating pln.247-pBI plasmid with Lipofectamine 2000 in a 2:1 ratio for 30 minutes. Sucrose, plasmid, rhBMP-2, Lipofectamine 2000, TCP crystals, and gelatin are each manufactured separately according to cGMP and combined under sterile conditions in a clean room.

- rhBMP-2 is marketed in the US as a component of the InFuse™ device kit by Medtronic, Inc. This kit was approved under PMA P050053 on 03/13/2007. The rhBMP-2 is manufactured in a human CHO cell line.
- Tricalcium phosphate crystals are marketed in the US as Osteogen by Impladent. This product was approved under 510(k) Premarket Notification on 5/31/1995.
- Sucrose and gelatin are common excipients and are purchased cGMP quality.
- Lipofectamine 2000 is purchased from Life Technologies as a cGMP grade cationic lipid.
- The pln.247-pBI expression plasmid is manufactured by Aldevron in a cGMP manner derived from *E. coli* fermentation.

5.2 Regenerating Bone in Large Critical Size Defects

Mature bone consists of inorganic calcium phosphate (70% wt.), proteins such as collagen and proteoglycans (25% wt.), water (4% wt.) and of course cells, blood vessels, and neurons (1% wt.). Normal bone is constantly remodeling, or turning over at a rate of up to 15-35%/year in some areas. Therefore, the therapeutic approach to bone regeneration must be considered in this light, and, indeed, bone growth, (apposition) is always accompanied by some degree of bone loss (resorption) in a process that is cyclic. It is the balance between bone apposition and resorption that dictates the state of the bone in a given region of the body.

New bone growth is complex and requires bone forming cells called osteoblasts which derive from the surfaces of existing bone or from pleuripotent circulating cells (adult stem cells) that originate in the marrow. The normal adult process of bone apposition occurs on the surfaces of existing bone structure where bone-resorbing cells called osteoclasts remove a layer of bone, and osteoblasts subsequently deposit new bone matrix. The cycle favors osteoblast activity in periods of net apposition.



Regenerating bone in a space where bone surfaces and a source of migrating osteoblasts are absent or distant, such as an attempt to rebuild the jaw to support implants in a patient whose jaw bone has resorbed severely or has been otherwise lost to disease or trauma, one must supplement the site with an osteoinductive graft material. Osteoinduction is the process whereby bone formation is induced beyond the normal bone-regenerating capacity of the site. By seeding bone defects with fresh bone from another source in the body, or by actively promoting 1) migration of pre-osteoblasts and osteoblasts, 2) differentiation (maturation) of the circulating adult stem cells into pre-osteoblasts then osteoblasts, and 3) proliferation (multiplication) of osteoblasts and pre-osteoblasts throughout the wound or graft, new bone can be regenerated before a mature non-mineralizing connective tissue occupies the space.

All three of these processes – migration, differentiation, and proliferation - are enhanced by the activity of a class of proteins called growth factors. Growth factors that are thought to have some role in bone formation include the bone morphogenetic proteins (BMP) 2 and 7, platelet derived growth factor (PDGF), fibroblast growth factor (FGF), insulin growth factor (IGF), and vascular endothelial growth factor (VEGF). Of these and other known growth factors, BMP-2 may hold the most potential for osteoinduction as it is known to promote differentiation of circulating stem cells into pre-osteoblasts and away from other non-bone forming cell types. Other growth factors, such as FGF-2, have a known ability to enhance the proliferation of osteoblasts and pre-osteoblasts, so they too have a potential supporting role in effective osteoinduction.

Augmenting growth factor activity is critical in treating larger bone defects that have an inherently limited potential to regenerate bone because these three processes cannot occur extensively and rapidly enough throughout the larger bone defect. The addition of growth factors alone, however, is not enough.

Bone is not all mineral, as pointed out above, and actually begins as a soft organ consisting of protein aggregates such as the collagens, with other bone-specific proteins, and proteoglycans. As this non-calcified matrix (termed osteoid) is laid down by the osteoblasts, the bone-specific nature of the osteoid allows the gradual accumulation of mineral and hardening of the osteoid into bone.

Proteoglycans were long considered only structural components of tissues such as bone but now are now known to have very important biological activities. Proteoglycans are composed of a protein core, to which is attached long and diverse co-polymeric chains consisting of hexuronic acid and hexosamine sugars. These oligosaccharides are known as glycosaminoglycans (GAGs) and vary in chain length and charge, due to the addition of sulfate groups to various positions on the constituent monosaccharides. The class of heparan sulfate proteoglycans are now being focused on for their role in tissue and cell regulation, particularly angiogenesis, growth factor activation, and wound healing.

Perlecan, originally named heparan sulfate proteoglycan-2, is an important component of all basement membranes and, with three potential sites for heparan sulfate GAG attachment, is an excellent example of a regulatory biomolecule. A body of evidence in the literature exists demonstrating a role for heparan sulfate proteoglycans, and perlecan in particular, in cell adhesion, cell proliferation, cell differentiation, and cell migration, all of which are crucial to osteoinduction.

The B-247 Cranial Bone Graft Implant generates a heparan sulfate proteoglycan that tightly binds FGF-2 or BMP-2 (0.3 nM estimated K_D) and significantly augments BMP activity in an osteoblast mineralization assay (or FGF-2 in a proliferation assay). Classic growth factor/receptor interaction mechanisms suggest that the heparan sulfate chains co-activate growth factors by promoting receptor dimerization through binding interactions between the growth factors and the glycosaminoglycans.

5.3 Advantages of the B-247 Cranial Bone Graft Implant

Bone grafts using bone harvested from elsewhere in the patient (autogenous), while effective, can create lasting and debilitating pain at the site of harvesting, and is naturally limited. Bone graft substitutes are necessary. However, products that use cadaver bone (allografts) have the potential for disease transmission and are not sufficiently effective, only slightly more effective than inert synthetic bone graft materials such as the calcium phosphates, which are used in approximately 30% of cases. Newer products combine growth factors with synthetic bone graft substitutes, constituting an approximately 2-3 billion dollar market (Windhover Information).

The growth factor called platelet derived growth factor, or PDGF, is available for periodontal and related oral surgery procedures in a device kit and is used in approximately 10-20 % of cases, despite its lack of clearly defined superiority.

Another biologically enhanced synthetic bone grafting gel, named Emdogain, is available for periodontal or related oral surgical use. This gel is a complex mix of porcine tissue that also brings the potential for growth factor activity to the bone defect and is also used in approximately 10-20 %of cases for periodontal therapy.

Currently, there are two BMP products on the market. One is marketed by Medtronics through its branded product InFuse®. InFuse® uses BMP-2. Another BMP, BMP-7, is used in OP-1® developed by Stryker. There are, however, inherent problems with the current use of BMPs because dangerously high levels of the growth factor are required for only moderate efficacy (moderate because of the lack of a necessary heparan sulfate co-activator that is the B-247 technology). Only InFuse® is approved for craniofacial use.

