NF PROTOCOL 107

A Study of INFUSE Bone Graft (recombinant human Bone Morphogenetic Protein-2/absorbable collagen sponge) in the treatment of Tibial Pseudarthrosis in Neurofibromatosis 1 (NF1)

Study Protocol & Statistical Analysis Plan

NCT02718131

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Bruce Korf, M.D., PhD, Principal Investigator
University of Alabama at Birmingham
Birmingham, AL 35294

Neurofibromatosis (NF) Consortium

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(Version for U.S. Sites)

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Protocol Chairs
Elizabeth K. Schorry, MD, Chair
B. Stephens Richards, MD, Co-Chair
David H. Viskochil, MD, PhD, Co-Chair
David G. Little, MBBS, FRACS, PhD, Co-Chair

Research Monitor
John P. Dormans, MD, FACS

PROTOCOL ROSTER:

Protocol PI/IDE-Holder:
Elizabeth K. Schorry, MD, Cincinnati Children's Hospital

Co-Investigators:
B. Stephens Richards, MD, Texas Scottish Rite Hospital for Children, Dallas, TX
Dave H Viskochil, MD, PhD, University of Utah
David G Little, MBBS, FRACS, PhD, The Children's Hospital at Westmead, AustraliaAU

Statistician / Data Management and Analysis Center:

Gary Cutter, PhD
Director, Department of Biostatistics
School of Public Health, University of Alabama at Birmingham
1665 University Boulevard, RPHB 410B
Birmingham, AL 35294
Phone: 205-934-4937
Fax: 205-975-2540
cutterg@uab.edu

Joan E Hilner, MPH, MA
Department of Biostatistics
School of Public Health, University of Alabama at Birmingham
1665 University Boulevard RPHB 514D
Birmingham, AL 35294
Phone: 205-934-4943
Fax: 205-975-2500
jhilner@uab.edu

Operations Center Research Nurse Manager:

Vivien Phillips, RN, BSN
Department of Genetics, University of Alabama at Birmingham
1918 University Boulevard, MCML 252
Birmingham, AL 35294
Phone: 205-934-5376
vphillip@uab.edu

Operations Center Program Director:

Karen A. Cole-Plourde, BS
Department of Genetics, University of Alabama at Birmingham
1918 University Boulevard, MCML 256
Birmingham, AL 35294
Phone: 205-934-5140
kplourde@uab.edu

Research Compliance Specialist:

Roy McDonald, MPH, CHRC, CCRP
Department of Genetics, University of Alabama at Birmingham
1918 University Boulevard, MCML 262A
Birmingham, AL 35294
Phone: 205-975-4075
rmcdonald@uab.edu

Research Monitor:

John P. Dormans, MD, FACS
Texas Children's Hospital
Baylor College of Medicine, Pediatric Orthopaedic Surgery & Scoliosis

Agent(s)/Device: INFUSE Bone Graft (recombinant human Bone Morphogenetic Protein-2 + absorbable collagen sponge)

Supplier:
Medtronic Sofamor Danek USA, Inc.
Drive1800 Pyramid Place
Memphis, TN 38132

Laboratory Support (antibody levels): Intertek USA, Inc. d/b/a Intertek Pharmaceutical Services

Consortium Participating Sites

Bruce R. Korf, MD, PhD
University of Alabama at Birmingham
720 20th Street South, KAUL 230
Birmingham, Alabama 35294-0024
Phone: 205-934-9411
bkorf@uabmc.edu

Elizabeth K. Schorry, MD
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue - ML 4006
Cincinnati, OH 45229
Phone: 513-636-4760
elizabeth.schorry@cchmc.org

Michael J. Fisher, MD
Children's Hospital of Philadelphia
34th Street & Civic Center Boulevard
Philadelphia, PA 19096
Phone: 215-590-2800/Lab: 215-590-2107
fisherm@email.chop.edu

James H. Tonsgard, MD
University of Chicago
5841 South Maryland Avenue, MC 3055
Chicago, IL 60637
Phone: 773-702-6488
tonsgard@midway.uchicago.edu

Roger J. Packer, MD
Children's National Medical Center
111 Michigan Avenue, NW
Washington, DC 20010
Phone: 202-884-2120
rpacker@cnmc.org

David Viskochil, MD, PhD
University of Utah
50 North Medical Drive – 2C412 SOM
Salt Lake City, UT 84132
Phone: 801-581-8943
dave.viskochil@hsc.utah.edu

D. Wade Clapp, MD
Indiana University
1044 West Walnut Street
Cancer Center, Room 402A
Indianapolis, IN 46202
Phone: 317-278-9290
dclapp@iupui.edu

David Gutmann, MD, PhD
Washington University School of Medicine
660 South Euclid Avenue – Box 811
St. Louis, MO 63110
Phone: 314-362-7379
gutmannd@neuro.wustl.edu

Tena Rosser, MD
Children's Hospital Los Angeles
300 Medical Plaza Box 696824
Los Angeles, CA 90095
Phone: 323-361-2471
trosser@chla.usc.edu

Jeffrey C. Allen, MD
New York University - Pediatrics
160 E. 32nd Street – 2nd Floor
Hassenfeld Children's Center
New York, NY 10016
Phone: 212-263-9907
Jeffrey.Allen@nyumc.org

Nicole J. Ullrich, MD, PhD
Children's Hospital Boston
300 Longwood Avenue- Fegan 11
Boston, MA 02115
Phone: 617-355-2066
Nicole.Ullrich@childrens.harvard.edu

Todd Mildbrandt, MD (ortho)
Dusica Babovic-Vuksanovic, MD (Medical Genetics)
Mayo Clinic
200 1st St SW
Rochester, MN 55905

Affiliate Sites:
Massachusetts General Hospital (Plotkin)/Dana Farber Cancer Institute (Kieran): Ullrich
Johns Hopkins (Blakeley): Packer
Lurie Children's Hospital of Chicago (Listernick and Goldman): Tonsgard

Shriners Hospitals Sites (5):

Shriners Hospitals for Children, Los Angeles
909 S. Fair Oaks Ave.
Pasadena, CA 91105
Robert Cho, MD
Dr. Selina Poon spoon@shrinenet.org

Shriners Hospitals for Children, Salt Lake City, Utah
Kristen Carroll, MD

Shriners Hospitals for Children, Chicago
Purnendu Gupta, MD

Shriners Hospitals for Children, St. Louis
Perry Schoenecker, MD (P.I. for all Shriners sites)

Shriners Hospital for Children, Philadelphia
Corinna Franklin, MD
Scott Kozin, MD

U. Texas Southwestern/ Texas Scottish Rite Hospital
Laura Klesse, MD, Ph.D., site P.I. (oncology)
B. Stephens Richards, MD (orthopaedics)
Texas Scottish Rite Hospital for Children
2222 Wellborn Street

Dallas, TX 75219
Phone: (214) 559-5000
Steve.richards@tsrh.org

Radiology Review Panel:

Molly Dempsey-Robertson, MD (head), TSRHC
B. Stephens Richards, MD, TSRHC
David Wilkes, MD, TSRHC
Pam Nuremberg, MD, TSRHC

Biospecimen Allocation Committee (BAC):

David Viskochil, MD, PhD, University of Utah
David Gutmann, MD, PhD, Washington University St Louis
Carol A. Wise, PhD, TSRHC
+CTF
Other Members to be determined

Data and Safety Monitoring Board (DSMB) Members

Laura Klesse, MD, PhD, Oncology, University of Texas Southwest, Dallas
Laura.Klesse@UTSouthwestern.edu
Benjamin Alman, MD, Orthopaedics, Duke University
Ben.alman@duke.edu
Richard Browne, PhD, Statistician (retired, Texas Scottish Rite Hospital)
Rbrowne46@att.net

Roles and Responsibilities Page:

Michael Fisher, MD: Executive Director of NF Consortium, oversight of trial coordination

Elizabeth Schorry, MD: Study PI, Protocol Chair / IDE-holder

B. Stephens Richards, MD: Protocol Co-Chair, oversight of orthopedic efforts in US sites

David H Viskochil, MD, PhD: Protocol Co-Chair

David Little, MBBS, FRACS, PhD: Protocol Co-Chair; oversight of international orthopedic efforts

Bruce Korf, MD, PhD: NF Consortium PI, NFC Operations Center PI, Study Committee

Gary Cutter, PhD: Deputy Director of NF Operations Center & Trial Statistician – NF Data Center

John P. Dormans, MD, FACS: Medical Monitor

Molly Dempsey-Robertson, MD: Head of Radiology Review Panel (RRP) for Tibial Xrays

Joan Hilner, MPH: Data Director – NF Data Center

Vivien Phillips, RN, BSN: Clinical Research Nurse Manager – NF Operations Center

Karen Cole-Plourde, BS: Program Director II – NF Operations Center

Roy McDonald, MPH, CHRC, CCRP: Regulatory Compliance – NF Operations Center

ABBREVIATIONS:

ACS – Absorbable collagen sponge

AE – Adverse event

BAC – Biospecimen Allocation Committee

BMP-2 – Bone morphogenetic protein-2

DMAC – Data Management and Analysis Center (at UAB)

DSMB – Data and Safety Monitoring Board

eDES – electronic data entry system

FDA – Federal Drug Administration

FFPE – formalin fixed, paraffin embedded

FPS-R – faces pain scale, revised

HRPO – Human Research Protection Office

IDE – Investigational Device Exemption

MPNST – malignant peripheral nerve sheath tumor

NF1 – neurofibromatosis type 1

NFCTC – Neurofibromatosis Clinical Trials Consortium

PODCI – Pediatric Outcome Data Collection Instrument

rhBMP-2 – recombinant human Bone Morphogenetic Protein-2

RRP – Radiology Review Panel

RUST score – Radiograph union score for tibial fractures

SAE – serious adverse event

TPA – tibial pseudarthrosis

TSF – Tissue Storage Facility

TSRHC – Texas Scottish Rite Hospital for Children

UAB – University of Alabama at Birmingham

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1.0 **ABSTRACT/ PROJECT SUMMARY**

Neurofibromatosis type 1 (NF1) is a dominantly inherited disorder with a prevalence of 1/3,000 individuals. Patients with NF1 have an increased risk of bone complications, including tibial dysplasia, a congenital deficiency making the bone prone to fracture and non-union (tibial pseudarthrosis, TPA). Between 2% and 5% of patients with NF1 have TPA, making this a rare condition with overall prevalence of about 1/60,000 and qualifying it as an orphan disorder. TPA causes significant morbidity to those affected, management is fraught with difficulty, and up to 1/3 will eventually require amputation. There is significant evidence from mouse models and human tissues that deficient bone formation occurs in NF1, resulting in decreased ability to form new bone as well as increased resorption of bone. Bone morphogenetic proteins (BMPs) are osteoinductive signaling proteins of the transforming growth factor-beta superfamily, and their mechanism of action involves the recruitment and differentiation of mesenchymal progenitor cells into osteoblasts. When applied to bone at the time of surgery, BMPs induce mesenchymal stem cells to infiltrate the fracture zone and to differentiate into osteoblasts, which subsequently form new bone. Bone morphogenetic protein-2 (BMP-2), as a collagen sponge device marketed as INFUSE, has shown efficacy in healing of complex traumatic tibial fractures in adult populations. The current study proposes adding BMP-2 (INFUSE), an anabolic agent, at the surgical site of TPA repair in children with NF1, compared to a control group of patients treated surgically without BMP-2. The following **Specific Aims** will be addressed: 1) to determine if use of an osteogenic agent (BMP-2) at the time of surgical repair of TPA in NF1 patients will result in improved bone healing; 2) to document safety of BMP-2 in a pediatric NF1 population; and 3) to collect, process, and preserve biologic specimens at the time of surgery for future studies.

Methods: A Phase 2 clinical trial will be performed by a multi-center group of the NF Consortium, to compare treatment with or without INFUSE Bone Graft at the time of surgical repair**. Parents of patients will have the option of choosing whether they have a preference for the BMP group or the Control group. Those with a preference will be placed in the group of their choice (Choice Arm). Those without a preference will be placed in a Randomized arm, and will be randomized at a ratio of 3:2 for Control group: BMP group.** For all patients, a standard surgical procedure will be used, including: resection of pseudarthrosis tissue; placement of a rigid intramedullary rod; and placement of autogenous bone graft from iliac crest. For patients in the BMP group, the INFUSE device containing BMP-2 will in addition be applied intraoperatively to the osteotomy site. A minimum of 36 and maximum of 52 patients will be enrolled. Fracture union will be determined by scoring of radiographs (RUST score) for cortical bone fusion and callus formation at the osteotomy site. Score will be by a group of radiologists blinded to the group assignment of the patients. RUST score at 12 months post-surgery will be the **primary outcome measure** to determine efficacy. Secondary measures will include determination of time to healing (months); quality of life measures; functional walking measures; and incidence of refracture after surgery. This study, once successfully completed, will determine if use of INFUSE Bone Graft improves healing of tibial pseudarthrosis in NF1 and will document safety issues. Regardless of results, the better performing of the two groups (control or BMP) will be able to serve as a much-needed control arm for future studies of additional targeted therapeutic agents for NF1-related bone disease. An international working group of orthopaedic surgeons and NF specialists has been formed and is committed to successful completion of this trial.

2. DEVICE DESCRIPTION AND PRIOR INVESTIGATIONS

2.1 Device description/specification:

INFUSE® is a device consisting of 2 components. The active component is recombinant human bone morphogenetic protein -2 (rhBMP-2) and it is reconstituted onto an absorbable collagen sponge (ACS) that serves as a carrier/scaffold. This device induces new bone tissue at the site of surgical implantation.

rhBMP-2 is a disulfide-linked dimeric protein with 2 subunits of 114 and 131 amino acids. Each subunit is glycosylated at one end with high-mannose-type glycans. The recombinant form is produced by genetically engineered Chinese hamster ovary cell line. rhBMP-2 and excipients are lyophilized and packed in the kit. It is reconstituted so that each milliliter of rhBMP-2 solution contains: 1.5 mg of rhBMP-2; 5mg sucrose, NF; 25mg glycine, USP; 3.7 mg L-glutamic acid, FCC; 0.1 mg sodium chloride, USP; 0.1 mg polysorbate 80, NF; and 1.0 ml of sterile water. The reconstituted rhBMP-2 solution has a pH of 4.5, and is clear, colorless, and essentially free from plainly visible particulate matter.

The absorbable collagen sponge (ACS) is a soft, white, pliable, absorbent implantable matrix for rhBMP-2. It is made from bovine Type I collagen obtained from deep flexor (Achilles) tendon. The ACS acts as a carrier for the rhBMP-2 and serves as a scaffold for new bone formation.

The INFUSE® Bone Graft device is designed to be used along with internal stabilization with an intramedullary rod or nail to help heal fractures of the lower leg bone (tibia). INFUSE® is supplied in a kit containing all the components necessary to prepare the device including: the collagen sponge, a vial with the lyophilized rhBMP-2, a vial with the sterile water for reconstituting the rhBMP-2, syringes and needles. rhBMP-2 is provided as a lyophilized powder in vials delivering either 4, 8, or 12 mg of protein, which is reconstituted at the time of surgery to 1.5 mg/mL. The solution is uniformly distributed across the entire sponge and no sooner than 15 minutes after applying rhBMP-2 the ACS is placed at the fracture site. Kits are stored at room temperature (15-25 degrees Centigrade).

Indications for Use: INFUSE kits are indicated for treating acute, open tibial shaft fractures that have been stabilized with intramedullary nail fixation after appropriate wound management.

Device Regulatory History: INFUSE was developed over a decade ago, and the Premarket approval application was approved in 2004 (PMA 000054). The original PMA for INFUSE® was filed December 20, 2000. There were 24 amendments, and it was formally approved as a device by the Centers for Devices and Radiological Health in the Food and Drug Administration. There were conditions of approval that included the following: premarket approval application (PMA) supplements, post-approval reports, adverse reaction and device defect reporting, and reporting under the medical device reporting (MDR) regulation. These documents were retrieved from the FDA website, and are provided as a 58-page document (PMA approval letter (7 pages), Summary of Safety and Effectiveness Data (29 pages), Important Medical information packet (15 pages), and a patient information brochure (7 pages). The approval was originally provided to Wyeth Pharmaceutical, Inc.; however, the present manufacturer is Medtronic Spinal and Biologics (2600 Sofamor Danek Dr., Memphis, TN 38132).

Source and Pharmacology [Summary of Safety and Effectiveness Data from premarket approval application].

Absorption
Animal studies using subcutaneous implantation in rats and implantation at orthotopic sites in rats and rabbits of radiolabeled rhBMP-2 embedded in absorbable collagen sponges provide the best assessment of absorption of rhBMP-2. In the femoral onlay model, 125I-rhBMP-2 was slowly released from the implant site with a mean residence time of approximately 8 days. The peak amount of radiolabeled rhBMP-2 detected in blood was 0.1% of the implanted dose, and consistent with other studies showing rapid clearance of IV-administered rhBMP-2 in non-human primates (t1/2=6.7 minutes).

Binding to ACS:
INFUSE has its effect by binding of BMP-2 to the collagen sponge (ACS) and retaining an effective local concentration of rhBMP-2 to induce bone formation. Studies of use of rhBMP-2 in vitro show that with a soak time of at least 15 minutes for the rhBMP-2 solution onto the ACS, an average of 95.1% of the initial rhBMP-2 protein was retained by the ACS after squeezing and rolling of the ACS strips. [Hsu et al, 2006]. This provides evidence that minimal amounts of rhBMP-2 are lost from the implant site. An in vivo study using rat and rabbit models confirmed that bone deposition occurred only when rhBMP-2 was associated with the ACS, and there was no significant ectopic bone formation when rhBMP-2 was injected without a carrier [Hsu et al., 2006]. This further indicates that there is little risk of forming bone at ectopic sites as a result of rhBMP-2 solution dripping from the implant during the surgical process.

Distribution
In animal studies, IV-administered radiolabeled rhBMP-2 is rapidly distributed to highly perfused tissue (1 minute after dosing, 82.4% of the dose was recovered in the liver, lung, kidney and spleen with the liver being the prominent site).

Metabolism
In animal studies, IV-administered radiolabeled rhBMP-2 primarily localized and was metabolized in the liver.

Excretion
In animal studies, 24 hours post IV administration of rhBMP-2 showed 92% recovery in the urine, which indicates that excretion is primarily renal.

Storage information
At room temperature (15-25 degrees Centigrade/50-77 degrees Farenheit).

Formulation and Stability
The rhBMP-2 and excipients are lyophilized, and upon reconstruction, each milliliter of rhBMP-2 solution contains: 1.5 mg of rhBMP-2, 5.0 mg sucrose (NF), 25 mg glycine (USP), 3.7 mg L-glutamic acid (FCC), 0.1 mg sodium chloride (USP), 0.1 polysorbate 80 (NF), and 1.0 mL of sterile water. The reconstituted rhBMP-2 solution has a pH of 4.5, and is clear, colorless, and essentially free of from visible particulate matter.

Supplier
The supplier of this device is Medtronic Sofamor Danek USA, Inc. located in Memphis, Tennessee. INFUSE® will be provided by the company with demonstration of FDA approval of this clinical trial and funding of the clinical trial from external sources. The INFUSE device has been approved by FDA through the premarket approval process (PMA #000054) for use in treatment of acute, open tibial shaft fractures that have been stabilized by intramedullary fixation. As outlined in the background, INFUSE has been applied in a physician-directed approach in conjunction with placement of an intramedullary rod combined with autogenous

bone grafting. The safety profile of INFUSE in the pediatric population [Oetgen and Richards, 2010] provides an acceptable benefit/risk ratio in those children with tibial pseudarthrosis, including those with NF1. We have recently received approval from the manufacturer of the device, Medtronic Spinal and Biologics for 28 Medium INFUSE Bone Graft kits with a current list price of $162,240. Except for this provision of INFUSE kits, there is no other sponsorship from Medtronic. Our formal application to the FDA for an IDE was on the basis of an investigator-initiated, government-sponsored clinical trial (this proposal). Medtronic provided a "Letter of Access" to the FDA which allowed the FDA permission to review Medtronic files in their effort to address questions regarding animal testing or other studies of this device. Formal FDA approval of IDE application for the use of INFUSE as outlined in this clinical trial proposal was obtained on September 30, 2014.

Toxicities

BMPs participate in embryologic development, and BMPs and their receptors have also been found to be present in various tumor types, including bone and soft-tissue sarcomas [Yoshikawa et al., 1994]. This has raised theoretical concerns about risks for carcinogenicity or reproductive toxicity. However, extensive studies have thus far found evidence for neither of these [Poynton & Lane, 2002]. Preclinical studies by manufacturers showed no proliferative effect of rhBMP-2 on human osteosarcoma, prostate, breast, tongue, or lung carcinoma cell lines [Poynton & Lane, 2002]. When applied to certain cancer cell lines, rhBMP-2 did not cause proliferation of cells, but rather had an inhibitory effect [Soda et al., 1998]. Data on use of BMP-7 (OP-1, a similar compound to BMP-2) in humans shows that only 5 of 571 patients treated subsequently developed malignancy, mostly non-osseous tumors in elderly patients, and not at a higher rate than in the general population [Poynton & Lane, 2002]. There is a single case report of a malignancy (MPNST) occurring in a 23 year old patient with NF1 and large paraspinal neurofibromas who was treated with BMP during spine surgery [Steib et al., 2010]. However, no other cases have been reported, and it is unclear if this is simply a chance association, as MPNST is known to occur with a lifetime frequency of 8- 13% in patients with NF1 [Evans et al., 2002]. In order to address the theoretical increased rate of malignancy, our proposed study will exclude patients with plexiform neurofibroma of the leg undergoing surgery.

