

CLINICAL PROTOCOL

PHASE III

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER
TRIAL OF THE SAFETY AND EFFICACY OF CEFTRIAXONE AND
DOXYCYCLINE IN THE TREATMENT OF PATIENTS WITH
SERONEGATIVE CHRONIC LYME DISEASE

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Version 1.5

6/10/1998

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1 **I. INTRODUCTION**

2

3 Lyme disease (LD) is the most common tick-borne disease in the United States¹. The
4 etiologic agent, *Borrelia burgdorferi*, is a spirochete, and is transmitted to humans and
5 other animals by tick vectors belonging to the genus Ixodes. The natural reservoir for the
6 etiologic agent is rodents; many other types of mammals and some birds may also
7 become infected².

8

9 As with many infectious diseases, the clinical manifestations of LD are variable and
10 unpredictable. Early manifestations include a rash (erythema migrans), general malaise,
11 and flu-like symptoms². Chronic manifestations include arthritis, cardiac and neurologic
12 manifestations that have been reported to spontaneously remit and recur even following
13 antibiotic therapy^{3 4 5 6 7 8}.

14

15 Recently, the term Chronic Lyme Disease has been used to describe a condition of
16 chronic or intermittent symptoms related to LD. The cause of CLD is not known, but
17 several possibilities have been suggested. The first is that it is the manifestation of a
18 chronic active infection by *B. burgdorferi* that has escaped control or eradication with the
19 use of conventional antibiotic regimens^{9 10}. A second possibility is that CLD may be due
20 to damage caused by the original infectious process, including triggering of post-
21 infectious immune phenomena, despite the eradication of the spirochete¹¹. A third
22 possibility is the presence of a co-infection with another organism transmitted by Ixodes
23 ticks.

24

25 **II. OBJECTIVES**

26

27 The objectives of this study are to determine whether: 1) intensive antibiotic treatment
28 benefits seronegative patients with CLD; 2) evidence of persistent infection with *Borrelia*
29 *burgdorferi* can be found in patients with CLD; 3) evidence of co-infection with other
30 microorganisms can be found in patients with CLD; 4) specific clinical or laboratory

1 parameters improve in patients who receive antibiotic therapy compared to patients who
2 receive placebo and 5) specific parameters are predictive of a response to therapy should
3 it be observed.

4 5 **III. DESIGN AND STUDY DESCRIPTION**

6
7 This study, which is being supported as contract through funds made available by the
8 NIAID, is a Phase III, randomized, double-blinded, placebo-controlled, multicenter trial
9 (two centers). Sixty six (66) patients will be enrolled in the study. Each patient will be
10 assigned to one of three strata based upon the duration of symptoms of chronic Lyme
11 disease (less than four years, four to less than eight years, or eight to less than twelve
12 years), and randomized to receive either antibiotic therapy or placebo in a 1:1 ratio.
13 Antibiotics and placebo will be administered both intravenously and orally. Separate
14 randomization schedules will be generated for each study center by NIAID or its
15 designate. The study population will include a defined cohort of patients with CLD who
16 meet the inclusion and exclusion criteria as defined for this study (see section V).

17
18 The antibiotic regimen will be ceftriaxone, 2.0 grams/ day, administered once daily via
19 the intravenous route for 30 consecutive days followed by doxycycline, 200 mg/day,
20 administered as 100mg twice daily via the oral route for 60 consecutive days. Placebos
21 identical to the intravenous and oral medications will be administered via the same route
22 and for the same duration to the patients randomized to the placebo treatment group.

23 24 **IV. MATERIALS AND SUPPLIES**

25 26 **A. Study Drugs**

27
28 Intravenous ceftriaxone at a dose of 2 grams daily or intravenous placebo (dextrose) will
29 be administered for the first 30 days of the treatment period to patients assigned to the
30 active treatment group (Group A) and the placebo treatment group (Group B)

1 respectively. Patients in group A will then receive doxycycline 100 mg every 12 hours
2 orally for 60 consecutive days and patients in group B will receive an identical placebo
3 every 12 hours orally for 60 consecutive days. Dr. Mark Klempner or a physician co-
4 investigator of New England Medical Center and Dr. Gary Wormser or a physician co-
5 investigator of New York Medical College, will supervise all aspects of the
6 administration of the study medications. Patients will receive intravenous therapy at home
7 with an indwelling short plastic venous access catheter.

8

9 There will be two groups in this study with 33 patients in each group:

10

11 1. IV ceftriaxone x 30 days followed by oral doxycycline x 60 days.

12

13 2. IV placebo (dextrose) x 30 days followed by oral placebo (dextrose) x 60 days.