Added to the safety issues there is one of cost. The ability to reduce the BMP content in any product, by a factor of as much as thirty, could have a demonstrable affect on usage and profitability. Currently, many

clinicians say that in spite of the relative effectiveness of these products, the price is simply too high. This is especially true in the case of periodontal and oral surgeries where reimbursement can be limited. InFuse® is used in approximately 10% of the craniofacial bone graft cases, though in spinal regeneration, InFuse® is used in more than 25% of the cases.

An additional concern is that InFuse® only allows the placement of a sponge which retains the rhBMP-2 for a very short period of time and has very little structural integrity or adaptability for irregular wounds. The B-247 Cranial Bone Graft Implant overcomes each of these shortcomings. First, the TCP crystals tightly bind and gradually release the pln.247-pBI plasmid and the rhBMP-2 so the risk of local seroma or systemic toxicity within 3 days after placement are reduced. Second, the cross-linked gelatin scaffold retains the crystals so the biologicals remain where placed. Third, the cross-linked gelatin scaffold allows the graft to be molded into the wound and is adaptable to irregular wounds. Fourth, the cross-linked gelatin scaffold is not a putty, as used by Stryker, but is a porous scaffold for immediate cell infiltration.

The clinical objective is to grow new bone where bone has been lost. In doing so, the **first requirement** is that space must be filled with a material (liquid, gel, solid, particles, or porous polymer) that allows/promotes cellular infiltration immediately. The **second requirement** is that the material should retain and protect the biologics (growth factor and co-activator) from pre-mature release in the first few days since the early wound is highly inflammatory. The material should gradually release the majority of biologic load between 4 and 14 days for most effect during the beginning of the regenerative phase.

The B-247 Cranial Bone Graft Implant generates a heparan sulfate proteoglycan that tightly binds FGF-2 or BMP-2 (1 nM estimated kD) and significantly augments BMP activity in an osteoblast mineralization assay (or FGF-2 in a proliferation assay). Classic growth factor/receptor interaction mechanisms suggest that the heparan sulfate chains co-activate growth factors by promoting receptor dimerization through binding interactions between the growth factors and the glycosaminoglycans.

5.4 Advantages of Plasmid DNA Pro-Drug Therapy

Pln.247-pBI is a plasmid DNA pro-drug that, when incorporated by the resident cells at the site of administration, results in the generation of recombinant proteoglycan as an *in situ* therapeutic, with crucial post-translational modifications to the glycosaminoglycan side chains that can be dictated by the wound environment by this method of delivery.

5.5 Rationale for Phase I Study of B-247 Cranial Bone Graft Implant

Agenta has performed a series of *in vitro* experiments clearly demonstrating the ability of the recombinant that is expressed by pln.247-pBI plasmid (rPln.247) to augment growth factor activities.

In vivo Safety Data: doses of *pln.247* from 40 μg/ml to 360 μg/ml graft volume have shown no signs of toxicity, or general morbidity. While TCP stimulated BMP-2 associated intramembranous bone formation that was juxta-proximal to the particles, (see (2)), and new bone that was contiguous with the maxilla, remnants of TCP particles remain embedded within the new bone for at least 12 weeks. The histopathology on >200 maxillary bone defects treated with doses ranging from 40 μg/ml to 360 μg/ml graft volume show no local pathology or acute bone resorption attributable to biologics. The CBC and clinical chemistry safety data showed hematology and clinical chemistry values within normal limits for aging rats.

Conclusion

- pln.247 plasmid more than doubles the dose-effectiveness of BMP-2 osteogenic activity for *in vivo de novo* bone generation
- B-247 formula of TCP, BMP-2, and pln.247 plasmid is superior to OsteoGen TCP bone graft
- B-247 provides more than 5 times as much maxillary ridge augmentation than InFuse™ using ≈1/50th of the BMP-2 dose
- B-247 formula generated new bone with 4 fold greater density than new bone generated by InFuse™.
- B-247 did not cause osteonecrosis in the first 3 weeks post-implant as did InFuse™.

5.5.4 Predicate Data

There is no previous human experience with plasmid DNA augmentation of the perlecan domain 1. There is a large body of data regarding the use of 1.5 mg/ml rhBMP-2 in craniofacial and orthopedic bone regeneration (InFuse®) and for rhBMP-7 (OP-1®). The data suggest that the dose of rhBMP required for the partial clinical efficacy is almost too great and poses a poor risk/benefit ratio. Therefore, the trial of an experimental product such as B-247 Cranial Bone Graft Implant, which has the potential to significantly lower the dose of rhBMP-2 while improving the efficacy with the safe addition of a relatively low level of plasmid DNA would be of great value to the field of medicine.

Subsequent clinical studies will evaluate the safety and efficacy of the B-247 Cranial Bone Graft Implant in direct comparison to other clinically accepted methods for treatment.

6. OBJECTIVES

6.1 Primary Objective

The primary objective is to evaluate the safety of the B-247 Cranial Bone Graft Implant when administered to adults with cranial bone defects in medical need of repair.

6.2 Secondary Objective

The secondary objective is to evaluate the efficacy of the B-247 Cranial Bone Graft Implant in the regeneration of bone in existing cranial bone defects.

7. STUDY DESIGN

7.1 Study Overview

This is a single center, unblinded, safety and efficacy study.

A total of 12 subjects will be enrolled.

All 12 subjects will receive the B-247 Cranial Bone Graft Implant.

Enrollment will begin with 3 subjects enrolled at a rate of no more than one subject per week. The second, and third subjects will only be treated with the experimental device if stopping rules (see Section 14.3) are not met and other safety concerns are absent. Following review of Day 7 safety data for each of the first 3 subjects, the SRC will determine whether to open enrollment to the remaining 9 subjects or to discontinue enrollment. The SRC will review the occurrence of AEs weekly, with 24 hour reporting for SAEs. Stopping rules are described in full in Section 14.3.

Safety will be assessed in all participants. Each participant will be requested to record solicited AEs daily for 2 years after their device placement using study diary cards. Additionally, unsolicited adverse events will be collected immediately following surgical device placement through two years via telephone contacts and at clinic visits. Serious adverse events (SAEs) will be collected throughout the 2 year period of safety surveillance.

Safety evaluation will include hematologic and biochemical toxicity evaluation.

Blood and urine samples will be collected at screening (Day -21 to -1), on Day 0 prior to administration of the device, and again on Days 7, and 42 to measure the effects of device on blood chemistry, urinalysis, and hematology. The urine and blood samples obtained on Day 0 will be used for the baseline comparison.

Subjects will be asked to undergo a physical examination at screening (Day –21 to -1) and a physical assessment including vital signs, lymph node exam, and symptom directed exams at all clinic visits following enrollment (Days 0, 3, 7, 42, 180, and 1 year).

Telephone contacts will occur on the first two days after surgery (Days 1, 2) for study staff follow-up on possible adverse events. In addition, telephone contacts will be made after two years to document the occurrence of SAEs and the resolution of AEs ongoing at the one-year visit.

The overall safety assessment of the device will be based on the incidence of local and systemic reactogenicity, the incidence of changes to laboratory parameters relative to baseline values, and solicited and unsolicited adverse events.