In initial animal studies, IV administration of rhBMP-2 failed to identify acute toxicities. Chronic toxicity of implanted rhBMP-2/ACS was evaluated as a 6-month mandibular/maxillary inlay in beagle dogs and as a 1-year femoral onlay in Sprague-Dawley rats. There were no systemic toxicities, and local effects were associated with the osteoinductive activity of rhBMP-2. Transient, low-titer immune responses were seen in the dog study. In human studies, there were no systemic toxicities associated with rhBMP-2/ACS in the adult (Govender et al., 2002) or pediatric populations (Oetgen and Richards, 2010); however, there were some adverse events or local toxicities reported that were potentially due to the osteoinductive effect of rhBMP-2. As with any surgery, surgical treatment of fracture is not without risk for adverse events. These include the adverse events noted in a clinical trial of INFUSE in open tibial fractures requiring surgical stabilization with an intramedullary rod or nail in adults. Even though the adverse event incidences were quite similar between controls without rhBMP-2 versus rhBMP-2/ACS implants at the surgical site for 2 different concentrations, the manufacturer of the device lists the following potential adverse events (alphabetized). Data on potential adverse events was obtained from adult studies, and may not apply to pediatric patients.

- Abnormal cellular growth
- Abnormal structure of the newly-formed bone
- Adhesion formation (sticking together of tissue surfaces)
- Aggressive bone resorption (breaking down of bone) or remodeling
- Allergic reaction

- Bone resorption (breaking down of bone) which may be transient
- Cyst or pseudocyst (collection of fluid) formation
- Delayed or failed healing
- Death
- Edema (swelling)
- Erythema (redness of the skin)
- Exuberant (too much) bone formation
- Failure of bone induction (bone growth)
- Fluid retention
- Foreign body (allergic) reaction
- Formation of antibodies (type of protein that helps protect the body against foreign matter)
- Formation of blood clots
- Hematoma or bruise (collection of blood caused by bleeding from a broken blood vessel)
- Heterotopic bone (bone in the wrong place) formation
- Implant fracture
- Implant migration (movement)
- Infection
- Inflammation (swelling, redness, and pain in tissues)
- Interference with wound healing when used in the closure of skin incisions
- Irritation
- Loss of purchase (loosening of the implant)
- Osteolysis (breaking down of bone tissue)
- Seroma (a collection of clear fluid), and/or fluid collections/ fluid-filled sacs
- Slow dissolving of the implant
- Spinal stenosis (narrowing of the spinal canal)
    - Incidences of spinal stenosis have only been reported when BMP-2 was used in spinal surgery. In this study, we will only utilize BMP-2 in tibial repair.

  and
- Swelling near the surgical site.

The review by Oetgen and Richards [2010] puts these potential complications in perspective for pediatric patients exposed to rhBMP-2. They performed a retrospective review of 81 patients less than 18 years of age in whom rhBMP-2 was used as a surgical adjunct to promote bone healing in spine (47), femur (7), cervical spine (5), tibia (21), and ribs (1). There were no instances of systemic toxicity defined as clinical deterioration of an organ system function. A total of 16 complications were identified (~20%), and of these 9 involved the local operative site. Only one case of dural fibrosis with subsequent weakness was possibly attributable to rhBMP-2 when used in spinal fusion [Oetgen and Richards, 2010].

2.2 Summary of Prior Studies with the Device:

2.2.1 Adults: There are several key studies showing the efficacy of INFUSE. Extensive data exists on use of rhBMPs for treatment of open tibial fractures in adults. Govender et al. [2002] studied 421 adults with open tibial fractures, who were randomized to receive one of the

two methods: routine surgical care with intramedullary nail fixation, or routine care plus an implant containing rhBMP-2. With the additional use of rhBMP, they found significant shortening of time to fracture healing, and decreased rate of infections. The group treated with rhBMP-2 also had 44% reduction in need for a secondary procedure for delayed union. There was no increase in soft-tissue calcifications or ectopic ossification in the treated group, and overall complications were lower in the BMP-treated group compared to the standard surgery group. Several additional large, multi-center studies performed since then have confirmed the efficacy and safety of rhBMP-2 for open tibial fractures in adults [Swiontkowski et al., 2006; Jones et al., 2006]. Recently, a clinical trial entitled Tibial Delayed Healing Pivotal Clinical Trial (NCT01016067; ClinicalTrials.gov identifier) was opened in 2010 to evaluate INFUSE/MASTERGRAFT™ Delayed Healing Device (Medtronic) as an alternative to autograft in the treatment of tibial delayed healing. Those with congenital pseudarthrosis of the tibia (NF1 patients with tibial pseudarthrosis) and patients less than 21 years of age were excluded.

Woo [2013] reviewed adverse events associated with use of rhBMP-2 in nonspinal orthopaedic procedures, many of which involved off-label use. There were a total of 62 events reported to the FDA through 2011, including: wound complications (15), heterotopic bone formation (12), pseudarthrosis (10), local inflammation (9), osteolysis or resorption (5), compartment syndrome (2), and peripheral nerve injury (2). Of the 12 reports of heterotopic ossification, 5 required surgery to remove extra bone. The author noted that this data could not be used to estimate incidence rates of adverse events, as the total number of surgical procedures using rhBMP-2 is unknown. It was additionally noted that occurrence of pseudarthrosis might simply reflect the refractory nature of certain fractures, regardless of use of BMP-2.

Use of BMP-2 during spinal fusion surgery in adults has received recent attention regarding safety and efficacy data. As reviewed by Carragee et al. [2011], initial industry-sponsored studies reported an extremely low rate of adverse events, despite significant events reported to the FDA. Carragee et al. undertook a review of the literature comparing the original industry-sponsored trials to data reported by the FDA and subsequent publications. From their review, they estimated the rate of adverse events associated with rhBMP-2 use in spinal fusion ranging from 10%-50%, depending on approach. Complications included swelling of neck and throat (associated with cervical spine fusion), inflammatory effect with increased rate of wound complications, urological complications including bladder retention and retrograde ejaculation in patients with lumbar spine fusions, and osteolytic defects. These data have likely influenced the approach of many orthopaedic surgeons in their use of rhBMP-2 during spinal fusion.

More recently, the FDA submission of a high dose rhBMP-2 product for spinal fusions, AMPLIFY™, which includes a total dose of 40 mg rhBMP-2 (10-fold higher than the dose in a small packet of INFUSE), reported a mild increase in cancer risk in elderly patients in a 5 year follow-up (3.9% of 239 patients). The increased cancer risk just reached significance (p=.05) according to the analysis of Carragee et al. [2011]. No increased cancer risk has been reported to date in patients treated with the lower-dose INFUSE product. The occurrence of tumors in this high dose BMP-2 group prevented the product being approved by the FDA, but the INFUSE product at a lower dose remains approved.

Recombinant human rhBMP-2 is currently approved by the FDA as a device for use in adults for lumbar spine fusion and open tibial fractures (INFUSE® Bone Graft). The FDA lists skeletal immaturity (age less than 18 years) as a contraindication to its use, primarily because there is limited data on its safety in children. Despite this, the device has been used on a physician-directed off-label basis in increasing numbers of children, and data are beginning to accumulate.

2.2.2 Pediatric use, including NF1

Chin et al. [2005] were among the first to report use of rhBMP-2 in children. They used rhBMP-2 instead of autogenous bone graft in repair of congenital facial clefts in 43 children (ages 6-14 years). Successful union of bone was achieved in 98% of patients, and no systemic adverse effects or ectopic bone formation occurred during up to two years of follow-up. Alonso et al. [2010] also reported use of rhBMP-2 in 16 patients who underwent repair of cleft lip and palate, between 8 and 12 years of age. Good bone union occurred in all patients, with no ectopic bone growth. Richards et al. [2010] performed a retrospective review of 7 patients, with congenital tibial pseudarthrosis (4 with NF1; age ranges 1.9-11 years) treated with rhBMP-2 applied locally at time of surgery with intramedullary rod. In 5/7 patients, there was a good healing after one surgery, with average time to union of 6.4 months. During a prolonged follow-up time of 4-9 years, there was no evidence of tumor growth or systemic effects noted from rhBMP-2 use.

Oetgen and Richards [2010] also performed a review of 81 children who have been treated with rhBMP-2 between 2001 and 2008, between the ages of 1 and 17 years. Of this group, 52 children received rhBMP-2 during spine surgery and 21 during tibial repair. Sixteen (16) of the children had NF1. One patient with NF1 subsequently had enlargement of a previously identified optic nerve glioma, but this was interpreted as unlikely to be related to rhBMP-2. There was a total complication rate of 17%, most of which were surgical wound inflammatory responses. Only one complication was postulated to be related to rhBMP-2, and that was a case of dural fibrosis developing after direct exposure of the dura to rhBMP-2. There were no reports of systemic toxicity, excessive bone growth, sarcomas, or other tumors.

2.3 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Neurofibromatosis type 1 (NF1) is a dominantly inherited disorder occurring with a prevalence of 1/3,000 individuals. Patients with NF1 have an increased risk of osseous complications, including tibial dysplasia and tibial pseudarthrosis (TPA) with chronic fracture and non-union. Although occurring in only 2-5% of NF1 patients, management of TPA is fraught with difficulty, with many patients requiring multiple surgeries and up to 33% eventually requiring amputation. Significant evidence from both animal models and human tissue documents a difference in bone biology in NF1, with decreased ability to form new bone as well as increased resorption and remodeling of bone. An international working group of NF specialists and orthopedic surgeons has been formed, and proposes a clinical trial to document efficacy of an osteogenic agent (INFUSE; recombinant human bone morphogenetic protein-2) as a supplement to surgical approaches to TPA in NF1. This international group is committed to successful completion of this trial.

PRIMARY AIMS:

Specific Aim 1: To determine if use of an osteogenic agent (rhBMP-2) at the time of surgical treatment of TPA in NF1 patients will result in improved bone healing when compared to patients treated with the same surgical protocol but without BMP-2.

Specific Aim 2: To document safety of BMP-2 in a pediatric NF1 population

Specific Aim 3: To collect, process, and preserve biologic specimens at the time of surgery for future studies related to NF1.

Primary Objectives:

Objective 1: To perform a phase 2 single-blind, randomized clinical trial to determine if use of INFUSE (rhBMP-2 on a collagen sponge) applied at time of surgical repair of TPA in NF1 patients will result in:

1a. Improved bone healing rates compared to patients treated without INFUSE, based on radiographic evidence of cortical bone union at 12 months after surgery (RUST score; **Primary Outcome Measure at 12 months)**

1b. Shorter time (months) to objective radiographic union than in patients treated without INFUSE.

Objective 2: To evaluate the feasibility and potential toxicities of BMP-2 administration in this population.

Objective 3: To collect, process, and preserve biologic specimens at the time of surgery for future studies.

SECONDARY AIMS/OBJECTIVES:

- To evaluate quality of life at the time of surgery, and at 6 months and 12 months after surgery.
- To determine the incidence and timing of re-fracture after surgery.
- To provide a cohort of patients with NF1 and TPA treated with a standardized surgical protocol, which can be used as a much-needed arm for future clinical trials.
- To provide annual long-term follow up of this cohort of patients for up to 10 years after surgery to monitor for rate of refracture and safety issues. (Note: this objective will not be funded by the current funding for the NF Clinical Trials Consortium).

3.0 BACKGROUND AND SIGNIFICANCE

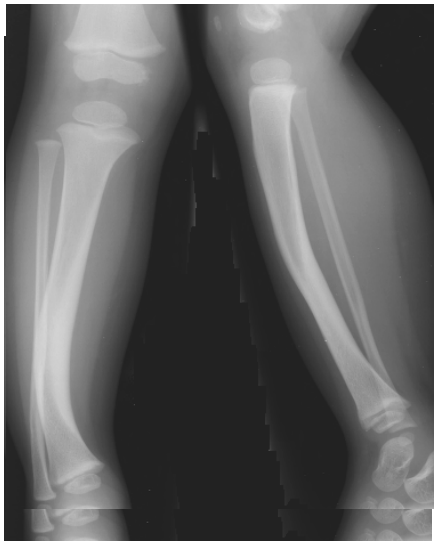
Neurofibromatosis Type 1 and Tibial Pseudarthrosis: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder whose features involve many systems, including skin (cafe-au-lait spots, cutaneous neurofibromas), central nervous system (learning disabilities), musculoskeletal, and tumor predisposition. Up to 38% of patients with NF1 have one or more skeletal abnormality [Crawford and Schorry, 1999], including long bone dysplasia, scoliosis, sphenoid wing dysplasia, and bone cysts. We have proposed a clinical trial for a rare osseous complication of NF1, tibial pseudarthrosis (TPA), which presents with significant morbidity for patients and extreme difficulty in surgical management.

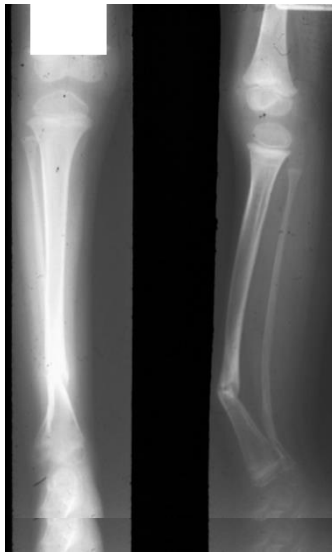
3.1 Prevalence of the Problem: NF1 occurs with a prevalence of 1/3000 in all populations regardless of ethnicity, race, or gender [Huson et al, 1988; Friedman and Riccardi, 1999]. Tibial pseudarthrosis (TPA) is a relatively rare complication of NF1; data from a large international NF1 database has documented tibial pseudarthrosis to occur in between 2-5% of patients with NF1[Friedman and Birch, 1997], resulting in a maximum estimated prevalence of 1/60,000 individuals. With a US population of 315 million, there are only estimated to be 5,250 individuals in the US with tibial pseudarthrosis related to NF1, qualifying it as an **orphan disease**.

Tibial pseudarthrosis is defined as a chronic non-union in a bone which has fractured. It can occur in the general population as well as in patients with NF1, although more than half of all individuals with TPA have NF1. In patients with NF1, TPA typically occurs in a bone which is congenitally dysplastic, presenting as an anterior-laterally bowed tibia and/or fibula which has a high risk for fracture. Stevenson et al. [1999] surveyed a multi-center cohort of patients with NF1 and tibial dysplasia and found that tibial dysplasia was 1) most often unilateral, 2) evident in the first year of life, and 3) affected males more often than females by a ~2:1 ratio. Additional findings demonstrated that tibial pseudarthrosis usually results as a progression from tibial anterolateral bowing. This anterolateral bowing is recognized as **tibial dysplasia**, whereas the non-union of bone after fracture is defined as **tibial pseudarthrosis** (Figure 1). Analyses in the report by Stevenson et al. [1999], which reviewed a total of 160 NF1 subjects, showed that 61-66% of individuals with tibial dysplasia progressed to fracture and pseudarthrosis. The average age of fracture was 4.6 years with a range of 0 to 28 years, and the average number of surgeries in cases with pseudarthrosis was 3, with some patients having as many as 10 surgical procedures.

Figure 1 A and B: Radiographs of tibial dysplasia

Figure 1. Image A shows typical tibial dysplasia with anterolateral bowing, thickening of cortex and narrowing of medullary canal. Image B demonstrates a tibia with chronic pseudarthrosis.





TPA in NF1 patients leads to major morbidity, including possible lower leg amputation. Presently, best care guidelines for the treatment of TPA in NF1 do not include medical management protocols. We have assembled a multidisciplinary team of clinicians who provide specialized care for patients with NF1. Principal investigators of this clinical trial participated in two clinical trial strategy meetings for treatment of bone abnormalities in NF1 (New York City, 02/2008 and 4/2011) [Elefteriou et al., 2009], which led to a consensus to prioritize the development of a clinical trial for the combined medical/surgical treatment of tibial pseudarthrosis (TPA). Drs. Viskochil, Schorry, and Little recruited Dr. Richards to jointly work with the NF Clinical Trials Consortium to develop a multi-center clinical trial that implements medical treatment combined with well-established surgical protocols to optimally manage tibial pseudarthrosis in NF1 patients.

3.2 Pathophysiology

The pathophysiology of bone manifestations in NF1 is not yet fully elucidated, although recent research with animal models and human tissues has made great progress in our understanding of this area. The *Nf1* gene is classified as a tumor suppressor gene [reviewed in Viskochil et al., 1993]. Its encoded product, neurofibromin, is a 240-kDa peptide [reviewed in Sherman et al., 1998] that stimulates the intrinsic hydrolysis of ras-bound guanosine triphosphate (GTP) [Martin et al., 1990]. Ras is a small intracellular protein that must be attached to the inner membrane of the cell to be active in signal transduction. When bound to GTP, the protein transduces growth signals to the cell's nucleus through both mitogen-activated protein kinase (MAPK) and AKT signaling. MAPK signaling cascades are operational in the key cells playing a role in bone homeostasis, remodeling, and fracture healing: osteoclasts and osteoblasts [Chaudhary and Avioli, 1998; Hipskind and Bilbe, 1998]. While *NF1*haploinsufficiency is associated with tumor predisposition, studies support the hypothesis that *NF1* haploinsufficiency also results in a range of non-tumor phenotypes [Wu et al., 2006], including skeletal abnormalities [Alwan et al, 2005]. Osteopenia has been reported in both children and adults with NF1, and is one of the recognized manifestations of this underlying bone disorder [Illes et al. 2001; Kuorilehto et al. 2004; Stevenson et al 2007]. More localized osseous manifestations also occur, including sphenoid wing dysplasia, bone cysts, pectus excavatum, and scoliosis (both idiopathic and dystrophic forms). Bi-allelic inactivation of the *NF1* gene in surgically resected pseudarthrosis tissue has been demonstrated in at least 6 NF1 patients to date [Stevenson et al., 2006; Lee et al., 2012], which suggests that somatic genetic changes contribute to abnormal bone

remodeling and pseudarthrosis in NF1. It is likely that generalized bone changes in NF1 (such as short stature and osteopenia) are related to haploinsufficiency of neurofibromin, whereas focal changes such as tibial dysplasia are related to bi-allelic inactivation of *Nf1*.
Bone is regulated by two competing forces: anabolism (new bone formation) and catabolism (bone resorption). The cell types driving these two processes are osteoblasts and osteoclasts, respectively. Osteoblasts, derived from mesenchymal stem cells, are stimulated to produce bone matrix by Bone Morphogenetic Proteins and other growth factors. Osteoclasts are derived from the macrophage/monocyte lineage, and play a major role in bone resorption and remodeling. Neurofibromin is expressed in multiple cell types involved in bone development, including osteoblasts, osteoclasts, chondrocytes, and vascular endothelial cells. Substantial evidence shows a role for dysfunction of both osteoblasts and osteoclasts in NF1-related bone disease [reviewed in Schindeler and Little, 2008]. This cumulating data in both human and animal tissues **documents significant differences in bone biology in NF1, leading to poor bone formation as well as overly active bone resorption**, **and justifying a therapeutic trial that addresses these issues in NF1 tibial pseudarthrosis.**

3.3. Animal models

The development of animal models that recapitulate the bone phenotype in NF1 has yielded insights for the role(s) of neurofibromin in bone development and remodeling. Murine models provide an opportunity to dissect the processes of union after fracture and are important in determining the most appropriate biologic compounds to enhance bone strength in long-bone dysplasia and healing in NF1-related TPA.

Several studies have shown reduced expression of bone markers and decreased mineralized matrix in cultured osteoblasts from NF1 mouse models and human *NF1* haploinsufficient embryonic bone cells [Klein et al., 1995; Schindeler et al., 2008; Wu et al., 2006; Yu et al., 2005]. *NF1* heterozygous mice showed elevated numbers of multinucleated osteoclasts compared to wild type mice, with increased migration capacity and increased capacity to resorb bone [Yang et al., 2007].

Mouse models for NF1 include $Nf1^{+/-}$ mice as well as conditional knockouts (somatic $Nf1^{-/-}$ cells) that target bone-specific cells The major characteristics of the skeletal phenotype of these *Nf1* genetically engineered mouse (GEM) models are highlighted in a modified table as reviewed by Elefteriou et al., [2009] (Table I).