14

15 **V. PATIENT SELECTION AND ENROLLMENT**

16

17 **A. Study Population**

18

19 This study will be conducted in 66 patients with CLD. Enrollment will begin in
20 approximately April, 1997 and proceed until the quota of patients is reached. We
21 anticipate an enrollment period not to exceed three years.

22

23 **B. Inclusion and Exclusion Criteria**

24

25 For the double-blind, placebo-controlled trial of antibiotics vs. placebo in seronegative
26 patients with chronic Lyme disease, the following criteria for inclusion will be used.

27

1 Inclusion Criteria:

- 2 1. 18 years of age or older.
- 3 2. Able to give informed consent.
- 4 3. Physician documented history of prior antibiotic treatment with a currently
5 recommended antibiotic regimen that was appropriate for the patient's clinical
6 features of Lyme disease at the time of presentation. (table on page 8 of the
7 technical proposal).
- 8 4. A past history of physician documented erythema migrans defined as an
9 erythematous skin lesion that expands over a period of days to weeks to form
10 an annular lesion estimated by the patient to be approximately 5 cm in size or
11 greater.
- 12 5. A past history of one or more of the following clinical features typical of
13 Lyme disease acquired in the United States:
- 14 a) Multiple erythema migrans lesions indicative of disseminated disease.
- 15 b) Early neurologic disease that includes lymphocytic meningitis, cranial
16 neuritis (e.g., facial palsy), or radiculoneuropathy not attributable to other
17 causes.
- 18 c) Acute cardiac illness consisting of signs and symptoms associated with
19 various degrees of A-V block not attributable to other causes.
- 20 d) Lyme arthritis defined as recurrent, brief attacks of objective joint swelling
21 in one or a few joints, especially the knees, sometimes followed by chronic
22 monoarthritis not attributable to other causes.

1 6. One or more of the following symptoms that have persisted for at least 6
2 months and are not attributable to another cause or condition:

3 a) Widespread musculoskeletal pain and fatigue that interferes with usual
4 function and which began coincident with or within 6 months following
5 initial infection with *B. burgdorferi*.

6 b) Symptoms of memory impairment that interfere with usual function and
7 which began coincident with or within 6 months following initial infection
8 with *B. burgdorferi*.

9 c) Symptoms of radicular pain, paresthesias and/or dysesthesias that interfere
10 with usual function and which began coincident with or within 6 months
11 following initial infection with *B. burgdorferi*.

12 7. Seronegative for antibodies against *B. burgdorferi* antigens by Western blot at
13 time of enrollment¹².

14 Exclusion criteria:

15 1. A history of hypersensitivity to ceftriaxone or doxycycline.

16 2. Currently, or within the last 7 days, taking beta lactam, tetracycline or
17 macrolide antibiotics.

18 3. Previously received a total of ≥ 60 days of parenteral ceftriaxone or
19 cefotaxime therapy for:

20 a) Widespread musculoskeletal pain and fatigue that interferes with usual
21 function and which began coincident with or soon after initial infection
22 with *B. burgdorferi*.

23 b) Symptoms of memory impairment that interfere with usual function and
24 which began coincident with or soon after initial infection with *B.*
25 *burgdorferi*.

- 1 c) Symptoms of radicular pain that interfere with usual function and which
2 began coincident with or soon after initial infection with *B. burgdorferi*.
- 3 4. Having received \geq 14 days of parenteral ceftriaxone or cefotaxime therapy
4 within the last 60 days.
- 5 5. Patients with active inflammatory synovitis.
- 6 6. Patients whose symptoms of CLD (a, b, c above) have been present for \geq 12
7 years.
- 8 7. Patients who have comorbid disease(s) that could account for symptoms of
9 chronic Lyme disease (a, b, and c above). Examples include severe clinical
10 depression, rheumatic illness such as rheumatoid arthritis or SLE, other
11 potential causes of radiculopathic pain such as intervertebral disc disease, etc..
- 12 8. Patients who have a serious comorbid disease (e.g., hematologic malignancy,
13 cirrhosis, metastatic cancer, etc.) or an active infection (e.g., HIV,
14 tuberculosis, etc.).
- 15 9. Patients who are receiving chronic medication therapy that could interfere
16 with the evaluation of symptoms in a, b, c above (e.g., narcotic analgesics,
17 prednisone \geq 10mg/day).
- 18 10. Patients who cannot tolerate or do not have adequate venous access for an
19 indwelling venous access catheter or are at increased risk of acquiring an
20 intravenous catheter related infection.
- 21 11. Patients who are pregnant, lactating, or unable to use birth control measures
22 during the treatment period of the study.
- 23 12. Patients who have previously enrolled in this study.
- 24 13. Patients who have positive PCR for *Borrelia* DNA in plasma or cerebrospinal
25 fluid at the time of initial evaluation for this study.

