MBN-101-201

Study to Assess Safety and Clinical Activity of Local MBN-101 in Treatment of Infected Bone Sites

NCT No.: NCT02436876

Date: December 13, 2017

16. APPENDICES

APPENDIX 16.1: STUDY INFORMATION

APPENDIX 16.1.1: PROTOCOL AND PROTOCOL AMENDMENTS

MICROBION | Chemistry for Life

December 13, 2017

To Whom It May Concern,

Microbion wishes to clarify a specific aspect of the conduct for Study MBN-101-201.

As currently written in Section 3.2 of the protocol, “Patients who are randomized but do not receive study drug may be replaced.” In order to better achieve the stated protocol objective of “having a total of 6 patients per dose level in the MBN-101-treated group”, Microbion asserts that they may also choose to replace a patient who is randomized but is discontinued from the study for reasons other than safety or is lost to follow up.

Sincerely,

Brett Baker, Microbion President and CSO

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CLINICAL TRIAL PROTOCOL: MBN-101-201

Version 5.0, 27 July 2017
Summary of Changes

Study Title: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 12-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Bone Sites

Study Phase: 2a

Product Name: MBN-101

Sponsor: Microbion Corporation

REASON(S) FOR CHANGES(S):

1. Expand target population.
2. Expand eligibility criteria.
3. Administrative changes and corrections.

CHANGES(S):

Note – All changes refer to page numbers in the revised version.

(1) **Cover page (and where appropriate)**

From	***To***
Version 4.0 Issue Date: 18 January 2017 Replaces Version 3.0 Issue Date: 05 May 2016	Version 5.0 Issue Date: 27 July 2017 Replaces Version 4.0 Issue Date: 18 January 2017
A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 12-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Bone Sites

Rationale: Administrative change.

(2) **Page 4: Synopsis (Investigators/Study Centers)**

From	***To***
Up to eight Study Centers in the United States	Up to twelve Study Centers in the United States

Rationale: Administrative change.

(3) Pages 4-5, 22: Synopsis (Study Design and Methodology) and Section 3.1 (Study Description)

From	***To***
This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection.	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection or to sites of chronic or acute-on-chronic osteomyelitis of the long bone extremities or residual amputated limbs.
Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections.	Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their infections.
Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated.	Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection or osteomyelitis that includes systemic antibacterial treatment per institutional standard of care guidelines and debridement/revision surgery with or without hardware removal and placement/replacement as indicated.
A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure.	A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected bone sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure.
In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure.	In cases where hardware is placed or replaced, or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure.
Added	
In cases where hardware is not required, MBN-101 will be applied to affected areas of bone only prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure.	
From	***To***
The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used.	The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected bone site will be used.
Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.	Patients will undergo study visits at Hour 48 and Weeks 2, 6 and 12. All patients will be followed for a total of 12 weeks after surgery.

Rationale: Expand target population.

(4) **Pages 5-6, 25: Synopsis (Inclusion Criteria) and Section 4.1 (Inclusion Criteria)**

From	***To***
1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection and have at least one of the following: • require revision surgery with or without removal and replacement of existing hardware	• have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), or have undergone arthrodesis, and have subsequently been diagnosed with an apparent fracture site infection **or** are diagnosed with chronic or acute-on-chronic osteomyelitis of the long bone extremities (including residual amputated limbs) • have at least one of the following: • require surgical debridement of infected soft tissue and/or bone, with or without removal and/or placement/replacement of hardware
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery	4. Patients requiring postoperative hospitalization for at least 48 hours after surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)	5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 12 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
5. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Agree to use a condom with spermicide from Baseline through Week 24	• Be sexually abstinent from Baseline through Week 12 • Agree to use a condom with spermicide from Baseline through Week 12

Rationale: Expand target population.

(5) Pages 6-7 and 26: Synopsis (Exclusion Criteria) and Section 4.2 (Exclusion Criteria)

Deleted	
1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement	
From	***To***
2. Patients with multiple, non-contiguous fracture site infections	1. Patients with multiple, non-contiguous sites of infection
5. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory	4. Serum creatinine, ALT, AST or Alkaline Phosphatase >2.0 times the upper limit of the normal range of the local testing laboratory
10. Individuals undergoing surgical treatment for more than one infected fracture	9. Individuals undergoing surgical treatment for more than one infected site
14. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site	13. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected bone site

Rationale: Expand target population; expand eligibility criteria.

(6) Page 7: Synopsis (Investigational Product, Dose and Mode of Administration)

From	***To***
MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement or directly to the immediate soft tissue and bone in patients with chronic or acute-on-chronic osteomyelitis of the long bone extremities, both as an adjunct to standard care systemic antimicrobial therapy.

Rationale: Expand target population; administrative change.

(7) Page 7: Synopsis (Reference Therapy, Dose and Mode of Administration)

From	*To*
MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement or directly to the immediate soft tissue and bone in patients with chronic or acute-on-chronic osteomyelitis of the long bone extremities, both as an adjunct to standard care systemic antimicrobial therapy.

Rationale: Expand target population; administrative change.

(8) Page 7: Synopsis (Study Duration)

From	*To*
24 weeks	12 weeks

Rationale: Expand eligibility criteria; administrative change.

(9) Pages 8, 37 and 49: Synopsis (Criteria for Evaluation: Efficacy Endpoints), Section 8.2 (Efficacy Endpoints) and Section 12.1 (Efficacy Endpoints)

From	*To*
• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site.	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 12. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed bone site by Week 12. • Time to removal of orthopedic hardware due to a healed bone site.

Rationale: Expand eligibility criteria; administrative change.

(10) **Page 18: Section 1.1 (Clinical Problem)**

From	***To***
The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).	The high incidence of postoperative orthopedic device-related infections and of osteomyelitis, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012; Sheehy, 2010). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Rationale: Administrative change.

(11) **Page 20: Section 1.3 (Value of MBN-101 for the Treatment of the Clinical Problem)**

From	***To***
With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.	With comparatively elevated rates of both postoperative infection associated with repair of traumatic orthopedic wounds, and antibiotic-resistance associated with osteomyelitis, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

Rationale: Administrative change.

(12) **Page 23: Section 3.3 (Number of Sites)**

From	***To***
Up to eight U.S. study sites are anticipated to enroll an adequate number of eligible patients.	Up to twelve U.S. study sites are anticipated to enroll an adequate number of eligible patients.

Rationale: Administrative change.

(13) **Page 24: Section 3.5 (Dose)**

From	***To***
With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.	With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected bone site will be applied. Following application, OR personnel will record in the Case Report Form (CRF) the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Rationale: Administrative change.

(14) **Page 27: Section 5.2 (Physical Exam)**

From	***To***
Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.	Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index infection site and involved extremity.

Rationale: Administrative change.

(15) **Page 28: Section 6 (Study Procedures)**

From	***To***
See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ±8 days will be allowed.	See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 and 12, a visit window of ±8 days will be allowed.

Rationale: Expand eligibility criteria; administrative change.

(16) **Page 29: Section 6.1.7 (Pain Assessments)**

From	***To***
Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.	Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6 and 12.

Rationale: Expand eligibility criteria; administrative change.

(17) Page 29: Section 6.1.9.1 (Specimen Collection)

From	*To*
Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site.	Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the infected bone site (if accessed) or involved tissue adjacent to any implant(s), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure/sinus tract site.

Rationale: Administrative change.

(18) Page 31: Section 6.1.10 (Radiographic Evaluation)

From	*To*
Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.	Radiographic evaluation will be performed at baseline and Weeks 2, 6 and 12. Radiographs (at least two orthogonal views) will be reviewed for bone morphology and integrity, periosteal reaction, union, interval callus formation, loss or change in reduction, and hardware integrity/failure.

Rationale: Expand eligibility criteria; administrative change.

(19) Page 32: Section 6.5.1 (Surgical Site Identification)

From	*To*
Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.	Individuals undergoing surgical treatment for more than one infected bone site are excluded from participation in this study.

Rationale: Expand target population; administrative change.

(20) **Page 32: Section 6.5.2 (Study Drug Administration)**

From	***To***
In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used.	In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected site should be used.
The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.	The approximate area of the infected site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the infected site. Recommended maximum volumes of MBN-101 for various areas of the infected site are provided in the table below. For infected bone surface areas that fall between the specified areas, the applied volume should be rounded up to the volume for the next area specified in the table (e.g., an 80 cm^2 wound would be rounded up to 100 cm^2, and the volume of MBN-101 Study Dose administered would be 2.0 mL).
Area of Osteosynthesis Site	**Area of Infected Bone Site**
The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site.	The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected site.
The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the eCRF.	The use of drains and vacuum assisted closure devices will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the CRF.

Rationale: Expand target population; administrative change.

(21) **Page 35: Section (6.6 Study Assessments)**

From	***To***
Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.	Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6 and 12. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 12 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 12 assessment will be followed until resolution of the event or stabilization of the condition.

Rationale: Expand eligibility criteria; administrative change.

(22) **Page 35: Section (6.9 Patient Withdrawals)**

From	***To***
All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.	All efforts should be made to have subjects complete the Week 12 (end of study) procedures prior to withdrawal from the study.

Rationale: Expand eligibility criteria; administrative change.

(23) **Page 36: Section 7 (Concomitant Medications)**

From	***To***
Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study.	Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 12 week study.

Rationale: Expand eligibility criteria; administrative change.

(24) **Page 40: Section 9.2 (Severity)**

From	***To***
A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).	A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the CRF.

Rationale: Administrative change and correction.

(25) **Page 48: Section 11.9 (Independent Medical Monitor)**

From	***To***
The independent medical monitor should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety.	The independent medical monitor should be a physician, dentist, psychologist, nurse, or other healthcare provider capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety.

Rationale: Administrative change and correction.

(26) **Page 51: Section 12.5 (Disposition and Study Population Characteristics)**

From	***To***
Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.	Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis or osteomyelitis site, with or without hardware placement/replacement, etc.), and medical history.

Rationale: Administrative change.

(27) **Page 52: Section 12.6.1 (Treatment Failure)**

From	***To***
A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.	A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12.

Rationale: Expand eligibility criteria; administrative change.

(28) **Page 52: Section 12.6.2 (Incidence of Serious Interventions)**

From	***To***
• Readmission (exclusive of readmissions associated with a healed fracture site) • Reoperation (exclusive of reoperations associated with a healed fracture site)	• Readmission (exclusive of readmissions associated with a healed bone site) • Reoperation (exclusive of reoperations associated with a healed bone site)
The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:	The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site) and total number of serious interventions will be tabulated for the following periods:
• from week 4 to week 8 • from week 8 to week 12 • from week 12 to week 24	• from week 4 to week 8; and • from week 8 to week 12.

Rationale: Expand eligibility criteria; administrative change.

(29) **Page 53: Section 12.6.3 (Time to First Serious Interventions)**

From	***To***
Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived.	Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site will be derived.

Rationale: Administrative change.

(30) **Page 53: Section 12.6.4 (Subjects Undergoing Removal of Stabilizing Orthopedic Hardware)**

From	***To***
The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived.	The number of subjects undergoing removal of hardware due to a healed bone site and the time to removal of hardware due to a healed bone site will be derived.

Rationale: Administrative change.

(31) **Page 54: Section 12.6.7 (Pain Assessments)**

From	***To***
Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.	Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6 and 12.

Rationale: Expand eligibility criteria; administrative change.

(32) **Page 54: Section 12.6.8 (Microbiology)**

From	***To***
Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible.	Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the infected bone site (if accessed) or involved tissue adjacent to any implant(s), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure/sinus tract site.

Rationale: Administrative change.

(33) **Page 55: Section 12.6.9 (Radiographic Evaluation)**

From	***To***
Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.	Radiographic evaluation will be performed at baseline and Weeks 2, 6 and 12.

Rationale: Expand eligibility criteria; administrative change.

(34) **Page 55: Section 12.6.10 (Serologic Markers)**

From	***To***
Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.	Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6 and 12.

Rationale: Expand eligibility criteria; administrative change.

(35) **Page 55: Section 12.6.11 (Subgroup Analyses for Efficacy Endpoints)**

From	***To***
1. Area of the osteosynthesis site	1. Area of the infected site

Rationale: Administrative change.

(36) **Page 57: Section 13 (References)**

Added
Sheehy SH, Atkins BA, Bejon P, Byren I, Wyllie D, Athanasou NA, Berendt AR, McNally MA. The microbiology of chronic osteomyelitis: Prevalence of resistance to common empirical anti-microbial regimens. Journal of Infection. 2010;60:338-43.

Rationale: Administrative change.

(37) **Page 62: Appendix 1 (Table of Study Events)**

From	***To***
Week 12 Day 84 ±8 days	Week 12, EOT Day 84 ±8 days
Deleted	
Week 24, EOT Day 168 ±8 days	

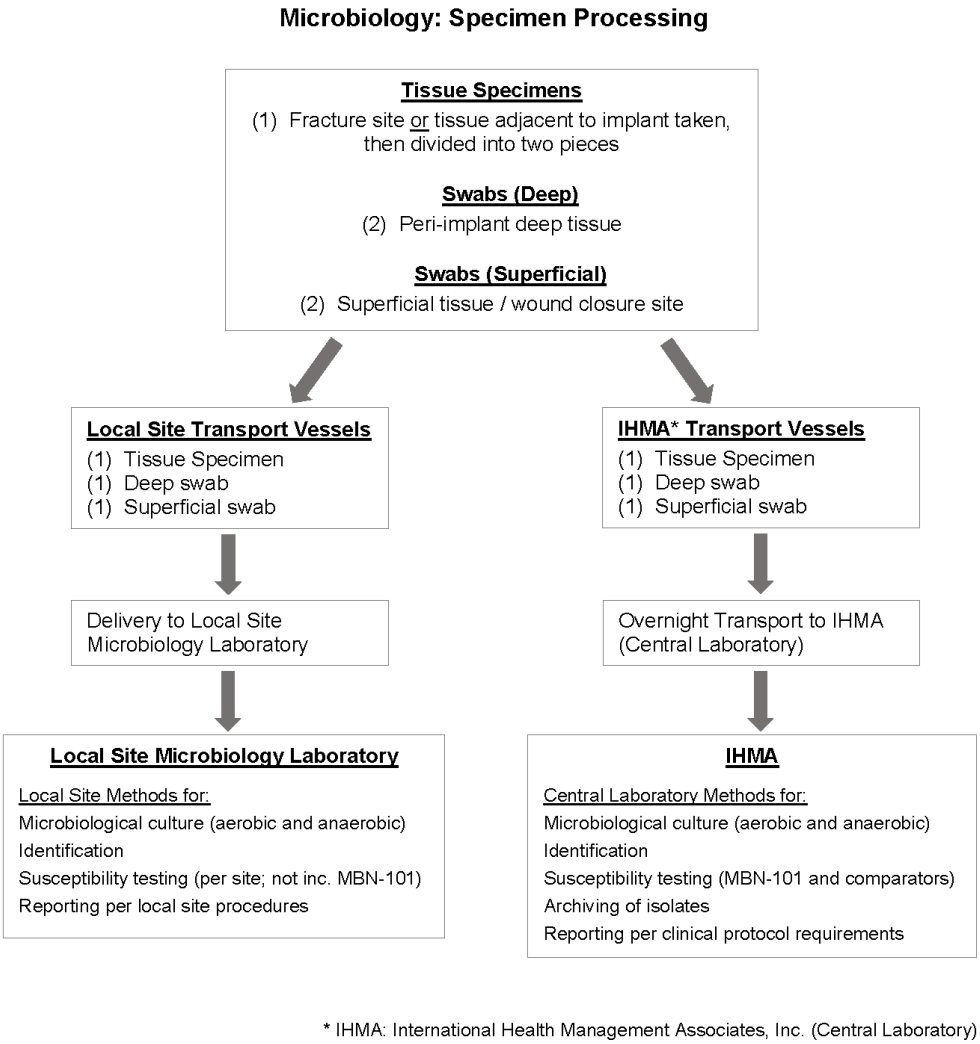
Rationale: Administrative change.

(38) **Page 63: Appendix 2 (Flowchart for Microbiological Specimen Processing)**

From	***To***
see Page 14	see Page 15

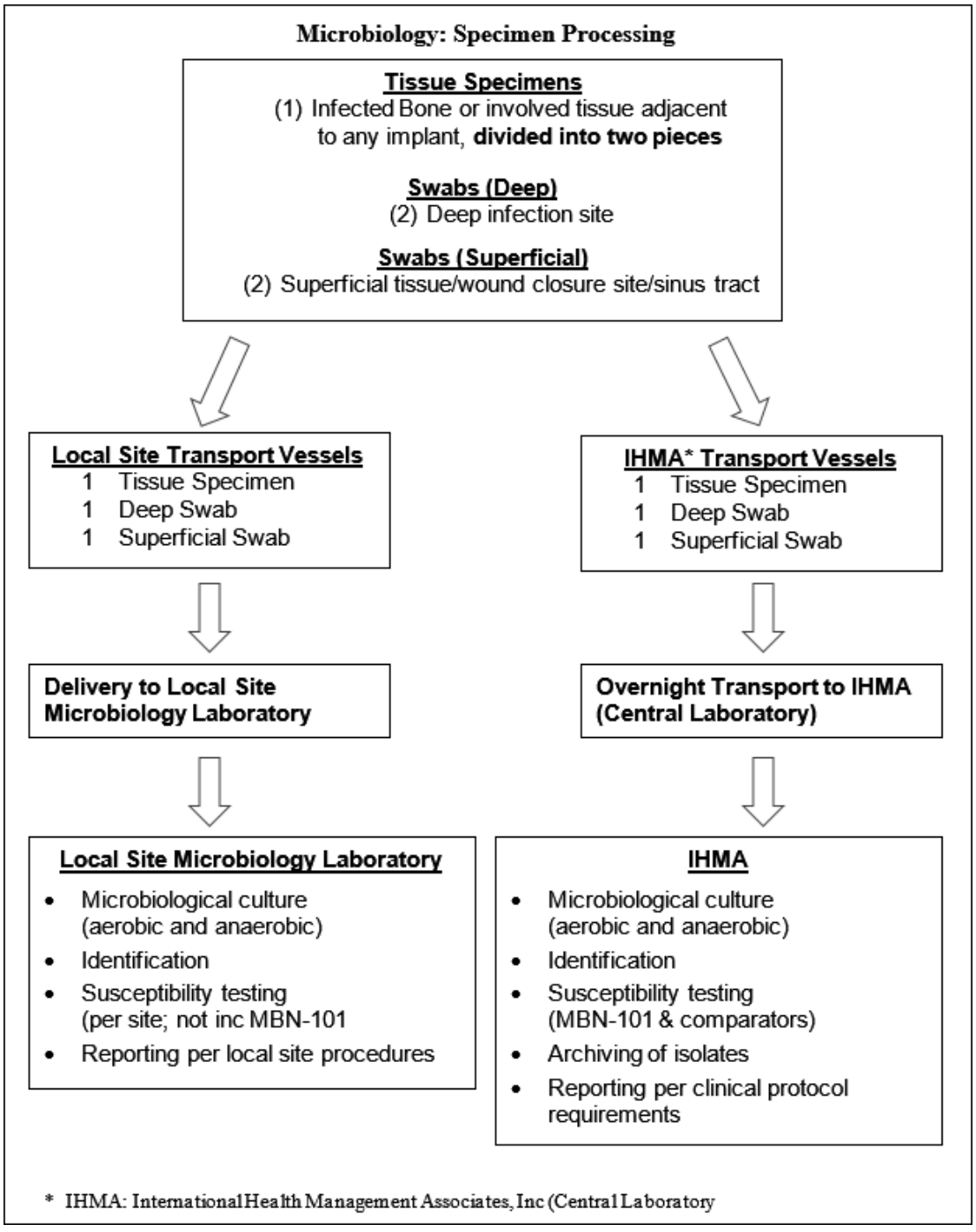
Rationale: Administrative change.