Table I. Osseous characteristics of the *Nf1* mouse models. (adapted from Elefteriou et al., 2009)

	$Nf1^{+/-}$ [Yu et al. 2005]	$Nf1^{ob-/-}$ [Elefteriou et al. 2006]	$Nf1^{Prx-/-}$ [Kolanczyk et al. 2007]	*NF1 Col2.3 Cre [zhang et al., 2011]*
Loss of *Nf1* activity	Whole body	Mature osteoblasts	Limb mesenchymal osteoprogenitors	Osteoblast lineage
Osteoblast differentiation	Decreased	Normal	Decreased	Decreased
Osteoclast differentiation	Increased	Increased	Increased	Increased
Bone quality	Not determined	Decreased cristalinity	Increased cortical porosity	Decreased bone density
Mechanical property	Decreased stiffness	Decreased stiffness	Not determined	

Parallels with human NF1 skeletal phenotype	- Increased bone resorption - Delayed bone healing	- Increased bone resorption - Delayed bone healing - Increased osteoidosis	- Increased bone resorption - Tibial bowing - Low bone density - Delayed bone healing	-low bone mass -tibial nonunion -shortened vertebrae

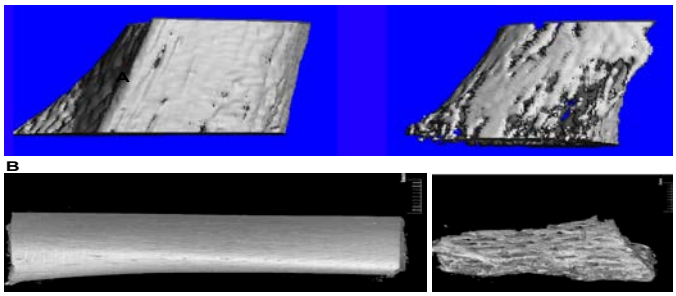
No mouse model fully replicates the human skeletal manifestations, but *Nf1*-deficient mice are highly valuable for the pre-clinical testing of candidate therapies for NF1 skeletal defects. Bone healing in the distal tibia, where TPA is most common, is abnormal in $Nf1^{+/-}$ mice [Schindeler et al., 2008a; Schindeler et al., 2008b]. The cortical porosity of the $Nf1^{Msc-/-}$ GEM mouse model and human long bone from an NF1 patient are remarkably similar, as shown in preliminary studies kindly shared by colleagues Elefteriou and Stevenson (see Figure 2).

The $Nf1^{prx-/-}$ model, which produces bi-allelic inactivation of *Nf1* in mesenchymal cells of the developing limbs, probably most closely parallels that of tibial pseudarthrosis in humans with NF1 [Kolanczyk et al, 2007].The resulting $Nf1^{prx-/-}$ mice showed stunted growth, tibial bowing, joint abnormalities, and highly porous cortical bone in the limbs. **Osteoblasts from these mice showed increased proliferation but decreased ability to differentiate and mineralize.**

Another bone phenotype was created by bi-allelic inactivation of *Nf1* in committed osteoblasts ($Nf1^{ob-/-}$). These mice had delayed mineralization of bone, increased numbers of osteoclasts, and increased bone resorption. A more recent mouse model of NF-1 bone disease, $NF1^{col2-/-}$ GEM, demonstrated neurofibromin loss in osteochondroprogenitor cells affecting both axial and appendicular skeleton [Zhang et al., 2011]. These mice showed progressive scoliosis and kyphosis, tibial bowing, chest wall deformities, severe growth retardation, and vertebral disc defects. There were also abnormal bone biomechanical properties, including a significant reduction in stiffness of bone. Studies of these Col2.3 Cre mice by Rhodes et al. [2013] recently demonstrated that the transforming growth factor beta (TGF-*B*1) pathway may also play a significant role in NF1 bone disease, and may represent additional targets for future therapeutics.

Lastly, none of these models actually demonstrated the classic features of TPA. Based on the findings by Stevenson et al. and Lee et al. that loss of heterozygosity of *Nf1* may be present in the fibrous tissue in TPA, El-Hoss et al [2012] used $NF1^{flox/flox}$ mice and a cre-expressing adenovirus to produce bi-allelic inactivation in a fracture site. This was performed in mice of $Nf1^{+/-}$ and $Nf1^{+/+}$ background. When *Nf1* was deleted at the fracture site, fewer than half of the mice showed effective union of bone when an open osteotomy was performed with periosteal stripping. The model featured the anabolic deficiency noted in TPA, as well as the fibrous tissue containing multiple osteoclast-like cells, typical of TPA. **This model confirms that neurofibromin is essential for fracture healing, and that its induced absence leads to a TPA-like picture.** These mouse bone data clarified that total loss of *NF1* function is likely the underlying molecular cause of the NF1 focal dysplastic defects, and correlates with the human data from tibial pseudarthrosis tissue [Stevenson et al., 2006; Lee et al. 2012].

Figure 2. 3-D reconstruction of microCT images of tibia from mice (A) and human (B)



(A) Image of tibial bone on left taken from wild-type mouse; image of tibial bone on right taken from an *Nf1*Msc-/- mouse showing cortical porosity. (B) Image on left taken from the tibia of a deceased child without NF1, while the image on the right is taken from a tibial sample during surgical repair of an NF1 child with tibial pseudarthrosis showing cortical porosity similar to what is observed in the mouse model. The tibial sample was cut longitudinally for the NF1 child but control tibial sample is left intact [courtesy F. Elefteriou (mouse images) and D. Stevenson (human images)].

Several animal studies have investigated use of BMPs in in bone healing. Earlier studies showed that BMPs were capable of restoring function with large diaphyseal bone defects, and in preventing non-unions in severe open fractures [Lane et al., 1999; Little et al. 2005; Hak et al. 2006]. Schindeler et al. [2008] investigated osteoblast response from cultured calvarial osteoblasts of NF1-deficient mice, and found that $NF1^{+/-}$ cells responded to BMP-2 in culture with a 2-fold increase in alkaline phosphatase expression, although the final effect was less than that seen in wild type mice. Similarly, BMP documented osteogenic effect by producing heterotopic bone when injected into mouse quadriceps muscle, although again to a lesser extent for *NF1* deficient mice compared to wild type [Schindeler et al., 2008].

<u>Innovation:</u> These preclinical findings from animal models encourage clinical trials using a medical approach to treat NF1-related TPA. Confirmation of NF1 target pathways in bone-forming cells provides a compelling justification for a medical approach aimed at increasing local bone anabolism. This approach can be used in combination with surgical techniques to promote fracture healing and to diminish non-union complications in repair of tibial pseudarthrosis.

3.4 Surgical Management of TPA: Background

In the general population, open fractures of the tibia are one of the most difficult fractures to heal, with approximately 60% of all non-unions occurring in the tibia. This is true when bone is perceived to be normal prior to fracture. Individuals with congenital pseudarthrosis of the tibia are unique in that the underlying bone is abnormal, which likely contributed to the fracture as well as the non-union [Johnston and Birch, 2008]. Thus, surgical management for these patients requires extraordinary approaches to establish bone union.

Surgical stabilization of tibial pseudarthrosis has evolved over the last 2 decades [Coleman et al., 1995; Grill et al., 2000]. External distraction procedures, such as the Ilizarov approach, are rarely implemented in the initial management of congenital pseudarthrosis of the tibia. An alternative approach is preferred by most pediatric orthopedic surgeons whereby the tibia is stabilized with placement of an intramedullary rod. Various institutions use different types of intramedullary rods, and the surgical management goal is to optimize both tibial healing and ankle function. The technique now used by most experienced NF surgeons includes; 1) excision of the pseudarthrosis site, 2) intramedullary rod placement (with or without transfixing the ankle

joint), and 3) placement of autogenous bone harvested from the iliac crest around the pseudarthrosis site [Johnston, 2002; Dobbs et al, 2005].

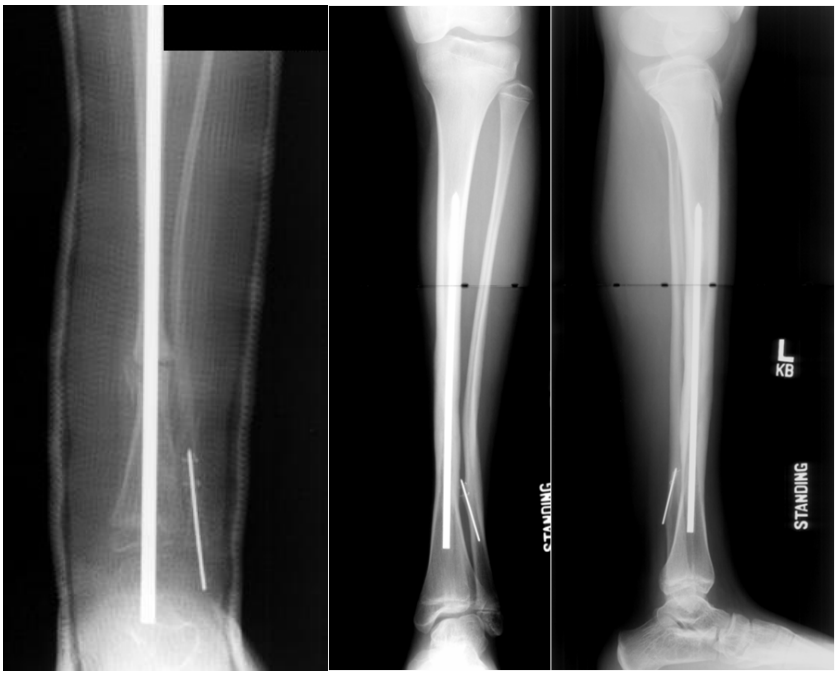
Johnston [2002] reported the results of 23 consecutive patients with congenital pseudarthrosis of the tibia (12 with NF1) who were treated between 1978 and 1992 by 7 different pediatric orthopaedic surgeons using an intramedullary rod (Charnley-Williams procedure [Charnley J, 1956]) for fixation and bone graft, with and without transfixation of the ankle. Outcomes were reviewed 4-14 years after surgical intervention and scored on a scale of 1 to 3. Eleven of 23 (48%) had a grade 1 outcome, which represents unequivocal union of the tibia; however, only 5 patients had this outcome with a single operation (5 had 2 surgeries and 1 required 3 surgeries) [Johnston, 2002]. Outcome was not presented separately for patients with NF1.

Dobbs et al., [2004] reported using this surgical technique in a cohort of 21 patients with TPA (12 with NF1) who were followed for 14 years. Patients were reported to have an eventual healing rate of 86% with mean time to union of 16 months; however, multiple surgeries were required for the majority of these patients. The healing rate after a single surgical procedure was not reported. In addition, 57% of patients re-fractured over time at the site of the initial pseudarthrosis. Of the 12 with NF1, 3 required amputation. In many of these studies, it has become apparent that healing rates in patients with NF1 and TPA are poorer than in those without NF1.

Other treatment methods used for TPA include free-vascularized fibular graft, McFarland bypass graft, and Ilizarov bone transport, with reported union rates ranging from 14% to 75% [Grill et al., 2000]. The Ilizarov technique of external fixation in experienced hands showed an overall 75% fusion rate [Grill et al., 2000]; however, given the high frequency of repeat surgeries needed and extensive time to final healing, this technique of external fixation is not an optimal initial procedure for the young patient population with TPA.

It is clear from the above data that surgical management of TPA, particularly in patients with NF1, has not yet been optimized and that significant numbers of patients continue to require multiple surgeries and/or progress to need for amputation. Subsequently, adjunctive treatments have been sought to improve healing and lower re-fracture rate. Orthopedic surgeons have begun to use **bone morphogenetic proteins** as adjunctive anabolic agents at the time of surgery for patients with open tibial fractures [Govender et al, 2002] and tibial fractures with cortical defects [Jones et al., 2006]. In a small case series conducted by one of the co-PIs, Richards et al., [2010] recently reported a group of 7 patients with TPA (4 with NF1) who were treated with intramedullary rod fixation; autogenous graft from the iliac crest; and placement of rhBMP-2 saturated absorbable collagen sponges (INFUSE™; Medtronic) around the site of the tibial defect site intraoperatively. Five of 7 patients (71%) had radiographic union at a mean time of 6.4 months post-operatively (see figure 3). Although a number of orthopaedists now routinely use BMP-2 in NF1 patients with TPA, organized clinical trials have not yet been implemented to prospectively evaluate its utility.

Figure 3. Williams rod procedure



Radiographs after Williams rod placement across the ankle joint, autogenous bone-grafting, and rhBMP-2 application, showing excellent early alignment (left panel). The pseudarthrosis was healed at 6.7 months and remained so 7.8 years later (from figure 2B and 2C in Richards et al, 2009). The fibula has been fixed with a nail.

The improved rate of radiologic union with use of rhBMP-2 is highly relevant to the NF1 population. Even though tibial dysplasia with fracture is a rare complication of NF1, it leads to significant morbidity due to poor bone union and high likelihood for re-fracture, regardless of surgical approach. A better understanding of bone remodeling in NF1 opens a new avenue of medical treatment to enhance fracture healing. **Abnormal bone formation seen in both animal models and human NF1 tissues raises the opportunity to improve outcome in NF1-related TPA by treatment with a protocol that utilizes a biologically-based anabolic agent (Bone Morphogenetic Protein-2) to improve bone union.**

It is also clear from the previously reviewed studies, that results from individual surgeons or groups in TPA techniques and outcomes are not directly comparable. Many surgeons have lumped NF1 patients with non-NF1 patients in their reports of surgical success; when data are extracted separately, it is clear that the outcome is poorer with all approaches for patients with NF1. This underscores the need to perform a trial specifically for NF1-related CPT, utilizing a standard surgical protocol and standard outcome measurements.

3.5 Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) are signaling molecules which are part of the transforming growth factor-beta (TGF-*B*) superfamily of proteins. They play a role in organogenesis of many cell types, including skeletogenesis. There are 14 or more known BMPs; one of the most well-studied is BMP-2, which is known to play a major role in bone development. Although present in high levels during embryogenesis, very minute amounts of BMP-2 occur naturally in bone in children and adults. BMPs are expressed during normal fracture repair. Gerstenfeld and colleagues [2003] noted early expression of BMP-2 during fracture repair, with expression of BMP-4, -5 and -6 coming later and BMP-7 later still. Recently

it has been proven that BMP-2 is an essential factor in the initiation of fracture healing [Tsuji et al., 2006].

BMPs bind to BMP receptors in target cells, resulting in a signal transduction cascade through the SMAD family of proteins. Of interest, other pathways, including the Ras-MAPK pathway important in NF1, can also be activated by BMPs. For example, M-Ras is activated by BMP-2 in transdifferentiation of C2C12 muscle cell lines to the osteoblastic lineage [Watanabe et al., 2010]. However, in a circumstance such as NF1 where Ras is abnormally regulated, the balance between SMAD mediated signaling and any further stimulation of the Ras/MAPK pathway may be important in determining cell fate decisions.

The ultimate effect of BMP signaling is to induce mesenchymal progenitors to infiltrate the fracture zone and to differentiate into osteoblasts, which subsequently form new bone. A concomitant increase in vascularity is also notable. BMPs can stimulate new bone formation even in extra-skeletal sites in a host, leading to their use in treatment of skeletal injuries, spinal fusions, and facial clefting repairs.

Through molecular technology, recombinant forms of human BMP-2 and BMP-7 are currently commercially available. The largest amount of data regarding safety and efficacy exists for rhBMP-2, which is currently approved by the FDA for use in adults for lumbar spine fusion and open tibial fractures. The device is used in the form of an absorbable collagen sponge impregnated with rhBMP-2 (INFUSE).

Extensive data exists on use of rhBMPs for treatment of open tibial fractures in adults. Govender et al. [2002] studied 421 adults with open tibial fractures, randomized to receive one of two methods: routine surgical care with intramedullary nail fixation, or routine care plus an implant containing rhBMP-2. With the additional use of rhBMP, they found significant shortening of time to fracture healing, and decreased rate of infections. The group treated with rhBMP-2 also had a 44% reduction in need for a secondary procedure for delayed union. There was no increase in soft-tissue calcifications or ectopic ossification in the treated group, and overall complications were lower in the BMP-treated group compared to the standard surgery group. Several additional large, multi-center studies performed since then have confirmed the efficacy and safety of rhBMP-2 for open tibial fractures in adults [Swiontkowski et al., 2006; Jones et al., 2006].

Of importance, BMPs under certain conditions, such as in high doses, can also induce activity of osteoclasts involved in bone resorption [Kanatani et al., 1995; Tsuji et al., 2006]. Therefore, appropriate doses must be used to achieve the correct balance between osteoblast and osteoclast activity. It appears that although rhBMP-2 may cause some increased osteoclastic activity, it is coupled with an osteoblastic upregulation of greater magnitude, with a purported end result of increased volume of bone fusion mass [Poynton & Lane, 2002].

3.5.1 Safety of rhBMP-2

BMPs participate in embryologic development, and BMPs and their receptors have also been found to be present in various tumor types, including bone and soft-tissue sarcomas [Yoshikawa et al., 1994]. This has raised theoretical concerns about risks for carcinogenicity or reproductive toxicity. However, extensive studies have thus far found evidence for neither of these [Poynton & Lane, 2002]. Preclinical studies by manufacturers showed no proliferative effect of rhBMP-2 on human osteosarcoma, prostate, breast, tongue, or lung carcinoma cell lines [Poynton & Lane, 2002]. When applied to certain cancer cell lines, rhBMP-2 did not cause proliferation of cells, but rather had an inhibitory effect [Soda et al., 1998]. Data on use of BMP-7 (OP-1, a similar compound to BMP-2) in humans shows that only 5 of 571 patients treated subsequently developed malignancy, mostly non-osseous tumors in elderly patients, and not at a higher rate

than in the general population [Poynton & Lane, 2002]. There is a single case report of a malignancy (MPNST) occurring in a 23 year old patient with NF1 and large paraspinal neurofibromas who was treated with BMP during spine surgery [Steib et al., 2010]. However, no other cases have been reported, and it is unclear if this is simply a chance association as MPNST is known occur with a lifetime frequency of 8- 13% in patients with NF1 [Evans et al., 2002]. In order to address the theoretical increased rate of malignancy, our study will exclude patients with plexiform neurofibroma of the leg undergoing surgery.

Recently, the FDA submission of a high dose rhBMP-2 product for spinal fusions, AMPLIFY™, which includes a total dose of 40mg rhBMP-2 (10-fold higher than the dose in INFUSE), revealed a mild increase in cancer risk in elderly patients in a 5 year follow-up. The increased cancer risk just reached significance according to the analysis of Carragee et al. [2011]. No increased cancer risk has been reported to date in patients treated with the lower-dose INFUSE product. The occurrence of tumors in this high dose BMP-2 group prevented the product being approved by the FDA, but the INFUSE product at a lower dose remains approved.

Human and animal studies have not demonstrated any evidence of systemic toxicity from rhBMP-2. When applied locally or by IV in animal studies, rhBMP-2 was found to have a very rapid systemic clearance, with a half-life of 6 minutes in primates, making the risk from systemic exposure low [Poynton & Lane, 2002]. Despite its role in embryologic development, there has been no evidence of embryolethality or teratogenicity in animal studies. Reproductive toxicity has not been studied in humans, however. There is also little evidence of excessive immune response to rhBMP-2: studies have shown that only 0.7% of patients (same as controls) mounted an antibody response to rhBMP-2 [McKay and Sandhu, 2002].

Woo [2013] reviewed adverse events associated with use of rhBMP-2 in nonspinal orthopaedic procedures. There were a total of 62 events reported to the FDA through 2011, including: wound complications (15), heterotopic bone formation (12), pseudarthrosis (10), local inflammation (9), osteolysis or resorption (5), compartment syndrome (2), and peripheral nerve injury (2). Of the 12 reports of heterotopic ossification, 5 required surgery to remove extra bone. The author noted that this data could not be used to estimate incidence rates of adverse events, as the total number of surgical procedures using rhBMP-2 is unknown. It was additionally noted that occurrence of pseudarthrosis might simply reflect the refractory nature of certain fractures, regardless of use of BMP-2.

3.5.2 Use of rhBMP-2 in children:

The INFUSE Bone graft (rhBMP-2 plus absorbable collagen sponge) is currently approved by the FDA as a device for use in adults for lumbar spine fusion and open tibial fractures. The FDA lists skeletal immaturity (age less than 18 years) as a contraindication to its use, primarily because there is limited data on its safety in children. Despite this, the device has been used on a physician-directed off-label or compassionate basis in increasing numbers of children, and data are beginning to accumulate.

Chin et al. [2005] were among the first to report use of rhBMP-2 in children. They used rhBMP-2 instead of autogenous bone graft in repair of congenital facial clefts in 43 children (ages 6-14 years). Successful union of bone was achieved in 98% of patients, and no systemic adverse effects or ectopic bone formation occurred during up to two years of follow-up. Richards et al. [2010] performed a retrospective review of 7 patients with congenital tibial pseudarthrosis (4 with NF1; age ranges 1.9-11 years) treated with rhBMP-2 applied locally at time of surgery with intramedullary rod. In 5/7 patients, there was good healing after one surgery, with average time to union of 6.4 months. During a prolonged follow-up time of 4 – 9 years, there was no evidence of tumor growth or systemic effects noted from rhBMP-2 use.