- 1 14. Patients who are seropositive by Western blot. (These patients may be offered
2 to enroll in seropositive study.)

3 **VI. STUDY PROCEDURES**

4
5 A. Initial Evaluation Procedures (Up to Two Weeks Prior to Dosing)

6
7 Within four weeks prior to administration of antibiotics or placebo, the following will be
8 obtained to assess compliance with the subject selection criteria outlined in Section V.
9 Patients will have a complete history and physical examination including detailed
10 neurologic examination and evaluation of tender points at 18 sites. Standardized history
11 and physical examination will be used to assess each patient for collection of
12 demographic data, a history of exposure to ticks in geographic regions with endemic *B.*
13 *burgdorferi* infection, clinical manifestations of acute Lyme disease, clinical
14 manifestations of chronic Lyme disease, prior treatment, history of previous serologic
15 tests for Lyme disease (although serologic status will be determined in the central
16 laboratory at the time of initial testing on entry to the study), medication allergies, recent
17 (last 2 months) and current medications. If the patient meets all the inclusion criteria and
18 none of the exclusion criteria, the study protocol will be explained and the patient will be
19 offered participation in the study.

20
21 Patients who, upon initial evaluation, have (a) a positive PCR for *Borrelia* DNA in
22 plasma or cerebrospinal fluid (CSF) or (b) evidence of synovitis will not be randomized
23 (see exclusion criteria #13). Such patients will be referred to either the NIAID Intramural
24 Clinical Study on Chronic Lyme Disease or elsewhere for appropriate treatment.

25
26 To determine whether spirochetes may persist in the skin of patients with chronic Lyme
27 disease, a skin biopsy will be obtained at the initial erythema migrans site from a subset
28 of up to 30 patients with a history of erythema migrans at New England Medical Center.
29 These specimens will be obtained from study subjects who volunteer to have the biopsy

1 procedure performed. This procedure is not a mandatory part of the Seropositive protocol.

2 There will be one biopsy performed per volunteer subject prior to initiating treatment.

3 Initial Evaluation:

4
5 1. Complete medical history (including all questions on data base forms related to
6 manifestations of acute Lyme disease and to current symptoms of chronic Lyme
7 disease).

8 2. Complete physical examination including detailed examinations of the
9 musculoskeletal and neurologic systems.

10 3. Administration of the SF-36 Health Survey¹³ and the Fibromyalgia Impact
11 Questionnaire¹⁴.

12 4. Vital signs - blood pressure, pulse rate, respiratory rates and temperature.

13 5. Lumbar puncture will be performed on all patients according to standard sterile
14 procedures. CSF will be examined for cell count, total protein, glucose,
15 production of antibody to *B. burgdorferi* antigens¹⁵ and PCR for detection of DNA
16 from *B. burgdorferi*¹⁶ and Ehrlichia sp.¹⁷.

17 6. Laboratory tests as follows: CBC and differential counts, ESR, ANA, total
18 bilirubin, serum glutamic-oxaloacetic transaminase (SGOT/AST), lactate
19 dehydrogenase (LDH), alkaline phosphatase, creatinine, blood urea nitrogen
20 (BUN), total protein, glucose, albumin, TSH, PCR for detection of DNA from
21 *B. burgdorferi* in serum, and total IgG. A CSF IgG index can be calculated
22 according to the following formula: (CSF IgG x serum albumin)/(CSF albumin x
23 serum IgG). The rate of IgG synthesis is calculated as follows: $5 \times \text{CSF IgG} - \text{IgG}$
24 $\text{serum} / 68.2 \times 2.15 (\text{CSF albumin} - \text{serum albumin} / 109) (\text{serum IgG} / \text{serum albumin})$.

25 7. Neuropsychological testing: to provide measures of immediate and delayed
26 memory, conceptualization, copying, perceptual discrimination, and language.

1 Each of these tests will be administered at baseline, 90 days and 180 days except
2 the MMPI that will be administered at baseline and 180 days only.

3 a) Rey Auditory - Verbal Learning Test (RAVLT)

4 Rationale: it is similar to those tests (i.e., California Verbal Learning
5 Test¹⁸, and Selective Reminding Test) shown sensitive to Lyme-related
6 memory loss and it has several alternate forms. (time = 20 minutes)

7

8 b) Symbol-Digit Modalities Test A test that requires attention and
9 concentration in addition to visual perception.