Old text:



* IHMA: International Health Management Associates, Inc. (Central Laboratory)

New text:


Microbiology: Specimen Processing
Tissue Specimens
(1) Infected Bone or involved tissue adjacent to any implant, divided into two pieces
Swabs (Deep)
(2) Deep infection site
Swabs (Superficial)
(2) Superficial tissue/wound closure site/sinus tract
Local Site Transport Vessels
1 Tissue Specimen
1 Deep Swab
1 Superficial Swab
IHMA* Transport Vessels
1 Tissue Specimen
1 Deep Swab
1 Superficial Swab
Delivery to Local Site Microbiology Laboratory
Overnight Transport to IHMA (Central Laboratory)
Local Site Microbiology Laboratory
• Microbiological culture (aerobic and anaerobic)
• Identification
• Susceptibility testing (per site; not inc MBN-101
• Reporting per local site procedures
IHMA
• Microbiological culture (aerobic and anaerobic)
• Identification
• Susceptibility testing (MBN-101 & comparators)
• Archiving of isolates
• Reporting per clinical protocol requirements
* IHMA: International Health Management Associates, Inc (Central Laboratory

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 12-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Bone Sites

PROTOCOL NUMBER: MBN-101-201

Version 5.0 Issue Date: 27 July 2017

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Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 5.0

Microbion Corporation

Reviewed and Approved by:

27 July 2017

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

GRANT SUPPORT

This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100.

The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.

Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site.

The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 12-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Bone Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____

Investigator's Printed Name

_____ _____

Investigator's Signature | Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 12-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Bone Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	Up to twelve Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	24
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection or to sites of chronic or acute-on-chronic osteomyelitis of the long bone extremities or residual amputated limbs. Three successive cohorts of 8 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection or osteomyelitis that includes systemic antibacterial treatment per institutional standard of care guidelines and debridement/revision surgery with or without hardware removal and placement/replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected bone sites, will be performed following the final irrigation

	and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is placed or replaced, or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. In cases where hardware is not required, MBN-101 will be applied to affected areas of bone only prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected bone site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6 and 12. All patients will be followed for a total of 12 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), or have undergone arthrodesis, and have subsequently been diagnosed with an apparent fracture site infection **or** are diagnosed with chronic or acute-on-chronic osteomyelitis of the long bone extremities (including residual amputated limbs) • have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery

	– Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require surgical debridement of infected soft tissue and/or bone, with or without removal and/or placement/replacement of hardware 2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 12 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 12 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 12 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients with multiple, non-contiguous sites of infection 2. Pathologic fracture (not including osteoporosis) 3. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 4. Serum creatinine, ALT, AST or Alkaline Phosphatase >2.0 times the upper limit of the normal range of the local testing laboratory 5. Absolute neutrophil count <1000 6. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration

	7. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol) 8. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 9. Individuals undergoing surgical treatment for more than one infected site 10. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 11. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 12. Immunocompromised due to illness or organ transplant 13. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected bone site 14. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 15. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician) 16. History of medical noncompliance 17. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results 18. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement or directly to the immediate soft tissue and bone in patients with chronic or acute-on-chronic osteomyelitis of the long bone extremities, both as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement or directly to the immediate soft tissue and bone in patients with chronic or acute-on-chronic osteomyelitis of the long bone extremities, both as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	12 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 12. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed bone site by Week 12. • Time to removal of orthopedic hardware due to a healed bone site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status.
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 24 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (2 per cohort; 6 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (6 subjects per dose) and combined (a total of 18 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, ‘as assigned’ sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

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If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice

GGT	Gamma glutamyl transferase
hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IWRS	Interactive Web Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter

OR	Operating room
ORP	Office of Research Protections
PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

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1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections and of osteomyelitis, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic-resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012; Sheehy, 2010). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases, synergy has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of both postoperative infection associated with repair of traumatic orthopedic wounds, and antibiotic-resistance associated with osteomyelitis, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection or to sites of chronic or acute-on-chronic osteomyelitis of the long bone extremities or residual amputated limbs. Three successive cohorts of 8 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection or osteomyelitis that includes systemic antibacterial treatment per institutional standard of care guidelines and debridement/revision surgery with or without hardware removal and placement/replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected bone sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is placed or replaced, or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. In cases where hardware is not required, MBN-101 will be applied to affected areas of bone only prior to definitive closure. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected bone site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6 and 12. All patients will be followed for a minimum of 12 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Twenty-four adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 6 patients per dose level in the MBN-101-treated group and 6 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

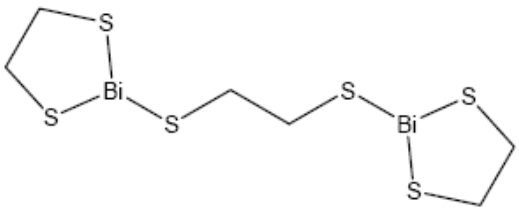
Up to twelve U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected bone site will be applied. Following application, OR personnel will record in the Case Report Form (CRF) the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 24 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), or have undergone arthrodesis, and have subsequently been diagnosed with an apparent fracture site infection

     or

   - are diagnosed with chronic or acute-on-chronic osteomyelitis of the long bone extremities (including residual amputated limbs)
   - have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require surgical debridement of infected soft tissue and/or bone, with or without removal and/or placement/replacement of hardware
2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 12 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 12
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 12

7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients with multiple, non-contiguous sites of infection
2. Pathologic fracture (not including osteoporosis)
3. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
4. Serum creatinine, ALT, AST or Alkaline Phosphatase >2.0 times the upper limit of the normal range of the local testing laboratory
5. Absolute neutrophil count <1000
6. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
7. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
8. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
9. Individuals undergoing surgical treatment for more than one infected site
10. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
11. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
12. Immunocompromised due to illness or organ transplant
13. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected bone site
14. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
15. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)
16. History of medical noncompliance
17. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results
18. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed, additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IWRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index infection site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 and 12, a visit window of ± 8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6 and 12.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the infected bone site (if accessed) or involved tissue adjacent to any implant(s), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure/sinus tract site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc.; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests for aerobic/facultative bacteria will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility tests for anaerobic bacteria will also include CLSI reference quality control strains, however, since quality control ranges for anaerobic bacteria are not yet established, the MIC values for the quality control strains will be collected then retrospectively compared to the quality control ranges once they are available. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6 and 12. Radiographs (at least two orthogonal views) will be reviewed for bone morphology and integrity, periosteal reaction, union, interval callus formation, loss or change in reduction, and hardware integrity/failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IWRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of an individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected bone site are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the approximate surface area of the surgical site as well as the amount and location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.
5. Alcohol wipes
6. One pre-printed "Diluted MBN-101 Drug Product" label
7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”)

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the infected site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the infected site. Recommended maximum volumes of MBN-101 for various areas of the infected site are provided in the table below. For infected bone surface areas that fall between the specified areas, the applied volume should be rounded up to the volume for the next area specified in the table (e.g., an 80 cm^2 wound would be rounded up to 100 cm^2, and the volume of MBN-101 Study Dose administered would be 2.0 mL).

Area of Infected Bone Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected site. By following these application requirements, the administered doses will be:

- Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the CRF.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6 and 12. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 12 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 12 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 12 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will receive their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 12 week study. Patients will also receive standard of care treatment for their infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 12.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed bone site by Week 12.
- Time to removal of orthopedic hardware due to a healed bone site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (4 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with nitric acid and H_2O_2 for 2 hours, followed by centrifugation and dilution in water with subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.4 or higher, using an extravascular administration model and actual sampling times. Interim analyses may use nominal sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis (as feasible):

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
V_z/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the CRF.

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. A statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC. A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. One pre-printed "Diluted MBN-101 Drug Product" label

7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Moreover:

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion and/or its designee will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitor should be a physician, dentist, psychologist, nurse, or other healthcare provider capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. The medical monitor must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 12.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed bone site by Week 12.
- Time to removal of orthopedic hardware due to a healed bone site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Changes in serologic markers CRP and ESR at each post baseline time point.

12.2 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Six subjects will receive active drug in each dose cohort. With 6 subjects receiving active drug, the probability of observing at least one of 6 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.385 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 6 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.385 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 18 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.153 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis or osteomyelitis site, with or without hardware placement/replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 12.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed bone site)
- Reoperation (exclusive of reoperations associated with a healed bone site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8; and
- from week 8 to week 12.

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed bone site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed bone site and the time to removal of hardware due to a healed bone site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patient's perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6 and 12.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the infected bone site (if accessed) or involved tissue adjacent to any implant(s), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure/sinus tract site. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6 and 12.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10 Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6 and 12.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11 Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the infected site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12 Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1 Table of Study Events

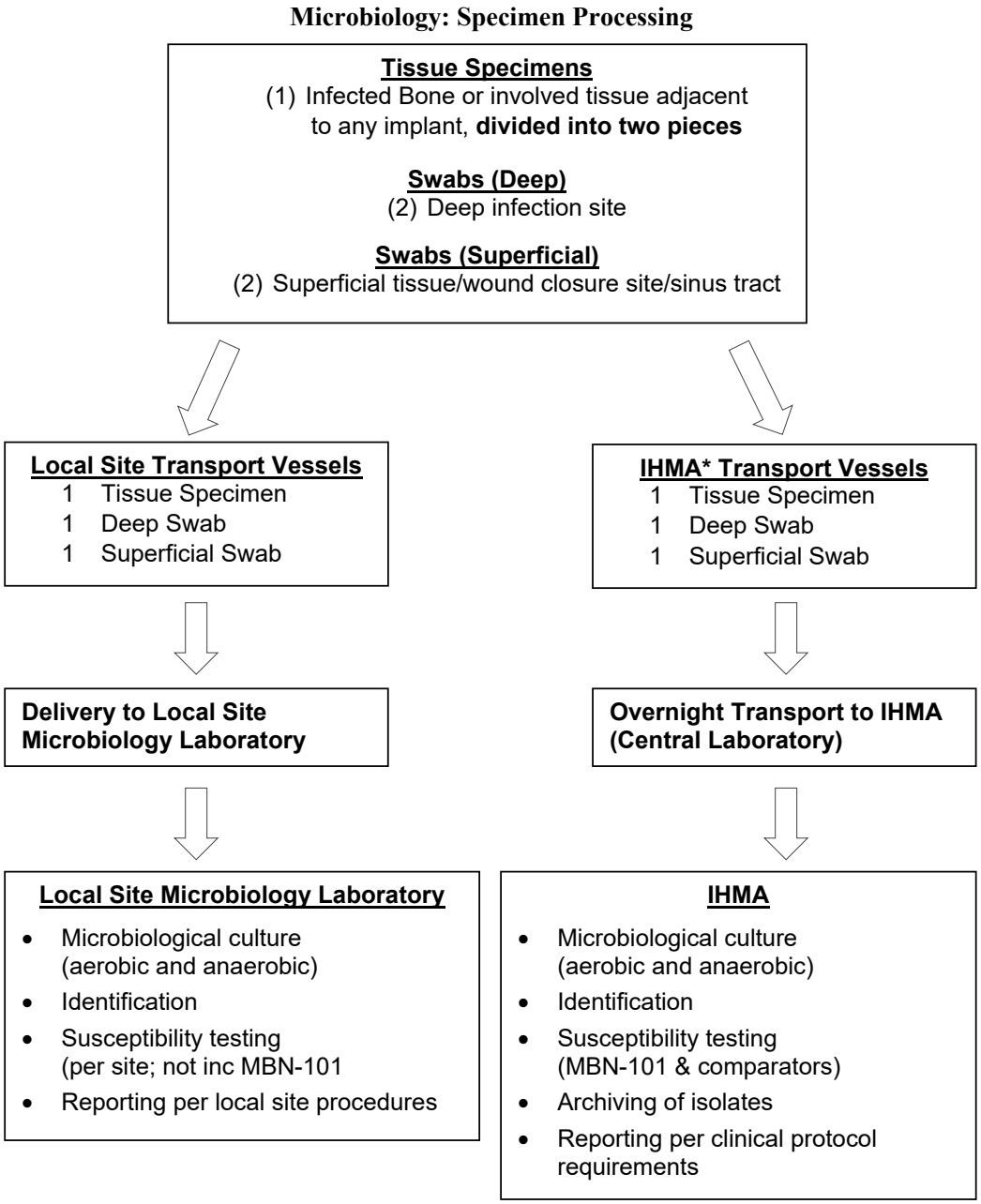
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±4 days	Day 42 ±8 days	Day 84 ±8 days	
Review of eligibility criteria	X	X							
Informed consent	X								
Randomization		X							
Surgery/Administration of the Investigational Product		X							
Medical history	X								
Physical exam	X								
Interval physical exam		X	X			X	X	X	X
Hematology	X		X				X	X	X
Serology		X				X	X	X	X
Serum chemistry	X		X				X	X	X
Urinalysis	X		X				X	X	X
Pregnancy test	X	X						X	X
BisEDT Blood levels[1]		X	X	X	X	X			
12-lead ECG	X	X	X					X	X
Vital signs	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2 Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc (Central Laboratory

PROTOCOL AMENDMENT FORM

DATE: 01/18/17 **AMENDMENT NUMBER:** 04

PROTOCOL NUMBER: MBN-101-201 (Version 4)

SPONSOR: Microbion Corporation

PROTOCOL TITLE: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

CHANGE(S):

Note – All changes refer to page numbers in the clean Version 4.0

Location	From	To	Comment
Header	Version 3.0, Issue Date 05 May 2016	Version 4.0, Issue Date 18 January 2017	Update version
Page 1	Version 3.0, Issue Date 05 May 2016 Replaces Version 2.1 Dated 29 December 2015	Version 4.0, Issue Date 18 January 2017 Replaces Version 3.0 Dated 05 May 2016	Update version
Page 1	Version: 2.1	Version: 3.0	Update version
Pages 4, 9, 25	**Synopsis (Number of Subjects), Synopsis (Statistical Analysis Consideration) and Section 4 (Study Population)** 36	24	Decrease cohort size
Pages 4, 22	**Synopsis (Study Design and Methodology) and Section 3.1 (Study Description)** Three successive cohorts of 12 patients will be enrolled in this trial.	Three successive cohorts of 8 patients will be enrolled in this trial.	Decrease cohort size
Page 9	**Synopsis (Statistical Analysis Consideration)** All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (3 per cohort; 9 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (9 subjects per dose) and combined (a total of 27 subjects).	All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (2 per cohort; 6 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (6 subjects per dose) and combined (a total of 18 subjects).	Decrease cohort size

PROTOCOL AMENDMENT FORM

DATE: 01/18/17 **AMENDMENT NUMBER:** 04

PROTOCOL NUMBER: MBN-101-201 (Version 4)

Location	From	To	Comment
Page 10	**Other Appropriate Trial Contact Personnel** Ronnie McAdams Clinical Trial Manager, Associate Director, Medpace 513-579-9911, ext. 2845 513-394-0516 r.mcadams@medpace.com	Tracy Robertson Clinical Trial Manager, Medpace 513-579-9911, ext. 12140 859-512-5183 t.robertson@medpace.com	Update information
Page 10	**Medpace Clinical Safety** Added: 866-336-0930 or		Update information
Pages 12, 27, 31	**List of Abbreviations, Section 5 (Subject Enrollment) and Section 6.3 (Randomization Procedure)** IVRS: Interactive Voice Response System	IWRS: Interactive Web Response System	Update randomization procedure
Page 23	**Section 3.2 (Number of Subjects)** Thirty-six adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 9 patients per dose level in the MBN-101-treated group and 9 patients in a pooled placebo-treated group.	Twenty-four adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 6 patients per dose level in the MBN-101-treated group and 6 patients in a pooled placebo-treated group.	Decrease cohort size
Page 50	**Section 12.3 (Sample Size Considerations)** Nine subjects will receive active drug in each dose cohort. With 9 subjects receiving active drug, the probability of observing at least one of 9 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.283 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 9 subjects receiving active study drug at a given dose, one would "rule-out" this AE as having an incidence rate of more than 0.283 with 95% confidence.	Six subjects will receive active drug in each dose cohort. With 6 subjects receiving active drug, the probability of observing at least one of 6 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.385 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 6 subjects receiving active study drug at a given dose, one would "rule-out" this AE as having an incidence rate of more than 0.385 with 95% confidence.	Decrease cohort size

PROTOCOL AMENDMENT FORM

DATE: 01/18/17 **AMENDMENT NUMBER:** 04

PROTOCOL NUMBER: MBN-101-201 (Version 4)

Location	From	To	Comment
	Similarly, if a rare event of interest is not reported in the 27 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.105 or less with 95% confidence.	Similarly, if a rare event of interest is not reported in the 18 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.153 or less with 95% confidence.	

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

PROTOCOL NUMBER: MBN-101-201

Version 4.0 Issue Date: 18 January 2017

Replaces Version 3.0 Issue Date: 05 May 2016

Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 4.0

Microbion Corporation

Reviewed and Approved by:

18 January 2017

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

GRANT SUPPORT

This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100.

The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.

Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site.

The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans (“Ethical Principles for Medical Research Involving Human Subjects,” Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____

Investigator’s Printed Name

_____ _____

Investigator’s Signature Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	Up to eight Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	24
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection. Three successive cohorts of 8 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original

	hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection and have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery – Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require revision surgery with or without removal and replacement of existing hardware

	2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 24 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement 2. Patients with multiple, non-contiguous fracture site infections 3. Pathologic fracture (not including osteoporosis) 4. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 5. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory 6. Absolute neutrophil count <1000 7. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration 8. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)

	9. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 10. Individuals undergoing surgical treatment for more than one infected fracture 11. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 12. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 13. Immunocompromised due to illness or organ transplant 14. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site 15. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 16. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician) 17. History of medical noncompliance 18. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results. 19. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	24 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 24 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (2 per cohort; 6 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (6 subjects per dose) and combined (a total of 18 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, 'as assigned' sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

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If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase

hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IWRS	Interactive Web Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter
OR	Operating room
ORP	Office of Research Protections

PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the

shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases, synergy has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection. Three successive cohorts of 8 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a minimum of 24 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Twenty-four adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 6 patients per dose level in the MBN-101-treated group and 6 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

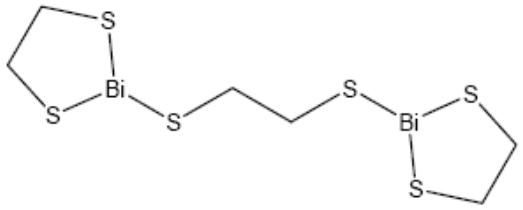
Up to eight U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 24 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis
   - are diagnosed with an apparent fracture site infection and have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require revision surgery with or without removal and replacement of existing hardware
2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 24
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 24
7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal
2. Patients with multiple, non-contiguous fracture site infections
3. Pathologic fracture (not including osteoporosis)
4. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
5. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory
6. Absolute neutrophil count <1000
7. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
8. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
9. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
10. Individuals undergoing surgical treatment for more than one infected fracture
11. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
12. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
13. Immunocompromised due to illness or organ transplant
14. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site
15. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
16. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)
17. History of medical noncompliance
18. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results.
19. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IWRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ± 8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc.; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests for aerobic/facultative bacteria will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility tests for anaerobic bacteria will also include CLSI reference quality control strains, however, since quality control ranges for anaerobic bacteria are not yet established, the MIC values for the quality control strains will be collected then retrospectively compared to the quality control ranges once they are available. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are

for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IWRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of an individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the approximate surface area of the surgical site as well as the amount and location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.
4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes
6. One pre-printed "Diluted MBN-101 Drug Product" label
7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”)

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.