Oetgen and Richards [2010] also performed a review of 81 children (ages 1-17 years; 16 with NF1) treated with rhBMP-2 between 2001 and 2008. Of this group, 52 children received rhBMP-2 during spine surgery, and 21 during tibial repair. One patient with NF1 subsequently had enlargement of a previously identified optic nerve glioma, but this was interpreted as unlikely to be related to rhBMP-2. There was a total complication rate of 17%, most of which were surgical wound inflammatory responses. Only one complication was postulated to be related to rhBMP-2, and that was a case of dural fibrosis developing after direct exposure of the dura to rhBMP-2. There were no reports of systemic toxicity, excessive bone growth, sarcomas, or other tumors.

3.5.3 Use of rhBMP-2 in NF1:

The role of the *NF1* gene raises concerns that NF1 patients might respond differently to rhBMP-2 compared to non-NF patients in terms of clinical response and risks. This potential difference was assessed in a mouse model, whereby BMP-2 application in long bone and calvarial cell culture system showed that $Nf1^{+/-}$ cells have a reduced osteogenic response compared to wild-type cells [Schindeler et al., 2008c]. Even though this response (in terms of alkaline phosphatase expression and matrix mineralization) was greatly enhanced by BMP-2 application, it still fell short of wild-type responses, and when 20µg BMP-2 was implanted intramusclularly, about half as much bone formed in $Nf1^{+/-}$ animals as wild-type littermates [Schindeler et al., 2008c]. These studies highlight that bone cell differentiation responses are abnormal in NF1, but also highlight that $Nf1^{+/-}$ osteoblasts can respond to BMP-2 stimulation.

Several studies reporting use of rhBMP-2 in humans have included individuals with NF1; however, in general the data have not been analyzed separately comparing NF1 patients to those without NF. Patients with NF1 have distinct differences in bone biology, including osteopenia [Stevenson et al. 2007], as well as an increased susceptibility to tumors due to neurofibromin's role in regulation of the Ras pathway. Animal studies with *Nf1* heterozygous mice showed abnormal proliferation and apoptosis of osteoblasts, and impaired matrix synthesis [Yu et al., 2005]. There is also evidence for increased activity of osteoclasts [Yang et al., 2006]. **These differences in bone biology, when applied to humans, may significantly impact response to BMP-2, and therefore study of the NF1 population as a separate group is crucial.**

3.6 Radiographic Measures of Tibial Fracture Healing (RUST; Primary Outcome Measure).

The determination of success for surgical intervention in tibial pseudarthrosis repair (ie. tibial fracture union) has not been standardized. The two key outcomes studies of surgical treatment of TPA without rhBMP-2, by Johnston [2002] and Dobbs et al. [2004], used different measures of success. Johnston [2002] used weight-bearing status, use of orthotic support, presence of pain, level of physical activity, and radiographic presence of cortical bone defects across the operative site and signs of remodeling. Dobbs et al. [2004] used radiographic osseous union, limb-length discrepancy, range of motion of the ankle, and angular deformity at the ankle.

The literature has documented significant subjectivity in interpretation of radiographic evidence of tibial fracture healing. Govender et al. [2002] reported that radiologists interpreted tibial healing on average 3 months later than did orthopedic surgeons. Such observer variation in the assessment of tibial fracture healing after intramedullary fixation [Whelan et al., 2002] led Whelen et al., [2010] to devise a novel **Radiographic Union Score for Tibial fractures (RUST)** based on the cortical bone as viewed on AP and lateral radiographs. This assessment tool includes scoring of fracture line on the medial and lateral cortices on the anteroposterior radiograph, and anterior and posterior cortices on the lateral radiograph, and scoring of callus as none, visible or bridging [Whelan et al., 2010]. This study included 7 reviewers who

independently scored 45 radiographs, and overall agreement was demonstrated by an inter-observer ICC (intra-class correlation coefficient) of ~86% with the 95% confidence interval of 0.79-0.91 and intra-observer ICC of 0.88 with 95% confidence interval of 0.80-0.96. **The primary outcome measure for the proposed study is post-surgical tibial union using the RUST scale.** RUST scores of NF1 patients randomized to the BMP-2 group will be compared to those treated without BMP-2. The same group of 4 reviewers will review all images and assign scores while blinded to the group assignment of the patients.

3.7 Pilot Data:

As an extension of the study by Stevenson et al. [1999], in preliminary studies we collected data from a multi-center natural history study of patients with tibial dysplasia and/or tibial pseudarthrosis (PI-Carey, Co-Investigators-Viskochil and Schorry, Shriner research grant #9165; 2004) in an attempt to better define the outcomes. The centers included NF clinics in the USA and Canada and Shrine Hospitals in North America, and included 27 patients with tibial dysplasia (anterolateral bowing) and 31 with tibial pseudarthrosis. Of the 31 patients with tibial pseudarthrosis, 24 had surgery involving the placement of a rod for fixation and tissue grafting, and 7 had an amputation as their initial surgical intervention. Fourteen of the 24 (58%) with rod/graft had a successful union without requiring a second surgery (at the time of survey). The range of follow-up in these 14 patients was 1 year to 16 years. The other 10 of 24 (42%) did not have a complete union after the first surgery (or re-fractured) and required a second surgery, 4 of which had amputations. In this preliminary assessment, of those with pseudarthrosis, 11 of 31 or just over 33% had amputations

Direct comparison of efficacy of rhBMP-2 in the NF1 population with tibial pseudarthrosis is limited because of small sample sizes. Richards et al. [2010] reported improved average time to initial union in a group of 7 patients with congenital pseudarthrosis of the tibia (4 with NF1) who were treated with intramedullary rod fixation; autogenous bone graft from the iliac crest; and rhBMP-2 (INFUSE™). Five of 7 patients (71%) had radiographic union at a mean time of 6.4 months post-operatively (range, 3.7-8.1 months). In a recent update to this study that was presented at the 2013 Pediatric Orthopaedic Society of North America meeting, Richards reported 23 patients with CPT (16 with NF1) have now been treated using BMP-2. Radiographic healing was achieved in 16 of the 23 patients at an average of 9.5 months postop (7/7 idiopathic and 9/16 NF). Of these 16 patients who healed primarily after the addition of BMP-2, those with fixation across the ankle had no refractures, whereas three of six patients whose fixation did not cross the ankle subsequently refractured. The overall refracture rate was 25% in these 16 patients. Two of these four refracture patients subsequently healed, one persisted, and one had amputation. Of the 7 patients who did not heal after this first operation using BMP-2, three healed after revision, two had persistent nonunions, and two had subsequent amputations. Three cases were complicated by deep infection (2 at tibia, 1 at iliac crest). No adverse effects attributable to BMP-2 were encountered. This update suggests that the addition of BMP-2 in the NF1 tibial pseudarthrosis population improves the chance of persistent union, but does not result in as high a success rate as seen in patients without NF1.

When compared to published data by Dobbs et al. [2004] performed without rhBMP-2 in 18 patients, the average time to union was 16 months (range, 5-43 months), many required multiple surgeries and 5 of 12 (41%) of NF1 patients refractured. **Although small numbers, the potential benefit of rhBMP-2 as an adjunct to surgical management of NF1-related tibial pseudarthrosis clearly deserves further assessment.**

As additional **pilot data** for the proposed project, radiologists at Texas Scottish Rite Hospital used the RUST scale to retrospectively score radiographs of 12 individuals with NF1 and TPA

treated without BMP-2 [Wilkes et al, unpublished data]. A group of 4 reviewers independently reviewed 36 sets of radiographs (3 per patient) and assigned RUST scores for degree of tibial healing (see Figure 4). There were several challenges in scoring these patients, such as eccentric position of internal rods which may have made one cortex inevaluable. However, they were able to successfully score the patients **with good inter-observer and intra-observer reliability** (kappa of 0.73 and 0.88 respectively). The group concluded that the RUST scale is reliable and reproducible, and can be modified to measure outcome in tibial pseudarthrosis. Modifications made for using the RUST in NF1 patients included:

Scoring system:
1 = no new calcification, or rod eccentricity precludes evaluation
2 = new calcification or callus present
3 = smooth continuous outer cortex, even if underlying lucency
Total score ranges from 4 to 12.
For the purpose of this study, a RUST score of 9-12, with at least 2 evaluable cortices having a score of 3, will be considered to be healed.

These preliminary data are crucially important as they document that the RUST scale can be used reliably in the NF1 population with TPA.

Figure 4a. and 4b. RUST score examples

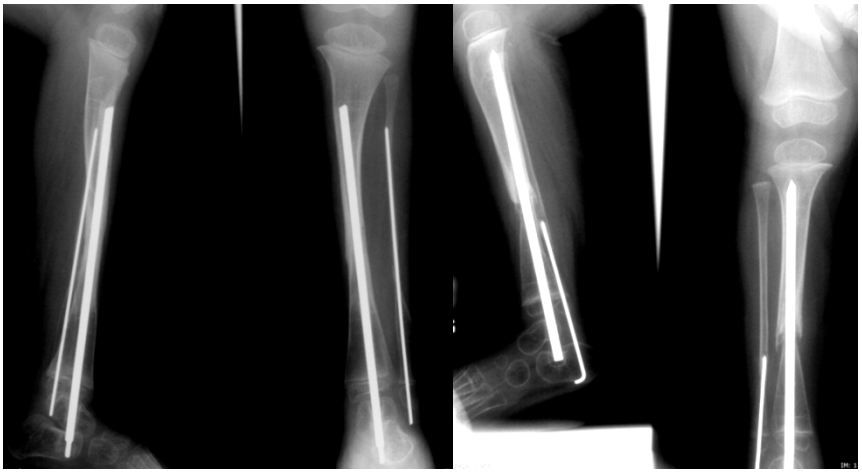


Figure 4a. NF1-TPA patient with complete union in all 4 cortices, RUST score of 3+3+3+3 = 12

Figure 4b.NF1-TPA patient with partial bridging/ callus formation of 4 cortices. Rust score 2+2+2+2 = 8

3.8 Summary of Preliminary Data

A number of key preliminary studies have contributed to the development of this clinical trial. Bone in NF1 patients and *Nf1* mice is abnormal, as summarized in the proceedings of a clinical trial strategy meeting whereby key investigators of this proposal (Viskochil, Schorry, Little, Richards) significantly contributed [Elefteriou et al., 2009]. NF1-related pseudarthrosis tissue has been harvested from surgery and shown to have double inactivation of the *NF1* gene [Viskochil as senior author; Stevenson et al., 2006] suggesting that biochemical properties of this tissue make it different from routine tibial fractures and likely more difficult to achieve healing. A better understanding of the natural history of tibial dysplasia and tibial pseudarthrosis

has been provided by a Shriners-funded study on outcomes of tibial pseudarthrosis in which Schorry and Viskochil were co-investigators with Dr. John Carey as PI (Shriner research grant #9165, 2004). These studies provide us with an estimate of eventual amputation rate of 33% in patients with tibial pseudarthrosis, and provide quality of life information that demonstrates the PODCI is an effective Quality of Life (QOL) measure that can be used to investigate outcome for treatment of TPA in the NF1 population. The most significant preliminary studies for the sake of this clinical trial are those from Dr. Richards at Scottish Rite Hospital in Texas. His recent presentation of 16 NF1 patients treated with a surgical protocol including BMP-2 suggests that patients with NF1 have an improved outcome with addition of BMP-2, although their response is not as good as in the non-NF population. Safety of BMP-2 has been documented by Oetgen and Richards [2010], who reviewed 81 children treated with BMP-2 between 2001 and 2008, and identified no systemic toxicity, excessive bone growth, sarcomas, or other tumors in association with rhBMP-2.

3.9 Rationale for Clinical Trial

Tibial pseudarthrosis is a rare childhood complication of neurofibromatosis type 1, however it carries significant morbidity for those affected. When fracture occurs, surgical intervention is required to optimize bone union; however, long-term success is achieved in only half of the cases who undergo surgery. The NF Bone Consortium group, a group of basic bone biologists, NF mouse modelers, clinicians and orthopedists which met in 2008 and 2011, has provided guidance on development of the proposed clinical trial for TPA.

A phase II study of BMP-2 versus control is needed to better assess the potential improved rate of union in the NF1 population with tibial pseudarthrosis. Small series and anecdotal reports have not enabled the orthopedic community to confirm the best approach toward management of this complicated condition. The implementation of a clinical trial to prospectively evaluate efficacy and safety of rhBMP-2 as a medical adjuvant to surgical intervention could potentially direct future therapy and have significant implication to affected patients. This trial also has the ability to serve as a foundation for additional medical interventions of other compounds which affect bone anabolism or catabolism in NF1. **The best performing arm from this study (BMP-2 or Control) can be subsequently used as a future control arm for studies involving additional targeted therapies for NF1, such as inhibitors of osteoclast activity or newly developed Ras pathway inhibitors.**

4.0 METHODOLOGY

4.1 Overview of Proposed Study

This phase II, single-blind, randomized study will evaluate children with neurofibromatosis type 1 (NF1) who undergo surgical management of tibial pseudarthrosis. The rationale is predicated on the observation that union of tibial fracture in patients with NF1 is difficult to achieve without extraordinary measures to optimize bone healing, and that deficits in bone formation as well as excessive bone resorption occur in NF1. The preliminary success in improved rate of tibial fracture healing when rhBMP-2 is locally applied intra-operatively, as outlined by Richards et al. (2010), has influenced the need for a carefully controlled clinical trial to document efficacy and safety of this device. Outcomes of this trial will likely influence surgical decisions in management of patients with NF1 and TPA.

Children with NF1 and TPA whose primary orthopedic surgeon deems surgical intervention is required are eligible for this trial. At time of enrollment, participants will be offered participation in a preference arm or randomized arm, and will be assigned to the BMP-2 or control group based on these choices. Surgery must take place at one of the participating consortium sites, and the standard surgical approach will include: extensive resection of abnormal pseudarthrotic

material, tibial osteotomy, placement of an intramedullary rod, and autogenous bone graft from the iliac crest to the osteotomy tibial gap. Those in the BMP-2 group will in addition have INFUSE in a collagen sponge wrapped around the tibia during the surgical process.

All patients will be immobilized for a minimum of 3 months after surgery in either a spica or above knee cast; surgeons will use clinical discretion to determine if further casting is needed on an individual basis. After the cast is removed, patients will be permanently braced (24 hours per day) in a custom-made above- or below-the-knee orthosis. **The primary outcome measure is radiographic evidence of union by the RUST scoring system at 12 months after surgery.** The rate of tibial healing will be assessed by application of the RUST scoring criteria for radiographs obtained at 6 weeks, 3 months, 6 months, 9 months, and 12 months after surgery. Quality of life will be assessed by the Pediatric Outcome Data Collection Instrument, and walking function will be assessed by the 10 meter timed walk, performed at baseline, 6 mos, and 12 mos. Biologic specimens including discarded pseudarthrosis tissue and bone fragments from the iliac crest will be collected at the time of surgery and sent to the Texas Scottish Rite Hospital.

The assessment of effectiveness of BMP-2 will be made by comparing the RUST union score at 12 months after surgery between the two groups (BMP-2 and Control). In addition, the rate of healing will be assessed by comparing the RUST score from radiographs at designated intervals in the post-operative period (6 weeks, 3 months, 6 months, and 12 months). Patients will continue to be followed in a registry with annual clinical assessments to determine long-term outcome, especially re-fracture and amputation.
Patients entered on the trial will be carefully monitored for the development of toxicities associated with BMP-2. BMP-2 and collagen antibody levels in serum will be measured preoperatively in all participants and results will be generated prior to enrollment for participants with documented prior exposure. All participants will be monitored at multiple intervals after surgery to test for antibody formation to the components of INFUSE.

Secondary Objectives:
Quality of life assessments for the 2 experimental groups will be obtained at baseline, and at 6 and 12 months after surgery. Pain assessment will be made at baseline and at clinical visits at 6 weeks, 3 mos, 6 mos, and12 mos post-op). Functional walking outcome will be measured by the 10 meter walking test, performed at baseline, 6 and 12 months.

Biological specimens (**optional studies**) will be collected and processed from those patients enrolled in this study who consent for their samples to be stored for future NF1 research. Pseudarthrosis tissue will be excised at surgery, and excess tissue not needed for pathology will be stored for future research study and sent to TSRHC. A sample of the pseudarthrosis tissue will be sectioned for histochemical analysis while the remainder of tissue will be frozen at TSRHC. Excess Iliac crest bone fragments will also be frozen at TSRHC for bone histomorphometry. Blood, serum, urine and tissue specimens for participants will be stored at the Tissue Processing Facility at Texas Scottish Rite Hospital. Access to the tissue will be provided to the NF1 research community through committee approval of applications submitted by investigators whose research is supported independently from this clinical trial. The Biospecimen Allocation Committee, composed of 4 members, will review and make decisions on all applications for use of these biospecimens. (Details for processing of biological research specimens are contained in Appendix II.

4.2 STUDY POPULATION, SCREENING AND ENROLLMENT PROCEDURES

The NF Clinical Trials Consortium Operations Center (UAB) will oversee recruitment, screening and enrollment procedures.

4.2.1 Study population

The target population for this study is limited to patients with NF1 who have tibial pseudarthrosis amenable to surgical intervention by intramedullary rod and autogenous bone graft. In general, approximately 2-5% of NF1 patients clinically present with tibial dysplasia in infancy through early childhood. All ethnic groups are affected equally, although more boys are affected with TPA than girls. The decision to surgically intervene is most often made in childhood, typically before age 10 years. Historically, participating members of this multi-center study have treated a significant portion of those afflicted with tibial pseudarthrosis. Participants will be recruited from the 13 NF Clinical Trials Consortium Centers (funded by DOD), with additional sites including the Texas Scottish Rite Hospital in Dallas, Texas. Five Shriners' Hospital sites in the US have also been added, and the Mayo Clinic site was added when the NF Consortium expanded in 2017. The involvement of a large group of centers (20 total sites) invested in orthopaedic care of NF1 patients is crucial for enrollment of patients with this relatively rare, but serious, complication.

Table 2: Possible participating sites and patient numbers.

A table outlining the accessible study population is shown below:

Sites	TPA Patients	NF1 Patients	Surgeries/ 4 yrs
Cincinnati Children's Hospital (Jain; Schorry)	30	1000	6
University of Utah (D'Astous; Viskochil)	10	200	7
University of Chicago (Sullivan; Tonsgard)	20	1400	0
Lurie Children's, Chicago (Goldman)			6
Boston Children's Hospital (Kasser, Spencer, Ullrich)	14	845	8
University of Alabama at Birmingham (Gilbert; Reddy)	6	300	4
St. Louis Childrens (Dobbs; Gutmann)	12	302	16
DC Childrens (Oetgen; Packer)	17	1200	4
Hopkins, Baltimore (Blakeley, Sponseller)			4
Childrens Hospital of Philadelphia (Davidson; Fisher)	20	545	
Indiana University (Loder; Robertson)	16	268	
Children's Hospital Los Angeles (Arkader, Rosser)	10-15	195	8
New York University (Feldman; Allen)	3	170	
Texas Scottish-Rite (Richards)	20	N/A	20+
Mayo Clinic			n/a
Shriner's Hospitals (5 sites)			10+
Total	208	>6,000	90+

Additional candidates will also be drawn from families who are members of support group organizations (i.e. Children's Tumor Foundation) that routinely provide ongoing information on clinical trials and studies to their members.

Subject Accrual
Subjects of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section 4.3. To date, there is no information that suggests differences in drug metabolism or disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Most tibial pseudarthrosis arises in early childhood, with fewer cases after age 11, thus we anticipate an enrollment of younger children. If differences in outcome that correlate to gender, age, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences.

4.2.2 Recruitment
The primary recruitment for this study will be through participating NF Consortium Centers and orthopaedic centers. This prospective clinic trial will be posted on websites and included in the newsletters of the NF support organizations (CTF and NF Network). Directors and coordinators at each of the 42 clinics affiliated with the CTF NF Clinic Network will be notified of the trial and eligibility.

A total of between 36-52 patients will be enrolled prospectively in this trial. The maximum number of patients that will be enrolled in the BMP arm is 26, based on the numbers of Infuse kits supplied by the sponsor. The ideal number of Control patients is also 26, but may range from 10-26, based on preferences of enrollees. This is further discussed in the Study Modification section below, 4.2.3. We expect that dropout rate of participants, once they have been enrolled and had their surgical procedure performed, should be very low, in the range of 5%. The experience of participating surgeons is that their TPA patients who have had surgery are extremely likely to attend subsequent follow-up visits. The final endpoint of the trial is at the one-year assessment of the last patient enrolled. Due to slow enrollment in the first year of the trial, we have added 5 Shriners orthopedic centers in the U.S. as participants in the trial. Funding has been included to help support family travel as outlined, in the Informed Consent forms (range of $200-$500 per patient, with the greater amount for patients living further than 50 miles from the participating center). Participants who live further than 50 miles from a participating center may have an option to elect to have several of the follow-up visits and Xrays (3 and 9 months post-op) performed by their local orthopedic surgeon, with results sent to the DMAC and radiographic team at TSRH. However, the surgical procedure and follow-up visits at 6 and 12 months must be at a participating center with either the orthopaedic surgeon or the NF specialist. All centers performing any subject evaluations for the study must have completed the appropriate mandatory electronic training modules prior to performing any assessments.