10 Rationale: shown to be sensitive by Halperin¹⁹ to Lyme-related decline
11 performance. (time = 2-3 minutes)

12

13 c) MMPI A personality inventory and psychopathology screen.

14 Rationale: previously used to compare Lyme and fibromyalgia patients by
15 Kaplan²⁰ and may reveal differences in personality profile of chronic
16 Lyme patient.^{21 22}(time = 45 minutes)

17

18 d) Beck Depression Inventory A depression index.

19 Rationale: previously used in several Lyme studies to assess depression
20 and compare depression to other patient groups. (time = 5-10 minutes)

21

22 e) California Computerized Assessment Package (CalCAP) A series of
23 continuous performance reaction time tests with large normative
24 sample.

25 Rationale: this is a more sensitive test of attention and concentration than
26 tests previously used. (time = 10-15 minutes)

27 f) Controlled Oral Word Association (COWA) A word generation test.

28 Rationale: Krupp²³ showed differences on this test between controls and
29 previously treated Lyme patients and it has several alternate forms. (time =
30 5 minutes)

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g) Benton Visual Retention Test (BVRT).

Rationale: Halperin and Kaplan showed Lyme effects using this or a similar test (Weckler) of memory. There are 3 alternate forms of the BVRT and only two of the Weckler. (time = 15 minutes)

All test scores will be transformed into standard scores that will be calculated from published, age-corrected normative data. According to a previously described system, evidence of memory impairment is defined as scores that are 2 SD below the normative mean on any one of the tests of memory or more than 1 SD below the mean on two of the tests. A score of 70 or above on the Minnesota Multiphasic Personality Inventory is considered indicative of depression.

8. For patients whose persistent symptoms are of peripheral neuropathy including paresthesias or radicular pain, a detailed electromyographic examination of limb and paraspinal muscles will be performed with concentric needle electrodes²⁴. Motor nerve and sensory-nerve conduction studies of the medial ulnar, peroneal, and tibial nerves will be performed with 10-mm surface recording and stimulating electrodes.
9. Testing for immune response to *B. burgdorferi* antigens in serum²⁵ and cerebrospinal fluid²⁶.
10. Cerebrospinal fluid and plasma specimens from patients enrolled in this study will be tested for the presence of *B. burgdorferi* DNA that encodes outer-surface protein (OspA). After extracting the DNA from the fluid, the samples are tested with a primer-probe set (called OspA 2,4) that targets a C-terminal portion of OspA (see methods in the technical proposal). The samples are then amplified by 45 cycles of PCR. The amplicons are resolved by agarose gel electrophoresis and stained with ethidium bromide. After transfer to a nitrocellulose membrane, the membrane is hybridized with a P32-labelled DNA probe by southern blotting. The membrane is

1 then exposed to autoradiography for 6-72 hr. Positive and negative controls are
2 used throughout this procedure.

3 11. Culture. All CSF samples will be cultured for the presence of *B. burgdorferi* in
4 BSK II medium and monitored by dark field microscopy for 6 weeks. Details of
5 the methods are contained in ²⁷.

6 12. For those women of child bearing age, urine pregnancy test will be performed.
7 Menstrual cycle dates will be recorded in the patient diary.

8 13. Informed consent obtained in writing.

9 14. Serologic test for antibodies against *Babesia microti* ²⁸.

10 15. Urine sample for subsequent testing of *B.burgdorferi* urine antigen ²⁹.

11 B. Patient Number Assignment

12

13 After the investigator determines that the patient qualifies for the study, explains the
14 nature of the study to the patient, and obtains informed consent documented in writing,
15 the investigator or designee will assign each patient to the appropriate stratum based on
16 the duration of their symptoms of chronic Lyme disease (less than four years, four to less
17 than eight years, or eight to less than twelve years) and then assign a patient number for
18 the study to the patient in numerical sequence within that stratum. The
19 investigator/designee will record the patient's initials and date of administration of the
20 first dose of the study medication on the case report form.

21

22 C. Medication Administration

23

24 The investigator will be given a blinded infusion bag or a pre-mixed 30 ml. syringe which
25 contains the study drug required for the patient's first infusion. The treatments
26 (ceftriaxone 2.0 grams in 100 ml. of 5% dextrose in water or placebo consisting of 100
27 ml. 5% dextrose in water with multi-vitamin to match the study medication for color) will

1 be contained in identical looking infusion bags that will have patient numbers noted on
2 the label and will be infused by gravity over 15 minutes. Syringes (containing either
3 ceftriaxone 2.0 grams in 30 ml. of 5% dextrose in water or placebo consisting of 30 ml.
4 5% dextrose in water with multi-vitamin to match the study medication for color) will
5 have patient numbers noted on the label and will be infused by I.V. push over 2-4
6 minutes. Approximate doses of multi-vitamin for color matching are:

7

| 8 | | <u>Infusion bag</u> | <u>Syringe</u> |
|----|---------------|---------------------|----------------|
| 9 | Ascorbic Acid | 10 mg | 3 mg |
| 10 | Vitamin A | 200 mg | 60 mg |
| 11 | Vitamin D | 20 mg | 6.0 mg |
| 12 | Thiamin | 1.0 mg | 0.3 mg |
| 13 | Riboflavin | 0.8 mg | 0.06 mg |
| 14 | Pyridoxine | 0.3 mg | 0.09 mg |
| 15 | Niacinamide | 2.0 mg | 0.6 mg |
| 16 | Vitamin E | 0.01 I.U. | 0.03 I.U. |

17

18 All first infusions of study medication will be monitored by one of the
19 physician/investigators. Subsequent infusions will be made under the supervision of the
20 principal investigator or designee (study nurse). We expect that most of these infusions
21 will take place in the patient's home. Following completion of the course of parenteral
22 therapy patients will be dispensed oral medication containing doxycycline 100 mg. or
23 identically appearing placebo (dextrose) which will be administered as one capsule every
24 12 hours for 60 consecutive days. Patients will be instructed that the oral medication may
25 be taken with or without food.

26

27 D. Patient Instructions

28

29 All patients will receive instructions regarding possible side effects of treatment. Known
30 side effects from ceftriaxone include but are not limited to: rash, diarrhea, GI upset,

1 cholestasis, and anaphylaxis; known side effects from doxycycline include but are not
2 limited to rash, photosensitization, diarrhea, GI upset, anaphylaxis, embryotoxicity and
3 teratogenicity. Patients will also be instructed on signs of intravenous catheter infection.
4 Patients will be able to reach the principal investigator or designee at any time to discuss
5 any symptoms they may be experiencing during the administration of antibiotics or
6 placebo.

7

8 E. Evaluations During Dosing Period

9

10 Ceftriaxone/Doxycycline or Placebo(Study Days 1 - 90).

11

12 1. Vital Signs Vital signs will be obtained on Days 1, 3, 5, 9,13,17, 21, 25 and 30.

13

14 2. Laboratory Tests Blood samples (15ml) will be obtained on Days 3, 5, 13, 21, 30,
15 45 and 75. CBC and liver function (as described in Section VI. A.4) will be
16 performed on samples from days 5, 13, 21, 30, 45 and 75 for safety monitoring.
17 Portions of these samples (from days 3, 5, 21 and 45) will be used for serial PCR
18 testing to detect the appearance of borrelial DNA during treatment. Aliquots from
19 these samples will also be stored at -70C as part of a specimen bank in patients
20 with chronic Lyme disease for future studies. Urine samples (10 ml) will be
21 collected on days 3, 5, 13, 21, 30, 45, and 75, and stored in aliquots at -70C.
22 These specimens will be used for serial antigen testing and as part of the specimen
23 bank for future studies of patients with chronic Lyme disease. No additional blood
24 studies will be obtained from these patients except as noted below.

25

26 3. Compliance The patient will be queried at each visit regarding all medications
27 taken, including all background medication. The information will be recorded in
28 the Case Report Form.

29

1 A capsule count for the trial medication will be done at the day 45 and day 75
2 visit to monitor compliance. All unused medication should be returned to the
3 Coordinating Center upon completion of the study. Any deviation from the
4 regimen designated by the protocol must be explained; the patient should explain
5 any apparent non-compliance. This will be recorded in the Case Report Form.

6

7 F. Post-Treatment Evaluations

8

9 Follow up evaluation by one of the physician investigators will occur at the completion of
10 intravenous therapy (day 30) and then at the completion of oral therapy (day 90). Post-
11 treatment evaluations will follow on days 180, and 360. At each of these visits all
12 patients will repeat the SF™ 36 Health Survey and the Fibrositis [fibromyalgia] Impact
13 Questionnaire. Physical examination with standardized recording of findings will also be
14 recorded. Repeat neuropsychological testing to provide measures of immediate and
15 delayed memory, conceptualization, copying, perceptual discrimination, depression and
16 language will be performed at 90, 180 and 360 days. While all tests will be administered
17 at the 90 day visit, only those tests which are not affected by "learning" will be repeated at
18 180 and 360 days (see methods for details). Blood (10cc) and urine will be collected at
19 90, 180 and 360 day follow up visits. Samples will be compared to baseline for an IgG
20 response to borrelial antigens. In those patients in whom initial CSF is abnormal for any
21 of the baseline parameters, follow-up lumbar puncture and CSF evaluations will be
22 performed on day 90. Since patients will be enrolled over the first 3.0 years we will have
23 the opportunity for long term follow-up. Therefore, we will also do telephone follow-up
24 every 6 months of patients after the 360 day follow visit.