Information will be collected on the osteoinduction associated with the device. A CT scan will be performed on each subject at baseline prior to placement of the device, then again at day 42, day 180, and at one year.

7.2 Trial Center

This trial will be conducted throughout the UAB Health System through the UAB Dept. of Neurosurgery at the University of Alabama, Birmingham under the direction of Melissa R. Chambers, M.D.

7.3 Reference Committees

A Safety Review Committee (SRC) will be utilized throughout this trial. The SRC is composed of the Principle Investigator, Agenta's Medical Monitor, and a third member appointed by the Principle Investigator. The SRC will assess the progress of the clinical trial weekly and will determine whether to continue, modify, or stop the trial at protocol-specified intervals. The safety data review procedure used by the SRC is described in Section 9.3. The SRC is responsible for the clinical evaluation of the safety of the experimental bone graft device based on medical and ethical considerations. A detailed description of the responsibilities of the SRC is provided in Section 14.

8. STUDY POPULATION

8.1 Inclusion Criteria

1. Males, 19 to 59 years of age, in need of cranial bone repair.
2. Subjects must provide written informed consent (attached to grant).
3. Subjects must be willing to participate through study completion.
4. Subjects must be willing to undergo CT examination at day 42, 180, and 360 after surgery for safety and efficacy analyses.
5. Screening Laboratory Inclusion Criteria (normal ranges determined by institutional criteria):

Hematology	CBC (hemoglobin, hematocrit, WBC count, Differential, Platelets)
Chemistry*	ALT, AST, alkaline phosphatase
	Creatinine
	Blood Urea Nitrogen (BUN)
	Sodium
	Potassium
	Chloride
	Bicarbonate
Urinalysis	Urine glucose
	Urine protein

8.2 Exclusion Criteria

1. Female, due to the long-term nature of the study and potential complications or risks pregnancy may introduce.
2. History of metabolic bone disease of any type, including osteoporosis.
3. Any previous cranial surgery
4. Any illicit drug use.
5. HIV positive at screening, due to the long-term nature of the study and potential complications or risks this may introduce.
6. Known or suspected malignancy, leukemia, or lymphoma. Malignancy not excluded: A participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained remission and/or is unlikely to recur during the period of the study.
7. Immunosuppressed, including altered or compromised immune status as a consequence of disease or treatment with systemic corticosteroids (including inhaled or intranasally-administered drugs), alkylating drugs, anti-metabolites, radiation, or other immunosuppressive therapies.
8. Receipt of any investigational drug in the past 30 days.
9. Known Diabetes mellitus.
10. Hypertension that is not well controlled: Blood Pressure ≥ 150/100 (either or both values) at screening or enrollment.
11. Any medical, psychiatric, or social condition, or occupational or other responsibility that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a participant's ability to give informed consent.

8.3 Prior and Concomitant Therapy

Notwithstanding above exclusions (section 8.2), standard medical protocol for pre-existing medications and post-operative management will apply, including post-operative instructions by the physician.

Participants will be advised that they are not to receive other investigational products (drug or vaccine) for 180 days post vaccination.

9. STUDY PROCEDURES

Recruitment for this trial will begin following receipt of all regulatory and ethics committee approvals and will conclude when 12 subjects have completed the 6-week visit.

When the site pharmacist is notified that the subject is available for device placement, the unblinded pharmacist will withdraw a device package from the refrigerator. The principle investigator will prepare the experimental device by cutting or shaping to fit the subject's cranial defect. The experimental device will be wetted, then placed into the defect in the same manner as placing an autologous bone graft, taking care to fit the device to the margins of the defect in all dimensions. The experimental site may or may not be covered with a vicryl screen for tissue support, and the wound will be closed by conventional means.

The preliminary safety assessment will be available approximately four months after enrollment has been completed. Follow-up of subjects will continue through two years.

Source documents will include subject diary cards, subject medical charts, telephone contact records, reports from clinical laboratory tests, and analytical results from tests conducted on clinical samples by Agenta and its contractors. Results of in-clinic assessments, as well as AE and SAE information, will be recorded directly onto

protocol Case Report Forms (CRFs) or will be transferred from source documents onto CRFs. Results of tests on clinical samples will be transferred directly from the source documents into the study database.

9.1 Study Visits

Screening Visit (Day -21 to -1)
Participants will be screened for eligibility to participate in the study. The following procedures must be performed during the screening period:

a. Obtain Informed Consent prior to any study procedures.
b. Check eligibility criteria.
c. Obtain medical history.
d. Record concomitant medication
e. Perform physical examination.
f. Obtain blood samples (10 mL + 5 mL) for screening clinical laboratory assessments.
g. Obtain a blood sample (4 mL) for HIV serology and illicit drug screen.
h. Obtain CT image of cranial defect.

Visit 1 (Day 0, Baseline)

a. Confirm that the subject wishes to remain in the trial.
i. Confirm eligibility criteria as needed.
j. Obtain interim medical history as needed.
k. Record concomitant medication as needed.
l. Perform physical assessment as needed.
m. Obtain blood samples (10 mL + 5 mL) and urine sample for clinical laboratory assessments if more than 3 days since prior screening.
n. Obtain blood sample (4 mL) for PT and PTT tests if more than 3 days since prior screening.
o. Administer experimental device.
p. Keep the subject under observation for 30 minutes post administration and record any reaction in the source documents and CRF.
q. Give participant the diary card with instructions for use.
r. Record AEs and SAEs.
s. Set up appointment for Visit 2 (Day 3).

Contact 1 (Day 1)

a. Record symptoms and symptom severity on Telephone Contact Symptom Assessment Form.
b. Record AEs and SAEs.
c. Schedule in-clinic evaluation for moderate or severe AEs or SAEs, as clinically appropriate.

Contact 2 (Day 2)

a. Record symptoms and symptom severity on Telephone Contact Symptom Assessment Form (Appendix D).
b. Record AEs and SAEs.
c. Schedule in-clinic evaluation for moderate or severe AEs or SAEs, as clinically appropriate.

Visit 2 (Day 3)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Review diary card with subject.
d. Record concomitant medication.
e. Perform physical assessment (vital signs, lymph node exam, and symptom directed exam).
f. Record AEs and SAEs.
g. Set up appointment for Visit 3 (Day 7).

Visit 3 (Day 7)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Record concomitant medication.

d. Perform physical assessment (vital signs, lymph node exam, and symptom directed exam).
e. Obtain blood samples (10 mL + 5 mL) and urine sample for clinical laboratory assessments.
f. Obtain blood sample (4 mL) for PT and PTT tests.
g. Collect and discuss diary card / distribute new diary card.
h. Record AEs and SAEs.
i. Set up appointment for Visit 4 (Day 42).

Visit 4 (Day 42)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Review diary card with subject.
d. Record concomitant medication.
e. Perform physical assessment (vital signs, lymph node exam, and symptom directed exam).
f. Obtain blood samples (10 mL + 5 mL) and urine sample for clinical laboratory assessments.
g. Obtain blood sample (4 mL) for PT and PTT tests.
h. Record AEs and SAEs.
i. Obtain radiographs and CT images of experimentally treated cranial defect.
j. Set up appointment for Visit 5 (Day 180).