4.2.3 Enrollment procedures
The NF Clinical Trials Consortium will enroll subjects in this trial. Selected subjects with NF1 and tibial pseudarthrosis will be enrolled at the participating sites where surgery is anticipated. Personnel at the UAB NF Clinical Trials Consortium Operations Center will coordinate enrollment with the participating site, and ensure that eligibility requirements are met, prior to scheduling of surgery and shipment of rhBMP-2. As part of the consent process, patients and families will be provided information about the research aspect of this prospective clinical trial.

Study Modification: Enrollment: Patients may be enrolled on the study once all eligibility requirements for the study have been met. All patients/parents will discuss the study in detail with their surgeon, and will view a Powerpoint presentation about the study with a study coordinator present. **At the time of enrollment, parents/patients will be offered to choose either the Choice Arm or Randomized Arm of the study**. Those selecting the Choice Arm will be assigned to their treatment of choice (either BMP or Control). Those who choose the Randomized arm will be randomly assigned at a 2:3 ratio to BMP or Control group. It is

expected based on prior experience that more patients are likely to prefer the BMP arm. Therefore, the mildly unequal randomization of 2 BMP: 3 Controls is planned to improve the ability to achieve adequate controls for the study. For those in the Randomized arm, assignment will be accomplished by accessing a password controlled website maintained by the DMAC. The website is 21 CFR 11.10(d) compliant for FDA clinical trials.

That website will assign a new subject to a treatment group determined by a randomization list produced by the study statisticians employing a permuted block design. Randomization will be stratified by age (≤ 8 vs > 8 years) and prior surgery (Y/N). Enrollment and randomized treatment assignment must occur before the date of surgery but should be as close to the date of surgery as feasible.

Blinding: For subjects in the Choice Arm, all subjects, parents, and surgeons will of necessity be **unblinded** to the patient's treatment status of BMP versus no BMP. For those in the Randomized group, all subjects, parents, radiologists, data recorders, and (non-surgeon) site PIs will be **blinded** with respect to the treatment status of each participant. Only the surgeon and one study coordinator or OR tech (designated to deliver the device to the OR) at the site will be aware of the treatment status. If the surgeon is also the site PI, then that site PI will be unblinded. For the measurement of outcome measures, the radiology panel calculating the RUST score and all data recorders will be blinded to the treatment status of all participants. Therefore, **calculation of the primary outcome measure will be blinded for all participants, ensuring a non-biased measure for the primary study outcome.**

4.2.4 Informed Consent/Assent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parent(s) or guardian if the patient is a child, and a signed informed consent and assent will be obtained according to both institutional and U.S. Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office (USAMRMC ORP HRPO) guidelines. Patients between the ages of 2 – 18 years at time of surgery will be eligible for consent. The principal investigator at the enrolling site will be responsible for obtaining informed consent. The study trial coordinator can provide information on the study and obtain consent if qualified as determined by the PI of the site. Most patients in this study will be less than 7 years of age. Those older than 7 will undergo age-appropriate assent tailored for both children aged 7-12 years, and for those aged 13-17 years (or until the age of majority in the state of the clinical center).
A study participant should sign the institution's HIPAA Consent.
A study participant should sign the institution's Release of Medical Information Waiver.

4.2.5 Rationale for Subject Selection

Neurofibromatosis type 1 is a pan-ethnic genetic disorder with an incidence of 1 in 3,000 people. Subject accrual in regards to gender, and racial and ethnic groups is described in Section 3.6. None of these groups are excluded from participation in the trial. Females who are pregnant or breast feeding will not be eligible for entry onto the trial because of the potential risks that rhBMP-2 could pose to the fetus or newborn. This trial is designed to determine the activity of BMP-2 in children with NF1 and surgically treated pseudarthrosis; therefore children will be entered onto this research trial. Almost all individuals with NF1-related congenital pseudarthrosis of the tibia will have their first or second surgery within the first 18 years of life. Individuals will be enrolled at one of the participating sites that collaborate with the NF Clinical Trials Consortium with additional affiliate sites at TSRHC and possibly Australia, and the United Kingdom. Patients may be referred from outside centers but treatment will be directed at a participating consortium site.

4.2.6 Participation of Children

Since tibial pseudarthrosis typically requires surgical intervention before age 10 years of age, children who meet eligibility criteria for this trial will be entered in the study. Children will be evaluated and cared for by physicians trained in pediatrics and in the orthopedic management of children with NF1.

4.2.7 Screening Procedures

No diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done before informed consent has been obtained.

Diagnostic or laboratory studies to determine candidacy for surgical intervention will be performed by the patient's orthopaedic surgeon on a clinical basis, prior to obtaining written informed consent**.** Once a patient's surgeon determines the need for surgical intervention for tibial pseudarthrosis, the patient and his family will be offered an opportunity to enroll in the clinical trial and written informed consent will be obtained. Documentation of informed consent will be maintained in the patient's research chart. **After consent has been obtained, pre-operative tibial Xrays and an Eligibility Form will be sent to the DMAC and forwarded electronically to the group of 4 protocol co-chairs for final eligibility determination.** The co-chairs will review eligibility data and will make a decision by phone call or email, with at least 2 of the 4 co-chairs participating.

4.3 PATIENT ELIGIBILITY/ INCLUSION AND EXCLUSION CRITERIA

Any individual with NF1 based on either NIH-diagnostic criteria or congenital pseudarthrosis of the tibia plus documented mutation in the *Nf1* gene is eligible for this study. Furthermore, decisions for surgical intervention must be made on a clinical basis with a determination that intramedullary rod plus autogenous bone graft is the most appropriate management approach for that individual patient. Patients may be enrolled only at the time of 1st or 2nd surgery for tibial pseudarthrosis, not subsequent surgeries. Candidates must be able to travel to an orthopedic surgical site that is participating in the study. Patient insurance must approve coverage of the surgical procedure and short-term post-operative care at the orthopedic center performing the operation, or patients can use a self-pay procedure to cover the surgical and hospital costs associated with the surgical procedure.

Inclusion Criteria for all patients:

- All patients who do not have a documented *NF1* mutation must meet the clinical diagnosis of NF1 using the NIH Consensus Conference criteria. In addition to tibial pseudarthrosis, one or more of the following diagnostic criteria for NF1 must be present:
  - Six or more café-au-lait spots (≥0.5cm prepubertal; ≥1.5cm postpubertal)
  - Freckling in the axilla or groin
  - Optic pathway glioma
  - Two or more iris Lisch nodules
  - Two or more neurofibromas or one plexiform neurofibroma
  - A first-degree relative with NF1
- Patients must have tibial pseudarthrosis that has the potential to cause significant morbidity. Radiographic findings (AP & lateral leg radiographs) must support the diagnosis of tibial pseudarthrosis with chronic non-union.
- Age between 2 years and 18 years of age at time of study entry.
- Performance Level: Karnofsky ≥ 50% for patients > 10 years of age and Lansky ≥ 50 for patients ≤ 10 years of age (Appendix III).
- Prior Therapy:
  - Patients who have undergone 1 previous surgery for tibial pseudarthrosis repair will be eligible to enter the study if they have refracture. Use of BMP-2 in the prior surgery is permitted, however patients with prior exposure must be screened for antibodies to BMP-2, bovine collagen, and rhBMP-2 neutralizing antibodies.

Prior use of BMP-2 is allowed but will be recorded as a possible compounding factor.
- Patients who have had 2 or more prior surgeries for pseudarthrosis repair are ineligible

- Absence of Tumors:
  - Patients must undergo thorough physical examination of the leg undergoing surgery. If physical exam is equivocal for presence of tumors, then a normal MRI of the lower extremity will be required before eligibility is met.
  - If there is evidence of plexiform neurofibroma or nodular neurofibroma of > 3 cm diameter on the ipsilateral leg, then they are ineligible for the study.
- Organ Function Requirements

  Adequate bone marrow function defined as:

  Absolute neutrophil count (ANC) > 1500/μl

  Platelet count > 100,000/μl

  Hemoglobin ≥ 10.0 gm/dl

  Adequate renal function defined as: maximum serum creatinine of 1.5 mg/dL OR a creatinine clearance≥70ml/min/1.73m^2.

  Adequate liver function defined as:

  Total bilirubin < 1.5 X upper limit of normal for age, and

  SGPT (ALT) < 2 X upper limit of normal for age

  Serum Vitamin D level ≥ 10 ng/ml

Exclusion Criteria:

- Lack of documentation for a diagnosis of NF1
- Tibial fracture without evidence of pseudarthrosis or tibial dysplasia
- Tibial dysplasia/bowing without fracture or pseudarthrosis
- Plexiform neurofibroma of any size, or nodular neurofibroma of > 3 cm diameter involving the ipsilateral leg, including the hip
  If presence of plexiform is suspected but not certain on physical exam, MRI of the leg may be indicated to rule this out.
- History of MPNST (malignant peripheral nerve sheath tumor) or any malignancy other than asymptomatic and stable optic nerve glioma
- Optic nerve glioma that has resulted in precocious puberty or visual impairment of any degree
- Visual impairment from any cause
- Precocious puberty from any cause
- Hypertension other than mild essential hypertension controlled with medication
- Metastatic disease of any kind
- Inadequate neurovascular status in the involved limb that may jeopardize healing
- Active or known prior infection at the pseudarthrosis site
- Active systemic infection
- Other injury or condition that prevents ambulation or completion of study assessments
- Two or more prior surgeries for tibial pseudarthrosis
- Bilateral tibial dysplasia
- Selection of a surgical approach that does not include prescribed surgical intervention, which must include removal of pseudarthrosis tissue, placement of an intramedullary rod using the Williams approach, and autogenous bone graft from the iliac crest distributed at the osteotomy site
- Normal ipsilateral fibula without planned fibular osteotomy at time of surgery
- Allergy to bone morphogenetic protein
- Allergy to bovine collagen products

- Positive antibody titers to BMP-2, bovine collagen, or BMP-2 neutralizing antibodies prior to surgery
- History of using any of the following medications, regardless of dose, for at least 1 month, within 3 months of enrollment: Anabolic agents, Glucocorticoids (does not include inhaled glucocorticoids), Growth hormone, Parathyroid hormone (PTH)
- Need for postoperative medications that could interfere with bone healing of the implant, such as steroids, (but not including low-dose aspirin or routine perioperative anti-inflammatory drugs)
- Untreated endocrine abnormality, such as hypothyroidism, parathyroid hormone disorder
- Severe Vitamin D deficiency with serum 25-OH Vitamin D < 10 ng/ml (25 nmol/l) Patients with Vitamin D levels < 10 ng/ml may be treated with Vitamin D and reconsidered for enrollment when levels are sufficient
- Females who are sexually active without use of effective contraception
- Females who are pregnant or breastfeeding

4.3.1 VITAMIN D SUPPLEMENTATION

It is recommended that all patients be sufficient for Vitamin D (25-OH Vitamin D) throughout the trial, as standard of care. Those with severe Vitamin D deficiency (< 10 ng/ml or 25 nmol/L) are excluded from the trial, although they can be re-screened after treatment with vitamin D. For all other patients, the following Vitamin D supplementation is recommended:

- Vit D insufficient (11-30 ng/ml) treat with 1,000 IU Vit D daily
- Vit D sufficient (31-60 ng/ml) treat with 400 IU Vit D daily
- Vit D level > 60 ng/ml, no need for additional supplementation
- Physicians will have the option to use differing doses of Vitamin D if they deem to be clinically indicated.

4.4 ADMINISTRATIVE ROLE OF OPERATIONS CENTER (UAB)

The Operations Center for the NF Clinical Trials Consortium, funded by the DOD, will provide administrative support for this clinical trial. In addition to what is described throughout this protocol, it will organize and oversee the clinical trial initiation meeting wherein all aspects of the protocol will be finalized and standard operating procedures fully elucidated. The Operations Center will continue to organize annual face-to-face meetings, and administer all teleconferences. There will be a minimum of one teleconference call per month, and the co-PIs on this protocol will also join the executive committee monthly teleconference call for the NF Clinical Trials Consortium. Data-sharing between sites will be overseen by the DMAC, and approvals for human subjects research will be orchestrated between the institutional review boards from the University of Alabama at Birmingham and the Department of Defense prior to distributing to all other participating sites for approval at their respective institutions.

Each participating institution is required to maintain a current MPA or FWA in order to participate in government-sponsored Group research. The files will be copied or made available for review by authorized persons as required for conduct of this trial.

Continuing Reviews, Amendments and Consents

The PI from each participating institution will provide the NF Consortium Operations Center with a copy of IRB approval of all amendments to the protocol or consent. The NF Consortium Operations Center will provide these institutional reviews to the USAMRMC ORP HRPO. As this trial receives funding by the US Army, approval of all protocol amendments will be obtained from the USAMRMC ORP HRPO in addition to the institutional IRB prior to implementation.

4.5 Data Collection and Data Handling

The trial is being conducted by the NF Clinical Trials Consortium. Electronic Case Report Forms (eCRFs) developed by the NF Consortium Operations Center will be used for submitting

clinical data to the DMAC. Data must be submitted to the DMAC within two weeks of completing each required evaluation while the patient is on study.

The **Radiology Review Panel (RRP**), comprised of a group of 4 physicians (3 radiologists and 1 orthopaedic surgeon) from Scottish Rite Hospital in Dallas will independently review all radiographs using the RUST scoring system. The RRP will receive radiographic studies with clinical trial study identifiers on a CD, which will then be uploaded with only study identifiers onto the reading system that allows each member to score the radiographs, blinded to other panel member's scores. After individual scoring is completed, the panel will meet as a group and assign a consensus score to each radiograph. The score sheets will be entered into a database interface created by the data management center.

The **Biospecimen Allocation Committee (BAC**) will oversee the review of applications for use of biological specimens. Once decisions have been made by the BAC to approve access to samples, the TSRH Tissue Processing Facility will distribute to the investigator using standard operating procedures. The BAC will be comprised of participants of the rhBMP-2 tibial pseudarthrosis clinical trial, one representative from TSRHC, members of the steering committee of the NF Clinical Trials Consortium**, operating independently of the Operations Center, and other members from outside the Consortium, as deemed appropriate.** Guidelines for the BAC review process will be finalized prior to enrollment of patients for the study.

Representatives from the FDA, and the U.S. Army Medical Research and Materiel Command will have access to the data and research records.

Role of Data Management

Instructions concerning the recording of study data on CRFs will be provided by the Data Management Center (DMAC) of the NF Operations Center. It is the responsibility of the DMAC to assure the quality of data for their study. This role extends from protocol development to generation of the final study database.

The DMAC has extensive experience in data management, currently managing 18 national/international collaborative trials/studies. For the purpose of the proposed trial, they will be using an existing electronic Data Entry System (eDES) that serves as a shared platform that seamlessly supports the data management needs across the spectrum of these projects. This system has a common administrative site and a consistent platform that reduces the enormous effort required to initiate, manage, maintain and validate multiple individual applications used, particularly those involved with FDA oversight, where extra requirements apply. The electronic Data Entry Management Systems (eDEMS) and methodologies employed for the other NF studies will be built on the existing highly successful MITS Suite.

The MITS Suite consists of the MITS Studio (electronic Data Entry Management System [eDES] Authoring Tool) and the Testing Framework. The architecture is designed to efficiently accommodate trial organizations, that have multiple projects operating across a common set of clinics. The MITS Studio provides a structured design environment that ensures repeatable results across trials by utilizing a common set of vetted software components. Our systems are designed to adhere to FDA 21 CFR Part 11 guidelines in developing the authoring and testing framework components and are compliant with the evolving FDA CDISK standards.

All centers participating in subject evaluation for this trial will complete mandatory electronic training modules developed by the DMAC prior to performing any of the study assessments to ensure compliance with 21 CRF11.10(i).

Data and Center Audits
The trial will be audited by the NF Consortium Operations Center for compliance and safety. Independent monitors will visit participating sites and review case report forms and source documentation. Missing or spurious information and protocol deviations will be communicated in a report to the clinical site and trial coordinating center. Protocol deviations, which may result in compromise to safely administer medical device, or to determine study endpoints will be reported promptly to the USAMRMC ORP HRPO.

All unexpected and serious adverse events will be forwarded to the Medical Monitor, the Study PI and the USAMRMC ORP HRPO by the NF Clinical Trials Consortium Operations Center.

5.0 TREATMENT PROGRAM

5.1 Agent Information: Bone Morphogenetic Protein -2
rhBMP-2 is the active agent of the INFUSE® device, which consists of two parts:
a recombinant human protein (rhBMP-2) to stimulate bone healing, and
an absorbable collagen sponge (ACS) made from cow (bovine) collagen that is soaked with the protein. rhBMP-2 is the active agent in INFUSE®. It is a disulfide-linked dimeric protein molecule with 2 major subunit species of 114 and 131 amino acids. Each subunit is glycosylated at one site with high-mannose-type glycans. rhBMP-2 is produced by a genetically engineered Chinese hamster ovary cell line. The INFUSE® Bone Graft device is designed to be used along with internal stabilization with an intramedullary rod or nail to help heal fractures of the lower leg bone (tibia).
Further details are in Sections 2.1 and 2.2

5.2 Surgical Protocol for Treatment of Tibial Pseudarthrosis
The surgical protocol to be used is outlined in Richards et al. [2010] and includes tibial osteotomy with resection of the pseudarthrosis tissue, followed by placement of an intramedullary rod which provides rigid fixation. The exact type of rod used will vary based on availability in different centers and different countries, but will be a rigid rod similar in size and configuration to a Williams or Rush type rod. In no cases will the intramedullary canal be reamed. Fixation of the ankle will be at the discretion of the surgeon regardless of the patient's age. Bone grafting will be performed with autogenous bone from iliac crest placed into the excised pseudarthrosis site. As per instructions provided by the provider of the INFUSE® kit device (Medtronic), rhBMP-2 will be applied to an absorbable collagen sponge. The sponge is applied as an onlay, bridging the area of pseudarthrosis. The application of the collagen sponge saturated with rhBMP-2 is shown below.

Figure 5: Application of INFUSE at surgery.

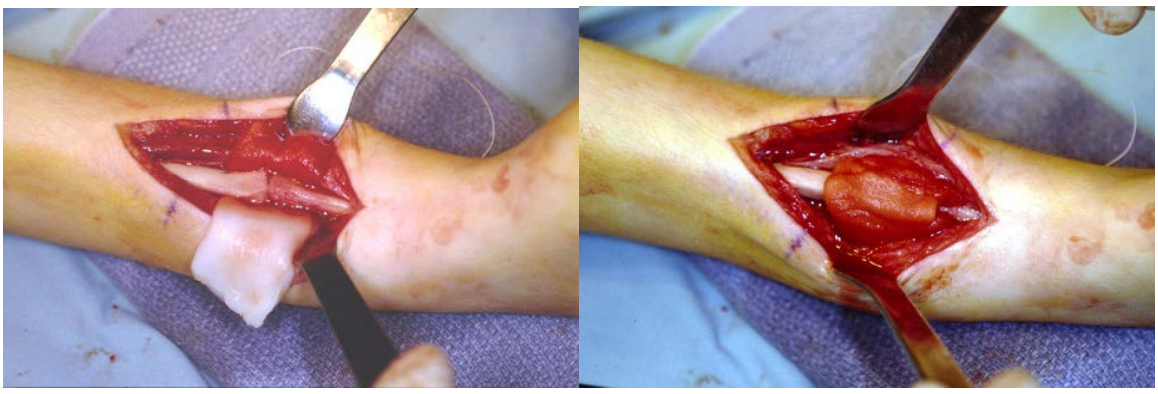


FIG 1A. FIG 1B.

The BMP-2 soaked collagen sponge is placed around bone junction at the tibial osteotomy site. After autogenous bone graft has been placed in the tibial osteotomy site, the collagen sponge is wrapped around both the tibia and the bone graft.

The dosing of rhBMP-2 is restricted to the size of absorbable collagen sponge used in the surgical field. The concentration of rhBMP-2 in the sponge is standardized to 1.5 mg/cc sterile water on the sponge. For the purposes of this trial, the size of INFUSE bone graft used will be adjusted for weight. Patients less than 30 kilogram weight will receive a small INFUSE package (4.2mg of rhBMP-2 on 2 collagen sponges, with concentration of 1.5 mg/cc); those greater than or equal to 30kg weight will receive a medium INFUSE package (8.4 mg BMP-2 and 4 collagen sponges, with concentration of 1.5 mg/cc). Higher doses were avoided due to the concern that higher doses of rhBMP-2 can induce osteoclasts that initiate bone resorption, and to decrease theoretical risk of carcinogenicity possibly seen with very high doses of BMP-2.

In addition to surgical management of the tibia, the ipsilateral fibula will be assessed radiologically and decisions for surgical intervention will be incorporated into the operative strategy to stabilize the tibia. In cases where the fibula is intact, the fibula will undergo osteotomy with intramedullary fixation in order to shorten the fibula and prevent distraction by the intact fibula. In cases where the fibula was severely resorbed ("sucked candy" configuration on Xray), an osteotomy is not necessary and intramedullary fibular fixation is impossible to perform.