25

26 **VII. MANAGEMENT OF INTERCURRENT EVENTS**

27

28 A. Adverse Events

29

1 Throughout the duration of the study, the investigator will monitor each patient for
2 evidence of drug intolerance and for the development of clinical and laboratory evidence
3 of an adverse event. All adverse events, including those which have previously been
4 reported as attributable to ceftriaxone, doxycycline or the presence of an intravenous
5 catheter or which are judged by the investigator to be related to the therapy will be
6 recorded on the case report form and followed to a satisfactory conclusion. The
7 description of the adverse event will include the date, time of onset, duration, severity,
8 etiology, relationship of the event to the study drug and any treatment or action required.
9 The investigator will rate the severity of the adverse event according to the following
10 definitions:

11
12 Severity:

- 13
14 1. Mild: The adverse event is transient and easily tolerated by patient.
15
16 2. Moderate: The adverse event causes the patient discomfort and interrupts the
17 patient ' s usual activities.
18
19 3. Severe: The adverse event causes considerable interference with the patient's
20 usual activities, and may be incapacitating or life threatening.

21
22 Likelihood that event is related to medication:

- 23
24 1. Concurrent Condition: An event, illness or effect of another drug not related to
25 study drug (e.g., has not been reported for this drug, transient, or has no temporal
26 relationship to study drug, or has a definite alternative etiology).
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28 2. Remote ADE: An adverse event not commonly associated with this drug class,
29 has little or no temporal relationship to the study drug, and a probable alternative
30 etiology exists.

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- 3. Possible ADE: An adverse event not commonly associated with this drug class, has a temporal relationship to the study drug, and a possible alternative etiology exists.
- 4. Probable ADE: An adverse event commonly associated with this drug class, has a temporal relationship to the study drug, lessened after the drug was discontinued, and no other etiology is apparent.
- 5. Definite ADE: An adverse event commonly associated with this drug class, has a temporal relationship to the study drug, reappeared on rechallenge, and no other etiology is apparent.

All deaths and serious adverse experiences which occur during the study or the post-therapy period, regardless of treatment group or relationship to drug, must be reported IMMEDIATELY BY TELEPHONE to the Clinical and Regulatory Affairs Branch (CRAB), DMID, NIAID. A serious adverse experience includes, but is not necessarily restricted to, events which are: [a] fatal; [b] life-threatening or potentially life-threatening; [c] permanently disabling; [d] an event requiring hospitalization; or [e] a congenital anomaly, cancer, or the result of overdose. It should be emphasized that, regardless of these criteria, any additional adverse experience which the investigator considers significant enough to merit immediate reporting should be so reported.

Every three months a report tabulating the adverse events by masked treatment assignment will be prepared by the statistical unit and transmitted in confidence to the to Medical Officer, NIAID, and the DSMB Safety Officer. These reports will include all events and indicate for each whether or not they are considered attributable to the study medication.

B. Concomitant Drugs

1 If administration of any concurrent medication is necessary during the course of this
2 study, dosage information, dates of administration, and indication for use must be
3 reported on the appropriate case report form. Administration of additional antibiotics to
4 treat intercurrent infectious diseases is acceptable provided the agent is not administered
5 for longer than 7 days and/or is not active against *B. burgdorferi*. Administration of these
6 agents must be documented on the case report form.

7

8 C. Premature Termination

9

10 All patients have the right to withdraw from the study at any time without prejudice. The
11 investigator may discontinue any patient's participation when he feels it is necessary for
12 any reason. Should a patient withdraw from the study, the reason must be documented on
13 the case report form, and a final evaluation of the patient must be performed, as defined
14 in Section VI.D.

15

16 D. Modification of Protocol

17

18 The investigators will not modify this protocol without first obtaining the concurrence of
19 the project officer. The modification must be documented in writing. Any change in the
20 protocol, except those necessary to remove an apparent immediate hazard to the subject,
21 must be reviewed and approved by the Institutional Review Board prior to
22 implementation. The investigators must submit protocol amendments to governmental
23 agencies, and protocol modifications may be subject to Institutional Review Board review
24 and approval.

25

26 E. Variation from Protocol for the Individual Patient

27

28 When significant variation from the protocol is deemed necessary for an individual patient, the
29 investigator or other physician in attendance will inform the project officer. Any
30 departure from the protocol will be authorized only for that one patient. A description of

1 the departure from the protocol and the reason for it will be recorded on the appropriate
2 case report form.