Area of Osteosynthesis Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site. By following these application requirements, the administered doses will be:

- Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the eCRF.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will receive their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study. Patients will also receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (4 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with nitric acid and H_2O_2 for 2 hours, followed by centrifugation and dilution in water with subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.4 or higher, using an extravascular administration model and actual sampling times. Interim analyses may use nominal sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis (as feasible):

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
V_z/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. A statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC. A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. One pre-printed "Diluted MBN-101 Drug Product" label

7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally, each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Moreover:

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion and/or its designee will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitor should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. The medical monitor must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.

- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Changes in serologic markers CRP and ESR at each post baseline time point.

12.2 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Six subjects will receive active drug in each dose cohort. With 6 subjects receiving active drug, the probability of observing at least one of 6 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.385 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 6 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.385 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 18 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.153 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed fracture site)
- Reoperation (exclusive of reoperations associated with a healed fracture site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8
- from week 8 to week 12
- from week 12 to week 24

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patient's perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10 Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11 Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the osteosynthesis site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12 Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters (AUC_{0-t}, AUC_{0-inf}, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1: Table of Study Events

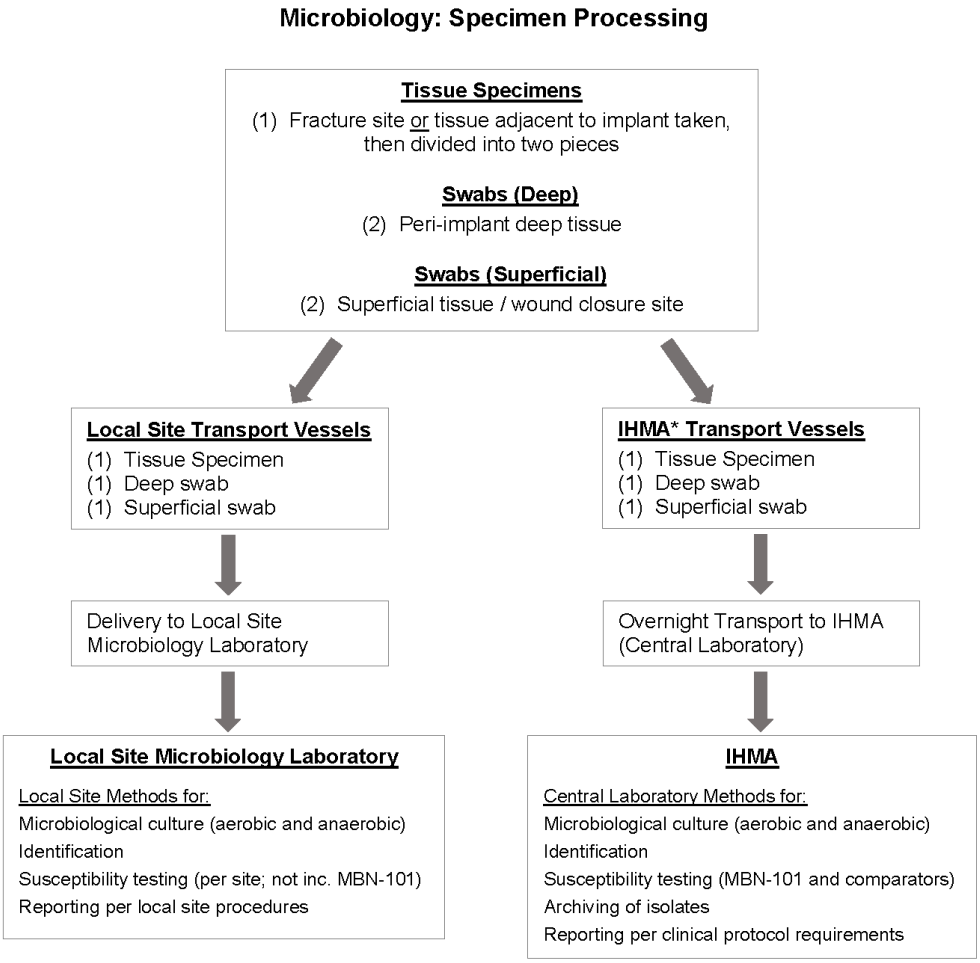
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12	Week 24, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±4 days	Day 42 ±8 days	Day 84 ±8 days	Day 168 ±8 days	
Review of eligibility criteria	X	X								
Informed consent	X									
Randomization		X								
Surgery/Administration of the Investigational Product		X								
Medical history	X									
Physical exam	X									
Interval physical exam		X	X			X	X	X	X	X
Hematology	X		X				X	X	X	X
Serology		X				X	X	X	X	X
Serum chemistry	X		X				X	X	X	X
Urinalysis	X		X				X	X	X	X
Pregnancy test	X	X							X	X
BisEDT Blood levels[1]		X	X	X	X	X				
12-lead ECG	X	X	X						X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2: Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc. (Central Laboratory)

PROTOCOL AMENDMENT FORM

DATE: 05/09/16 **AMENDMENT NUMBER:** 03

PROTOCOL NUMBER: MBN-101-201 (Version 3)

SPONSOR: Microbion Corporation

PROTOCOL TITLE: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

REASON(S) FOR CHANGE(S): Revise protocol with administrative changes and updated methodologies. Modified eligibility criteria to increase enrollment rate.

CHANGE(S):

Note – All changes refer to page numbers in the clean Version 3.0

Location	From	To	Comment
Header	Version 2.1, Issue Date 29 December 2015	Version 3.0, Issue Date 05 May 2016	Update version
Page 1	Version 2.1 Issue Date: 29 December 2015 Replaces Version 2.0 Dated 08 October 2015	Version 3.0, Issue Date 05 May 2016 Replaces Version 2.1 Dated 29 December 2015	Update version
Page 1	Version: 2.1	Version: 3.0	Update version
Pages 4, 23	**Number of Sites** 3-4	 Up to eight	Increase enrollment rate
Pages 4, 22	**Study Design and Methodology** This is a randomized, single-blind, … with an apparent fracture site infection within one year of the last surgical intervention.	 This is a randomized, single-blind, … with an apparent fracture site infection.	Increase enrollment rate
Pages 5, 25	**Inclusion Criteria** • are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following:	 • are diagnosed with an apparent fracture site infection and have at least one of the following:	Increase enrollment rate
Pages 6, 26	**Exclusion Criteria** *deleted* 2. Greater than one year time lapse from last operative procedure 3. Patients with a previous revision surgery at the site		Increase enrollment rate

PROTOCOL AMENDMENT FORM

DATE: 05/09/16 **AMENDMENT NUMBER:** 03

PROTOCOL NUMBER: MBN-101-201 (Version 3)

Location	From	To	Comment
Page 38	**8.3.2 Assay Methodology** The samples are prepared for analysis by digestion with 2% nitric acid and H2O2, followed by centrifugation and dilution with water into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.	The samples are prepared for analysis by digestion with nitric acid and H_2O_2 for 2 hours, followed by centrifugation and dilution in water with subsequent quantitation of Bi by ICP-MS analysis.	Update methodology
Page 38	**8.3.3 PK Analysis** Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.3 or higher, using an extravascular administration model and actual sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis:	Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.4 or higher, using an extravascular administration model and actual sampling times. Interim analyses may use nominal sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis (as feasible):	Update methodology
Page 50	**12.1 Efficacy Endpoints and 12.2 Safety Endpoints** 12.2 Changes in serologic markers CRP and ESR at each post baseline time point. Safety Endpoints	• Changes in serologic markers CRP and ESR at each post baseline time point. **12.2 Safety Endpoints**	Correct placement of last efficacy endpoint and section heading for safety endpoints.
Page 63	**Appendix 2: Flowchart for Microbiological Specimen Processing** **Swabs (Deep)** (2) Superficial tissue / wound closure site	**Swabs (Deep)** (2) Peri-implant deep tissue	Correct description of specimen for deep swab to be consistent with description in 6.1.9.1 Specimen Collection

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

PROTOCOL NUMBER: MBN-101-201

Version 3.0 Issue Date: 05 May 2016

Replaces Version 2.1 Dated 29 December 2015

Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 3.0

Microbion Corporation

Reviewed and Approved by:

05 May 2016

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

GRANT SUPPORT

This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100.

The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.

Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site.

The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans (“Ethical Principles for Medical Research Involving Human Subjects,” Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____

Investigator’s Printed Name

_____ _____

Investigator’s Signature Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	Up to eight Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	36
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original

	hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection and have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery – Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require revision surgery with or without removal and replacement of existing hardware

	2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 24 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement 2. Patients with multiple, non-contiguous fracture site infections 3. Pathologic fracture (not including osteoporosis) 4. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 5. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory 6. Absolute neutrophil count <1000 7. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration 8. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)

	9. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 10. Individuals undergoing surgical treatment for more than one infected fracture 11. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 12. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 13. Immunocompromised due to illness or organ transplant 14. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site 15. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 16. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician) 17. History of medical noncompliance 18. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results. 19. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	24 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 36 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (3 per cohort; 9 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (9 subjects per dose) and combined (a total of 27 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, ‘as assigned’ sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

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If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase

hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IVRS	Interactive Voice Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter
OR	Operating room
ORP	Office of Research Protections

PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the

shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases synergy, has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a minimum of 24 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Thirty-six adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 9 patients per dose level in the MBN-101-treated group and 9 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

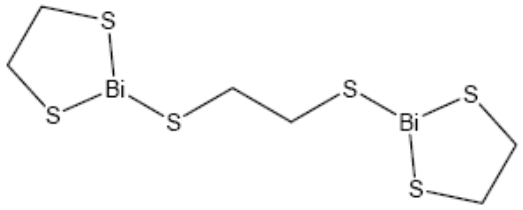
Up to eight U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 36 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis
   - are diagnosed with an apparent fracture site infection and have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require revision surgery with or without removal and replacement of existing hardware
2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 24
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 24
7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal
2. Patients with multiple, non-contiguous fracture site infections
3. Pathologic fracture (not including osteoporosis)
4. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
5. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory
6. Absolute neutrophil count <1000
7. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
8. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
9. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
10. Individuals undergoing surgical treatment for more than one infected fracture
11. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
12. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
13. Immunocompromised due to illness or organ transplant
14. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site
15. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
16. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)
17. History of medical noncompliance
18. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results.
19. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IVRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ± 8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc.; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests for aerobic/facultative bacteria will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility tests for anaerobic bacteria will also include CLSI reference quality control strains, however, since quality control ranges for anaerobic bacteria are not yet established, the MIC values for the quality control strains will be collected then retrospectively compared to the quality control ranges once they are available. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are

for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IVRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of an individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the approximate surface area of the surgical site as well as the amount and location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.
4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes
6. One pre-printed "Diluted MBN-101 Drug Product" label
7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”)

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.

Area of Osteosynthesis Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site. By following these application requirements, the administered doses will be:

- Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the eCRF.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will received their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study. Patients will also receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (4 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with nitric acid and H_2O_2 for 2 hours, followed by centrifugation and dilution in water with subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.4 or higher, using an extravascular administration model and actual sampling times. Interim analyses may use nominal sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis (as feasible):

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
V_z/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. A statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC. A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. One pre-printed "Diluted MBN-101 Drug Product" label

7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Moreover:

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion and/or its designee will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitor should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. The medical monitor must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.

- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Changes in serologic markers CRP and ESR at each post baseline time point.

12.2 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Nine subjects will receive active drug in each dose cohort. With 9 subjects receiving active drug, the probability of observing at least one of 9 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.283 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 9 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.283 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 27 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.105 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed fracture site)
- Reoperation (exclusive of reoperations associated with a healed fracture site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8
- from week 8 to week 12
- from week 12 to week 24

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patients perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10 Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11 Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the osteosynthesis site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12 Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1: Table of Study Events

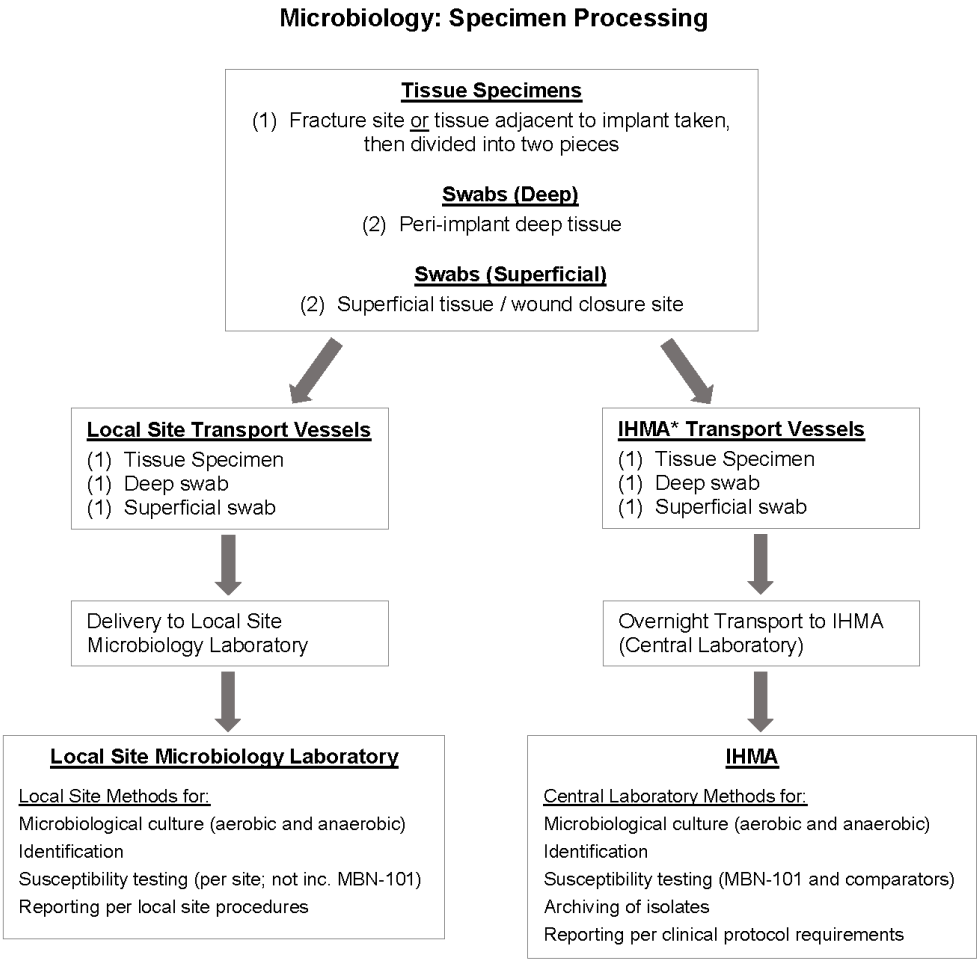
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12	Week 24, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±4 days	Day 42 ±8 days	Day 84 ±8 days	Day 168 ±8 days	
Review of eligibility criteria	X	X								
Informed consent	X									
Randomization		X								
Surgery/Administration of the Investigational Product		X								
Medical history	X									
Physical exam	X									
Interval physical exam		X	X			X	X	X	X	X
Hematology	X		X				X	X	X	X
Serology		X				X	X	X	X	X
Serum chemistry	X		X				X	X	X	X
Urinalysis	X		X				X	X	X	X
Pregnancy test	X	X							X	X
BisEDT Blood levels[1]		X	X	X	X	X				
12-lead ECG	X	X	X						X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2: Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc. (Central Laboratory)

PROTOCOL AMENDMENT FORM

DATE: 12/29/15 **AMENDMENT NUMBER:** 02

PROTOCOL NUMBER: MBN-101-201 (Version 2.1)

SPONSOR: Microbion Corporation

PROTOCOL TITLE: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

REASON(S) FOR CHANGE(S): Revise protocol with administrative changes and updated methodologies.

CHANGE(S):

Note – All changes refer to page numbers in the Clean Version 2.1

Location	From	To	Comment
Header	Version 2.0, Issue Date 08 October 2015	Version 2.1, Issue Date 29 December 2015	Update version
Page 1	Version 2.0 Issue Date: 08 October 2015 Replaces Version 1.0 Dated 06 May 2015	Version 2.1 Issue Date: 29 December 2015 Replaces Version 2.0 Dated 08 October 2015	Update version
Page 1	Version: 2.0	Version: 2.1	Update version
Page 8, Page 37 and Page 49	Protocol Synopsis – Criteria for Evaluation: Efficacy Endpoints Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm).	Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).	Cite updated VAS scale template
Page 10	Other Appropriate Trial Contact Personnel deleted Name Office Phone Number E-mail:	 Markus Heep, MD Medical Director Infectious Diseases Medpace Germany GmbH +49 174 190 6865 M.Heep@Medpace.com	Administrative change

PROTOCOL AMENDMENT FORM

DATE: 12/29/15 **AMENDMENT NUMBER:** 02

PROTOCOL NUMBER: MBN-101-201 (Version 2.1)

Location	From	To	Comment
Page 29	6.1.7 Pain Assessments Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-100 mm, where 0=no pain at all and 100 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.	Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.	Cite updated VAS scale template
Page 30	6.1.9.3 Specimen Processing: IHMA (Central Laboratory) The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria.	The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by microbiological culture for both aerobic and anaerobic bacteria.	Delete Central Laboratory Gram Stain, since only Local Laboratory Gram Stain will be performed and utilized for patient care
	Susceptibility tests will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators.	Susceptibility tests for aerobic/facultative bacteria will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility tests for anaerobic bacteria will also include CLSI reference quality control strains, however, since quality control ranges for anaerobic bacteria are not yet established, the MIC values for the quality control strains will be collected then retrospectively compared to the quality control ranges once they are available.	Cite updated testing strategy
Page 38	8.3.1 Sample Collection and Handling Documentation stating the exact time of blood sampling (5 mL per timepoint) in relation to the time of study drug administration will be collected and provided.	Documentation stating the exact time of blood sampling (4 mL per timepoint) in relation to the time of study drug administration will be collected and provided.	Cite correct tube size

PROTOCOL AMENDMENT FORM

DATE: 12/29/15 **AMENDMENT NUMBER:** 02

PROTOCOL NUMBER: MBN-101-201 (Version 2.1)

Location	From	To	Comment
Page 38	8.3.2 Assay Methodology The samples are prepared for analysis by digestion with 2% nitric acid followed by dilution into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.	The samples are prepared for analysis by digestion with 2% nitric acid and H_2O_2, followed by centrifugation and dilution with water into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.	Cite updated methodology

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

PROTOCOL NUMBER: MBN-101-201

Version 2.1 Issue Date: 29 December 2015

Replaces Version 2.0 Dated 08 October 2015

Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 2.1

Microbion Corporation

Reviewed and Approved by:

29 December2015

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

GRANT SUPPORT

This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100.

The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.

Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site.