Visit 5 (Day 180)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Review diary card with subject.
d. Record concomitant medication.
e. Perform physical assessment (vital signs, lymph node exam, and symptom directed exam).
f. Record AEs and SAEs.
g. Obtain CT images of experimentally treated cranial defect.
h. Set up appointment for Visit 6 (Day 360).

Visit 6 (Day 360)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Review diary card with subject.
d. Record concomitant medication.
e. Perform physical assessment (vital signs, lymph node exam, and symptom directed exam).
f. Record AEs and SAEs.
g. Obtain CT images of experimentally treated cranial defect.
h. Set up appointment for Contact 3 (Day 730).

Telephone Follow-up (Day 730)

Study personnel (study coordinator or other personnel assigned by the investigator) will contact the participant to collect information on ongoing, previously recorded adverse events and new or ongoing SAEs. Reminder postcards will be mailed to each participant approximately one month prior to this contact. Study personnel will contact the participant by telephone approximately two weeks in advance of the follow-up target date to schedule a time for the telephone contact. During the telephone conversation, study personnel will collect interim medical history as well as update contact information.

Contact 3 (Day 730)

a. Confirm that the subject wishes to remain in the trial.
b. Obtain interim medical history.
c. Record AEs and SAEs.
d. Verify telephone contact information and mailing address and provide instructions to participant in the event of a change in contact information.

Additional Clinic Visits

Any subject with an abnormal laboratory result or who experiences an adverse event may be requested to come into the clinic for additional follow-up evaluation not specified by the protocol if this is deemed medically prudent by the investigator.

9.2 Obtaining, Handling, Storage, and Shipment of Specimens

9.2.1 Blood Samples for Safety Laboratory Tests

Blood samples collected for safety analysis will be labeled and handled per the local reference laboratory standard operating procedures for hematology and biochemistry profiles. Blood samples for safety laboratory tests will include one 5 mL and one 10 mL sample, as well as one 4 mL sample for PT and PTT testing where specified.

9.2.2 Urine Samples

A clean-catch urine sample will be collected by each participant for safety evaluation. Study personnel will be responsible for instructing subjects on this technique. Urine samples will be handled and labeled per the local reference laboratory standard operating procedures for urinalysis.

9.3 Safety Data Review

The SRC will perform a review of the safety data through seven days post-administration for the first three subjects, and through seven days post-administration for all subsequent subjects. The SRC will use the following evaluation criteria to approve the completion of enrollment after the first 3 subjects:

a) Absence of SAEs or severe AEs "definitely", "probably" or "possibly" related to the experimental device.

b) No evidence of severe hematologic or biochemical toxicity.

c) No consistent pattern of AEs that, in the judgment of the SRC, represents a significant threat to the safety of the subjects with continued enrollment.

Approval from Agenta will be obtained before proceeding with treatment of subjects 4-12.

9.4 Compliance with Protocol

All participants who receive the experimental device will be included in the safety evaluation.

The following events are to be considered sufficient reason for discontinuation of a participant from the study:

1. Poor or non-compliance with visit schedule. In cases where a participant is outside the recommended visit schedule, the participant may be continued in the study as a protocol violator and will be analyzed as such as long as the study personnel receives approval from both Agenta and the Principal Investigator.
2. Any situation where, in the opinion of the investigator, continuation of the study would not be in the best interest of the participant, including, but not limited to, illness requiring immunosuppressive therapy.
3. Individual request of the study participant.
4. Decision of Agenta.

The collection of adverse events including subject diaries, in-clinic assessments, and the 6, 12, and 24 month evaluations will continue for subjects who are discontinued but agree to continue study follow up.

In the case of participants who fail to appear for a follow-up visit or are not able to be contacted by telephone, extensive effort (i.e. documented telephone calls and certified mail) should be undertaken to locate or re-call them to determine their health status. These efforts must be documented in the participant's record.

If a certified letter is sent to an unresponsive participant and then returned to the study site unopened, or if the letter is received and signed for but the participant cannot be contacted, the returned certified letter or receipt must be stored with the participant's records. These subjects will be terminated from the trial and their data will be analyzed as "lost to follow-up."

Subjects who fail to complete the study or withdraw for any reason prior to completion of the day 42 visit and examination will be replaced with new enrollment if feasible.

All subjects will be classified at the end of the trial according to the following design:

1. Subjects who complete all the visits according to the protocol through Day 180 will be classified as normal study completion.
2. Subjects who receive the study device but missed study visits or had visits performed outside the protocol windows will be classified as study completion not according to protocol.
3. Any participant that is withdrawn from the study at any time either at the discretion of the Investigator or Agenta will be classified as "withdrawal."
4. Any participant that requests to be withdrawn will be classified as "drop-out." The reason for this must be clearly documented in the participant's record.
5. Any participant that does not finish the trial and with whom the study staff is unable to make contact after extensive effort will be classified as "lost to follow-up."

10. ADVERSE EVENTS

Adverse event (AE):

Any untoward medical occurrence in a patient or clinical trial subject administered a pharmaceutical product or device and which does not necessarily have to have a causal relationship with this treatment.

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product or device.

The intensity (severity) of a specific event, as perceived by the patient, is typically described as mild, moderate or severe (although a severe event itself may be of minor medical significance). The term serious is based on the event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness serves as a guide for defining regulatory reporting obligation.

Serious Adverse Event (SAE):

A serious adverse event (experience) is any untoward medical occurrence that at any dose:

1. results in death,
2. is life-threatening,
3. requires inpatient hospitalization or prolongation of existing hospitalization,
4. results in persistent or significant disability/incapacity

It should be noted that 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 that hypothetically might cause death if it were more severe.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations. These include important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Unexpected adverse event:

An unexpected AE is defined as "any adverse event, the specificity or severity of which is not consistent with the current investigator's brochure; or, if an investigator brochure isn't required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or

elsewhere in the current application, as amended" (adopted from ICH E6 guideline, "Good Clinical Practice: Consolidated Guideline").

10.1 Recording of Adverse Events

Adverse events will be recorded for all subjects who receive the study device. Adverse events will be documented in subject diaries, during in-clinic assessments, and during telephone contacts through Contact 3 (Day 730) of the study. All adverse events will be graded by severity and relationship to the experimental device and followed until resolution or the completion of subject participation. All SAEs will be recorded throughout the 2 year period of safety surveillance.

Any subject who was discontinued due to an AE or non-adherence to the protocol windows for study agent administration will be followed for safety and efficacy endpoints by clinic visits, telephone contacts, and subject diaries as specified by the protocol. All safety evaluations will continue to be completed according to the protocol, if possible.

All AEs that are moderate or mild will be submitted within 7 days from the site to the designated Medical Monitor, Dr. Marks.