Patients will be immobilized with above-the-knee cast or spica cast for a minimum of 3 months post-operatively. Managing surgeons can decide on an individual basis whether a patient requires casting for longer than 3 months. After the cast is removed, patients will be permanently braced (24 hours per day) in a custom-made orthosis, either above-the-knee or below-the-knee, at the discretion of the surgeon. Radiographs of the lower leg and ankle will be performed at 6 weeks, 3, 6, 9, and 12 months after surgery at times corresponding to follow-up evaluations and cast changes.

Summary of Surgical Protocol:

- Tibial osteotomy
- Resection of pseudarthrosis tissue
- Fibular osteotomy if intact fibula
- Placement of intramedullary rod providing rigid fixation
    - Williams, Rush, or other type
- Ankle fixation at discretion of the surgeon
- Placement of iliac crest autograft at the surgical site
- If randomized to BMP-2 treatment group:
    - Reconstitution of BMP-2 in sterile water as per package instructions (at least 15 min before application) and applied to the collagen sponge(s).
    - A small INFUSE package (4.2 mg total BMP-2 and 2 sponges with concentration of 1.5 mg/ml) will be used for patients weighing < 30 kg
    - A Medium INFUSE package (8.4 mg total BMP-2 and 4 sponges, with concentration of 1.5 mg/ml) will be used for patients weighing ≥ 30 kg. INFUSE bone graft sponge is wrapped around the tibial osteotomy site and autograft.
- Incision is closed
- Above-the-knee or spica cast x minimum of 3 months

Rehabilitation Protocol

- All patients will be in an above-the-knee or spica cast for a minimum of 3 months
- Recommend no weight-bearing on affected leg for 3 months post-op
- Further cast immobilization may be used if the surgeon interprets the radiographs as having too little callus formation at the tibial pseudarthrosis site
    - Cast time may be extended in 4-6 week intervals, followed by radiographic re-evaluation until satisfactory healing has been achieved.
    - If casting is required beyond 3 months, it may be below-the-knee instead of above-the-knee.
- Braced (above or below knee) with partial weight-bearing beginning at 3 months
- Knee mobilization exercises beginning at 3 months (or after removal of cast if later than 3 months).
- Full weight-bearing (braced) based on X-ray appearance (RUST score of 9 or greater)
- No sports participation until 1 year post-operative and with brace in situ.
- Individual questions about rehabilitation protocol may be addressed to the two study surgeons (Drs. Richards and Little).

5.3 Protocol to Radiographically Score Tibial Healing (RUST score, Primary Outcome Measure; Specific Aim 1)

The assessment of tibial healing in patients with tibial pseudarthrosis will be based on radiographic evidence of union by determination of cortical continuity on Xray and scoring by a novel system called the Radiographic Union Score for Tibial fractures (RUST) score [Whelan et al., 2010]. This validated method assigns a score to a set of anteroposterior (AP) and lateral (Lat) radiographs based on healing at each of the 4 cortices. The lateral and medial cortices are denoted on the AP radiograph and the anterior and posterior cortices on the lateral radiograph. A score of 1-3 is assigned based on the persistence of the fracture line and the presence or absence of callus formation. If all 4 cortices are evaluable then scores range from 4 (non-union all bone columns) to 12 (complete union of all 4 bone columns). As this method was validated in fractured normal tibias, any lucency was considered residual fracture. However, in these patients there is known underlying dysplasia affecting the appearance of the bone underlying the osteotomy; therefore an intact peripheral cortex with residual intramedullary dysplasia will be considered healed. A cortex that is not visualized due to eccentric overlying hardware will be given a value of 1.
Scoring system:
1 = no new calcification, or rod eccentricity precludes evaluation
2 = new calcification or callus present
3 = smooth continuous outer cortex, even if underlying lucency

Total score ranges from 4 to 12.

For the purpose of this study, a RUST score of 9-12, with at least 2 evaluable cortices having a score of 3, will be considered to be healed, and a successful outcome.

Figure 6: RUST scoring

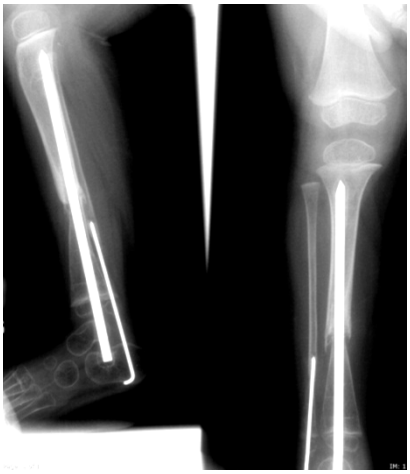


Figure showing partial bridging and callus formation of all 4 cortices.

RUST score = 2+2+2+2 = 8

Implementation of the RUST scoring method as the sole criterion for tibial fracture healing will enable the same radiology review panel (RRP) of 4 individuals to score both prospective cases and historic controls. The total RUST point score range is 4 to 12. Additionally, information on functional status will be collected before surgery, and at each subsequent evaluation after surgery, as outlined in Johnston [2002] and Richards et al., [2010]. This includes status of the ability to walk with weight-bearing, use of orthotic support, presence of pain, point tenderness at the site, level of physical activity, additional surgery of the ipsilateral leg in addition to radiographic evidence of union, knee and ankle motion, foot position, leg-length discrepancy, and overall clinician satisfaction.

5.4 QUALITY OF LIFE ASSESSMENTS

Pediatric Outcome Data Collection Instrument (PODCI)
An important outcome measure of clinical trials in treatment of tibial pseudarthrosis is quality of life. Many individuals with TPA undergo multiple surgical interventions with long rehabilitation times, precluding their participation in school and recreational activities. In preliminary studies performed by collaborators Viskochil and Schorry, an outcomes trial led by John Carey (Shriners Research Grant #9165; Multi-Center Study of Outcome of Tibial Dysplasia in NF1 Patients) used the pediatric outcome data collection instrument (PODCI) to evaluate the quality of life of individuals with NF1, with and without tibial dysplasia. The PODCI consists of five domains, with a number of items in each domain [Daltroy et al, 1998; Vitale et al, 2001]. Twenty-three individuals with NF1 with tibial dysplasia were compared to 63 NF1 individuals without tibial dysplasia. Notably, the Basic Mobility normative scores, the Sports and Physical Function scores, and Global Functioning were significantly decreased ($p<0.001$) in NF1 with tibial dysplasia with the mean score for cases about one-third of controls [Stevenson and Carey, 2009]. The PODCI has also been reviewed by the REINS committee (Response Evaluation in Neurofibromatosis and Schwannomatosis), and found to be an appropriate outcome measure for this population. **These data demonstrate that the PODCI is an effective QOL measure that can be used to investigate outcome for treatment of TPA in the NF1 population**, and can accompany more traditional clinical measures such as union, range of motion, leg length discrepancy, and limitation of walking distance. Other measures of quality of life have been implemented for the NF1 population [Krab et al., 2009] and the orthopedic community [Vitale et al., 2001; Furlong et al., 2005]; however, these measures have not been applied to the pediatric NF1 population with orthopedic issues.

- Quality of Life will be assessed using the Pediatric Outcome Data Collection Instrument (PODCI) questionnaire The PODCI is a comprehensive orthopedic-specific instrument (Pencharz et al., 2001) comprised of over 100 questions and takes 15-20 minutes to

complete. Quality of Life Questionnaires are to be completed by patient and parents as follows at baseline, at 6 months, and at 12 months.

- PODCI Pediatrics-parent/child: baseline and follow-up (completed by the parent for all children 2-10 years old).
- PODCI Pediatrics-parent/adolescent: baseline and follow-up (completed by the parent for all adolescents 11-18 years old).
- PODCI Pediatrics-adolescent baseline and follow-up (completed by patient between 11 and 18 years old)
- In addition to the PODCI, pain intensity will be assessed using the Faces Pain Scale-Revised (FPS-R) for patients aged 4 years and older, at baseline and at 6 weeks, 3 mos, 6 mos, and 12 mos after surgery. This is a self-report pain scale which has been validated for use in research, in patients as young as 4 years. Children chose one of 6 faces as indicating their current pain. There are no validated self-report pain scales for children between 2 and 4 years. Additional pain measures, including pain interference, are included in the PODCI for all ages participating in the study.

5.5 FUNCTIONAL ASSESSMENT: Ten Meter Timed Walk

The **timed 10 meter walk test** is a functional outcome measure that has been used extensively in the fields of rehabilitation medicine, neurology, and orthopaedics. In this well-validated, easily administered test, a 10 meter (32.8 feet) hallway is measured and marked, with starting line and end line. The patient is given instructions either to walk the distance at his/her normal, comfortable speed, or as quickly as safely possible. The walk is timed using a standard stopwatch, and results are recorded in seconds. The patient performs the test 3 times, and the mean value of the 3 trials is used as the result. Results from this test will be compared for individuals at baseline, 6 mos after surgery, and 12 mos after surgery, and will be used as a secondary outcome measure. The timed 10 meter walk is easy to perform in a clinic setting without special equipment such as a gait analysis lab. The test has been used extensively in studies of muscular dystrophy, spinal cord injury and cerebral palsy, and has been validated from ages 4 ears through adults [Pirpis et al, 2003]. It has also been used as an outcome measure in a Phase 3 clinical trial for multiple sclerosis [Cohen et al., 2001]. A 20% change in walking speed is considered to be significant. The test can be performed while a patient is in a brace or other adaptive device. This test and other functional outcome measures have recently been reviewed extensively by the REINS committee, and based on its reliability and ease of use was recommended as a secondary outcome measure for NF trials assessing ambulation.

The 10 meter Timed Walking Test will be performed before surgery, and at 6 months and 12 months post surgery. The test can be performed with the leg braced, and any bracing or other assistive device will be recorded at each time point. The test will be performed 3 times, and the mean of the 3 times (in seconds) will be recorded as the final score at each visit.

Data will be entered into eDES at the clinical sites and sent electronically to the DMAC for subsequent analysis.

5.6 Protocol to Collect, Process, and Store Biologic Specimens (Specific Aim 3)

Numerous biologic specimens will be collected for future NF1 studies funded independently and performed by investigators who are formally approved to access the specimens from the Tissue Storage Facility at the Texas Scottish Rite Hospital, Dallas, TX. The purpose of this collection is to preserve tissue for appropriate biologic screening, when techniques and funding become available. The patient or patient's parents must initial the informed consent document indicating that tissue can be collected for this processing. Depending on level of consent for use of tissue and/or DNA specimens, samples will be coded with the patient's study trial registration number prior to shipment.

Any consortium member (including TSRH members) can request access to the specimens for research purposes by contacting the Operations Center of the Consortium with a valid proposal for their use. The **Biospecimens Allocation Committee (BAC)** will make decisions regarding future use of tissues. Researchers will be encouraged to request outside funding for further study of these biologic specimens, such as through a CTF research award.

5.7 RISK/ BENEFIT ANALYSIS

Evaluation of benefits and risks/discomforts

The potential benefit from participation in this trial is improved union of surgically treated tibial pseudarthrosis, faster time to union, and improved quality of life. There are risks for surgery that exist regardless of enrollment on the clinical trial. The primary risks to the subjects from participation in this clinical trial are complications related to BMP-2. Toxicities and anticipated adverse events are outlined in Sections 2.1 and 2.2. Patients enrolled on this trial will be carefully monitored for the development of toxicities, including development of antibodies to BMP-2, and a toxicity stopping rule is in place to prevent additional patients from exposure to BMP-2. Clinical report forms, radiographic studies, and biologic specimens for future research studies, will have identifiers that can be traced to the patient only by contacting the NF Clinical Trials Consortium Operations Center. However, as the samples are linked to the patient's name, a small risk persists that unauthorized persons could gain access to information. Some testing may eventually reveal information that could result in discrimination with health or life insurance or employment. We believe that these risks are minimal since it is already known that patients enrolled on the study have NF1. All research results will be kept confidential.
Patients also have the right to withdraw the blood specimens obtained for research purposes at any time.

Potential benefits to all participants of this trial include: 1) receiving their surgery from an experienced surgeon with expressed interest in neurofibromatosis; 2) screening and treatment for factors such as low Vitamin D levels which may affect bone healing; 3) use of a standardized protocol for intramedullary rod and iliac crest bone graft; and 4) use of a standardized rehabilitation protocol. In addition, all participants will have Xray assessment of bone healing by a group of radiologists experienced in NF1 bone disease, using the RUST score, which may reflect a more accurate measure of bone healing than local radiographic reading.

Risk/benefit analysis

The primary objective of this phase II trial is to determine integrity of tibial union at 12 months after surgery for NF1-related tibial pseudarthrosis. Thus, patients entered on the trial will be treated with therapeutic intent and response to the therapy will be closely monitored. Therefore, this protocol involves greater than minimal risk to children, but presents the potential for direct benefit to individual subjects. The potential benefit of this treatment with BMP-2 is that it may improve healing of tibial pseudarthrosis. In addition, treatment may lessen the symptoms, such as pain, that are caused by the pseudarthrosis. The radiographic union scoring (RUST) used as the outcome measure will provide more precise assessment of union, and therefore will give more complete and objective information on which to base any possible future treatment decisions. Radiographic assessment by the RUST criteria is currently not available on a routine clinical basis.

Potential Risks:

a. Device dropped or deemed unsterile
   Mitigation strategy: For centers that do not have a backup clinical supply, an additional sterile package provided by Medtronic to prevent procedure termination.

b. Pain at surgical site

Discussion: Post-operative pain is expected in both control and experimental groups.
Mitigation strategy: standard post-operative pain control with close monitoring of patient's pain. This risk is included on the patient consent form.

c. Inflammation or tissue swelling at surgical site
Discussion: Some studies suggest increase rate of inflammation at surgical site with use of BMP-2.
Mitigation strategy: Anti-inflammatory medications as needed post-operatively, close monitoring. Monitoring for fever or signs of infection. This risk is included on the patient consent form.

d. Infection at surgical site
Discussion: This is a risk with any surgical procedure
Mitigation strategy: Monitor clinically for infection in post-op period. Treat with antibiotics if clinically indicated. This risk is included on the patient consent form.

e. Allergic/ antibody response
Discussion: Studies in adults show very low rate of antibody formation to BMP-2, and no clinical allergic reactions. However, antibody formation has not yet been tested in a pediatric population.
Mitigation strategy: Information about management of antibody testing will be included as part of investigator training. Participants with prior exposure will have expedited antibody testing pre-operatively as part of eligibility screening. All participants (control and experimental) will be tested for antibodies to BMP-2 and bovine collagen before and at multiple intervals after surgery. Those that test positive to rhBMP-2 will have additional bloodwork drawn to check for neutralizing antibodies. Any clinically evident allergic response will be treated if clinically indicated with anti-histamines or other medications. Patients continuing to be antibody positive at the 12 month endpoint of the study will continue to have antibody levels checked on a yearly basis thereafter.

f. Failure of bone healing/ persistent pseudarthrosis
Discussion: This is a risk for both the control and experimental groups. The expectation (and purpose of this study) is that the BMP-2 group will have a better healing rate/ lower incidence of non-union post-surgery. This risk is included on the patient consent form.
Mitigation strategy: All patients will be followed closely by their participating orthopedic surgeons post-operatively, with Xrays obtained a minimum of every 3 mos to document healing of tibia. Patients with clear non-union after 12 months may be offered a second surgical procedure (outside of the research protocol), if clinically indicated.

g. Theoretic increased risk of malignancy
Discussion: BMP-2 is a potent osteogenic agent, and there is theoretical concern that it may increase risk of malignancy in a pediatric population or an NF1 population known to have an increased risk of tumors. In actuality, BMP-2 has been used successfully in over 81 reported children without occurrence of malignancy, and in over 30 pediatric NF1 patients. It has been well documented that BMP-2 is very rapidly cleared from the serum, and its effect is expected to be limited to the surgical site. The severity of continued pseudarthrosis and possible amputation justifies this small theoretic risk in our opinion. This risk is included in the patient consent form. Further details are in section 3.5, pages 23-25.
Mitigation strategy: Patients will be excluded from the study if they have a known malignancy, or plexiform neurofibroma of 3 cm diameter or larger on the surgical leg, to decrease risk of malignant transformation. Patients will be monitored throughout the study for any new tumor growths.

h. Theoretical increased risk of teratogenicity
Discussion: There is no information available about risk of exposure of pregnant women to BMP-2; however as a potent growth agent it could have potential to cause fetal harm.
Mitigation strategy: Young women of childbearing age must have a urine pregnancy test performed within 7 days before surgery and again at the 6 week post-op visit. Patients who are pregnant or who are sexually active without use of effective contraception are excluded from the study.

i. Ectopic bone formation.
Discussion: There have been several reports of heterotopic ossification in patients treated with INFUSE, although the frequency of this complication is not known.
Mitigation strategy: the INFUSE device will be placed only at the area of the tibial pseudarthrosis repair. Xrays obtained at standard intervals during the study will be monitored for signs of ectopic calcification. If asymptomatic, no intervention may be necessary. If causing symptoms or functional impairment, surgery may be performed to remove the ectopic bone.

j. Excessive bone resorption.
Discussion: In rare cases, excessive bone resorption has been noted when using high doses of BMP2, due to a potential stimulation of osteoclastic activity.
Mitigation strategy: the dosage of BMP-2 chosen for the study was selected in a range in which excessive bone resorption has not been reported in pediatric patients. In addition, patients are required to have adequate Vitamin D levels prior to surgery, which may help counteract bone loss. If excessive bone resorption of the affected tibia does occur, the patient may require prolonged bracing, or may require an additional surgery, to be determined by the attending surgeon.

k. Death.
Discussion: Although quite rare in this population, death is a possibility with any surgical procedure. All patients will have their surgery and post-operative care at a hospital with extensive experience in caring for medical and surgical pediatric patients. ICU care is available in the case of serious complications.

5.8 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Pre and on-study evaluations are described in table format in Appendix I.

Required Clinical, Laboratory and Disease Evaluation

All entry/eligibility studies must be performed within 2 weeks prior to entry onto the trial (unless otherwise specified).

History, physical examination and vital signs

Physical examination will be performed prior to surgery, 6 weeks (+/- 2 week) after surgery, 12 weeks (+/- 2 weeks), 6 months (+/- 4 weeks), 9 months (+/- 4 weeks), and 12 months (+/- 4 weeks). This exam includes total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and basic nervous system). The affected extremity must be palpated (when no longer casted) for evidence of point tenderness or growth of tumors or masses. Significant findings made after the use of INFUSE, which meet the definition of an Adverse Event must be recorded.
Vital sign assessment consists of height, weight, head circumference, pulse, blood pressure, temperature and weight.

Clinical information to be documented includes a description of any bracing device, ability to bear weight on the affected leg while walking, leg-length discrepancy, leg asymmetry, knee and ankle range of motion, foot position, tenderness at the site, pain, documentation of intervening surgeries subsequent to the initial surgery, and overall satisfaction of the surgeon regarding outcomes.

For the purposes of the study, end of study will be 12 months; however, additional data will be collected on an annual basis through a registry maintained by Texas Scottish Rite Hospital for Children.

Hematology

Hematology labs will include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. CBC, differential and platelets must be obtained within two weeks prior to surgery, and again at 3 months and 12 months after surgery.

Blood chemistry

Blood chemistry must include sodium, potassium, chloride, bicarbonate, calcium, phosphorous, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), GGT, uric acid, and alkaline phosphatase. The initial serum studies prior to surgery include calcium, phosphorus, magnesium, and Vitamin D (25-OH Vit D). Enrollment cannot proceed if any of the serum levels indicate that treatment for abnormal levels needs to be initiated. Blood chemistries should be drawn within two weeks prior to study entry, and at 3 months and 12 months post-surgery.

Urinalysis routine

Standard urinalysis dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed at the beginning of study, 3 months, and 12 months.

Urinalysis/Pregnancy

Standard pregnancy tests will be given to all females of childbearing age within 7 days prior to surgery to ensure that females are not pregnant at the time they are treated with BMP-2. This will be repeated at 6 weeks after surgery for females of childbearing age.

Antibody Studies

For safety purposes, all subjects will be assayed for antibodies to bovine type I collagen and rhBMP-2. Testing will be performed by Intertek Pharmaceutical Services, San Diego, CA, using a validated enzyme linked immunosorbent assay (ELISA). Intertek Pharmaceutical laboratory previously validated the BMP-2 assay for FDA approval of the INFUSE device in adult patients. The following methods were used for validation:

Samples are incubated with rhBMP-2 or bovine Type I collagen, which has been immobilized on an ELISA plate, to allow any rhBMP-2 (or bovine collagen I) specific immunoglobulins to bind. After incubation, the plates are washed and the bound antibody is detected using assay-specific peroxidase conjugated detection antibody which produced a color end-product when the substrate was added.