The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans (“Ethical Principles for Medical Research Involving Human Subjects,” Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____
Investigator’s Printed Name

_____ _____
Investigator’s Signature Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	3-4 Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	36
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original

	hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery – Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require revision surgery with or without removal and replacement of existing hardware

	2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 24 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement 2. Greater than one year time lapse from last operative procedure 3. Patients with a previous revision surgery at the site 4. Patients with multiple, non-contiguous fracture site infections 5. Pathologic fracture (not including osteoporosis) 6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory 8. Absolute neutrophil count <1000 9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration

	10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol) 11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 12. Individuals undergoing surgical treatment for more than one infected fracture 13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 15. Immunocompromised due to illness or organ transplant 16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site 17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 18. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician) 19. History of medical noncompliance 20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results. 21. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	24 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 36 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (3 per cohort; 9 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (9 subjects per dose) and combined (a total of 27 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, 'as assigned' sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

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If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase

hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IVRS	Interactive Voice Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter
OR	Operating room
ORP	Office of Research Protections

PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

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1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the

shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases synergy, has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a minimum of 24 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Thirty-six adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 9 patients per dose level in the MBN-101-treated group and 9 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

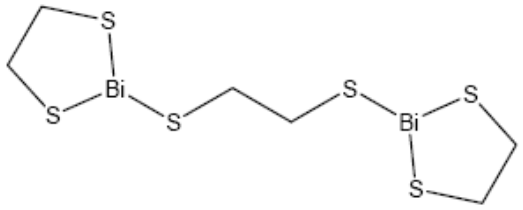
Three or four U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 36 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis
   - are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require revision surgery with or without removal and replacement of existing hardware
2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 24
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 24
7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal
2. Greater than one year time lapse from last operative procedure
3. Patients with a previous revision surgery at the site
4. Patients with multiple, non-contiguous fracture site infections
5. Pathologic fracture (not including osteoporosis)
6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory
8. Absolute neutrophil count <1000
9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
12. Individuals undergoing surgical treatment for more than one infected fracture
13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
15. Immunocompromised due to illness or organ transplant
16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site
17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
18. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)
19. History of medical noncompliance
20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results.
21. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IVRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ± 8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-10 scale, where 0=no pain at all and 10 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc.; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests for aerobic/facultative bacteria will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility tests for anaerobic bacteria will also include CLSI reference quality control strains, however, since quality control ranges for anaerobic bacteria are not yet established, the MIC values for the quality control strains will be collected then retrospectively compared to the quality control ranges once they are available. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are

for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IVRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of an individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the approximate surface area of the surgical site as well as the amount and location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.
4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes
6. One pre-printed "Diluted MBN-101 Drug Product" label
7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”)

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.

Area of Osteosynthesis Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site. By following these application requirements, the administered doses will be:

- Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the eCRF.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will received their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study. Patients will also receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (4 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with 2% nitric acid and H_2O_2, followed by centrifugation and dilution with water into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.3 or higher, using an extravascular administration model and actual sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis:

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
Vz/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. A statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC. A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. One pre-printed "Diluted MBN-101 Drug Product" label

7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Moreover:

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion and/or its designee will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitor should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. The medical monitor must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.

- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-10 scale).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.

12.2 Changes in serologic markers CRP and ESR at each post baseline time point.Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Nine subjects will receive active drug in each dose cohort. With 9 subjects receiving active drug, the probability of observing at least one of 9 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.283 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 9 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.283 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 27 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.105 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed fracture site)
- Reoperation (exclusive of reoperations associated with a healed fracture site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8
- from week 8 to week 12
- from week 12 to week 24

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patients perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the osteosynthesis site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1: Table of Study Events

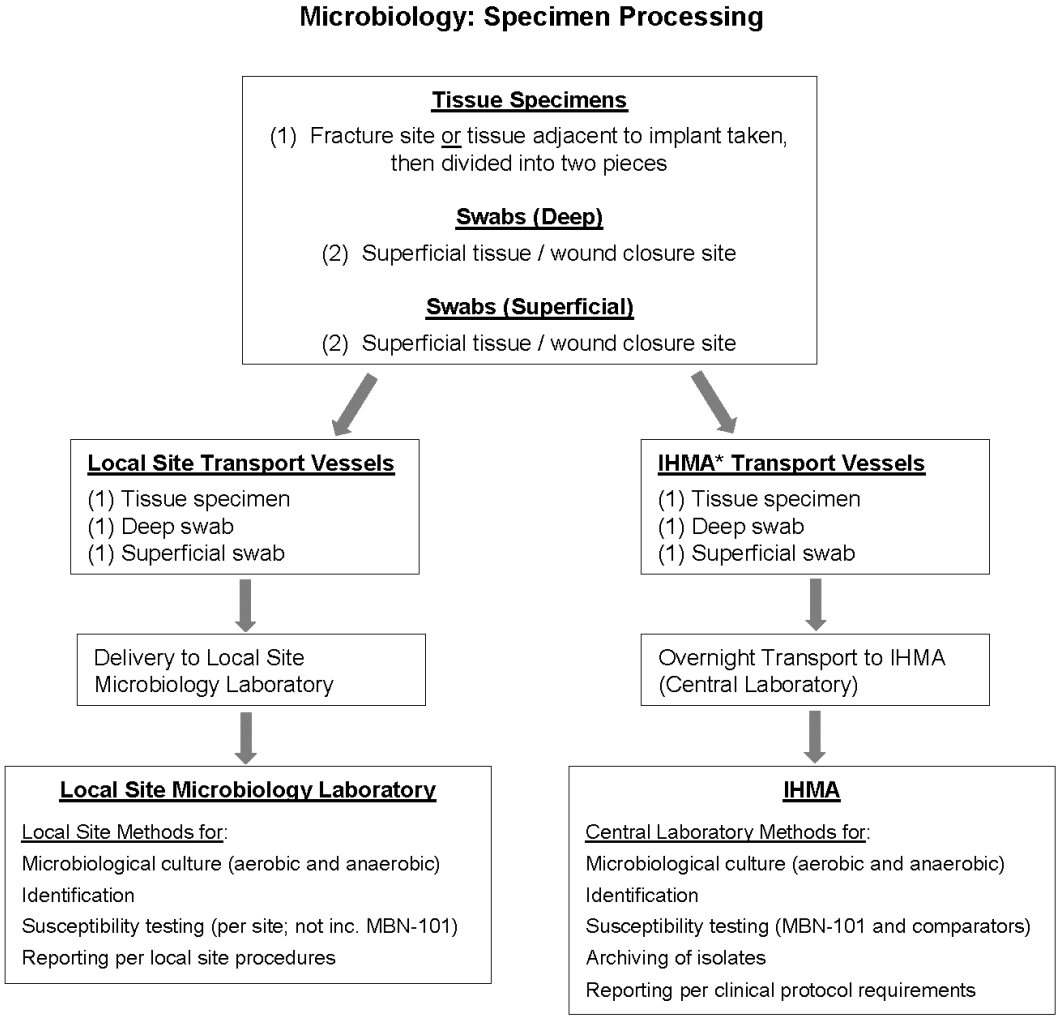
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12	Week 24, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±4 days	Day 42 ±8 days	Day 84 ±8 days	Day 168 ±8 days	
Review of eligibility criteria	X	X								
Informed consent	X									
Randomization		X								
Surgery/Administration of the Investigational Product		X								
Medical history	X									
Physical exam	X									
Interval physical exam		X	X			X	X	X	X	X
Hematology	X		X				X	X	X	X
Serology		X				X	X	X	X	X
Serum chemistry	X		X				X	X	X	X
Urinalysis	X		X				X	X	X	X
Pregnancy test	X	X							X	X
BisEDT Blood levels[1]		X	X	X	X	X				
12-lead ECG	X	X	X						X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2: Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc. (Central Laboratory)

PROTOCOL AMENDMENT FORM

DATE: 10/08/15 **AMENDMENT NUMBER:** 01

PROTOCOL NUMBER: MBN-101-201 (Version 2.0)

SPONSOR: Microbion Corporation

PROTOCOL TITLE: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

REASON(S) FOR CHANGE(S): Changes were implemented to add a citation of grant support, to correct the study drug application procedure in cases where hardware is replaced or in cases of two-stage procedures, to clarify exclusion criteria #16 and #18, to maintain consistent descriptions of study procedures across sections of the protocol, and to correct several administrative and editorial errors from Version 1.0.

CHANGE(S):
Note – All changes refer to page numbers in the Clean Version 2.0

Location	From	To	Comment
Header	Version 1.0, Issue Date 06 May 2015	Version 2.0, Issue Date 05 October 2015	Update version
Page 1	Issue Date: 06 May 2015	Version 2.0, Issue Date: 05 October 2015 Replaces Version 1.0 Dated 06 May 2015	Update version
Page 1	Version: 1.0	Version: 2.0	Update version
Page 2		Grant Support: This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100. The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.	Added grant support

PROTOCOL AMENDMENT FORM

DATE: 10/08/15 **AMENDMENT NUMBER:** 01

PROTOCOL NUMBER: MBN-101-201 (Version 2.0)

Location	From	To	Comment
		Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site. The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.	
Page 5	In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all surfaces of new hardware immediately prior to implantation, and following implantation to adjacent bone and accessible hardware surfaces.	In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure.	Correct study drug application procedure
Page 5		nominal	Administrative change
Page 7		other than the index infected osteosynthesis site	Clarify Exclusion #16
Page 7		(hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)	Clarify Exclusion #18
Page 22	In cases where hardware is replaced or in cases of two-stage procedures, the investigational product will be applied to all surfaces of new hardware immediately prior to implantation, and following implantation to adjacent bone and accessible hardware surfaces.	In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure.	Correct study drug application procedure

PROTOCOL AMENDMENT FORM

DATE: 10/08/15 **AMENDMENT NUMBER:** 01

PROTOCOL NUMBER: MBN-101-201 (Version 2.0)

Location	From	To	Comment
Page 24	The Treatment Dose will be provided to the surgeon in an 8 mL volume in a sterile 10 mL syringe.	The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe.	Correct study drug application procedure
Page 24	lightly	sparingly…actual	Correct study drug application procedure
Page 25	<75	≤75	Maintain consistency across sections of the protocol
Page 26		other than the index infected osteosynthesis site	Clarify Exclusion #16
Page 26		(hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)	Clarify Exclusion #18
Page 29	Inc	Inc.	Administrative change
Page 31	…absence of individual…	…absence of an individual…	Administrative change
Page 32		approximate surface area of the surgical site as well as the amount and	Correct study drug application procedure
Page 32	, including the dimensions and approximate surface area of the surgical incision,		Correct study drug application procedure
Page 32	15 gauge	18 gauge	Correct study drug application procedure
Page 32		6. One pre-printed "Diluted MBN-101 Drug Product" label	Correct study drug application procedure
Page 33	Prior to implantation, the surgeon will use a gloved finger to sparingly coat all hardware with a thin layer of the investigational product. Following surgical implantation of the coated hardware, a thin layer of the investigational product will be applied to surrounding bone using a gloved finger. Administration of the investigational product will therefore be limited to target areas, specifically orthopedic	In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.	Correct study drug application procedure

PROTOCOL AMENDMENT FORM

DATE: 10/08/15 **AMENDMENT NUMBER:** 01

PROTOCOL NUMBER: MBN-101-201 (Version 2.0)

Location	From	To	Comment
	hardware and the immediately adjacent, accessible bone (osseous) surfaces.		
Page 34	•Cohort 1: 25 µg/mL MBN-101 = 0.5 µg/cm^2 •Cohort 2: 75 µg/mL MBN-101 = 1.5 µg/cm^2 •Cohort 3: 250 µg/mL MBN-101 = 5.0 µg/cm^2	•Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2 •Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2 •Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2	Administrative change
Page 34	…assayed for BisEDT	…recorded in the eCRF	Maintain consistency across sections of the protocol
Page 38		nominal	Administrative change
Page 42	This DRC will include a Statistician, an orthopedic surgeon and a Medical Safety Officer. An independent statistician…	A statistician…	Maintain consistency across sections of the protocol
Page 42		A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.	Administrative change
Page 43	15 gauge	18 gauge	Correct study drug application procedure
Page 43		6. One pre-printed "Diluted MBN-101 Drug Product" label	Correct study drug application procedure
Page 47	Microbion	Microbion and/or its designee	Administrative change
Page 47		Moreover:	Administrative change
Page 48	monitors	monitor	Administrative change
Page 48	Medical monitors…	The medical monitor…	Administrative change
Page 62	±2 days	±4 days	Maintain consistency across sections of the protocol
Page 62	28	42	Correction to Week 6 Visit
Page 62		Added “X” for Concomitant medications Days 3 and 4	Maintain consistency across sections of the protocol
Page 62		Added “X” for Adverse events Days 3 and 4	Maintain consistency across sections of the protocol
Page 62		nominal	Administrative change

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

PROTOCOL NUMBER: MBN-101-201

Version 2.0 Issue Date: 08 October 2015

Replaces Version 1.0 Dated 06 May 2015

Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 2.0

Microbion Corporation

Reviewed and Approved by:

08 October 2015

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

GRANT SUPPORT

This Phase 2a clinical study is supported in part by a $2.5 million grant awarded through the "Defense Medical Research and Development Program (DMRDP) Military Infectious Diseases Clinical Trial Award, W81XWH-12-DMRDP-MID-CTA". The grant award number is W81XWH-12-2-0100.

The Awardee is the University of Pennsylvania, and the Principal Investigator (PI) for this grant award is Dr. Samir Mehta, Chief, Division of Orthopaedic Trauma, and Assistant Professor of Orthopaedic Surgery at the Hospital of the University of Pennsylvania. Dr. Mehta is also serving as the clinical PI for the University of Pennsylvania orthopaedic trauma clinical site.

Participating as a Co-Investigator in this grant award is Dr. Saam Morshed, attending orthopaedic trauma surgeon and Director of the Clinical Research Center at the University of California San Francisco (UCSF) and the San Francisco General Hospital Orthopaedic Trauma Institute (OTI). Dr. Morshed will also serve as the clinical PI for the UCSF orthopaedic trauma clinical site.

The Program Sponsor, Microbion Corporation, is represented by Dr. Brett Baker, President and Chief Scientific Officer.

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____

Investigator's Printed Name

_____ _____

Investigator's Signature Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	3-4 Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	36
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original

	hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery – Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require revision surgery with or without removal and replacement of existing hardware

	2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 24 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement 2. Greater than one year time lapse from last operative procedure 3. Patients with a previous revision surgery at the site 4. Patients with multiple, non-contiguous fracture site infections 5. Pathologic fracture (not including osteoporosis) 6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory 8. Absolute neutrophil count <1000 9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration

	10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol) 11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 12. Individuals undergoing surgical treatment for more than one infected fracture 13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 15. Immunocompromised due to illness or organ transplant 16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site 17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 18. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician) 19. History of medical noncompliance 20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results. 21. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	24 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 36 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (3 per cohort; 9 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (9 subjects per dose) and combined (a total of 27 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, 'as assigned' sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

Medical Monitor

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24 Hour Emergency Contact Information

Medpace Clinical Safety:

SAE hotline:	800-730-5779, ext. 2999
Facsimile:	866-336-5320
E-mail:	medpace-safetynotification@medpace.com

If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase

hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IVRS	Interactive Voice Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter
OR	Operating room
ORP	Office of Research Protections

PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

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1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the

shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases synergy, has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a minimum of 24 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Thirty-six adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 9 patients per dose level in the MBN-101-treated group and 9 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

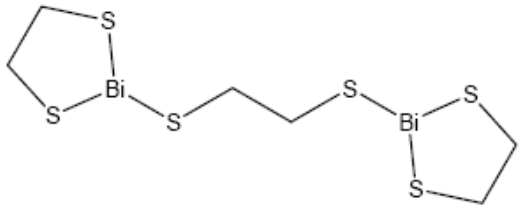
Three or four U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 36 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis
   - are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require revision surgery with or without removal and replacement of existing hardware
2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 24
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 24
7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal
2. Greater than one year time lapse from last operative procedure
3. Patients with a previous revision surgery at the site
4. Patients with multiple, non-contiguous fracture site infections
5. Pathologic fracture (not including osteoporosis)
6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory
8. Absolute neutrophil count <1000
9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
12. Individuals undergoing surgical treatment for more than one infected fracture
13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
15. Immunocompromised due to illness or organ transplant
16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) other than the index infected osteosynthesis site
17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
18. Poorly controlled diabetes mellitus (hemoglobin A1c > 9.0% for ≥ 6 months despite management by a physician)
19. History of medical noncompliance
20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results.
21. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IVRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ±8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-100 mm, where 0=no pain at all and 100 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc.; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IVRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of an

individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the approximate surface area of the surgical site as well as the amount and location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.
4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.
5. Alcohol wipes
6. One pre-printed "Diluted MBN-101 Drug Product" label
7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort ("Treatment Dose").

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose ("Treatment Dose")

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures) following implantation of hardware and immediately prior to definitive closure. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.

Area of Osteosynthesis Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site. By following these application requirements, the administered doses will be:

- Cohort 1: 0.025 mg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 0.075 mg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 0.250 mg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be recorded in the eCRF.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will received their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study. Patients will also receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (5 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with 2% nitric acid followed by dilution into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.3 or higher, using an extravascular administration model and actual sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis:

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
Vz/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. A statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC. A sponsor representative will also serve as a non-voting member of the DRC to facilitate sponsor internal planning.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 18 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. One pre-printed "Diluted MBN-101 Drug Product" label

7. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Moreover:

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion and/or its designee will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitor should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. The medical monitor must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.

- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.