10.2 Reporting of Serious Adverse Events

During the study period all SAEs must be reported to the sponsor immediately (within 24 hours). Any SAE occurring from the time of device placement until termination of the study, whether deemed device-related or not, must be reported by facsimile to Agenta immediately (usually within 24 hours) using a SAE Report Form.

SAE Contact Information (Agenta Medical Monitor):
Donald Marks, M.D.
Cell Phone (24 hours): 205-283-1688
FAX (24 hours): 603-372-4813

Additional contact information for the Medical Monitor is provided in Section 1 of this protocol, "Study Contacts."

The SAE Report must include the Investigator's written medical judgment as to the relation of the event to the experimental device (i.e., "definite/certain", "probable", "possible" or "unrelated"). This initial judgment can be modified later by the investigator, as more information relevant to the SAE becomes available.

Definite/Certain Related is applied to those SAEs which have a temporal relation to the study device and no alternative etiology is present. It must have occurred within a reasonable temporal sequence of the device administration (0 - 7 days), must not be reasonably explained, and must follow a known pattern of response.

Probable Related has a timely relation to the study device and a potential alternative etiology is not apparent (e.g., fever or malaise when no other symptoms suggestive of an illness are present).

Possible Related has a timely relation to the study device; however, a potential alternative etiology exists which may be responsible for the symptom (e.g., fever or malaise when other symptoms are present that suggest another etiology such as urinary tract infection).

Unrelated is applied to those SAEs where evidence exists that the event is definitely related to an etiology other than the study device (e.g., auto accident, or a symptom suggestive of another illness, which is not accepted to be a possible event due to the experimental device).

Participants will be instructed to call the study site to report SAEs at any time during the study period through 24 months. In addition, active surveillance of SAEs will be performed at every scheduled site visit or telephone call. In the event that a study participant reports a SAE, study personnel will be authorized to visit or telephone the participant until the problem is resolved. Any SAE is to be documented and fully described whether or not it is consider to be related to the study device.

A report of any SAE must be followed by a written report to the IRB, the SRC and to Agenta, by the Investigator, including a full description of the event and any sequelae. All deaths, whether considered device-related or not, must also be reported immediately to the IRB, the SRB, and to Agenta.

Agenta will report all SAEs to the government agencies, adhering to timelines for reporting outlined in Therapeutic Products Program Directorate Guidelines (ICH/GCP) and the Code of Federal Regulations (21CFR 312.32). The FDA will be notified of any SAE as soon as possible, not later than 15 days, and within 7 days for any death or life-threatening event associated with the drug substance (experimental device).

All SAEs must also be recorded in the appropriate section of the CRF. All reported SAEs will be reviewed periodically by the SRC.

11. TRIAL ASSESSMENTS

11.1 Safety

The safety profile to be assessed includes immediate adverse events, solicited local and systemic events, unsolicited adverse events, and hematologic and biochemical toxicity evaluation.

11.1.1 Immediate Adverse Events

All participants will be observed for 30 minutes after administration of the study treatment. Any immediate AE will be recorded on the AEs page.

11.1.2 Solicited Local and Systemic Events

All subjects will be given diary cards that are to be filled out for the purposes of recording any changes in health status after device administration.

- Diary Card #1 will be used to record all local and systemic events, as well as any other AEs that represent a change in the health status of the subject, from device placement through Visit 3 (Day 7).
- Diary Card #2 will be used to record unsolicited adverse events and SAEs through Visit 4 (Day 42).
- Diary Card #3 will be used to record unsolicited adverse events and SAEs after Visit 4 (Day 42) through Visit 5 (Day 180).
- Diary Card #4 will be used to record unsolicited adverse events and SAEs after Visit 5 (Day 180) through Visit 6 (Day 360).
- Diary Card #5 will be used to record unsolicited adverse events and SAEs after Visit 5 (Day 360) through Contact #3 (Day 720).

The diary cards will be considered the source document and will be transcribed into the case report form (CRF) by study site personnel. If the diary card is lost, this will be documented in the CRF, and the limited information collected during the clinic visit will serve as the source document. All other data points will be noted with "ND" (not determined).

For the purpose of collecting solicited local and systemic events, the following procedures will be implemented:

1. Study participants will be supplied with a digital thermometer on day 7 and shown how to use it to measure their oral temperature for weeks 1-6 following administration of the study product.
2. Participants will be asked to observe for AEs daily during the first 6 weeks post-implantation period. This includes systemic events including fever, chills, nausea/vomiting, weakness, muscle pain, and headache.
3. Diary Card #1 will be distributed during Visit 1 (Day 0) and collected during Visit 3 (Day 7). Diary Card #2 will be distributed during Visit 3 (Day 7) and collected during Visit 4 (Day 42). Diary Card #3 will be distributed during Visit 4 (Day 42) and collected during Visit 5 (Day 180). Diary Card #4 will be distributed during Visit 5 (Day 180) and collected during Visit 6 (Day 360). Diary Card #5 will be distributed during Visit 6 (Day 360) and collected by mail at Contact #3 (Day 720). Subjects will be asked

to bring their current diary card to each clinic visit. At each visit, the study staff will review with the participant the information found in the diary card for clarity and completeness but will not make changes to the subject's information. However, the participant may add to or amend the information on the card if appropriate. Any information crossed out by the participant should be deleted with a single strikethrough line in order to maintain the legibility of the original information, and should be initialed and dated by the participant. For any questions not completed, the participant should record "ND" (not determined) beside the question.

11.1.3 Unsolicited Adverse Events

All AEs and SAEs, including any condition that, according to the subject, represents a change in health status, and that occurs after the first administration of the study agent on Day 0 until the follow-up assessment at Contact #3 (Day 720) will be recorded. This information will be actively surveyed at every telephone contact or site visit with the subject during the period between Visit 1 and Contact #3. All SAEs that occur after the first administration of the study agent until the 2 year follow-up contact will be recorded. The source documents will be the subject diary cards, and the clinical records, from which AEs (as described above) will be transcribed to the CRF.

Any unresolved AE will be followed until its resolution or until the 24 month follow-up telephone contact, whichever occurs first.

11.1.4 Adverse Event Grading Scales

The severity of all adverse events will be graded as mild (Grade 1), moderate (Grade 2), severe (Grade 3), or potentially life threatening (Grade 4) according to the adverse event grading scale provided below. This grading scale will be used for local adverse events (those related to the cranial site of device administration) and systemic adverse events.

Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Symptom is present but does not interfere with activity	Symptom interferes with some activities but does not cause missed attendance at school or work	Incapacitating, unable to attend school or work or perform essential daily activities	Requires emergency room visit or hospitalization

Abnormal vital signs will be graded according to the above severity scale. This grading scale is based on the scale provided in the draft FDA guidance for industry, "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (April 2005)."

Any "severe" rating for local or systemic events requires the participant to call the study site for evaluation, the study site to notify the Medical Monitor (at the discretion of the investigator), and place comments on the AE page of the CRF that describe the event(s). Any severe adverse event that is deemed possibly, probably or definitely related to the study treatment will invoke a temporary stopping rule for the study to permit evaluation of the event by the SRC (see Section 14.3).