A normal human serum pool and an antibody positive serum which recognizes and binds to rhBMP-2 (or bovine I collagen) are included on each plate as negative and positive controls, respectively, to monitor assay performance. The optical density (OD) values of the samples were compared to the cutpoint OD of the plate to determine if antibodies specific for rhBMP-2 or bovine I collagen were present or absent. The cutpoint OD was defined as two times the mean of the negative control OD. Samples were initially tested in duplicate in a screening format at 2 dilutions. Any samples that generated an OD greater than or equal to the cutpoint were

reanalyzed in the titer format. In the titration format, the same samples were also incubated on plates that were uncoated (no rhBMP-2 or bovine I collagen) to determine if a positive signal generated was specific or non-specific. The antibody titer was defined as the reciprocal dilution of the sample which generated an OD equal to the cutpoint OD. This was reported as the arithmetic antibody titer.

Antibody testing will be performed on serum samples at the following timepoints:

- Pre-operatively; 6 weeks $\pm$ 2 weeks, 3 months $\pm$ 2 weeks, 6 months $\pm$ 4 weeks, and 12 months $\pm$ 4 weeks post-op.
- Any subject testing positive to antibodies to bovine collagen will be tested for antibodies to human collagen.
- Any subject testing positive to antibodies to rhBMP-2 will be tested for BMP-2 neutralizing antibodies

Patients with history of prior exposure to BMP-2 in a previous surgery, who are positive for antibodies to rhBMP-2, bovine collagen, or neutralizing antibodies pre-operatively are excluded from participation in the study.

Radiographs of tibial union

Standard anteroposterior (AP) and lateral (lat) radiographs of the tibia and fibula will be obtained within 2 weeks prior to surgical intervention and postoperatively at the following intervals: 6 weeks (+/- 2 weeks), 12 weeks (+/- 2 weeks), 6 months (+/- 4 weeks), 9 months (+/- 4 weeks) and 12 months (+/- 4 weeks). Postoperative exams will be acquired out of cast.

The imaging protocol of the lower leg is standard anteroposterior and lateral radiographs of lower leg and ankle. Images will be sent on CD to the Radiography Review Panel at TSRH.

Tissue Collection for Future Studies (US Centers; Further details in Appendix II and Lab Manual)

The following tissue will be collected following participant consent:

Tibial pseudarthrosis and adjacent bone: These samples will be obtained intraoperatively, and sent by overnight shipment to the Tissue Storage Facility (TSF) at TSRHC. In those circumstances where the orthopedic surgeon deems clinical pathology is needed, samples will be collected, cataloged, and shipped only after the clinical pathologist agrees to release the samples from the medical facility. Bone and pseudarthrosis samples will be shipped overnight in packages. Once received in the TSF, samples will be photographed before processing. The cataloging from the originating site will include documentation of orientation of the sample, which must include designation of the proximal tibial bone – tibial pseudarthrosis tissue – distal bone. After obtaining additional consent, provided clinical information will be identified with study participant number and will stay with the tissue sample at the TSF.

Iliac crest bone: with informed consent these samples will be obtained intraoperatively, and shipped overnight to the TSF for storage.

At the time of enrollment (pre-operatively), peripheral blood will be collected (1 EDTA tube, 1 citrate tube) and shipped at ambient temperature to the tissue processing facility at TSRH for DNA extraction and future lymphocyte immortalization. Samples will be cataloged and stored for future use. DNA concentrations will be denoted on each aliquot tube.

First morning void urine will be collected at the time of enrollment and shipped frozen in 5ml aliquots to the TSF.

Participants will be able to check a box on the informed consent form if they wish to participate in the optional research tissue collection. Details of research sample collection are in Appendix II.

Quality of Life and Pain Evaluations

Quality of Life will be assessed using the Pediatric Outcome Data Collection Instrument (PODCI) questionnaire and the Faces Pain Scale Revised (FPS-R) The PODCI is a comprehensive orthopedic-specific instrument (Pencharz et al., 2001) comprised of over 100 questions and takes 15-20 minutes to complete. As discussed in our preliminary data we have demonstrated that the PODCI is a valid tool for the NF1 population and has demonstrated differences in NF1 individuals with and without tibial dysplasia in expected domains (Stevenson and Carey, 2009).

Quality of Life Questionnaires are to be completed by patient and parents as follows at time of surgery (pre-op), at 6 months, and at 12 months post-surgery. In addition, the pain intensity scale (FPS-R) will also be obtained at baseline, 6 weeks, 3 months, 6 months and 12 months post-surgery, in order to assess post-operative pain.

PODCI Pediatrics-parent/child baseline and follow-up (completed by the parent for all children 2-10 years of age).

PODCI Pediatrics-parent/adolescent baseline and follow-up (completed by the parent for all adolescents 11-18 years of age).

PODCI Pediatrics-adolescent baseline and follow-up (completed by patient between 11 and 18 years of age)

FPS-R (completed by patients aged 4 – 18 years). Obtained at baseline, 6 weeks, 3 mos, 6 mos, and 12 mos post-surgery.

The 10 meter Timed Walking Test will be performed before surgery, and at 6 months and 12 months post-surgery. The test can be performed with the leg braced, and any bracing or other assistive device will be recorded at each time point. The test will be performed 3 times, and the mean of the 3 times (in seconds) will be recorded as the final score at each visit.

Forms will be forwarded to the UAB Operations Center for collation and subsequent analysis.

5.9 Adverse Reporting Requirements and unanticipated problems

Definitions

Adverse Events: An adverse event is any new, undesirable medical occurrence or change (worsening) of an existing condition in a subject that occurs during the study, whether or not considered to be related to BMP-2. Therefore, adverse events are treatment emergent signs or symptoms. Elective hospitalizations for pretreatment conditions (e.g., elective cosmetic procedures) are not considered adverse events for this study. Non-clinically significant abnormal laboratory values should not be reported as adverse events; however, any clinical consequences of the abnormality should be reported as adverse events. All adverse events must be entered into the data-entry system.

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification (within 24 hours) to the site PI and clinical coordinator, as well as the NF Operations Center and follow 21CFR812.140 investigator responsibilities. Follow-up of the adverse event, even after the date of therapy discontinuation, is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and sponsor.

Serious Adverse Events: A serious adverse event is defined by regulatory agencies as one that suggests a significant hazard or side effect, regardless of the investigator's or sponsor's opinion on the relationship to investigational product.

This includes, but may not be limited to, any event that (at any dose):
is FATAL
is LIFE THREATENING (places the subject at immediate risk of death);
requires HOSPITALIZATION or prolongation of existing hospitalization;
is a persistent or significant DISABILITY/INCAPACITY; or
is a CONGENITAL ANOMALY/BIRTH DEFECT

Important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the subject or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious. Examples include allergic bronchospasm, convulsions, and blood dyscrasias.

Unanticipated Adverse Device Effects: An unanticipated adverse device effect is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem or death was not previously identified in nature, severity or degree of incidence in the IDE application. Or, any other unanticipated serious problem associated with a device that relates to the rights, safety or welfare of participants.

Common Terminology Criteria for Adverse Events (CTCAE)
This study will utilize the CTCAE of the National Cancer Institute for reporting of adverse events. A copy of the current version of the CTCAE version 4.03 can be downloaded from the CTEP home page: http://ctep.cancer.gov/reporting/ctc.html

Attribution: Definitions of relationship to study medical device are as follows:

Unrelated: bears no relation to timing of medical device use, similar to symptoms or signs expected in the disease process, does not recur on rechallenge.

Unlikely: does not have temporal relationship to intervention, could readily have been produced by the subject's clinical state, environmental, or other interventions, does not reappear or worsen with reintroduction of intervention.

Possibly: bears relation to timing of medical device use, similar to symptoms or signs expected in the disease process, does not recur on rechallenge.

Probably: bears clear relation to timing of medical device use, distinct from symptoms or signs expected in the disease process, does not recur on rechallenge.

Definitely: bears clear relation to timing of medical device use, distinct from symptoms or signs expected in the disease process, occurs on rechallenge.

The expected adverse events related to administration of BMP-2 are listed in **Section 2.1 and in the Informed Consent Forms.** All other adverse events not attributed to BMP-2 will be considered unexpected. Adverse events attributable to BMP-2 will also be reported if the adverse events are at an intensity that is more severe than previously documented or considered significant by the investigator and noted in the product insert and/or protocol.

Reporting Procedures for All Adverse Events

All observed or volunteered adverse events, regardless of suspected causal relationship to study medical device will be recorded as Adverse Events in the electronic data system at the time of surgery and at each follow-up visit, and entered into the electric system within 2 weeks of the visit. Events involving adverse reactions to the device, illnesses with onset during the study, or exacerbation of pre-existing illnesses should be recorded. Objective test findings (e.g., abnormal laboratory test results) should also be recorded.

As the investigational intervention (INFUSE bone graft) occurs only at the time of surgery, it is not expected that adverse events noted at follow-up visits would require removal of a participant from the study. If a subject voluntarily withdraws from the study, he or she will be given appropriate care under medical supervision until symptoms cease or until the condition becomes stable.

The severity of toxicities will be graded in accordance with the Common Terminology Criteria for Adverse Event (CTCAE) version 4.03 (http://ctep.info.nih.gov).

The relationship of adverse events to the medical device will be assessed by the physician using the categories of “unrelated, unlikely, possibly, probably, or definitely” as listed above.

Expedited Reporting Guidelines

The following adverse events require expedited reporting as required by 21CFR812.150:

- All adverse events that are both serious and unexpected per section 2.1.
- Adverse events that might influence the benefit-risk assessment of administration of INFUSE as outlined in the protocol
- All grade 5 adverse events
- A serious adverse event that occurs within 30 days of the placement of an investigational device
- Expedited AE reporting timelines defined:

“24 hours; 3 calendar days” – The investigator must initially report the AE within 24 hours of learning of the event to Operations Center followed by a complete report within 7 calendar days of the initial 24-hour report. All adverse events must be entered into the electronic data system.

Adverse events requiring expedited reporting will be reported and documented on a designated Serious Adverse Event case report form developed for this protocol, “NF Clinical Trials Consortium Operations Center IDE Serious Adverse Event Form for IDE# G140004”and forwarded to:

NF Clinical Trials Consortium Operations Center
Research Nurse Manager: Vivien Phillips, RN BSN
Regulatory Compliance: Roy McDonald, MPH
Phone: Vivien: 205-934-5376 / Roy: 205-975-4075.
Email: NFConsortium@uab.edu

The Operations Center Research Nurse Manager will forward all related adverse events that are both serious and unexpected to the FDA. If paper forms are sent, they will be sent on the "NF Clinical Trials Consortium Operations Center IDE Serious Adverse Event Form for IDE# G140004."

The Operations Center will also forward to the Study sponsor within 3 working days all adverse events which require expedited reporting and subsequently to the Principal Investigators of participating sites.

Adverse Event Reporting to US Army

This trial receives funding by a grant from the U.S. Army. Per US Army Medical Research & Materiel Command (USAMRMC) Office of Research Protections Human Research Protections Regulatory requirements, adverse events will be reported to the USAMRMC by the NF Clinical Trials Consortium Operations Center regulatory compliance specialist as follows:

All adverse events, which require prompt reporting (as defined in Section 5.9), will be immediately reported by the sites to NF Clinical Trials Consortium Operations Center. NF Clinical Trials Consortium Operations Center representative will promptly report by email (usarmy.detrick.medcom-usamrmc.orther.hrpo@mail.mil), telephone (301-619-2165), or by facsimile (301-619-7803) to the USAMRMC Deputy Chief of Staff for Regulatory Compliance and Quality. An initial written report will follow the site's notification within three working days to NFConsortium@uab.edu. The NF Clinical Trials Consortium Operations Center will notify all appropriate study regulatory personnel and send written report to the U.S. Army Medical Research and Materiel Command within seven working days of the initial notification of the adverse event requiring prompt reporting. The written report will be sent to ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000 or email, usarmy.detrick.medcome-usamrmc.other.hrpo@mail.mil, using appropriate HRPO log number assignment.

Reporting of Protocol Violations/Deviations and Unanticipated Problems

Any protocol deviations or unanticipated problems will be compiled by the DMAC on a monthly basis. Sites should report all unanticipated problems as soon as possible in the data entry system. Sites will report deviations that impact subject safety or the scientific integrity of the study to the Operations Center promptly by email and into the data entry system. The Operations Center will provide this information to the protocol team, Medical Monitor, DSMB, and Sponsor, as needed.

The Operations Center will report deviations that impact subject safety or the scientific integrity of the study to the USAMRMC ORP HRPO promptly. Other protocol deviations will be provided annually to the Sponsor. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

Grading of Adverse Events

Adverse events (toxicities) will be graded according to the NCI Common Terminology Criteria for Adverse Events v4.03 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html

CRITERIA FOR REMOVAL FROM STUDY

a. Non-compliance with casting or bracing post-operatively

b. Development of a secondary diagnosis post-operatively which may affect bone metabolism (diabetes, renal disease, hyperparathyroidism, etc.)

c. Non-attendance at more than 2 scheduled follow-up visits

Off Study Criteria
a) Death
b) Lost to follow-up
c) Withdrawal of consent for any further data submission.
d) Initiation of medical treatment (e.g. chemotherapy, biologic therapy, radiation therapy) directed towards NF1-related tumor such as an optic pathway glioma or MPNST.

6.0 STATISTICAL AND ETHICAL CONSIDERATIONS

Subject Accrual
Subjects of both genders, from all racial and ethnic groups are eligible for this trial if they meet the criteria outlined in Section 4. To date, there is no information that suggests differences in drug metabolism or disease response among racial or ethnic groups or between the genders, indicating that results of the trial will be applicable to all groups. Most tibial pseudarthrosis arises in early childhood, with fewer cases after age 11; thus we anticipate an enrollment of younger children. If differences in outcome that correlate to gender, age, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences.

6.1 Statistics and Feasibility
The primary objective of this study is to determine if there is improved healing of tibia in patients treated with BMP-2, compared to those treated with standard surgical protocol alone. The primary outcome for the study will be radiographic evidence of tibial healing 12 months after surgery, using the RUST criteria as described in section 6.4. For each cortex a score of 1, 2, or 3 will be given. A non-visible cortex will be given a score of 1. The cortex scores will be added together for a total score. We will define three levels of healing: RUST score of 4-7, score 8-9, or score 10-12. The primary analysis will be to compare the distribution of scores across the 3 groups (4-7, 8-9, 10-12) at 12 months post-surgery using a Wilcoxon Test. The proportion of patients achieving **complete healing** (score 9-12, with at least 2 evaluable cortices having a score of 3) will also be calculated and an exact 95% confidence interval computed.

Analyses based on the actual 4 – 12 scores will also be performed and compared between the two groups. In addition, the % of patients having a **successful outcome** (defined as RUST score of 9 or greater, with at least 2 evaluable cortices having a score of 3) will be compared between the two groups. Time to union will be compared between the two groups based on Xrays obtained at 5 points after surgery (6 weeks, 3 mos, 6, 9, and 12 mos).

Subjects who experience a serious treatment-related adverse event, refracture, or need for reoperation will be considered to be **study failures**. The proportion of failures within each group will be compared to assess safety success.

6.2 Power Analysis/Sample Size Justification
The primary study endpoint will be the subject's RUST score 12 months after surgery. The radiology panel determining RUST score will be blinded to the treatment status of all patients, whether in the Choice or Randomized arms. The two treatment groups will be compared with

respect to the RUST score using the Wilcoxon Rank Sum Test at the one sided 0.05 significance level.

Data from the TSRH of 12 patients treated without BMP but an identical surgical protocol showed RUST scores at 12 mos post surgery of: Score 4-7 in 33%; Score of 8-9 in 42%; and score of 10-12 in 25%. If the use of BMP improves these scores to: 15% 4-7; 20% 8-9, and 65% 10-12, as suggested by our data from Dr. Richards' 16 patients treated with BMP, then it follows that for two patients randomly chosen, one from each treatment group, the probability that the patient in the BMP group will have a better score than the patient in the Control group is .691. Using a standard formula [Kolassa, 1995] implemented in nQuery Advisor® 7.0 this study with the initially planned sample size of 50 (25 per group) will have 86% power for a Wilcoxon Rank Sum Test at the one sided p.05 significance level.

Study Modification: Based on data from the first year of the trial, more eligible patients declined to participate than agreed to participate; however, in many of these cases the family would have agreed to a Parental Preference trial. Similar Parental Preference clinical trials have achieved acceptance within the orthopedic field, as demonstrated by the large BrAIST study of bracing versus no bracing for adolescent scoliosis [Weinstein et al, 2013; Schwieger et al., 2016]. The study was thus modified after initiation to allow a **Choice Group**, wherein after offering whether a potential participant had a preference, this would be honored. If there were no preference or if there is no option to obtain BMP at the institution where the participant is being seen, they would be **randomized** to BMP or Control using a 2:3 randomization. The rationale for this unbalanced randomization is to obtain or achieve enough controls to have a reasonable comparison of the effect of BMP. It is expected by participating surgeons that more patients would prefer the BMP arm to the Control arm if given the choice.

We anticipate that this would leave the study with a potential slight imbalance in BMP treatment and controls. Thus, we evaluated the impact on the primary analysis given various scenarios on recruitment and testing treatment effects assuming the results of choice and randomized allocation could be pooled.

The study is limited by the company's allocation of just 26 BMP devices, so we examined the power of the study under various scenarios of BMP treated to Controls. The table below shows the power, along with the sample sizes (shown in italics in the table in the 4th and 5th rows), if the study ends up with 5 and 26 patients in Usual Care versus BMP, up to comparison of 20 Usual Care versus 26 BMP across the columns of the table. The power ranges from 46% power to detect a difference in RUST scores using a 1 tailed test at 0.05 to over 80% (as per our original primary analysis and endpoint). This is testing the RUST scores assuming UC of: 33% (Rust Scores 4-7), 42% (8-9) and 25% (10-12) compared to 15% (4-7); 20% (8-9) and 65% (10-12). So if we enroll at least 10 -15 controls, we have approaching 70% power to declare a significant difference. Obviously, we need to have a sufficient number of controls to enhance the power, and to help ensure this result, we will randomize 3 controls to 2 BMP cases in the randomization to enhance our ability to achieve at least 10 controls.

Power using a Wilcoxon (Mann-Whitney) rank-sum test that P(X<Y) = .5 (ordered categories) (unequal n's), One sided Type I error=0.05
(Control Rust scores 33% (4-7), 42% (8-9), 25% (>=10) versus BMP 15%, 20%, 65% respectively)

	1	2	3	4	5	6	7	8
Number of categories, k	3	3	3	3	3	3	3	3
p_1 = P(X<Y)	0.702	0.702	0.702	0.702	0.702	0.702	0.702	0.702
Power (%)	*46*	*51*	*56*	*59*	*62*	*65*	*76*	*82*

n_1	26	26	26	26	26	26	26	26
n_2	5	6	7	8	9	10	15	20

Our analysis will be a primary analysis combining the Choice and randomized participants. We will perform sensitivity analyses within the Choice and Randomized cohorts separately. Given the limited sample sizes, we do not expect significant differences, but will examine descriptive statistics comparing the Choice Group to the Randomized Group. We will examine the RUST scores amongst those who choose BMP versus those who refuse and choose usual care. We will of course, consider comparisons to historical controls should the results prove clearly different between the Choice and Randomized Groups.

As noted above we are likely giving up some power with this change to a Choice Design. Our assumption based on the current rate of declining to participate is that more patients will chose the BMP arm above the Control arm, but if we are incorrect, we would have adequate power (76% - 82%) if we were able to enroll 15-20 patients in the Control arm and 26 patients in the BMP arm. Even in a worse scenario that we were only able to recruit 10 patients to the Control arm + 26 to BMP, we would still have reasonable power of 65% to show a difference between the groups.

It is important to note that, although participants and providers in the Choice Arm will no longer be blinded to the treatment status of the participants, the Primary Outcome Measure (RUST score), will be determined by a group of 4 radiologists, all blinded to the treatment status of all participants.

Missing data: Reason for any missing 12 month radiographic data will be recorded by treatment group. Data from subjects missing the 12 month radiograph will not be included, unless multiple imputation is applied. Although minimal data are expected to be missing, multiple imputation technique will be used if 5% or more data are missing. This multiple imputation will replace the missing value with a set of plausible values that represent the uncertainty about the right value to impute. The multiply imputed data sets will then be analyzed by using the Wilcoxon Rank Sum Test for complete data on each and combining the results from these analyses. The variables used for the imputation will be RUST score at 6 months, age, prior surgery (Yes=1, No=0), and timed 10 meter walk at 12 months post-surgery.