12.2 Changes in serologic markers CRP and ESR at each post baseline time point.Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Nine subjects will receive active drug in each dose cohort. With 9 subjects receiving active drug, the probability of observing at least one of 9 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.283 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 9 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.283 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 27 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.105 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed fracture site)
- Reoperation (exclusive of reoperations associated with a healed fracture site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8
- from week 8 to week 12
- from week 12 to week 24

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patients perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10 Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11 Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the osteosynthesis site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12 Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1: Table of Study Events

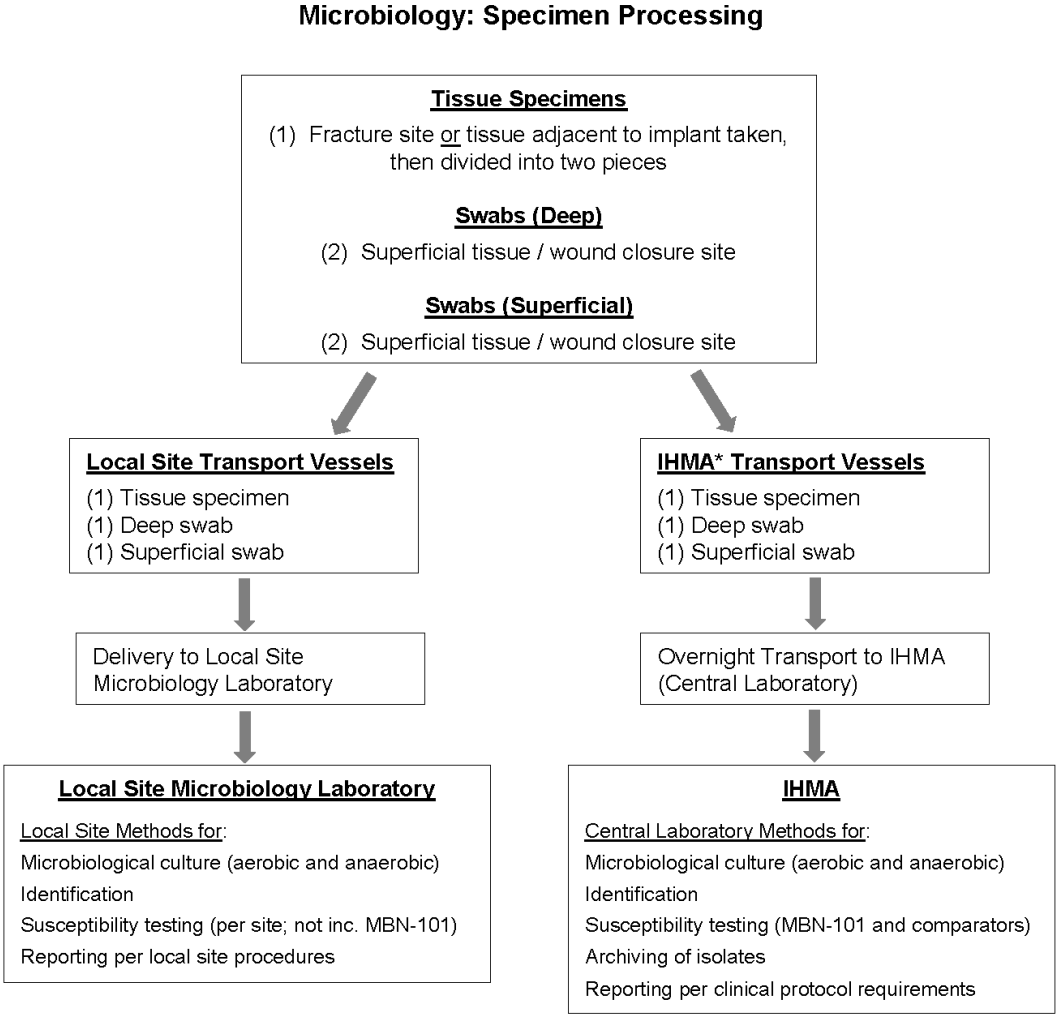
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12	Week 24, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±4 days	Day 42 ±8 days	Day 84 ±8 days	Day 168 ±8 days	
Review of eligibility criteria	X	X								
Informed consent	X									
Randomization		X								
Surgery/Administration of the Investigational Product		X								
Medical history	X									
Physical exam	X									
Interval physical exam		X	X			X	X	X	X	X
Hematology	X		X				X	X	X	X
Serology		X				X	X	X	X	X
Serum chemistry	X		X				X	X	X	X
Urinalysis	X		X				X	X	X	X
Pregnancy test	X	X							X	X
BisEDT Blood levels[1]		X	X	X	X	X				
12-lead ECG	X	X	X						X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at nominal 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2: Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc. (Central Laboratory)

PROTOCOL CHANGE FORM

DATE: 05/06/15

Changes in Version 1 April 14, 2015 to Version 1 May 6, 2015

PROTOCOL NUMBER: MBN-101-201 (Version 1.0)

SPONSOR: Microbion Corporation

PROTOCOL TITLE: A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

REASON(S) FOR CHANGE(S): Changes were implemented immediately prior to the IND filing to update the study drug application procedure, correct drug product description, change spelling within protocol for consistency with the rest of the IND filing.

CHANGE(S):
Note – All changes refer to page numbers in the Track Changes Version 1.0

Location	From	To	Comment
Page 1	Issue Date: 14 April 2015	Version 1.0 Issue Date: 06 May 2015 Replaces Version 1.0 Dated 14 April 2015	Update date of Version 1
Multiple pages	orthopaedic	orthopedic	Consistency of spelling throughout the protocol and rest of IND filing
Page 18	Unique mechanism of action	Apparently unique mechanism of action	Correct to improve accuracy of statement
Page 31	Adminstration	Administration	Spelling correction
Page 32	Application to retained hardware: Access to infected wound surfaces and implanted orthopaedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopaedic hardware, as well as surrounding bone and other connective tissue surfaces that are likely to come into contact with the orthopaedic hardware. Wound surfaces that are not in the immediate vicinity of the orthopaedic hardware will not be coated, resulting in administration of the investigational product to only a limited area of the wound.	Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.	Update study drug application procedure

PROTOCOL CHANGE FORM

DATE: 05/06/15

Changes in Version 1 April 14, 2015 to Version 1 May 6, 2015

PROTOCOL NUMBER: MBN-101-201 (Version 1.0)

Page 32	Application to new hardware: Prior to implantation, the surgeon will use a gloved finger to sparingly coat all hardware with a thin layer of the investigational product. Following surgical implantation of the coated hardware, a thin layer of the investigational product will be applied to surrounding bone and other connective tissue surfaces that are likely to come into contact with the orthopaedic hardware using a gloved finger. Wound surfaces that are not in the immediate vicinity of the orthopaedic hardware will not be coated, resulting in Administration of the investigational product being limited to target areas, specifically orthopaedic hardware and the immediately adjacent, accessible bone (osseous) surfaces.	Application to new hardware: Prior to implantation, the surgeon will use a gloved finger to sparingly coat all hardware with a thin layer of the investigational product. Following surgical implantation of the coated hardware, a thin layer of the investigational product will be applied to surrounding bone using a gloved finger. Administration of the investigational product will therefore be limited to target areas, specifically orthopedic hardware and the immediately adjacent, accessible bone (osseous) surfaces.	Update study drug application procedure
Page 33	The gel should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware (target structures). Direct application to muscle and other soft tissues (non-target structures) should be avoided.	The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided.	Correct drug product description from gel to suspension. Delete redundant description, i.e., target, non-target structures
Page 33	volumes of gel	volumes of MBN-101.	Correct drug product designation
Page 52	(Selim, 2009)	(Selim, 2009; Veterans Affairs website, 2014).	Add reference

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

PROTOCOL NUMBER: MBN-101-201

Issue Date: 06 May 2015

Regulatory Sponsor:
Microbion Corporation
1102 West Babcock, Suite B
Bozeman MT 59715

Version: 1.0

Microbion Corporation

Reviewed and Approved by:

12 May2015

Dr. Brett Baker
Microbion Corporation
President and Chief Scientific Officer

Date

INVESTIGATOR AGREEMENT

A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined therein and will make all reasonable efforts to complete the study within the designated time.

I will provide copies of the protocol and access to all information furnished by Microbion Corporation to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I understand that the study may be terminated or enrollment suspended at any time by Microbion Corporation with or without cause, or by me, if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in accordance with the requirements of this protocol and in accordance with the following:

- Good Clinical Practice: Consolidated Guideline International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996)
- United States (U.S.) Code of Federal Regulations (CFR) associated with clinical studies (21 CFR parts 50, 56, 312)
- Declaration of Helsinki, concerning medical research in humans (“Ethical Principles for Medical Research Involving Human Subjects,” Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004).

_____

Investigator’s Printed Name

_____ _____

Investigator’s Signature Date

PROTOCOL SYNOPSIS

Protocol Number	MBN-101-201
Title	A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety, Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites
Regulatory Sponsor	Microbion Corporation, 1102 West Babcock, Suite B, Bozeman MT 59715
Name of Investigational Product	MBN-101
Phase	2a
Investigators/Study Centers	3-4 Study Centers in the United States
Objectives: **Primary Objective**	To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy
Secondary Objectives	• To evaluate the clinical activity of single escalating doses of locally administered MBN-101 • To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101
Number of Subjects	36
Study Design and Methodology	This is a randomized, single-blind, placebo-controlled, multi-center study to assess the safety and tolerability of single escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. Patients meeting eligibility criteria on screening will be offered participation in the study. Following baseline evaluation, patients will receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. A single application of MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original

	hardware is retained, MBN-101 will be applied to all accessible surfaces of hardware and adjacent bone (target structures). In cases where hardware is replaced or in cases of two-stage procedures, MBN-101 will be applied to all surfaces of new hardware immediately prior to implantation, and following implantation to adjacent bone and accessible hardware surfaces. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used. Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. PK assessments will be performed predose and at 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14; Week 2) hours after administration of study drug. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a total of 24 weeks after surgery.
Study Population:	
Inclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable): 1. Patients who: • have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis • are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following: – Elevated ESR above the upper limit of normal – Elevated CRP above the upper limit of normal – Draining wound / sinus tract – Positive culture from site of prior surgery by aspirate or other modality – Local erythema or induration at the site of prior surgery – Exposed hardware – Periosteal reaction on x-ray – Loose or broken hardware • require revision surgery with or without removal and replacement of existing hardware

	2. Male or female ≥18 and ≤75 years of age at the time the ICF is reviewed and signed 3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care 4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery 5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum) 6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements: • Be sexually abstinent from Baseline through Week 24 • Be > 6 months post-vasectomy • Agree to use a condom with spermicide from Baseline through Week 24 7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained 8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)
Exclusion Criteria:	To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable): 1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal without replacement 2. Greater than one year time lapse from last operative procedure 3. Patients with a previous revision surgery at the site 4. Patients with multiple, non-contiguous fracture site infections 5. Pathologic fracture (not including osteoporosis) 6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted) 7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory 8. Absolute neutrophil count <1000 9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration

	10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol) 11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months 12. Individuals undergoing surgical treatment for more than one infected fracture 13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80)) 14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing 15. Immunocompromised due to illness or organ transplant 16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months) 17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix) 18. Poorly controlled diabetes mellitus 19. History of medical noncompliance 20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results. 21. Current incarceration
Investigational Product, Dose and Mode of Administration:	MBN-101: A suspension of 0.025, 0.075, or 0.25 mg/mL (w:v) BisEDT powder in 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Reference Therapy, Dose and Mode of Administration:	MBN-101 diluent (placebo): 3% methylcellulose / 0.5% Tween 80 / 10 mM sodium chloride / 10 mM sodium phosphate, pH 7.4 (up to 8 mL dose volume) applied directly to structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement as an adjunct to standard care systemic antimicrobial therapy.
Study Duration	24 weeks

Criteria for Evaluation:	
Efficacy Endpoints	• Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24. • Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24. • Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site. • Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24. • Time to removal of orthopedic hardware due to a healed fracture site. • Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing. • Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA). • Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm). • Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection. • Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination. • Changes in serologic markers CRP and ESR at each post baseline time point.
Safety Endpoints	• Treatment-emergent adverse events. • Treatment-emergent serious adverse events. • Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis). • Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature). • Changes in physical exams. • Clinical findings of 12-lead electrocardiograms. • Change in microbiology status
Pharmacokinetic Endpoints	• Whole blood concentrations of Bismuth (Bi) will be measured after administration of single escalating doses of MBN-101 (bismuth as a surrogate for BisEDT) and will be used to calculate pharmacokinetic parameters (i.e., T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, CL/F, V_z/F).

Statistical Methods:	
Statistical Analysis Consideration:	The study sample of size of 36 subjects was chosen empirically for the purpose of the study without considering any formal statistical hypothesis testing. The safety analysis set will include all subjects who are randomized and receive any study drug; the efficacy analysis set will include all randomized subject with at least one post treatment assessment. Pharmacokinetic analysis set will include all subjects who have sufficient plasma samples to allow for calculation of pharmacokinetic parameters. All safety and efficacy endpoints will be tabulated with descriptive statistics; data from all placebo subjects (3 per cohort; 9 subjects in total) will be pooled. Data from BisEDT treated subjects will be presented by dose (9 subjects per dose) and combined (a total of 27 subjects). If data permits, differences between the treatment groups and 95% confidence intervals for the difference will be presented. If data permit, exploratory dose-response analyses will be performed for selected efficacy and safety endpoints. The primary analyses of efficacy and safety endpoints will be based on the actual treatment received in the event that the received treatment is not the assigned treatment; however, ‘as assigned’ sensitivity analyses are planned to evaluate the robustness of the efficacy data.

SPONSOR CONTACT INFORMATION

Medical Monitor

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If any Sponsor contact information needs to be changed during the course of the study, this will be done by the Sponsor, with written notification to the Investigator, and will not require a protocol amendment.

LIST OF ABBREVIATIONS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase (Serum glutamic pyruvic transaminase [SGPT])
AO/OTA	Arbeitsgemeinschaft für Osteosynthesefragen/Orthopedic Trauma Association
AST	Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase [SGOT])
$AUC_{0-\infty}$	Area under the curve from time 0 to infinity
AUC_{0-t}	Area under the curve from time 0 to the time of the last measurable concentration
Bi	Bismuth, used as a surrogate in concentration and PK analyses after MBN-101 administration
BisEDT	Bismuth-1,2-ethanedithiol
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK	Creatinine kinase
CL/F	Apparent clearance after extravascular administration
CLSI	Clinical and Laboratory Standards Institute
Cm	Centimeter
C_{max}	Maximum observed concentration
CRF	Case report form
CRP	C-reactive protein
DFU	Directions for Use
DOD	Department of Defense
DRC	Data Review Committee
ECG	Electrocardiogram
ESBL	Extended spectrum beta lactamase
ESR	Erythrocyte sedimentation rate
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase

hCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICP-MS	Inductively coupled plasma mass spectrometry
IHMA	International Health Management Associates, Inc.
IRB	Institutional Review Board
ITT	Intent to treat
IUD	Intrauterine device
IV	Intravenous
IVRS	Interactive Voice Response System
Kg	Kilogram
MBN-101	Bismuth-1,2-ethanedithiol (BisEDT) suspension
MC	Methylcellulose
MCS	Mental health domain score
MCV	Mean corpuscular volume
MDR	Multidrug Resistant
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mg/mL	Milligrams per milliliter
MIC	Minimal inhibitory concentration
mITT	Modified intent to treat
mL	Milliliter
mm	Millimeter
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NCA	Non-compartmental (PK) analysis
ng/mL	Nanograms per milliliter
OR	Operating room
ORP	Office of Research Protections

PCS	Physical health domain score
PI	Principal investigator
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QIDP	Qualified Infectious Disease Product
RBC	Red blood cells
SAE	Serious Adverse Event
SAE CRF	Serious Adverse Event Case Report Form
SAP	Statistical Analysis Plan
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMFA	Short Musculoskeletal Function Assessment
SOC	System organ class
SOP	Standard Operating Procedure
$T_{1/2}$	Apparent half-life after extravascular administration
TEAE	Treatment-emergent adverse event
T_{max}	Time of the maximal observed concentration
USAMRMC	US Army Medical Research and Materiel Command
VAS	Visual Analog Scale
VR-12	Veterans Rand 12 Item Health Survey
V_z/F	Apparent volume of distribution after extravascular administration
w/v	Weight:volume
w:w	Weight:weight
WBC	White blood cells

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Clinical Problem

Postoperative orthopedic infections, particularly antibiotic-resistant infections, present a serious clinical challenge to surgeons and other treating physicians, since these infections involve foreign materials (stabilizing orthopedic hardware) which are frequently associated with persistent microbial biofilms (Hetrick, 2006). Surgical intervention, including irrigation, debridement and potential replacement of orthopedic hardware, combined with a prolonged course of systemic antibiotics, is the standard of care for postoperative infections (Schmidt, 2000; Patzakis, 2005). However, outcomes associated with these infections are often poor, including chronic/recurrent infections, repeated hospitalizations, repeated surgeries, multiple courses of systemic antibiotic treatment, loss of function, disability, amputation and death (Berkes, 2010).

Systemic antibiotics are poorly efficacious in the treatment of heavily contaminated or infected wounds (Zalavras, 2003; Robson, 1997) and often antibiotics "…have practical and potential value only if a therapeutic blood level or, more importantly, tissue level is achieved within the first 4 hours after wounding" (Robson, 1999), i.e., *before* biofilm formation. The 'contest' between bacteria and host tissues with respect to speed of colonization of foreign objects such as orthopedic medical devices has been described as the "race for the surface' (Gristina, 1988). Bacteria are able to adhere to foreign, implanted objects almost immediately, facilitating rapid formation of microbial biofilms which drastically increase the resistance of wound-associated bacteria and contribute significantly to persistence and virulence of the infection (Parra-Ruiz, 2012; Gristina, 1994). Such biofilms can serve as quiescent reservoirs of adherent, antibiotic-tolerant or antibiotic-resistant bacteria within the wound, or as foci of active infections, which helps to explain the chronic and/or recurrent nature of many device-related infections. In addition, biofilms are hypermutable (Ciofu, 2011), increasing the likelihood of developing genetically-based antibiotic resistance. Finally, biofilms serve as environments in which bacteria can exchange genetic material encoding antibiotic resistance genes (Ghigo, 2001). Recent studies have shown that the most highly multidrug resistant (MDR) bacteria are also the strongest biofilm-forming bacteria (Kwon, 2008), and similarly, that invasive MDR bacteria are usually biofilm-forming bacteria (Reiter, 2011).

The high incidence of postoperative orthopedic device-related infections, as well as the shortcomings of the standard of care treatment in resolving those infections, particularly considering the increasing prevalence of antibiotic-resistant pathogens, has resulted in an expanding unmet need for safe, innovative, new and effective therapies to reduce the infectious risks and consequences of these infections, which increasingly involve antibiotic- resistant strains (Gessman, 2012; Morris, 2013; Tosh, 2012). Antibiotic resistance adds significantly to the challenge of successfully resolving these orthopedic infections (Ferry, 2010; Salgado, 2007). For example, infection treatment failures are nine times more frequent in orthopedic patients with MRSA infections than methicillin-sensitive *S. aureus* (MSSA) infections (Salgado, 2007).

Development of local therapies that eradicate established biofilms or prevent the formation of biofilms (as a new target) is an innovative new clinical strategy, particularly considering the

shortcomings of systemically administered antibiotics for treatment of wound infections. For example, several groups have investigated the direct, local application of vancomycin powder on the surgical wound in combination with systemic prophylaxis to prevent or treat infection. The outcomes of these studies have been mixed, with some groups reporting a reduced infection rate and/or a reduction in patient treatment costs (Heller, 2013; Godil, 2013) and others reporting no benefit (Tubaki, 2013; Martin, 2013). Antibiotic-loaded bone cement containing tobramycin, gentamicin, or vancomycin/tobramycin or other antibiotic combinations have been investigated for their ability to reduce infection rates and /or reduce costs when applied locally in a variety of orthopedic surgical procedures (Cummins, 2009; Nowinski, 2012; Selhi, 2012). Evidence suggests that local therapies may reduce implant related surgical site infections.

1.2 Investigational Therapy

MBN-101 [Bismuth-1,2-ethanedithiol (BisEDT) suspension] represents the first drug product from a new class of antimicrobial agents with apparently unique mechanisms of action. In nonclinical models, BisEDT has been shown to be effective against a broad-spectrum of orthopedic device-associated bacteria including antibiotic-resistant strains.

BisEDT has several characteristics especially suited to the treatment of postoperative orthopedic infections:

a) Broad spectrum antimicrobial activity against the most critically important antibiotic-resistant bacteria, including MRSA, MRSE, antibiotic-resistant *Pseudomonas aeruginosa*, ESBL-positive *Klebsiella pneumoniae*, and antibiotic-resistant *Enterobacter* species.
b) Apparently unique mechanisms of action.
c) Anti-biofilm capabilities.
d) Extremely low spontaneous mutation frequency, superior to many conventional antibiotics.
e) Efficacy against both aerobic and anaerobic bacteria.
f) Demonstrated lack of interference with the activity of a wide range of antibiotics.
g) Enhanced activity in combination with specific antibiotics; in some cases synergy, has been demonstrated in vitro and in vivo.
h) Maintenance of antimicrobial activity in the presence of excessive protein.
i) A favorable safety and tolerability profile in nonclinical toxicology studies in a variety of species, including absence of effect on wound healing or bone repair
j) A favorable safety and tolerability profile in a clinical Phase 1 study evaluating topical administration.

Based in part on these characteristics, BisEDT has been granted Qualified Infectious Disease Product (QIDP) designation by the FDA for the local, intra-operative treatment of resistant post-surgical orthopedic implant infections.