11.1.5 Hematologic and Biochemical Toxicity Evaluation

Blood samples for evaluation of hematologic and biochemical toxicity, and urine samples for urinalysis will be obtained from each participant on Day -21 to -1 (screening period) and again on Day 0 prior to device placement, if screening samples were taken more than 3 days prior, then Days 3, and 42. If deemed necessary, other toxicity tests (to be determined) may be performed with these samples as well.

The samples will be prepared and shipped according to specifications provided by the testing laboratory at the investigator's site where the tests will be performed. The toxicity evaluation tests will include hematologic testing, serum biochemistry testing, and urinalysis as indicated in the table below.

Safety Laboratory Tests to be Conducted

Hematology (Complete Blood Count)	White Blood Count	Mean Corpuscular Hemoglobin Concentration
	Red Blood Count	RDW
	Hemoglobin	Platelet count
	Hematocrit	Prothrombin Time (PT)
	Mean Corpuscular Volume	Partial Thromboplastin Time (PTT)
	Mean Corpuscular Hemoglobin	
Blood Chemistry	Sodium	Creatinine
	Potassium	Calcium
	Chloride	Alanine Aminotransferase (ALT)
	Bicarbonate	Alkaline Phosphatase
	Glucose	Bilirubin
	Blood Urea Nitrogen (BUN)	
Urinalysis	Color	Nitrite
	Specific Gravity	Leukocyte esterase
	pH	Turbidity
	Protein	Crystals
	Glucose	Bacteria
	Ketones	WBC
	Bilirubin	RBC
	Blood	

Laboratory values that are outside the institutional range for normal lab values (Appendix A of this document) will be recorded as AEs only if they are deemed clinically significant in the judgment of the Investigator. In order to assign clinical significance of abnormal lab values, the Investigator will use as a guide the institutional lab range of normal values.

11.2 Osteoinduction

Radiographic and CT scan assessment for osteoinduction at the site of the experimental device will be performed according to protocol for all subjects, unless the investigator determines that it is not in the best interest of the subject to continue with imaging.

11.2.1 Osteoinduction Evaluation

The primary outcome for osteoinduction is assessed by CT analysis.

12. STUDY MEDICATION

12.1 Product Description

The B-247 Cranial Bone Graft Implant is an experimental device which consists of plasmid DNA (pln.247-pBI, 200 μg/ml of implant volume) for transient expression of human perlecan domain 1 with 100 μg/ml rhBMP-2 in a gelatin/tricalcium phosphate scaffold for cranial bone regeneration. The product is provided as a pliable solid single-phase unit consisting of 80% bioactivated tricalcium phosphate (TCP) crystals 200-600 μm particle size, evenly distributed within a 20% cross-linked gelatin polymer. The implant is bioactivated by incorporating plasmid DNA lipoplexes and rhBMP-2 in 1% sucrose excipient, then lyophilizing the loaded implant to a final concentration of 200 μg/ml of implant of pln.247-pBI plasmid and 100 μg/ml of implant of rhBMP-2. Plasmid lipoplexes are derived from incubating pln.247-pBI plasmid with Lipofectamine 2000 in a

2:1 ratio for 30 minutes. Sucrose, plasmid, rhBMP-2, Lipofectamine 2000, TCP crystals, and gelatin are each manufactured separately according to cGMP and combined under sterile conditions in a clean room.

12.2 Dosing

The device will deliver 200 μg/ml of graft material of the B-247 plasmid, and 100 μg/ml of graft material of the rhBMP-2. The amount of graft material that is required to fill the bone defect should be applied. The total dose to the participant will vary depending on the amount of the device required to fill the bone defect. However, the dose per volume of graft material will remain constant.

12.3 Labeling

All study devices will be provided by Agenta and labeled with an investigational product label. Examples of the investigational product label are reproduced below:

Cranial B-247 Investigational Bone Graft Implant:

B-247 Investigational Bone Graft
100 μg/ml rhBMP-2
200 μg/ml B-247 DNA plasmid
vol: 28 ml
Store at 4°C ± 3°C
Caution: Investigational Device – Limited by US Law to investigation al use.
Agenta Biotechnologies, Inc.

12.4 Packaging

The formulated, sterile drug product will be packaged in a sealed wrapper with label affixed. Upon use, the wrapper will be torn or cut open and the semi-solid experimental bone graft device will be dropped onto the surgical field for shaping and surgical placement.

12.5 Storage, Issue, and Return of Study Medication

All study device and placebo will be provided by Agenta and labeled with an investigational product label, as described in Section 12.3. Study device will be shipped to the study site pharmacy on ice and must be stored in a secured, monitored alarmed refrigerator at 4 ± 3°C. The study product temperature will be continuously monitored and any deviations from the acceptable range for the study product storage temperature will be noted by the pharmacist including the temperature, date and time.

The site pharmacist will maintain documentation showing receipt, shipment, and disposition of investigational device. This documentation will include the investigator's name, and product code/ lot number for each shipment. An investigational product dispensing and reconciliation form will be maintained for all study devices.

When the site pharmacist is notified that the subject is available for device placement, the unblinded pharmacist will withdraw a device package from the refrigerator. Unused product should be returned to Agenta with the appropriate documentation at the time of study close-out.

Once the investigational device accountability has been verified at the end of the study, all unused graft material must be returned to Agenta with the appropriate documentation.

13. STATISTICAL EVALUATION

13.1 Sample Size Calculation

The goal of the safety evaluation for this study is to identify safety concerns associated with device administration. Sample size calculations for safety are expressed in terms of the ability to detect rare events [e.g. serious adverse events (SAEs)]. The ability of the study to identify serious adverse experiences can be expressed by the maximum true rate of events that would be unlikely to be observed and the minimum true rate of events that would very likely be observed. Specifically, for a group size of 10, there is a 90% chance of observing at least 1 serious adverse experience if the true rate of such an event is at least 21%; there is a 90% chance that at least 1 SAE would not be observed if the true rate was no more than 1%.

Probabilities of observing 0, or 2 or more, SAEs among a group of 10 subjects are presented in the following table for a range of possible true event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the experimental device. Note that the planned group size for the study will be 12, i.e., 2 subjects more than the sample size calculations illustrated here.

Probability (%) of Response for Different Safety Scenarios

True Event Rate (%)	Pr (0/10)	Pr (2+/10)
1.0	90.4	0.4
3.4	70.0	4.6
5.0	59.9	8.6
10.0	34.9	26.4
20.0	10.7	62.4
30.0	2.8	85.1
40.0	0.6	95.4
50.0	<0.1	98.9
60.0	<0.1	99.8

13.1.1 Responsibility for the Analysis

The principal investigator will conduct the safety evaluations at the study site on Days 0, 3, 7, 42, 180, and 360. The SRC will review safety data for specific cohorts of subjects at protocol-specified intervals.

A full statistical analysis will be performed under the control of Agenta after all Day 180 data is received in-house, and again after all Day 360 data is received in -house. The study statistician will be responsible for completing the statistical analysis of all safety and efficacy data.