Generalizability of the Results:

As we noted above, we expect that most of the data from this study will be generated by the choice arm and hope to maintain at least 10 randomized controls. We will compare the participant characteristics as well as Rust Scores between any controls identified by choice to those controls by randomization and more importantly RUST scores between the choice and randomized BMP treated arms. We will look at the overlap of the confidence intervals and test the equivalence of the two sets of results using two one sided tests of equivalence at the 5% level with an equivalence margin of 0.513. The margin of equivalence of 0.513 was derived based on the hypothesized difference between treatment and control originally planned and assuming these scores are normal continuous variables. Here we used the midpoint of each Rust Scoring Interval (5.5, 8.5 and 11) and computed the average Rust score for each group based on the hypothesized distribution in each group and ended up with a mean of 9.675 in the BMP group compared to 8.135 in the control group yielding an average difference in Rust Score of 1.54. We took 1/3 of the difference between the treatment average Rust score and the usual care Rust score ending up with an equivalence margin of 0.513. Since these sample sizes are small, we don't expect to reject the null hypothesis of differences as the power is probably low, but if they are different we understand that pooled results will be difficult to interpret. If these

tests of Choice versus Randomized treatment arms are not able to reject nonequivalence, we understand that this is not strong evidence for a lack of poolability between choice and randomization. Nevertheless, it will be sufficient to allow a comparison and calculation of the differences between the treatments and an overall assessment of the benefit of BMP in healing especially if we can demonstrate equivalence. Sensitivity analyses will be conducted using pooled and unpooled results as no statistical evidence of a lack of nonequivalence is not absolute evidence for nonequivalence.

Analyses:
All statistical analyses will be performed using SAS (version 9.3). All analyses are based on evaluable patients, as defined in section 10.5 with sensitivity analyses considering inevaluable patients as failures (scores 4-7). Summary statistics will be used to describe the study population (such as ranges, medians of ages, gender, baseline performance characteristics). The quality of life assessment in this study is based on the Pediatric Outcomes Data Collection Instrument, the PedsQL Pediatric Pain Questionnaire, and the 10 meter Timed Walk. From data collected on these questionnaires, scales are formed that measure domains such as global functioning, extremity and physical function, transfers and basic mobility, sports/physical function, happiness, and pain/comfort. These QOL and pain measures will be given pre-surgery, 3 months, 6 months, and 12 months after surgery. Since the scales are continuous, summary statistics will be generated for each measure at each time-point. The paired t-test will be used to look at changes in these measures from pre-treatment levels through 12 months. Longitudinal data analysis methods will be used to evaluate long-term changes in these measure using regression techniques employing mixed models.

In order to determine factors which may be prognostic for the success of BMP-2 treatment, we will perform Wilcoxon Rank Sum Tests to within each group to compare:

1) age $\leq$ 8 years versus age > 8 years
2) prior surgery versus no prior surgery; and
3) prior rhBMP-2 exposure versus rhBMP-2 naïve.

Regarding dose of rhBMP-2, those with 4.2 mg of rhBMP-2 implanted will be compared to those with 8.4 mg of rhBMP-2 implanted with respect to success rate using a chi square contingency table and with respect to number of adverse events using the Wilcoxon Rank Sum Test.

Relationships between quality of life measures and other variables (i.e., pain) will be investigated using correlational analyses. Performance status will be assessed using the Karnofsky Performance Scale. Generalized linear models will be used to evaluate changes in these scales and scores over time.

Early Stopping Rule
While BMP-2 has been evaluated extensively in non-NF1 patients, it has not been administered as a clinical trial in children with NF1 who have tibial pseudarthrosis. An early stopping rule will be invoked to prevent accrual of patients onto the study in the event that BMP-2 is associated with a higher than acceptable rate of severe toxicity identified at 6 weeks and 12 weeks post surgery [types: Grade 3 opportunistic infection, Grade 4 rash, Grade 3 hypertension, grade $\geq$ 3 allergic reaction (determined by clinical assessment and antibody levels), grade 3 renal toxicity, severe ectopic bone formation requiring surgery, or the development of malignant peripheral nerve sheath tumor or other cancers]. Toxicity assessment will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) (v4.03). After 10 patients are enrolled in each arm, toxicity will be reviewed and if greater than the

number of patients shown in the table below experiences severe toxicity, accrual will be stopped. Based upon results calculated using the binomial distribution, the following holds true for the table as a whole:

True probability of toxicity	Chance of stopping early
.10	7%
.20	32%
.25	47%
.30	62%
.40	83%

Number of patients	Trial will be stopped if the number of patients with severe toxicity is greater than or equal to the number below
10	3

Should the number of patients showing severe toxicity during treatment with BMP-2 exceed 25% of the total number of patients enrolled, the study committee and medical monitor will review the toxicities and define whether a protocol revision is required. This 25% level was determined based on prior studies showing a total complication rate of 17% in pediatric patients treated with BMP-2 [Oetgen and Richards, 2010].

Likewise, the trial may be stopped early if at least 6 patients of the first 10 enrollees in the BMP-2 arm have complete failure of union by 12 months postop (RUST score of 5 or less). This will be determined after the first 10 enrollees in the BMP-2 arm have completed the 12 month assessment. The entire study will be terminated if the BMP-2 group is stopped early due to toxicity or failure of union.

Since subjects, once they have had surgery, will need to be seen regularly for follow-up, we expect little or no attrition or loss to follow-up. Therefore, we will plan to enroll a total of between 36-52 patients, with a minimum goal of 15 eligible patients in the control arm and 26 patients in the BMP arm.

6.3 HUMAN SUBJECTS PROTECTION

The PI from each participating institution will provide the NF Clinical Trials Consortium Operations Center with a copy of the initial IRB protocol approval and the yearly IRB continuing reviews. The Operations Center Regulatory Manager will submit these to the USAMRMC ORP HRPO. Enrollment will be halted at a participating institution if a current continuing approval is not on file at the NF Consortium Operations Center.

As the Operations Center receives funding by the US Army, approval of the protocol and of all protocol amendments will also be obtained from the USAMRMC Office of Research Protections (ORP), Human Research Protection Office (HRPO) in addition to the institutional IRB and the NF Consortium IRB prior to implementation.

Rationale for Subject Selection

Neurofibromatosis type 1 is a pan-ethnic genetic disorder with an incidence of 1 in 3,000 people. Subject accrual in regards to gender, and racial and ethnic groups is described in Section 3.6. None of these groups are excluded from participation in the trial. Females who are pregnant or breast feeding will not be eligible for entry onto the trial because of the potential risks that rhBMP-2 could pose to the fetus or newborn. This trial is designed to determine the

activity of BMP-2 in children with NF1 and surgically treated pseudarthrosis, therefore children will be entered onto this research trial. Almost all individuals with NF1-related congenital pseudarthrosis of the tibia will have their first or second surgery within the first 18 years of life. Individuals will be enrolled at one of 13 sites that collaborate with the NF Clinical Trials Consortium with additional affiliate sites at TSRH, Australia, and the United Kingdom. Patients may be referred from outside centers but treatment will be directed at a participating consortium site.

Participation of Children

Since tibial pseudarthrosis typically requires surgical intervention before age 10 years of age, children who meet eligibility criteria for this trial will be entered in the study. Children will be evaluated and cared for by physicians trained in pediatrics and in the orthopedic management of children with NF1.

Compensation of Participants

Participants who live further than 50 miles from the center where surgery will be performed can receive partial compensation for travel expenses. Maximum reimbursement will be $500 per patient (US dollars), with $250 payable at the 6 week post- surgical follow-up visit and the remainder after all follow-up visits have been completed at 12 months. As travel costs for families will often exceed this amount, this would not be considered unduly influential. Participants who live within 50 miles of the surgical center will be reimbursed a maximum total of (US) $200 per patient for time and travel involved ($100 payable at 6 week post-surgical follow-up visit and remainder at 12 months).

Consent and assent process and documentation

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient and the patient's parents or guardian. A signed informed consent document will be obtained. Consent will be obtained by the site PI or an associate investigator on the trial according to state and institutional guidelines. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial. Age appropriate assent forms for children from 7 through 12 years, and for children from 13 through 17 years have been developed for use in this trial. This is a multi-institutional trial, and the NF Operations Center will require evidence of local IRB approval and of USAMRMC ORP HRPO approval of the protocol prior to allowing for accrual of patients at that institution. This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

6.4 MONITORING PROCEDURES

Research Monitor and Data Safety Monitoring Plan

The trial PI and co-investigators will review the study progress regularly. Patients entered on the trial and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol. Monthly reports will be generated by the NF Consortium DMAC to assess completeness of data. There will be monthly phone conferences between the NF Consortium Operations Center and the Principal Investigators to address QA issues. An independent **Data Safety Monitoring Board** will be established for the purpose of ensuring data compliance and regular monitoring of this trial of adverse events and study timepoints.

All DOD funded studies that are greater than minimal risk requires that a research monitor be identified by name with their DOD specific role included (see * below). In the past NF Consortium trials, the medical monitor has been identified to function in this role.

The research monitor's duties should be based on specific risks or concerns about the research. The research monitor may perform oversight functions and report their observations and findings to the IRB or a designated official. The research monitor may be identified from within or outside the PI's institution.

*Research monitor functions may include: observing recruitment and enrollment procedures and the consent process for individuals, groups or units, overseeing study interventions and interactions, reviewing monitoring plans and UPIRTSO reports, overseeing data matching, data collection, and analysis

There may be more than one research monitor. The monitor may be an ombudsman or a member of the data safety monitoring board.

At a minimum, the research monitor: may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

John P. Dormans, MD, FACS (Pediatric Orthopaedic Surgery & Scoliosis, Baylor College of Medicine) will serve as the **research monitor**. He will serve as a patient advocate and is independent of the clinical study team. He will oversee the progress of the protocol, especially issues of individual subject/patient management and safety. The research monitor is required to review all unanticipated problems involving risks to subjects or others, including all serious and unexpected adverse events associated with the protocol as defined in Section 5.9. The monitor provides an unbiased written report of the event.

Research Monitor Language required by the DOD:

The research monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor should also indicate whether he concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the USAMRMC ORP HRPO.

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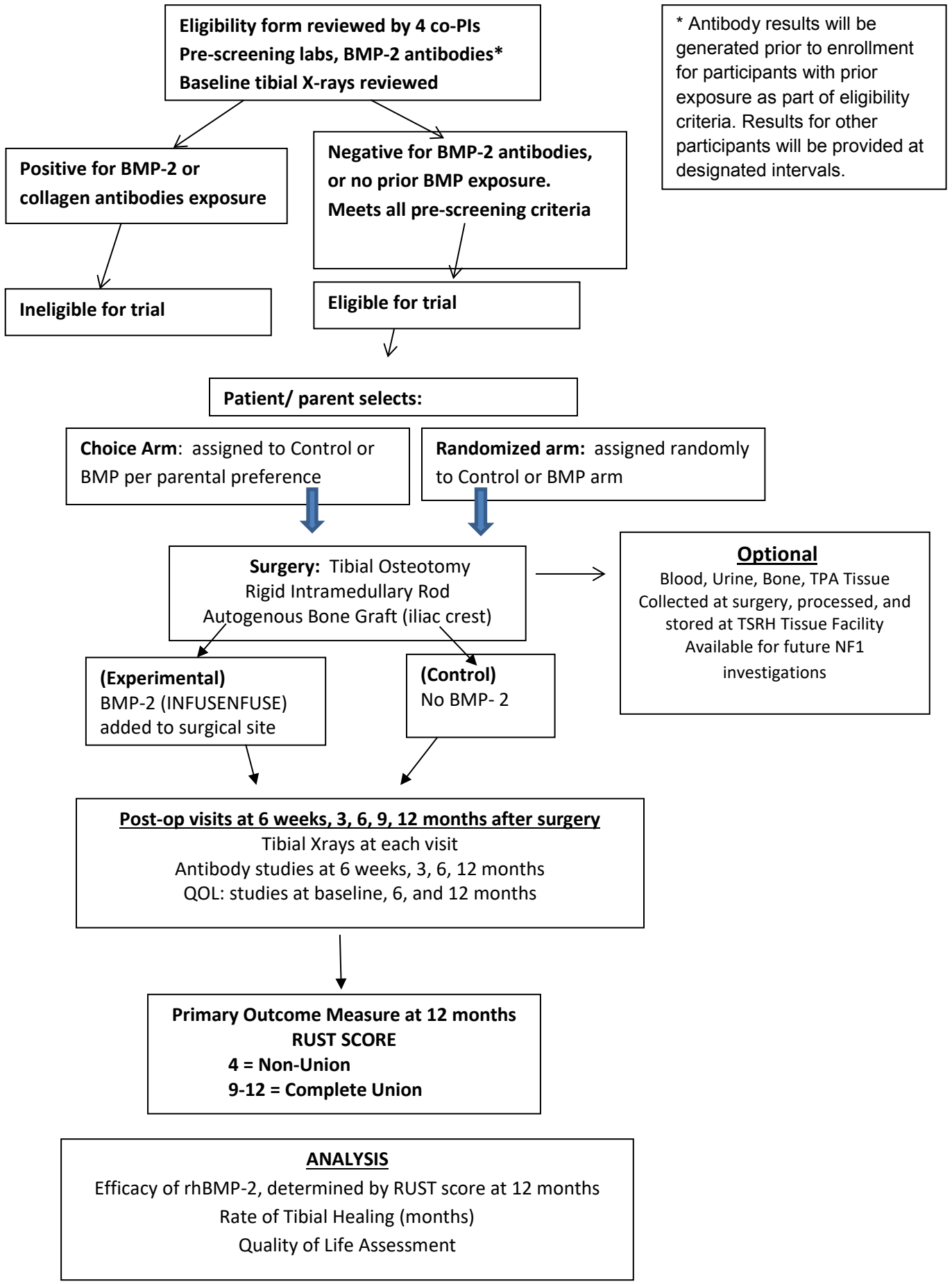
6.6 EXPERIMENTAL DESIGN SCHEMA



APPENDIX I: Schedule of Evaluations

STUDIES TO BE OBTAINED	Pre-Study	Surgery	Post-Surgical Follow-up	Post-Trial FU
Informed Consent/ Assent	X			
Eligibility Checklist	X			
History and Physical Exam	X		6 w, 12 w (± 2 wks) 6 m, 9 m, 12 m (± 4 wks)	Annual
PE with vital signs (including T, P, RR, BP), Ht, weight	X		6 w, 12 w (± 2 wks) 6 m, 9 m, 12 m (± 4 wks)	
Performance Status (Karnofsky, Lansky scales)	X			
Adverse Event Reporting		X	6 w, 12 w, 6 m, 9 m, 12 m	Annual
CBC, differential, platelets	X		3 m, 12 m	
Total bilirubin	X			
Urinalysis	X		3 m, 12 m	
Urine Pregnancy[2]	X		6 w	
Blood Electrolytes, BUN, creatinine, albumin, total protein, alkaline phosphatase, uric acid, SGOT, SGPT, GGT,	X		3 m, 12 m	
Serum calcium (ionized), magnesium, phosphorus	X		3 m, 12 m	
Serum Vitamin D	X		3 m, 12 m	
Bovine collagen I antibodies	X		6 w, 3m, (± 2 wks) 6 m, 12 m (± 4 wks)	Until negative
BMP-2 antibodies	X		6 w, 3 m, (± 2 wks) 6 m, 12 m (± 4 wks)	Until negative

Radiographs (PA/Lat) of the affected leg	X		6 w, 3 m, (± 2 wks) 6 m, 9 m, 12 m (± 4 wks)	Annual
PODCI (QOL scale)	X		6m, 12 m	
Faces pain scale	X		6 w, 3m, 6 m, 12 m	
10 Meter timed walk	X		6 m, 12 m	
OPTIONAL STUDIES TO BE OBTAINED (if participant consents)				
Blood for research studies		X		
Pseudarthrosis tissue		X		
Iliac crest bone		X		
Urine for research studies		X		

1. All studies to determine eligibility must be performed within 2 weeks prior to enrollment unless otherwise indicated below.
2. For females of childbearing age.

APPENDIX II: Biologic Specimen Collection

RESEARCH SAMPLES:

Bone/Pseudarthrosis samples:

Up to 3 small samples of excess bone or **pseudarthrosis tissue** removed at surgery will be obtained and sent for research studies.

Estimated size of each sample approx. 1 cm diameter

In addition, a small sample of excess bone from the **iliac crest bone** site may be sent (max size 5 mm).

All bone samples should be labeled in the OR by site (pseudarthrosis tissue, tibia, iliac crest)

1. The surgeon will excise the specimen and place it on a sterile container moist with saline.

2. Obtain up to 3 small pieces (maximum 1 cm diameter each) of excess pseudarthrosis tissue or bone and place each piece into a single 50 mL conical tube containing 40 mL L-15 media (provided by TSRHC)

3. Label each conical tube containing tissue with the following: appropriate collection site description (pseudoarthrosis tissue, tibia, iliac crest, etc.), study number, the subject's research number, collection date

4. Place all conical tubes containing tissue into a Ziploc plastic bag and store in the refrigerator (4°C). **DO NOT FREEZE**

Blood – Will be obtained before or on the day of surgery. Will be provided as maximum of 2 EDTA tubes of 5 ml each. These will be processed at TSRH to isolate and store DNA for future studies.

1. Prior to surgery, collect 5 mL* each into 1 or 2 EDTA purple top tubes
   *volume depends on subject size and institutional safety guidelines

2. Invert purple top tube 5 times. **DO NOT SHAKE VIGOROUSLY.**

3. Label all purple top tubes with the appropriate information: study number, the patient's research number, collection date, and collection timepoint (baseline, 6 weeks, 3 months, etc.)

4. Place in a Ziploc plastic bag and store all purple top tubes in the refrigerator (4°C)

Urine – obtained before or on the day of surgery, as a first morning void.

- Aliquot 5 ml of urine sample each into between 3 – 5 urine transport vials, labelled with the Participant's ID, date, volume, and "urine". Place all urine transport vials in a biohazard bag and freeze in -20C or -80C until shipping.

SHIPPING INFORMATION:

***TSRHC should be contacted prior to shipping. Tracking number and scanned copy of de-identified requisition form should be sent to** Reuel.Cornelia@tsrh.org and Jonathan.Rios@tsrh.org.

Test Requisition: NF Repository

a) Fresh **Bone/Pseudarthrosis samples** from above
Pack in biohazard bag and ship with 2 – 3 frozen ice packs in an insulated (styrofoam) container by overnight courier FedEx. Specimen should arrive in the laboratory within 48 hrs of collection. Do not heat or freeze.

b) **Blood sample** from above
Place in biohazard bag and ship with frozen ice packs in an insulated (Styrofoam) container by overnight courier FedEx. Specimen should arrive in the laboratory within 48 hrs of collection. Do not heat or freeze. This may be shipped with the fresh tissue sample.

c) **Urine** from above
Pack in biohazard bag and ship with 5 lbs dry ice pack in an insulated container (Styrofoam) by overnight courier FedEx. Specimen should arrive in the laboratory within 48 hrs of collection. May be shipped with frozen tissue sample.

All specimens must be properly packed and labeled to indicate the general nature of the materials transported. All shipments must comply with all applicable local, state and federal laws governing packaging, labeling and transportation. There will be one FedEx account number for NF repository. After packaging and shipment are done please inform the following with the FedEx account tracking number thru email Reuel.Cornelia@tsrh.org and Jonathan.Rios@tsrh.org to track down arrival of delivery.

All Shipment should be address to the following:

Shipping address:
REUEL CORNELIA OR JONATHAN RIOS
RESEARCH DEPARTMENT
TEXAS SCOTTISH RITE HOSPITAL FOR CHILDREN
2222 WELBORN ST.
DALLAS, TEXAS 75219
TELEPHONE NUMBER: 214-559-7766 OR 214-559-8532

ADDITIONAL DETAILS CAN BE FOUND IN THE SOP MANUAL.

BMP ANTIBODY TESTING PROTOCOL:

Samples will be obtained from each study participant for antibodies to BMP and collagen at 5 different timepoints.

Time of antibody tests: Baseline, 6 weeks, 3 months, 6 months, and 12 months after surgery.

Blood volume needed: 3 ml per blood draw.

INSTRUCTIONS FOR ANTIBODY BLOOD DRAW AND PROCESSING:

1. 3 ml blood is drawn in a red top serum separator tube.
2. After obtaining blood sample, invert the collection tube 5 times to allow the blood to clot for 30minutes at ambient temperature (19-24°C).
3. Centrifuge sample at approximately 1000-1300 RCF for 10 minutes at room temperature in a swinging bucket centrifuge.
4. Serum is then aliquotted to 3-separate 2 ml polypropylene screw cap tubes
    a. At least 0.5 serum per tube
5. Tubes are labeled with the study number (NF107), patient's research number, date, and timing of the test (i.e., baseline, 6 weeks, etc)
6. Within 90 minutes of collection, store aliquot samples upright at -20°C.
7. Tubes are frozen to at least -20 Celsius (-4 Fahrenheit). When ready for shipping, samples are packaged in dry ice. Samples from the pre-surgery blood draw should be shipped as soon as possible, as antibody levels are a pre-enrollment screening requirement. Samples post-surgery can be batched if desired, but must be marked with the appropriate research number, date, and timing.
8. Package sent by Federal Express overnight (next day delivery) Monday through Wednesday only, to Intertek Pharmaceutical Services, 10420 Wateridge Circle, San Diego, CA 92121; Attention: Trang Le. Phone : (858) 558-2599 Fax: (858) 558-2600; Direct Line: (858) 210-3413
9. Samples from international sites should be shipped using World Courier services.
10. A paper copy of the CRF should be sent in the package with the samples.

APPENDIX III: PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA Karnofsky and Lansky performance scores are intended to be multiples of 10			
Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.