1.3 Value of MBN-101 for the Treatment of the Clinical Problem

The broad-spectrum antimicrobial, anti-biofilm activity of BisEDT, its activity against relevant antibiotic-resistant pathogens, and its ability to enhance the activity of certain other antibiotics are properties that will promote more rapid and/or more complete eradication of infection, and reduce infectious risks to patients. With comparatively elevated rates of postoperative infection associated with repair of traumatic orthopedic wounds, the development of a new and innovative treatment strategy to complement the current standard of care would therefore be expected to result in a substantial reduction in mortality, amputation, morbidity, and disability, along with a reduction in patient treatment costs.

MBN-101 provides important potential advantages over current standard of care treatment for orthopedic infections. Direct, local contact of MBN-101 with infected target tissue and contaminated device surfaces immediately delivers a therapeutically active dose of BisEDT to the site of infection. Combined antimicrobial and anti-biofilm effects are achieved with minimal systemic exposure. Systemic antibiotics administered alone are frequently ineffective, in part because altered perfusion at the surgical wound site hinders effective and timely delivery of systemic antibiotics, making it difficult to reach therapeutic antibiotic levels at wound tissues/surfaces. The combined effect of IV administered antibiotics, which already serve as a pillar of the current standard of care for orthopedic device-related infections, along with the local administration of MBN-101, is expected to eradicate bacteria from postoperative orthopedic wounds, and will ultimately also serve to reduce the likelihood of development of antibiotic-resistant bacteria. By reducing the time to resolution of infection and improving the ability of current antibiotic therapies to effectively control and eliminate post-surgical orthopedic device-related infections, MBN-101 will contribute to reductions in the number of additional serious interventions needed to resolve infections including reduction in repeat surgeries, additional rounds of systemic antibiotics, patient hospitalization time, morbidity and mortality in both civilian and military populations.

2 OBJECTIVES

2.1 Primary

To evaluate the safety and tolerability of single escalating doses of locally administered MBN-101 or placebo as adjunct to standard of care antimicrobial and surgical therapy.

2.2 Secondary

- To evaluate the clinical activity of single escalating doses of locally administered MBN-101.
- To evaluate the pharmacokinetics of single escalating doses of locally administered MBN-101.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a randomized, single-blind, placebo-controlled multi-center study to assess the safety and tolerability of escalating doses of MBN-101 applied directly to target structures within infected osteosynthesis sites during revision surgery with or without hardware removal and replacement for patients diagnosed with an apparent fracture site infection within one year of the last surgical intervention. Three successive cohorts of 12 patients will be enrolled in this trial. Consecutive patients from each of the study sites will be screened for potential participation as they present to the orthopedic service for clinical care for their postoperative infections. After signing an Informed Consent Form (ICF), subjects will complete screening procedures. Patients meeting all eligibility criteria on screening will be offered participation in the study. Patients accepting participation in the study will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort. The DRC will monitor all safety data in an ongoing manner from all patients enrolled onto this study.

Following baseline evaluation, patients will receive standard of care treatment for their post-operative fracture site infection that includes systemic antibacterial treatment per institutional standard of care guidelines and revision surgery with or without hardware removal and replacement as indicated. Multiple debridements, soft tissue transfer, and revision fixation procedures may be performed prior to definitive closure. A single application of the investigational product, MBN-101 or placebo, applied intraoperatively directly to target structures within infected osteosynthesis sites, will be performed following the final irrigation and debridement procedure and immediately prior to definitive closure. In cases where original hardware is retained, the investigational product will be sparingly applied to all accessible surfaces of hardware and adjacent bone. In cases where hardware is replaced or in cases of two-stage procedures, the investigational product will be applied to all surfaces of new hardware immediately prior to implantation, and following implantation to adjacent bone and accessible hardware surfaces. The volume applied will be determined by the surgeon's assessment of the size (in cm^2) of the target area, and guided by the Table in Section 6.5.2. If wounds are left open, the investigational product will be applied immediately prior to definitive closure. The minimum amount of MBN-101 required to achieve a thin coat of the relevant target structures within the infected osteosynthesis site will be used (see Section 6.5.2).

All patients will receive standard postoperative care per institutional guidelines, and will be discharged from the hospital in accordance with local standards. Patients will undergo study visits at Hour 48 and Weeks 2, 6, 12, and 24. All patients will be followed for a minimum of 24 weeks after surgery.

See Appendix 1 for a Schedule of Activities.

3.2 Number of Subjects

Thirty-six adult male or female patients meeting protocol-specified eligibility criteria may participate in the study, with the objective of having a total of 9 patients per dose level in the MBN-101-treated group and 9 patients in a pooled placebo-treated group. Patients who are randomized but do not receive study drug may be replaced.

3.3 Number of Sites

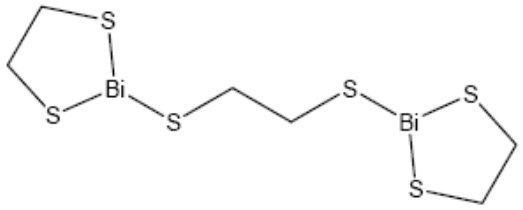
Three or four U.S. study sites are anticipated to enroll an adequate number of eligible patients.

3.4 Clinical Trial Material

Patients will be randomized 3:1 (active:control) to three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent). Enrollment to the next dose cohort will not commence until an evaluation by the Data Review Committee (DRC) of all available safety data on all patients through Week 6 of study supports escalation to the next cohort.

3.4.1 MBN-101

MBN-101 is Bismuth-1,2-ethanedithiol (BisEDT) suspension (0.025, 0.075, or 0.25 mg/mL, w:v) in diluent (3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4).

Structural Formula	
Molecular Formula	$C_6H_{12}S_6Bi_2$
Molecular Weight	694.51 g/mol
Stereochemistry/Chirality	BisEDT has no chiral centers.

3.4.2 Placebo (MBN-101 diluent)

Placebo is MBN-101 diluent comprised of 3% methylcellulose / 0.5% Tween 80 / 10mM sodium chloride / 10 mM sodium phosphate, pH 7.4.

3.5 Dose

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

Investigational product kits (MBN-101 and placebo) configured for each dose cohort will be provided and will contain the supplies necessary for preparation of the specified concentrations of MBN-101 or placebo for administration to each patient.

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the Stock Formulation and Diluent provided in the Drug Product Kit according to the Directions for Use (DFU) provided. The Treatment Dose will be provided to the surgeon in an 8 mL volume in a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided for the largest exposed target areas) to lightly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. With dose volume determined according to Section 6.5.2, the volume of MBN-101 required to achieve a thin coat of the relevant structures within the infected osteosynthesis site will be applied. Following application, OR personnel will record in the CRF the volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

4 STUDY POPULATION

The study population will include 36 male or female adults who meet all of the study entry requirements.

4.1 Inclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "YES" answer (unless not applicable):

1. Patients who:
   - have had operative fracture fixation of the upper extremity (AO/OTA class 15, 11-13, 21-23), lower extremity (AO/OTA class 31-34, 41-44, 81, 82) or pelvis (61, 62), **or** have undergone arthrodesis
   - are diagnosed with an apparent fracture site infection within one year of their last surgical intervention and have at least one of the following:
     - Elevated ESR above the upper limit of normal
     - Elevated CRP above the upper limit of normal
     - Draining wound / sinus tract
     - Positive culture from site of prior surgery by aspirate or other modality
     - Local erythema or induration at the site of prior surgery
     - Exposed hardware
     - Periosteal reaction on x-ray
     - Loose or broken hardware
   - require revision surgery with or without removal and replacement of existing hardware
2. Male or female ≥18 and <75 years of age at the time the ICF is reviewed and signed
3. Patients receiving or anticipated to receive systemic antibiotic therapy as per institution's standard of care
4. Patients requiring postoperative hospitalization for at least 48 hours after revision surgery
5. Female subjects of childbearing potential, defined as not surgically sterile or at least 2 years postmenopausal, must agree to use one of the following forms of contraception from screening through the Week 24 visit: hormonal (oral, implant, or injection) begun >30 days prior to screening, barrier (condom, diaphragm with spermicide), intrauterine device (IUD), or vasectomized partner (6 months minimum)
6. Male participants must meet at least one of the following specifications and they must ensure their female sexual partner complies with the contraception requirements:
   - Be sexually abstinent from Baseline through Week 24
   - Be > 6 months post-vasectomy
   - Agree to use a condom with spermicide from Baseline through Week 24
7. Have read and signed the Informed Consent Form (ICF) after the nature of the study has been fully explained
8. Be willing and able to provide authorization for the use and disclosure of personal health information in accordance with Health Insurance Portability and Accountability Act (HIPAA)

4.2 Exclusion Criteria

To be eligible for this study, each of the following criteria must be satisfied with a "NO" answer (unless not applicable):

1. Patients who are no longer hardware dependent or are definitively treated for their infection by hardware removal
2. Greater than one year time lapse from last operative procedure
3. Patients with a previous revision surgery at the site
4. Patients with multiple, non-contiguous fracture site infections
5. Pathologic fracture (not including osteoporosis)
6. Patient requires immunosuppressive therapy (Topical or inhaled corticosteroids are permitted)
7. Serum creatinine, ALT, AST or Alkaline Phosphatase >1.5 times the upper limit of the normal range of the local testing laboratory
8. Absolute neutrophil count <1000
9. Patients without definitive soft-tissue coverage over the surgical site at time of study product administration
10. Any condition that has required treatment with any other bismuth containing compound within the last 2 weeks (i.e., Kaopectate or Pepto-Bismol)
11. Participation in an investigational trial to evaluate pharmaceuticals or biologics within the past 3 months
12. Individuals undergoing surgical treatment for more than one infected fracture
13. Known allergy to metals or materials comprising the orthopedic hardware, bismuth and/or MBN-101 excipients (methylcellulose, Tween 80 (polysorbate 80))
14. Patients who are pregnant, lactating, or female patients who have a positive serum hCG as determined by laboratory testing
15. Immunocompromised due to illness or organ transplant
16. History of chronic or recurrent infections (≥ 3 infections at the same site within 12 months)
17. History of any type of cancer (excluding non-melanomatous localized skin cancer or completely excised and cured carcinoma-in-situ of uterine cervix)
18. Poorly controlled diabetes mellitus
19. History of medical noncompliance
20. Other medical conditions which, in the opinion of the Principal Investigator, would jeopardize the safety of the study subject or impact the validity of the study results.
21. Current incarceration

5 SUBJECT ENROLLMENT

Potential patients will undergo screening medical history and physical examination. If they are deemed appropriate candidates for study, they will be asked to provide appropriate informed consent. After informed consent is signed additional baseline evaluation will be performed, including but not limited to clinical laboratory assessment, radiographic evaluation, electrocardiogram (ECG) and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study. Randomization will occur using an IVRS system immediately after a patient has met all study entry criteria.

5.1 Medical History

Medical history and demographic data, including gender, date of birth, ethnicity, and race will be recorded.

5.2 Physical Exam

Each patient will receive a physical examination (head, ears, eyes, nose, throat, chest, heart, abdomen, and skin) including vital signs, weight (kg), and height (cm). Each patient will also receive a detailed evaluation of the index fracture site and involved extremity.

5.3 Studies

Following signing of the informed consent form, each patient will undergo all screening evaluations as provided in the Table of Study Events. These evaluations should be completed no more than 2 weeks prior to surgery date; after a screened subject is confirmed to be eligible for the study, this qualified subject will be randomized prior to surgery to receive the investigational product.

5.4 Laboratory Tests at Screening Visit

Hematology	White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6 STUDY PROCEDURES

See the Table of Study Events outlining study procedures in Appendix 1. For the study site visit at Week 2, a visit window of ± 4 days will be allowed; for study site visits Weeks 6 – 24, a visit window of ±8 days will be allowed.

6.1 Description of Study Procedures

6.1.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, including but not limited to past medical history, past surgical history, current medications, allergic history and psychosocial history.

6.1.2 Physical Exams

A comprehensive physical examination will be performed on all patients at Screening, including assessments of height, weight, head, ears, eyes, nose, throat, chest, heart, abdomen, and skin. Interval physical exams will be performed at all subsequent study visits according to the schedule in Appendix 1 and will focus on the surgical site, with assessments of local erythema, induration, drainage and degree of healing.

6.1.3 Vital Signs

Vital signs include blood pressure, pulse, respiratory rate and body temperature; vital signs will be assessed according to the schedule in Appendix 1.

6.1.4 12-lead ECG

Testing will be conducted at screening, baseline, and 48 hours. The ECG trace will be stored at the investigational sites as source document; clinical interpretation, including normal, abnormal but not clinically significant, and abnormal and clinically significant, will be determined by the investigator and reported on the CRFs.

6.1.5 Laboratory Tests

All clinical laboratory tests will be performed by the local clinical site laboratory.

Blood and urine will be collected for hematology, serology, serum chemistry, urinalysis, and pregnancy testing (as indicated) according to the schedule in Appendix 1.

Hematology	WBC, RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils, PT, PTT.
Serologies	ESR, CRP.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), AST (SGOT), ALT (SGPT), GGT, creatinine kinase (CK), calcium, magnesium, Vitamin D.
Other	Urine analysis Serum pregnancy test for women of childbearing potential

6.1.6 BisEDT (MBN-101) Blood Levels

BisEDT levels in whole blood after MBN-101 administration will be assessed with a qualified Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay method using bismuth as a surrogate for BisEDT according to the schedule in Appendix 1.

6.1.7 Pain Assessments

Pain related specifically to the surgical site (Surgical Site Pain Score) will be assessed via a Visual Analog Scale (VAS, 0-100 mm, where 0=no pain at all and 100 =the worst possible pain) utilizing a 24 hour recall at screening, baseline, Hour 48 and Weeks 2, 6, 12, and 24.

6.1.8 Patient Reported Outcomes

Patient-reported outcomes will be collected using the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA) according to the schedule in Appendix 1.

6.1.9 Microbiology

The microbiology of the index site will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2).

6.1.9.1 Specimen Collection

Samples collected will include at least one tissue site (bone when possible; eraser head size, suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the peri-implant deep tissue, and two swabs of the superficial tissue/wound closure site. One set of specimens (i.e. 1 tissue specimen, 1 deep swab, 1 superficial swab) will be placed into local site transport vessels. The duplicate set of samples will be placed into transport vessels provided by the central laboratory (International Health Management Associates, Inc; IHMA). One set of specimens will be delivered to the local site microbiology laboratory using the standard storage and transport procedures employed at each site. The duplicate set of specimens will be transported to IHMA. The details regarding specimen packaging, transport, and processing will be described in the central laboratory study manual that will be provided to each study site.

6.1.9.2 Specimen Processing: Local Laboratory

The tissue site sample and swabs will be analyzed immediately in the local laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level and susceptibility testing) per the standard methods of each local site microbiology laboratory. Following isolation and identification of each microorganism, the susceptibility of each to one or more standard antibacterial agents (but not including MBN-101), as specified by

the surgeon or per local site standard procedures, will be determined. It is recognized that the local site laboratory may be using one of a variety of susceptibility test methods, and therefore, the susceptibility result may be reported as a specific Minimal Inhibitory Concentration (MIC) value or as a categorical result (i.e. Susceptible, Intermediate or Resistant). Susceptibility to MBN-101 will not be determined at the local site laboratory; the susceptibility results determined locally are for the use of the surgeon in the care of the patient. All Gram stain and microbiology results from the local site laboratory will be recorded on the CRF.

6.1.9.3 Specimen Processing: IHMA (Central Laboratory)

The tissue site sample and swabs will be analyzed immediately in the central laboratory for the presence of infectious pathogens by Gram stain and microbiological culture for both aerobic and anaerobic bacteria. The specimens will be processed (primary isolation, identification to species level, and susceptibility testing) per the central laboratory methods, as described in the central laboratory study manual.

Following identification, the susceptibility of the isolates to MBN-101 and comparator agents will be determined using either the reference broth microdilution method (for aerobic/facultative bacteria) or the reference agar dilution method (for anaerobic bacteria) as detailed by the Clinical and Laboratory Standards Institute (CLSI). Susceptibility tests will be quality-controlled as detailed by CLSI using reference quality control strains and previously-established ranges for MBN-101 and comparators. Susceptibility testing will be conducted with batches of study isolates as each study cohort is completed. Patient and specimen identifier and microbiology results will be recorded in the database. The bacterial isolates recovered from the specimens will be propagated and archived at the central laboratory.

6.1.9.4 Integration of Local Site Laboratory and Central Laboratory Primary Isolation and Identification Results

There may be instances where the organism(s) isolated from a given specimen may be different at the local site laboratory and the central laboratory. In this instance, the central laboratory will first confirm the identification of the archived isolate. If the identification is confirmed, the pathogens present in the specimen will be defined as the total number of pathogens recovered by the local site laboratory and the central laboratory. For example, if the local site laboratory were to recover *S. aureus* and the central laboratory recovered *S. aureus* and *P. acnes*, the specimen would be defined as containing both *S. aureus* and *P. acnes* following merger of the data.

It is possible that local site laboratory may perform susceptibility tests with agents that are also present in the central laboratory test panel. The MIC value (and interpretation) provided by the central laboratory supersedes the local site determination for the purposes of clinical trial data analysis and inclusion in the Clinical Study Report. The local site susceptibility test results are for use by the surgeon in the local care of the patient; however, these data will also be captured in the study database.

6.1.10 Radiographic Evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24. Radiographs (at least two orthogonal views) will be reviewed for union, interval callus formation, loss or change in reduction, and hardware failure.

6.1.11 Surgical Site Signs and Symptoms Evaluation

The following signs and symptoms of the surgical site will be evaluated:

- local erythema extending beyond suture material or staples
- induration
- drainage from surgical incision
- degree of healing of the surgical incision

Patients with non-healing or worsening status of their surgical site may be considered for additional standard of care treatment, but should be encouraged to remain in the study in order to complete study evaluations. In case additional treatment is given, the start/stop dates and dose regimen of the new treatment will be recorded on the CRFs.

6.2 Screening and Baseline Assessments

Patients who meet initial screening criteria by medical history and physical exam will be offered participation on study. If the patient agrees to participate, they will be asked to provide written informed consent, after which additional evaluation may be performed, including but not limited to clinical laboratory assessments, electrocardiogram (ECG), radiographic evaluation and pregnancy test if applicable. Only patients with an acceptable medical history and physical exam, ECG without clinically significant abnormalities, and laboratory results within acceptable limits will be randomized in the study prior to surgery.

6.3 Randomization Procedure

Subjects will be randomized in a 3:1 (active:placebo) ratio up to 24 hours prior to surgery via a central randomization scheme. The central randomization will be accomplished using an IVRS system linked to study drug treatment kits securely stored under appropriate conditions at the study site pharmacy. The randomization number assigned to a subject will be captured on the CRF. The specific study drug treatment kit will be utilized by the study pharmacist or other qualified individual to perform the appropriate dilution of drug product under sterile conditions into a vial to be transported to the operating room. Verification of study patient and study drug treatment kit will take place in the operating room prior to treatment with the investigational product. The appropriately diluted drug product must be administered within 8 hours of preparation.

6.4 Unblinding Procedure

This study is a single blind study; however, knowledge of treatment assignment should remain limited to those directly involved with the patient's surgical procedure and other individuals on a need to know basis. In order to provide unblinding data in an emergency in the absence of

individual privy to treatment assignment, sites will be provided with a numbered unblinding envelope corresponding to each numbered test kit the site is delivered. These envelopes will be securely stored in the pharmacy. The unblinding envelope specifies the treatment group of the kit and should only be opened when absolutely necessary. For treatment of adverse events, it should be assumed that the patient has received study drug, thus avoiding the need to unblind the patient. However, in the event the Investigator determines the randomization assignment information is required to treat the patient for an adverse event, the Medical Monitor should be contacted. If the Medical Monitor cannot be reached, the blind may be broken by opening the envelope. The site should document the unblinding process in the patient's source documents.