13.2 Variables and Timepoints of Measurement

13.2.1 Safety Variables

In-clinic safety evaluation on Days 0, 3, 7, 42, 180, and 360 will be graded for severity and relationship to the study agents and will include:

a) Rates of SAEs and their relationship to device placement, through 2 years of long-term follow-up.

b) Rates of local or systemic solicited AEs and their categories (mild, moderate and severe), through Day 360 ± 28 days.

c) Rates of reported unsolicited AEs, through Day 360 ± 28 days.

d) Hematologic and biochemical toxicity evaluation following device placement.

13.2.2 Osteogenicity Variables

Osteogenicity will be evaluated using radiographs and CT scans obtained from participants on Day 0 (baseline, pre-implantation), Day 42 (± 7), 6 weeks after device placement), and Day 180 (± 14) 6 months after device placement).

13.3 Statistical Methods

13.3.1 Safety

The safety of device implantation in males 19-59 years of age will be evaluated.

Adverse events will be tabulated, graded for severity and relationship to the study products, and categorized by body system and the preferred term (MedDRA), and compared between groups. The number and percent of subjects with adverse events will be tabulated by body system, preferred term and treatment group. A similar summary will be provided for subjects with serious adverse events.

Hematologic and biochemical toxicity data will be summarized according to the criteria for toxicity described herin.

13.3.2 Osteogenicity

The osteoinductive nature of the experimental device will be evaluated.

13.4 DATA MANAGEMENT

Clinical data reported on CRFs and/or relevant serological/biological sample analysis results will be integrated into the internal clinical data management system at Agenta.

13.4.1 Data Processing

For each batch of data, double entry, quality control and triggers to computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis. Each step of this process will be monitored through the implementation of individual passwords and/or regular backups in order to maintain appropriate database access and guarantee database integrity.

14. SAFETY REVIEW COMMITTEE

The Safety Review Committee (SRC) will be established to review all safety data and make recommendations to Agenta regarding the continuation of the trial. This group may recommend trial continuation as scheduled, modifications to the current enrollment plan and/or cessation of device implantation.

14.1 Member Selection

The SRC will be comprised of 3 members, including the Principal Investigator, the Medical Monitor and a third member selected by the PI. Dr. DeCarlo, the sponsor's PI, will chair the SRC.

14.2 Meeting Schedule/ Adverse Event Review

The SRC will have a scheduled meeting at least once per week during the period of active enrollment and follow-up for each enrolled participant to 42 days. All members of the SRC will review the safety data weekly. Scheduled meetings may be cancelled at the agreement of all three SRC members if there are no safety issues to discuss. The SRC will hold a meeting to review the Day 7 data for the first 3 subjects, and the Day 7 safety data for each subject for the remaining 9 subjects. The SRC will recommend to either continue enrollment or termination of enrollment based on the safety and tolerability of the study product. All AEs that are moderate or mild will be submitted within 7 days from the site to the Study Medical Monitor. SAEs and severe adverse events will be reported within 24 hours after the clinical trial site is aware of the event.

The Medical Monitor will keep and maintain documentation for all SRC meetings with meeting minutes. Agenta personnel will tabulate the data for SRC review.

14.3 Stopping Rules

Stopping rules will be in place to arrest the enrollment process and suspend all experimental device placement with the occurrence of significant safety event(s). The stopping rules are:

- Any SAE or severe AE that is definitely, probably, or possibly related to the experimental device in the opinion of the investigator or Safety Review Committee.
- Occurrence of 3 or more moderate AEs of the same type that are definitely, probably, or possibly related to the study agent in the opinion of the investigator or Safety Review Committee.

The occurrence of AEs and SAEs will be monitored by the Medical Monitor who will notify the other members of the SRC if a condition is met to institute a stop rule. Enrollment and study agent administration cannot continue after a stop rule has been implemented until the SRC has reviewed the AEs and other subject safety data and made a recommendation to continue the trial as planned, modify the protocol or stop enrollment. The recommendation of the SRC must be reviewed and approved by Agenta.

15. MONITORING/ LABORATORY QUALITY ASSURANCE

15.1 Monitoring

Agenta personnel or their representatives will make periodic site visits to assess good clinical practice (GCP) and protocol compliance and review CRFs for completeness and accuracy. CRFs will be verified against the medical records (source documents and diary cards) and sent to Data Management. Confidentiality will be maintained within legal limits in the review of the medical records and consent forms, which may contain the identity of the subject.

15.2 Laboratory Quality Assurance

Agenta will conduct periodic reviews of the research laboratories in order to assess protocol compliance and review of records.

15.3 Auditing

Audits or inspections may be made by Agenta or regulatory agencies to ensure that the study has been conducted in accordance with the protocol, regulations and GCP.

16. INVESTIGATOR OBLIGATIONS

16.1 Ethics

16.1.1 Ethical Conduct of the Trial

This trial will be conducted in accordance with the latest version of the Declaration of Helsinki (Edinburgh, October 2000 revision, text available upon request). It will also follow GCP and international conference on harmonisation (ICH) regulatory guidelines and applicable country or local requirements regarding ethical committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research.

16.1.2 Independent Ethics Committee/ Institutional Review Board (IRB) Review

The investigator is responsible for submission of this protocol and the informed consent form developed for the study to an Institutional Review Board constituted in conformance with regulatory agency requirements. A copy of the IRB's letter of approval for the study will be provided to the sponsor along with a copy of the Informed Consent Form approved by the IRB for the study.

No study device will be released for shipment to the investigator until these requirements have been met.

16.2 Subject Benefits/ Potential Risks

16.2.1 Subject Benefits

It is possible that no benefit will be derived from participating in this trial. Subjects will be screened at enrollment using routine clinical hematological, serum biochemical measurements and urine analysis methods, and will have a limited physical exam performed at no cost to the participant. Participants will receive the study device free of charge and will have additional diagnostic evaluations to monitor safety and efficacy free of charge.

16.2.2 Potential Risks

There may be some discomfort or bruising from the blood draws. There may be discomfort in additional imaging by CT. This is a Phase I/IIa trial. Therefore, there may be other risks not yet identified.

16.3 Informed Consent Process and Documentation

The investigator will be responsible for presenting a full description of the research project including risks/benefits and then obtaining written informed consent from the subject prior to screening procedures or device implantation and maintaining up-to-date records of such consent. Subjects will be provided a copy of the signed informed consent form. Volunteers will have every opportunity to have their questions answered before and after consenting to participate. Participants are free to withdraw from the study at any time without giving a reason.

16.4 Modification of the Protocol

Adherence to the trial protocol is essential. If modification of the protocol becomes necessary, a clear description of the rationale for the modification should be provided in a protocol amendment. All amendments to this protocol will be made only after consultation with and agreement of Agenta and the investigator concurrently (except in the case of immediate hazard to human subjects). If agreement is reached concerning the need for an amendment, such an amendment will be produced in writing by the sponsor and/or the investigator, will be signed by Agenta and the investigator, and will be made a formal part of the protocol.