3
4 **VIII. EVALUATION OF EFFICACY AND SAFETY**

5
6 A. General Eligibility

7
8 The following conditions must be met if the patient is to be considered evaluable for the
9 purposes of this study. The patient must have completed both baseline and follow up
10 blood tests, CSF tests (where indicated), history and physical examinations and
11 neuropsychiatric testing.

12
13 B. Evaluation of Antibiotic Therapy Efficacy

14
15 Success of ceftriaxone/doxycycline in the treatment of the symptoms of chronic Lyme
16 disease will be determined by the investigator at the completion of the studies. The
17 primary outcome for this study is defined as an improvement in the patients' health-
18 related quality of life (HQL). Health-related quality of life will be measured using the
19 SF-36 Health Survey. The SF-36 Health Survey includes eight multi-item scales that
20 measure physical functioning, role-physical, bodily pain, general health, vitality, social
21 functioning, role-emotional and mental health. The eight multi-item scales are
22 hypothesized to form two distinct higher-order factors, physical and mental, which
23 provides the basis for scoring the SF-36 physical and mental health summary scales.
24 Three additional multi-item scales from the medical outcomes study (MOS) will be used
25 to measure cognitive functioning, pain, and role functioning but they will not be used for
26 the primary analysis of efficacy of antibiotic treatment. The SF-36 Health Survey (and the
27 additional MOS measures) will be administered to study participants four times: at
28 baseline, at one month (end of parenteral treatment), at three months (end of treatment),
29 at six months and at one year.

1 To document the burden of Lyme disease on health-related quality of life, baseline scores
2 for the SF-36 (and MOS scales) will be compared to general population norms. General
3 population norms will be age adjusted to correct for differences in age between the study
4 sample and the general population. Norm-based comparisons of SF-36 (and MOS scale
5 scores) will also be conducted with 1, 3- and 6- month follow-up scores in both treated
6 and placebo groups to evaluate change in the study participant HQL profile relative to
7 general population norms.

8

9 To assess changes in HQL over time for study participants, a change score for each SF-36
10 (and MOS scale) will be calculated by subtracting the baseline score from the 1 month
11 score, the 3 month score and the 6 month score. Using two standard errors of
12 measurement (SEM), individual patients will be classified into 3 change categories: (1)
13 those whose follow-up scores did not change more than would be expected by chance
14 (“same group”); 2) those whose follow-up scores improved (more than 2 SEM's) more
15 than would be expected by chance (“better group”); and 3) those whose follow-up scores
16 declined (more than 2 SEM's) more than would be expected by chance (“worse group”).
17 Based on prior studies with the SF-36, a 2 SEM change is 6.5 points for the SF-36
18 physical health summary scale and 7.9 points for the SF-36 mental health summary scale.

19

20 An overall determination of change in health status will be determined for each patient
21 from the results of the two SF-36 summary scales (and the three MOS scales). Patients
22 will be scored as improved if they are better on both scales or better on one scale and are
23 not worse on the other scale.

24 The primary analysis will be an intent-to-treat analysis of all patients enrolled in the
25 study. For secondary analysis, patients will be evaluated who have received at least 75%
26 of the course of study therapy. Both analyses will involve comparisons between the
27 proportion of patients improved at 6 months in the placebo and treated groups. A
28 secondary analysis will compare the groups at the end of parenteral (30 days) and total
29 active treatment (90 days). Comparison will consist of a χ^2 test at a significance level of
30 0.05, together with an estimate of treatment efficacy (risk ratio and risk difference) with

1 95% confidence intervals. As secondary and exploratory analyses, we will check to see if
2 efficacy differs across subgroups by examining interaction terms in a multivariate
3 analysis. We will also determine whether changes in patient performance on the
4 supplementary MOS scales correlate with changes in the SF-36. Such subgroup analyses
5 will need further confirmation in future studies. Nevertheless, their discovery may suggest
6 important treatment interactions.

7

8 Tests will be done to ensure that no important potential confounders are unevenly
9 distributed between patient groups. Regression analysis will be performed to determine
10 the effect on results if any confounders are identified. An analysis stratified by duration of
11 symptoms of chronic Lyme disease will be performed. An analysis stratified by site also
12 will be performed to ensure that the results are not dependent on site. Among potential
13 confounders and interaction variables are demographic variables (e.g., age, sex, race,
14 education and location), medical history variables (e.g., previous psychiatric illness, prior
15 history of arthralgia or arthritis, duration of symptoms, and interval between onset of
16 acute Lyme disease and chronic symptoms) and physical examination variables (e.g.,
17 tender points and findings on neurologic examination).