6.5 Investigational Product Administration

6.5.1 Surgical Site Identification

Individuals undergoing surgical treatment for more than one infected fracture are excluded from participation in this study.

6.5.2 Study Drug Administration

On the day of surgery, randomized subjects will receive the investigational product during their surgical procedure. Investigational product is only administered during the surgical procedure by the operating surgeon; therefore, 100% compliance with investigational product administration is assured. The operating surgeon will record the location of investigational product administration in the operative note and on the CRF. All other details of the surgical procedure, including the dimensions and approximate surface area of the surgical incision, will be recorded in the operative note.

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 ("Stock Formulation") rubber stoppered and sealed.
2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed ("Diluent").
3. Sterile polypropylene syringe (1 mL) and 15 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.
4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.
5. Alcohol wipes
6. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort ("Treatment Dose").

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.
2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.
3. Alcohol wipes
4. Detailed directions-for-use (DFU) for preparation of the target dose ("Treatment Dose")

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

The Treatment Dose for each patient will be prepared at the clinical site under sterile conditions no more than 8 hours prior to dose administration using the MBN-101 Stock Formulation and Diluent provided in the Drug Product Kit according to the DFU provided. The Treatment Dose will be provided to the surgeon in a vial from which an 8 mL volume will be drawn up in the operating room into a sterile 10 mL syringe. Surgeons will be instructed to use sufficient volume (up to the full 8 mL provided) to sparingly coat the bone at the site of infection, the exposed surfaces of any retained hardware, and the surfaces of any new hardware to be implanted. Following application, OR personnel will record in the CRF the actual volume (in tenths of milliliters) of any unapplied Treatment Dose remaining in the syringe.

Application to retained hardware: Access to infected wound surfaces and implanted orthopedic hardware will be provided by surgical intervention. The surgeon will use a 10 mL syringe to apply, followed by a gloved finger to spread, a thin layer of the investigational product directly onto all accessible surfaces of the orthopedic hardware, as well as surrounding bone. Other wound surfaces will not be coated, resulting in administration of the investigational product to only a limited area of the wound.

Application to new hardware: Prior to implantation, the surgeon will use a gloved finger to sparingly coat all hardware with a thin layer of the investigational product. Following surgical implantation of the coated hardware, a thin layer of the investigational product will be applied to surrounding bone using a gloved finger. Administration of the investigational product will therefore be limited to target areas, specifically orthopedic hardware and the immediately adjacent, accessible bone (osseous) surfaces.

In all cases, the minimum amount of MBN-101 required to coat the relevant structures within the infected osteosynthesis site should be used. The suspension should be applied in a thin layer to cover all affected areas of bone as well as any exposed hardware. Direct application to muscle and other soft tissues should be avoided. The approximate area of the osteosynthesis site should be calculated based on the length of exposed bone multiplied by the width of bone exposed; the surface area of the hardware should be added to this value to derive the approximate area of the osteosynthesis site. Recommended maximum volumes of MBN-101 for various areas of the osteosynthesis site are provided in the table below.

Area of Osteosynthesis Site	Recommended Volume of MBN-101
25 cm^2	0.5 mL
50 cm^2	1.0 mL
75 cm^2	1.5 mL
100 cm^2	2.0 mL
125 cm^2	2.5 mL
150 cm^2	3.0 mL
175 cm^2	3.5 mL
200 cm^2	4.0 mL
225 cm^2	4.5 mL
250 cm^2	5.0 mL
275 cm^2	5.5 mL
300 cm^2	6.0 mL
325 cm^2	6.5 mL
350 cm^2	7.0 mL
375 cm^2	7.5 mL
400 cm^2	8.0 mL

The table shows the specified volume of MBN-101 to be applied based on the area (in cm^2) of the target infected osteosynthesis site. By following these application requirements, the administered doses will be:

- Cohort 1: 25 µg/mL MBN-101 = 0.5 µg/cm^2
- Cohort 2: 75 µg/mL MBN-101 = 1.5 µg/cm^2
- Cohort 3: 250 µg/mL MBN-101 = 5.0 µg/cm^2

The use of drains and vacuum assisted closure devices at the site of hardware will be allowed per surgeon preference. The drainage volume over the first 24 hours or prior to discontinuation of the drain will be assayed for BisEDT.

6.6 Study Assessments

Each patient enrolled in the study will have an assessment at 48 hours following surgery and at Weeks 2, 6, 12, and 24. Study assessments will be performed according to Section 6.1 and Appendix 1. The Week 24 assessment will be the final assessment for adverse events. Any adverse events ongoing at the Week 24 assessment will be followed until resolution of the event or stabilization of the condition.

6.7 Safety Monitoring

Patients will be instructed to inform the Investigator and/or a member of the study staff of any adverse events that occur at any time during the study. Patients will be asked a general health question at each clinic visit to identify changes in the state of their health since their last study visit.

6.8 Study Monitoring

The Sponsor or the Sponsor's designee will provide training on the protocol for the study site personnel prior to giving the site permission to begin enrollment. Once the enrollment and dosing phases of the study have begun, the Sponsor or the Sponsor's designee will visit the site to monitor the clinical conduct of the trial, the adequacy of study documentation and compliance with safety reporting. Clinical site monitoring will continue on a periodic basis until the site has been closed or all patients at the site have completed the study. The frequency of monitoring visits may vary depending on enrollment at the study site.

6.9 Patient Withdrawals

Patients will be free to withdraw at any time for any reason, or they may be withdrawn if necessary to protect their health and safety or the integrity of the study. Any patient that is randomized but not treated will be replaced. The final report will include reasons for withdrawals. As this study involves a single administration of study drug, significant patient withdrawals are not anticipated.

All efforts should be made to have subjects complete the Week 24 (end of study) procedures prior to withdrawal from the study.

6.10 Individual Patient Stopping Rules

Patients who develop adverse events at any time during the study will be treated appropriately according to standard of care. Adverse events will be assessed and followed as described in Section 9. As this is a single dose study with the investigational product applied directly into a surgical site, specific stopping rules for individual patients have not been established.

6.11 Study Stopping Rules

Patient enrollment on study will be stopped if the number of individual patients experiencing possibly-related or probably-related adverse events $\geq$ Grade 2 exceeds 2 in any dose cohort or if there is any death on study. Should the study stopping rules be invoked, all safety data on all patients will be assembled for review by the Data Review Committee (DRC). The study will not resume until this information has been reviewed by the DRC and the Sponsor, discussed with the FDA, and concurrence reached with the FDA as to the resumption of the study.

7 CONCOMITANT MEDICATIONS

All patients will received their usual concomitant medications for any underlying medical conditions without interruption. Efforts should be made to maintain all concomitant medications at a stable dose for the duration of the 24 week study. Patients will also receive standard of care treatment for their postoperative fracture site infection that includes systemic antibacterial treatment per prevailing standard of care guidelines (i.e., national, institutional, or physician preference) and other indicated postoperative medications, including pain medications. All concomitant medications will be recorded in the CRF. Pain medication usage, including medication, dose, route, day and time of administration, will be recorded on a separate CRF. Use of any other bismuth-containing compounds is prohibited from Screening through Week 4.

8 STUDY ENDPOINTS

8.1 Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

8.2 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.
- Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.
- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm).
- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.
- Change in serologic markers CRP and ESR at each post baseline time point.

8.3 Pharmacokinetic Endpoint

8.3.1 Sample Collection and Handling

Blood samples will be obtained to measure blood concentrations of Bismuth (Bi) as a surrogate for BisEDT. A pre-dose sample will be collected prior to administration of the investigational product. Blood will be drawn for PK samples at 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4), and 336 (Day 14) hr after application of MBN-101 to the surgical site. Documentation stating the exact time of blood sampling (5 mL per timepoint) in relation to the time of study drug administration will be collected and provided.

Primary and back-up blood samples will be stored at -70°C.

8.3.2 Assay Methodology

Bismuth (Bi) levels, a surrogate for BisEDT, will be assayed in whole blood using a validated inductively coupled plasma mass spectrometry (ICP-MS) assay performed by Medpace Bioanalytical Laboratories (Cincinnati, Ohio). The samples are prepared for analysis by digestion with 2% nitric acid followed by dilution into 2% Tetramethyl Ammonium Hydroxide (TMAH) with 0.02% Triton X-100, and subsequent quantitation of Bi by ICP-MS analysis.

A detailed method description, including validation, calibration and quality assurance procedures, will be included in the analytical report which will be part of the Final Study Report.

8.3.3 PK Analysis

Bismuth (Bi) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.3 or higher, using an extravascular administration model and actual sampling times. The following PK parameters will be derived from blood concentrations of Bi using extravascular noncompartmental PK analysis:

T_{max}	Time to maximum observed concentrations of Bi
C_{max}	Maximum observed concentrations of Bi
AUC_{0-t}	Area under the Bi concentration vs time curve from time zero to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the Bi concentration vs time curve from time zero and extrapolated to infinity.
$T_{1/2}$	The apparent half-life of Bi after extravascular administration of BisEDT
CL/F	Apparent clearance after extravascular administration of BisEDT
Vz/F	Apparent volume of distribution after extravascular administration of BisEDT

9 ADVERSE EVENTS

An adverse event is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes) that occurs after initiation of the investigational study whether or not it is considered to be related to the investigational product. A worsening of an existing medical condition is one that was present at Day 1 (e.g., diabetes) and became more severe, more frequent, or increased in duration during investigational product treatment. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

All adverse events (AEs) will be recorded throughout the study, beginning when the subject signs the Informed Consent Form.

A treatment-emergent adverse event (TEAE) is defined as any AE starting after the first dose of the investigational agent or placebo is administered. If the AE is present prior to the administration of the first dose of the investigational agent or placebo but increases in severity, it will also be considered a TEAE.

Treatment-emergent adverse events will be recorded beginning with the first exposure to investigational product and continuing until the subject is discharged from the study due to completion or early termination.

Adverse events will be categorized as local adverse events and as systemic adverse events.

The Principal Investigator, or medically qualified designee, must completely and promptly record each AE on the appropriate CRF. The Principal Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually. Once a diagnosis is established, the CRFs should be updated with the final diagnosis.

Any adverse event ongoing at the time of study completion will be recorded and followed-up according to the safety procedures described in the following sections.

9.1 Reporting and Following Adverse Events

All AEs encountered during the study will be reported on the AE page of the CRF in a timely manner. All AEs should be followed in accordance with good medical practice until resolved or fully characterized.

Serious adverse events: Section 9.4 presents the definition and reporting obligations for Serious Adverse Events (SAEs). After the initial report, a follow-up SAE Report Form should be filled out and sent to Medpace Clinical Safety within 24 hours. Updates are to be provided by the site as soon as relevant information (especially regarding outcome) is available.

9.2 Severity

The Investigator must indicate the severity of the adverse event in the description of the adverse event. A severity category of mild, moderate, or severe, as defined below, should be determined and entered on the Adverse Events page of the Case Report Form (CRF).

- Mild (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's baseline functioning level. It may be an annoyance.
- Moderate (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of baseline functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- Potentially Life-Threatening (Grade 4): Symptoms cause inability to perform basic self-care functions immediately necessary to sustain life or require medical or operative intervention to prevent permanent impairment, persistent disability or death.

9.3 Relationship to Clinical Trial Material

The Investigator must document their opinion of the relationship of the event to the investigational product as follows:

- NONE: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or other cause they believe to be the cause of the adverse event.
- UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure or cause they believe may have contributed to the adverse event.
- POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.
- PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

9.4 Serious Adverse Events

To report SAEs in a timely manner, monitor subject care, and to fulfill regulatory requirements, SAEs (regardless of their relationship to the investigational product) must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event by the Investigator or members of the study staff.

9.4.1 Definition

SAEs are defined as those adverse events that meet any of the following criteria:

- Life threatening, that is, any event that, in the opinion of the Investigator, poses an immediate risk of death from that event
- Results in death
- Results in or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect

In addition, events which may not meet these criteria, but, in the opinion of the Investigator, are very unusual, potentially serious, or require medical or surgical intervention to prevent one of the outcomes listed above, should also be reported in the same manner as events which meet the serious adverse event criteria.

9.4.2 Reporting

Serious AEs require immediate reporting (within 24 hours of the site's knowledge of the event) to Medpace Clinical Safety whether or not the Investigator believes that the experience is related to the investigational product. A completed SAE CRF signed by the Investigator must be faxed to Medpace Clinical Safety. Criteria for documenting the relationship to study product and severity will be the same as those previously described in Sections 9.2 and 9.3.

SAEs must also be reported by the study site to the responsible IRB immediately. The Investigator is responsible for complying with the local IRB regulations regarding the reporting of AEs.

Reporting of a suspected SAE should not be delayed in order to obtain additional information. Any additional information, if collected, can be reported as a follow-up to the initial report. Any SAE that occurs at any time during the study, whether or not related to the investigational product, must be reported to Medpace Clinical Safety.

All possibly- and probably-related serious adverse events must be followed until the outcome is known or the event is otherwise explained. All serious adverse events that are not resolved at the end of the study are to be followed until resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

9.5 Pregnancies

Pregnancies occurring after the first dose of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The investigator should complete the pregnancy report form and fax it to Medpace Clinical Safety within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report form should be completed and submitted via fax to Medpace Clinical Safety. Spontaneous abortions should always be reported as SAEs. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

9.6 Data Review Committee (DRC)

An independent Data Review Committee will be established to review unblinded clinical trial data prior to dose escalation and on an as-needed basis. This DRC will include a Statistician, an orthopedic surgeon and a Medical Safety Officer. An independent statistician, serving as a non-voting member to the DRC, will provide data preparation support to the DRC.

The primary role of the DRC will be to assess unblinded safety data on all patients in a dose cohort completing the Week 6 visit, and to determine whether dose escalation should occur. The DRC may recommend dose escalation, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

The DRC will also be asked to assess unblinded safety data on an as-needed basis. The Chair of the DRC will be informed by Microbion Corporation of the potential need for ad hoc meetings and will coordinate any ad hoc meeting with the remainder of the DRC members. The DRC may recommend no modifications to the study, specific modification to the study protocol, or study termination. In each case, the recommendation will be provided to Microbion Corporation for final disposition.

A formal DRC Charter will be prepared and finalized prior to study enrollment. The DRC will meet prior to initiation of the clinical trial in order to review, revise and reach concurrence on the content of a DRC charter. The draft charter will be provided by Microbion Corporation to the DRC prior to the initial meeting.

10 INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Study Drug

Three successive doses (0.025, 0.075, or 0.25 mg/mL w:v) of MBN-101 (up to 8 mL, for a dose of up to 0.2, 0.6, or 2.0 mg, respectively) or placebo (diluent) will be studied.

The MBN-101 Kit for administration to patients randomized to treatment with MBN-101 will contain the following:

1. One 5 mL clear glass vial of MBN-101 containing 2.5 mL of sterile 2.5 mg/mL MBN-101 (“Stock Formulation”) rubber stoppered and sealed.

2. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed (“Diluent”).

3. Sterile polypropylene syringe (1 mL) and 15 gauge needle(s) for preparation of Treatment Dose by dilution of Stock Formulation with Diluent in the clinical site pharmacy.

4. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product (Treatment Dose) in the operating room.

5. Alcohol wipes

6. Detailed directions-for-use (DFU) for preparation of the specified concentration of drug product for each cohort (“Treatment Dose”).

The Placebo Kit for administration to patients randomized to treatment with Placebo will contain the following:

1. One 20 mL clear glass vial of MBN-101 diluent containing 9 mL sterile diluent solution rubber-stoppered and sealed.

2. Sterile 10 mL polypropylene syringe and 14 gauge needle for administration of diluted drug product in the operating room.

3. Alcohol wipes

4. Detailed directions-for-use (DFU) for preparation of the target dose (“Treatment Dose”

Additionally each clinical site will be provided with a vortex mixer designated to be used for dilution and resuspension of Stock Formulation and of the final Treatment Dose preparation.

10.2 Study Drug Packaging and Labeling

All shipments of the investigational product will be accompanied by an inventory form. The contents of the shipments should be inventoried immediately upon receipt and confirmation of inventory shall be performed according to instructions provided by the clinical supplies distributor.

The following information will be provided with each shipment of study drug: Sponsor name and contact information (telephone number), study protocol number, description of the contents of the container, conditions for storage, and a statement regarding the investigational (clinical trial) use of the study drug.

Each package unit of the investigational product will be labeled with the following information: Sponsor name, study protocol number, description of the contents, a statement regarding the investigational (clinical trial) use of the study drug, and a packaging lot number.

10.3 Study Drug Storage

The investigational product should be kept in a limited-access area at 15-30 degrees C at the study site.

10.4 Study Drug Accountability

It is the responsibility of the Principal Investigator or his/her designee at each site to ensure that all investigational product quantities received at the site will be inventoried and reconciled throughout the study and the result recorded on the drug accountability form maintained in the study file.

10.5 Study Drug Handling and Disposal

Only qualified study personnel familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the handling of pharmaceutical agents.

The study monitor will verify that the disposition of all investigational materials at the trial site is in accordance with Sponsor specifications and applicable regulatory requirements. Requirements for disposition of used, unused, damaged/quarantined, and expired investigational materials will be provided to applicable site personnel. The study monitor will confirm associated documentation is filed in the Investigator site file and copies retrieved for the Sponsor or Sponsor-designee Investigator File.

Used, damaged, and expired investigational materials will be destroyed as directed by the Study Monitoring Plan. Specific instructions of on-site destruction, return of materials to a 3rd party vendor, or any other mechanism of removal of the investigational materials from the site will be detailed in the Study Monitoring Plan.

The study monitor will not take possession of investigational materials. Any deviations from this process must be approved in advance by the Sponsor or Sponsor-designee.

11 GENERAL CONSIDERATIONS

11.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans ("Ethical Principles for Medical Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

11.2 Institutional Review Board

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at each clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse product per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per their Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

11.3 Informed Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollment, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

11.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

11.5 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Signed and dated Investigator Agreement
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

11.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the U.S. Food and Drug Administration (FDA).

11.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, Microbion will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance
- Maintain SOPs for using these systems
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)
- Maintain a security system that prevents unauthorized access to the data
- Maintain a list of the individuals who are authorized to make data changes
- Maintain adequate backup of the data
- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)
- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject
- Microbion will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved and/or where it intends to apply for approval
- Specific essential documents will be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirement(s) or if needed by the Sponsor.
- Microbion will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed

11.8 Use of Information and Publication

All information concerning BisEDT, MBN-101, MBN-101 diluent, Microbion operations, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Sponsor to the investigator and not previously published, is considered confidential and remains the sole property of Microbion Corporation. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without the Sponsor's written consent.

The investigator understands that the information developed in the clinical study will be used by Microbion in connection with the continued development of MBN-101 and BisEDT, and thus may be disclosed as required to other clinical investigators or government regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the Sponsor with all data obtained in the study.

Any publication or other public presentation of results from this study requires prior review and written approval of Microbion Corporation. Draft abstracts, manuscripts, and materials for presentation at scientific meetings should be provided to the Sponsor at least 30 working days prior to abstract or other relevant submission deadlines. Authorship of publications resulting from this study will be based on generally accepted criteria for major medical journals.