The investigator is responsible for submitting the amendment to the appropriate IRB/Ethics Committee for approval. Agenta is responsible for submitting the amendment to the appropriate government regulatory body. An administrative change to the protocol is one that is editorial and does not affect subject risks or benefits, nor changes their desire to continue to participate in the trial. An administrative change only requires IRB notification, not approval.

16.5 Interruption of the Trial

The trial may be prematurely discontinued as the results of new data generated from this or other trials regarding the experimental device being evaluated in this trial become available.

16.6 Confidentiality of Data/Access to Subject Records

Prior to initiation of the study, the principle investigator will sign a confidentiality agreement with Agenta.

Confidentiality will be maintained within legal limits in the review of the medical records and consent forms, which may contain the identity of the subject. Any records sent to Agenta will not identify the subjects. Such records will be coded with the subject's PIN and study number.

16.7 Archiving

The investigator will retain all source documents, consent forms, and copies of CRFs for 7 years after completion of the study. The investigator will contact Agenta for instructions regarding the disposition of the documents at the end of this period. Agenta will ensure that all records of the study, including test results on clinical samples produced by Agenta or its contract testing facilities, will be archived for a period consistent with regulatory requirements.

16.8 Stipends for Participation

Participants will be provided a stipend according to local practice to compensate for their time and travel required for each visit to the study site.

16.9 Adverse Event Compensation and Insurance

In the occurrence of an AE, the participant will be evaluated and treated in accordance with local regulations and good medical practice protocol by the investigator. If the investigator deems that the AE is directly related to the administration of the study product, Agenta will provide reimbursement for all reasonable outpatient costs of the medical evaluation or treatment or hospitalization, to the extent such costs are not covered by the subject's hospital insurance or by third party or government programs providing such coverage. No additional form of compensation is available.

16.10 Publication Policy

Data derived from the trial are the exclusive property of Agenta. Agenta must approve any publication or presentation related to the trial before submission of the manuscript. After publication of the results of the trial, any participating center may publish or otherwise use its own data provided that any publication of data from the trial gives recognition to the trial group. In addition, Agenta shall be associated with all such publications, it being understood that Agenta is entitled to refuse the association.

Agenta must have the opportunity to review all proposed abstracts, manuscripts or presentations regarding this trial at least 60 days prior to submission for publication/presentation. Any information identified by Agenta as confidential must be deleted prior to submission, it being understood that the results of this trial are not to be considered confidential.

Agenta review can be expedited to meet publication guidelines.

17. LIST OF APPENDICES

A. Normal Laboratory Values

APPENDIX A:

UAB Hospital Normal Laboratory Values

(Revised May 2007)

UAB Hospital Laboratories Bulletin of Information

CBC

Synonyms:
Complete Blood Count

Reference Range:

CBC:
White Blood Count:
4.0-11.0 x10^3/microL

Red Blood Count:
Female: 3.8-5.2 x10^6/microL
Male: 4.4-5.8 x10^6/microL

Hemoglobin:
Female: 11.3-15.2 gm/dL
Male: 13.5-17.0 gm/dL

Hematocrit:
Female: 33-45%
Male: 39-50%
Critical: <15% and >60%

Mean Corpuscular Volume:
80-96 fL

Mean Corpuscular Hemoglobin:
27-33 pg

Mean Corpuscular Hemoglobin Concentration: 32-36 g/dL

RDW: 11-16%

Platelet Count:
150-400 x10^3/microL
Critical: <20 x10^3/microL
(UAB BMT <10 x10^3/microL)

Container:
Lavender or Pink Top Vacutainer Tube containing EDTA additive. (Minimum volume: 2 mL Lavender Top Tube)

Infants:
One Lavender Top Microtainer (Minimum volume: 300 microliters)

Availability:
TAT: 2-4 hours
STAT: 1 hour

Basic Metabolic Panel

AMA approved panel available for out-patient, referred, and UAB Hospital in-patient testing.

Synonyms:
BMP

Reference Range:

Sodium: 133-145 mEq/L
Critical: <120 and >160 mEq/L

Potassium: 3.3-5.1 mEq/L
Critical: <3.0 and >6.5 mEq/L

Chloride: 96-108 mEq/L

Bicarbonate: 23-29 mEq/L
Critical: <10 and >35 mEq/L

Glucose: 70-100 mg/dL
Critical: <40 and >450 mg/dL

BUN: 6-19 mg/dL

Creatinine
Male: 0.7-1.3 mg/dL
Female: 0.4-1.2 mg/dL

Calcium: 8.4-10.2 mg/dL
Critical: <6.0 and > 14.0 mg/dL

Container:
Vacutainer SST with Gel Barrier (Minimum volume: Gold Top SST with 3.5 mL blood)

Tube contains clot activator additive. Mix by 5 complete inversions to activate clotting. Store filled tubes upright, not on side.

Availability:
TAT: 2 hours
STAT: 1 hour

Alanine Aminotransferase, Serum

Synonyms:	*Reference Range:*	*Container:*	*Availability:*
ALT SGPT	Male: 6-45 Units/L Female: 6-30 Units/L	Vacutainer SST with Gel Barrier (Minimum volume: Gold Top SST with 3.5 mL blood) Tube contains clot activator additive. Mix by 5 complete inversions to activate clotting. Store filled tubes upright, not on side.	TAT: 2 hours STAT: 1 hour

Alkaline Phosphatase, Serum

Synonyms:	*Reference Range:*	*Container:*	*Availability:*
Alk Phos	39-117 Units/L	Vacutainer SST with Gel Barrier (Minimum Volume: Gold Top SST with 3.5 mL blood) Tube contains clot activator additive. Mix by 5 complete inversions to activate clotting. Store filled tubes upright, not on side.	TAT: 2 hours STAT: 1 hour

Bilirubin, Adult, Serum

Synonyms:	*Reference Range:*	*Container:*	*Availability:*
	Total: 0.0-1.0 mg/dL Direct: 0.1-0.3 mg/dL Indirect: 0.0-0.9 mg/dL	Vacutainer SST with Gel Barrier (Minimum volume: Gold Top SST with 3.5 mL blood) Tube contains clot activator additive. Mix by 5 complete inversions to activate clotting. Store filled tubes upright, not on side.	TAT: 2 hours STAT: 1 hour

Urinalysis

Synonyms:

Reference Range:

Color:	Yellow, Straw, Amber
Specific Gravity:	1.003-1.035
pH:	4.6-8.0
Protein: Critical: 4+	Negative
Glucose:	Negative
Ketones:	Negative
Bilirubin:	Negative
Blood:	Negative
Nitrite:	Negative
Leukocyte Esterase:	Negative
Turbidity:	None
Crystals:	Negative
Bacteria:	Negative
WBC:	0-5/hpf
RBC:	0-2/hpf

Container:

10 mL fresh Urine in Screw-capped Container (Minimum volume: 2 mL for macroscopic;
5 mL required for centrifuged microscopic exam)

Deliver to lab within 2 hours of collection. Refrigerate until delivery.

Do not use any blood collection tube including the plain red top tube as a urine container.

Availability:

TAT: 2 hours
STAT: 1 hour