11.9 Independent Medical Monitor

In accordance with US Army Medical Research and Materiel Command (USAMRMC) and Department of Defense (DOD) requirements, an independent medical monitor will be identified. The independent medical monitors should be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer management and safety. Medical monitors must be independent of the investigative team and possess sufficient educational and professional experience to serve as the volunteer advocate. Depending on the nature of the study, the medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor provides an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the Office of Research Protections (ORP). The medical monitor may be assigned to discuss research progress with the PI, interview volunteers, consult on individual cases, or evaluate adverse event reports. The medical monitor must promptly report discrepancies or problems to the IRB and the ORP. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor report.

12 STATISTICAL ANALYSIS METHODOLOGY

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

In general, safety and efficacy data will be summarized with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.

Data from all subjects randomized and treated with placebo in each cohort will be pooled for the analysis; data from the subjects randomized and treated with MBN-101 will be presented by dose as well as combined.

Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by treatment group and time point.

12.1 Efficacy Endpoints

Clinical activity of locally administered MBN-101 will be assessed by:

- Proportion of treatment failures. A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

Cumulative number of serious interventions (as defined in Section 12.6.2) at Week 24.

- Time to first serious intervention, time to readmission and time to reoperation, exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site.
- Number of subjects undergoing removal of stabilizing orthopedic hardware due to a healed fracture site by Week 24.
- Time to removal of orthopedic hardware due to a healed fracture site.
- Incidence and intensity of surgical site signs and symptoms, including local erythema, induration, drainage and degree of healing.
- Change from baseline in patient-reported outcomes at each post baseline time point. Patient-reported outcomes will include Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA).
- Surgical site pain score and change from baseline in pain score at each post baseline time point. Pain intensity at the surgical site will be assessed by Visual Analog Scale (VAS, 0-100 mm).

- Findings of microbiology evaluations at the surgical site. Microbiologic success is assessed by clearance of infection.
- Findings of radiographic evaluations at the surgical site. Radiographic success is assessed by healing on radiologic examination.

12.2 Changes in serologic markers CRP and ESR at each post baseline time point.Safety Endpoints

The safety and tolerability of locally administered MBN-101 will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology, and urinalysis).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams.
- Clinical findings of 12-lead electrocardiograms.
- Change in microbiology status.

12.3 Sample Size Considerations

Sample size for this study was chosen empirically for the purpose of the study without any formal statistical hypothesis to be tested.

Nine subjects will receive active drug in each dose cohort. With 9 subjects receiving active drug, the probability of observing at least one of 9 subjects in a dose cohort exhibiting an AE of a specific type is 95% if the true background incidence rate for that event-type were approximately 0.283 or greater. Hence, if an AE of a specific type is not observed in the study in a sample size of 9 subjects receiving active study drug at a given dose, one would “rule-out” this AE as having an incidence rate of more than 0.283 with 95% confidence.

Similarly, if a rare event of interest is not reported in the 27 subjects treated with the active study drug, one could conclude that the rate of the event would be 0.105 or less with 95% confidence.

12.4 Analysis Datasets

The following analysis datasets will be identified for the purpose of analysis:

Screened Subjects: All subjects who signed informed consent for the study will be included in the Screened Subjects analysis set.

Randomized Subjects: All subjects who were randomized in the study will be included in the Randomized Subjects analysis set. A randomized subject may or may not be treated with the assigned study drug. This is the classic Intent-to-Treat (ITT) analysis set.

Treated Subjects: All subjects who received any amount of study drug (active or placebo) will be included in the Treated Subjects analysis set. All treated subject will be included in the safety analysis. This analysis set is also referenced as the Safety Analysis Set.

Modified Intent-to-Treat (mITT) Subjects: the ITT subjects who received any amount of study (active or placebo) and have at least one post-treatment assessment will be included in the mITT analysis set. The mITT analysis set is the primary dataset for efficacy analysis.

PK Concentration Subjects (i.e., PK Concentration Population): The PK Concentration Population will include all subjects with any measurable bismuth blood concentrations.

PK Evaluable Subjects (i.e., PK Evaluable Population): The PK Evaluable Population will include all subjects providing sufficient measurable bismuth blood concentrations to facilitate determination of PK parameters.

In the event that a subject received study drug treatment that is not the assigned/randomized treatment group, the subject has a major protocol deviation. The subject, however, will be included in the analysis for safety and efficacy in the actual treatment group received.

Subjects randomized but not treated for any reason will be included in the disposition tabulation and will be excluded from the safety and efficacy analysis.

12.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, randomized, treated, subjects that completed the study and reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), surgery site characteristics (type of injuries, type of fraction fixation, surgical site location), microbiology analysis results, time from previous surgery to study enrollment, and type of previous treatment), current surgery characteristics (area of the osteosynthesis site, with or without hardware replacement, etc.), and medical history.

Disposition summary will be based on randomized treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

12.6 Efficacy Analysis

12.6.1 Treatment Failure

A treatment failure is defined as a subject with non-healing or worsening status of their surgical site requiring serious intervention by Week 24.

The number of subjects meeting treatment failure criteria during the study will be tabulated by treatment group. If data permit, the difference in proportion of treatment failures between the groups and the corresponding 95% confidence intervals for the difference will be presented. Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived. Dose-response assessment may be performed.

12.6.2 Incidence of Serious Interventions

All interventions meeting the following criteria are serious interventions:

- Initiation of antibiotic treatment in patient not previously on antibiotics
- Change in the route of antibiotic administration from oral to intravenous for escalation of treatment
- Change in the type or dosage of antibiotic drug for escalation of treatment
- Readmission (exclusive of readmissions associated with a healed fracture site)
- Reoperation (exclusive of reoperations associated with a healed fracture site)

The number of subjects with at least one serious intervention (exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site) and total number of serious interventions will be tabulated for the following periods:

- duration of the study;
- within the first 4 weeks after the surgery;
- from week 4 to week 8
- from week 8 to week 12
- from week 12 to week 24

The difference between the placebo arm and the active arms will be evaluated as follows if data permits: Fisher's exact test may be used to test the difference between the groups; difference in rates and 95% confidence intervals for the relative risk will be derived.

Similar summaries will be prepared for incidence of re-admission and incidence of re-operation. Time elapsed between the initial hospital discharge to hospital readmission and between the operations will be derived and tabulated.

12.6.3 Time to First Serious Interventions

Time to the first serious intervention exclusive of serious interventions, readmissions, and reoperations associated with a healed fracture site will be derived. Time to first serious intervention will be calculated as the number of study days from the study surgery date to the event onset date plus 1. Subjects who do not have any serious intervention will be censored to the last observation date.

No formal inferential statistics will be carried out for this endpoint.

12.6.4 Subjects Undergoing Removal of Stabilizing Orthopedic Hardware

The number of subjects undergoing removal of hardware due to a healed fracture site and the time to removal of hardware due to a healed fracture site will be derived. Time to removal of hardware will be calculated as the number of study days from the study surgery date to the event date plus 1.

12.6.5 Surgical Site Signs and Symptoms

The surgical site will be examined for local erythema, induration, drainage and degree of healing.

Subjects with non-healing or worsening status of their surgical sites will be tabulated.

12.6.6 Patient-Report Outcomes

12.6.6.1 The Veterans RAND 12 Item Health Survey (VR-12) Questionnaire

The Veterans RAND 12 Item Health Survey (VR-12) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health related quality of life, to estimate disease burden and to evaluate disease-specific benchmarks with other populations. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions; physical functioning; role limitations due to physical and emotional problems; bodily pain; energy-fatigue, social functioning and mental health (Selim, 2009; Veterans Affairs website, 2014).

VR-12 total score, physical health domain score (PCS), and mental health domain score (MCS) will be tabulated by visit; change from baseline in total score, physical health domain score and mental health domain score will also be derived and tabulated.

12.6.6.2 The Short Musculoskeletal Function Assessment Questionnaire (SMFA)

The 46-item SMFA questionnaire comprises two parts: the dysfunction index with 34 items and the bother index with 12 items. The dysfunction index assesses the patients perceptions of the amount of difficulty they have in the performance of certain functions (25 items) and how often the patients have difficulty when performing certain functions (9 items). The dysfunction items are grouped into four categories: daily activities, emotional status, function of the arm and hand, and mobility. Each item has a 5-point response format (1 point for good function and 5 points for

poor function). The bother index asks the patients to assess how much they are bothered by problems in various areas of life (e.g., recreation, work, sleep and rest). These items also have a 5-point response format (1 point for not at all bothered and 5 points for extremely bothered).

The scores of the dysfunction and the bother indices are calculated by summing up the responses to the items and then transforming the scores according to the formula: (actual raw score - lowest possible raw score)/(possible range of raw score) ×100.

This transformation formula gives the final scores, which ranged from 0 to 100. The higher scores indicate poorer function. In the case of the dysfunction index, unanswered items in a category are replaced by the individual's mean score for that category, as long as more than 50 per cent of the items in that category have been answered. Substitution with the mean is not appropriate for the bother index as each item addresses a unique area of function (Swiontkowski, 1999; Short Musculoskeletal Function Assessment Injury and Arthritis Survey, www.grossortho.com/forms/injury.pdf).

SMFA results and change from baseline will be summarized by visit and treatment group with descriptive statistics.

12.6.7 Pain Assessments

Surgical site pain score utilizing a 24 hour recall will be assessed via Visual Analog Scale (VAS) (Burckhardt, 2003; Brokelman, 2012; Briggs, 1999) at baseline, and Weeks 2, 6, 12, and 24.

Change in pain from baseline will be derived; observed pain score and change from baseline will be tabulated by visit and treatment group.

12.6.8 Microbiology

Microbiology will be assessed at baseline and at the time of any subsequent surgical procedure at the index site. The presence of viable microorganisms will be determined by standard microbiological culture methods and include speciation and antimicrobial susceptibility testing of any bacterial isolates found (see flowchart, Appendix 2). Samples collected will include at least one tissue site (eraser head size; suitable for dividing into two samples) from the fracture site (if accessed) or involved tissue adjacent to the implant(s), two swabs of the infected hardware (in situ or ex vivo), two swabs of the deep infection site, and two swabs of the superficial tissue/wound closure site when possible. Duplicate specimens will be transported aerobically and anaerobically, respectively. Specimens will be processed by the local laboratory and the central laboratory as presented in Sections 6.1.9.3 and 6.1.9.2.

Microorganisms isolated from each assessment will be listed. This information may also be used to identify potential subgroups.

Microbiological success is assessed by clearance of infection. The proportion of subjects that meet the treatment success criteria per microbiologic evaluations will be identified and tabulated by each treatment group.

12.6.9 Radiographic evaluation

Radiographic evaluation will be performed at baseline and Weeks 2, 6, 12, and 24.

Radiographic success is assessed by healing on radiographic examination. The proportion of subjects that met the treatment success criteria per radiographic examination will be identified and tabulated by each treatment group.

12.6.10 Serologic Markers

Serology markers CRP and ESR will be assessed at baseline and Weeks 2, 6, 12, and 24.

Serology markers CRP and ESR have been used together with clinical signs and symptoms for periprosthetic infection diagnosis. The values for each serologic marker and the number of subjects with normal or abnormal serologic marker values at each visit will be tabulated by treatment group.

12.6.11 Subgroup Analyses for Efficacy Endpoints

If data permit, the following potential covariates will be identified and subgroup analysis may be performed for those subgroups.

1. Area of the osteosynthesis site
2. Method of fracture fixation
3. Type of infection (per identified microorganism)
4. Anatomic location of the surgery site
5. Hardware retention status (retained vs. exchanged)
6. Influence of host factors
7. Method of wound closure / wound management

12.6.12 Sensitivity Analyses for Efficacy Endpoints

The primary efficacy analysis set (mITT) will included all randomized subjects who received any amount of study drug and have at least one post-treatment assessment. In addition, in the event that a subject received treatment that is different from the assigned treatment, the subject will be included in the actual treatment received in the mITT analysis.

An ‘as assigned’ analysis will be performed on the mITT subjects as a sensitivity analysis for the primary efficacy endpoint. In this analysis, subjects who received a treatment that is not the assigned treatment will be included in the ‘assigned’ treatment group for this analysis.

12.7 Safety Analysis

12.7.1 Study Drug Exposure and Concentrations

Study drug exposure will be presented by treatment group. Descriptive statistics will be provided without any formal inferential statistics.

12.7.2 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA Version 16 or higher) will be used to classify all AEs with respect to system organ class (SOC) and preferred term. Summary of adverse events will include:

1. Treatment emergent adverse events by SOC, preferred term, and intensity
2. Treatment emergent adverse events by SOC, preferred term, and relationship to study drug
3. All AEs leading to study discontinuation by SOC and preferred term
4. All serious adverse events by SOC and preferred term

Summary tables will provide total number of events and number of subjects with the event.

The incidence and intensity of each pre-specified local AEs will be tabulated by visit and treatment groups.

12.7.3 Clinical Laboratory Tests

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to surgery will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits shift in result class from baseline to post baseline may also be tabulated.

12.7.4 12-lead ECG

ECG findings at each time point will be tabulated by treatment group without inferential statistics.

12.7.5 Vital Sign Measurements

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

12.7.6 Physical Examinations

Findings of physical examinations will be tabulated by treatment groups without inferential statistics.

12.7.7 Subgroup Analyses for Safety Endpoints

No subgroup analysis is planned for safety endpoints.

12.8 PK Analysis

Blood bismuth concentration vs. time profiles after administration of MBN-101 will be summarized for each nominal sampling time point and by dose group using descriptive statistics generated by WinNonlin. The NCA PK parameters ($AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, T_{max}, C_{max}, $T_{1/2}$, CL/F, V_z/F) will also be summarized with descriptive statistics (generated by WinNonlin) for each dose group as well as for all treated subjects in the PK Evaluable Population.

If data permit, the correlation between Bismuth concentration and efficacy and/or safety endpoints may be explored.

12.9 Interim Evaluation

Interim analyses are planned for this study for the purpose of DRC. The purpose of the interim analysis is to allow the DRC to review the data and to make recommendation to the Sponsor on whether or not the next cohort of the subjects should be studied as planned.

An analysis plan for the DRC will be prepared as an appendix of the DRC charter. The scope of the interim analyses for the DRC will be defined in this analysis plan.

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14 APPENDICES

Appendix 1: Table of Study Events

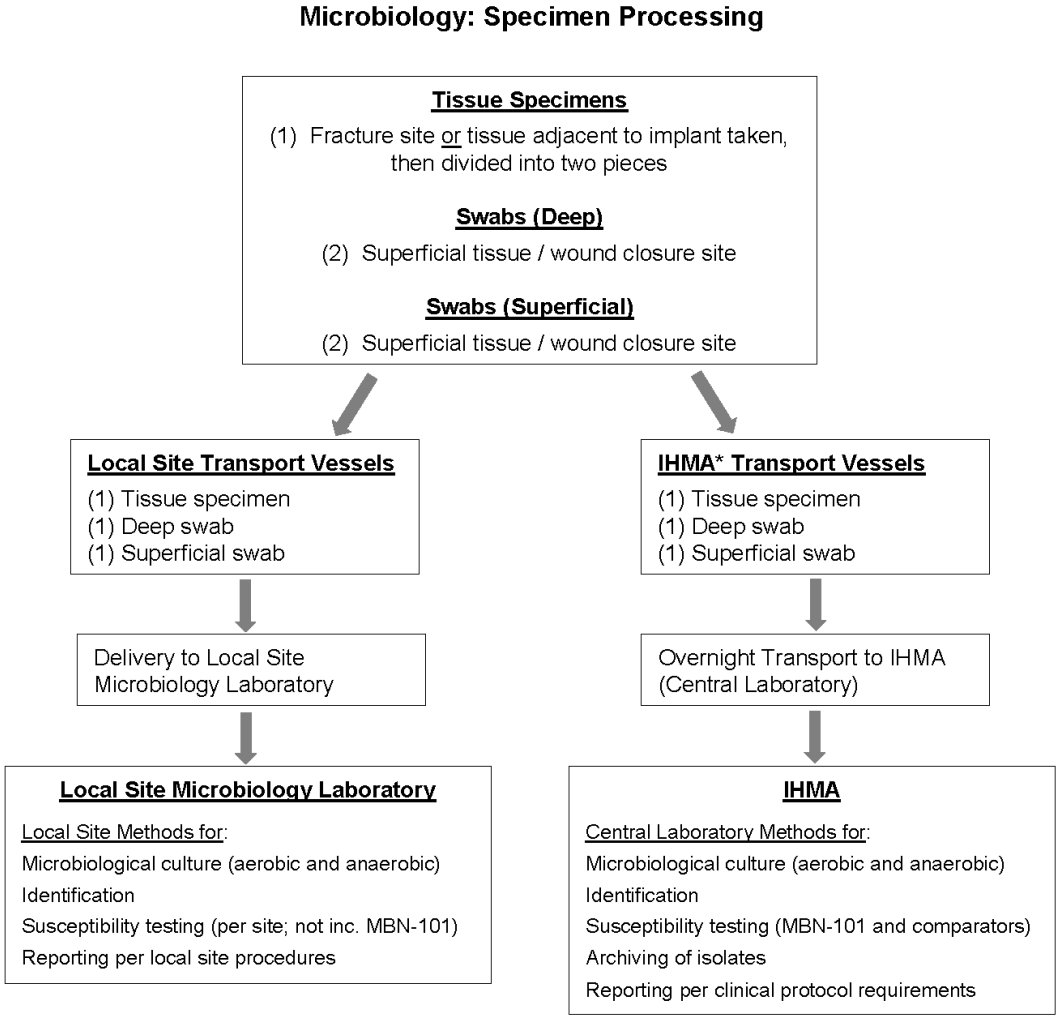
Procedure	Screening	Baseline/ Day of Surgery	Hour 48- 60	Hour 72	Hour 96	Week 2	Week 6	Week 12	Week 24, EOT	Early Term / Tx Failure
		Day 1	Day 2	Day 3	Day 4	Day 14 ±2 days	Day 28 ±8 days	Day 84 ±8 days	Day 168 ±8 days	
Review of eligibility criteria	X	X								
Informed consent	X									
Randomization		X								
Surgery/Administration of the Investigational Product		X								
Medical history	X									
Physical exam	X									
Interval physical exam		X	X			X	X	X	X	X
Hematology	X		X				X	X	X	X
Serology		X				X	X	X	X	X
Serum chemistry	X		X				X	X	X	X
Urinalysis	X		X				X	X	X	X
Pregnancy test	X	X							X	X
BisEDT Blood levels[1]		X	X	X	X	X				
12-lead ECG	X	X	X						X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes[2]	X					X	X	X	X	X
Surgical site signs and symptoms		X	X	X	X	X	X	X	X	X
Surgical site pain score		X	X	X	X	X	X	X	X	X
Microbiology		X	(X)[3]			(X)	(X)	(X)	(X)	(X)
Radiographic evaluation		X				X	X	X	X	X
Concomitant medications	X	X	X			X	X	X	X	X
Adverse events		X	X			X	X	X	X	X

[1] PK blood samples will be collected pre-dose and at 1, 6, 12, 24, 36, 48 (Day 2), 60, 72 (Day 3), 96 (Day 4) and 336 (Day 14; Week 2) hours after administration of the investigational product

[2] Patient-reported outcomes include the Veterans Rand 12 Item Health Survey (VR-12) and Short Musculoskeletal Function Assessment (SMFA)

[3] Microbiology will be performed at any time that a new surgical intervention is required.

Appendix 2: Flowchart for Microbiological Specimen Processing



* IHMA: International Health Management Associates, Inc. (Central Laboratory)