18

19 C. Statistical Analysis

20

21 Study Sample Size

22

23 For a long course of high dose antibiotics to show efficacy there should be a substantial,
24 durable difference between the antibiotic treated and the placebo groups. For an efficacy
25 study to achieve 80% power, assuming a natural healing rate of 0.4 (40%) and 30 patients
26 per group, a difference of 35% between the natural healing rate and the treated rate must
27 occur³⁰. A natural healing rate of 0.2 (20%) and a treated improvement rate of 0.55
28 (55%) gives 81% power. A placebo rate of 0.4 (40%) and a treated rate of 0.76 (76%)
29 gives 82% power. Multiplying by 1.10 for 10% dropouts and then by 2 groups gives a
30 total of 66 patients.

1

2 Safety Evaluations

3

4 Safety will be assessed primarily by summarizing adverse event data. Adverse event
5 incidence rates will be computed for each adverse event. The analysis of safety will be
6 analyzed with Fisher's Exact Test. Laboratory data for each patient will be reviewed by
7 the Data Safety Monitoring Board and the Medical Officer for clinically significant
8 changes and will be summarized with descriptive statistics or other appropriate statistical
9 methods.

10

11 Data Collection Procedures

12

13 Based on the extensive experience of the study Investigators in conducting clinical trials,
14 a centralized data entry system has been selected to ensure the most effective approach.
15 Data collection will be done at the study sites by a research nurse using multi-copy case
16 report forms (CRFs). The study site will detach and retain a copy and send the remaining
17 copies directly to the study coordinator at New England Medical Center. Upon receipt of
18 the CRFs, each subject will be entered into the study log book and the CRF checked for
19 completeness. When data are missing, an initial hand-written query will be generated, a
20 copy of the form retained, and the incomplete form returned to the study site research
21 nurses. Data from the originally complete CRFs and returned completed CRFs will be
22 entered into a computer database, using a program with built-in checks for ranges and
23 limits of values and logic checks. A computerized query will then be generated on each
24 CRF and will be sent back to the study site hospital nurse for any needed completion and
25 verification. The answers to these queries will in turn be sent back to the Study
26 Coordinator where all data from the CRF including new information will then be entered
27 into the computerized database for a second time. The program will once again run the
28 data checks for additional problems. There, following checking, they will be combined
29 with the data from the CRFs. A SAS database will be created and analyses will be done

1 on microcomputers on the NEMC IBM mainframe computer. The initial and follow-up
2 data to be collected on all patients is described above.

3 **IX. CASE REPORT FORMS**

4

5 The investigators will review the case report forms for completeness and accuracy, and
6 will sign and date the forms where indicated. The investigator and the Data Safety
7 Monitoring Board will meet to review all completed case report forms for completeness
8 and legibility.

9

10 All records which support case reports of this study must be retained in the files of the
11 principal investigator.

12

13 **X. INFORMED CONSENT**

14

15 Each subject must voluntarily sign the informed consent form before participating in this
16 study. Obligation for obtaining the informed consents for both studies of each subject is
17 assumed by the investigator. The study protocols and informed consent forms must be
18 approved by the investigator's IRB. Each original will be retained by the investigator and
19 a copy will be given to each patient.

20

21 **XI. PATIENT CONFIDENTIALITY**

22

23 All reports and communications relating to patients in the study will identify each subject
24 only by the patient's initials and by the patient's study number. The investigator agrees to
25 retain complete patient identification on the confidential patient follow-up form, which
26 will be used for the purpose of long-term follow-up if needed. This information will be
27 treated with strict adherence to professional standards of confidentiality.

28 Case report forms will be reviewed for completeness and acceptability by the principal
29 investigator or designee at the study site. Portions of the patients medical records

1 pertinent to the study will be reviewed by these personnel and possibly by FDA personnel
2 to assure accuracy.

3

4 **XII. USE OF INFORMATION AND PUBLICATION**

5

6 To allow for the use of the information derived from this clinical study and to insure
7 complete and thorough analysis, the investigator is obligated to provide the NIAID with
8 complete test results and all data developed in this study. Included in the data will be
9 results of testing for concordance for seroreactivity by a second laboratory. Should the
10 investigator choose to publish the results of this study, a copy of the manuscript will be
11 provided to NIAID at least 30 days prior to submission to the intended publisher.

12

13 **XIII. COMPLETION OF STUDY**

14

15 The investigator will complete and report this study in satisfactory compliance with the
16 protocol within 12 months of final data collection.

17

18

19 If, at the completion of the study, the protocol treatment is found to be effective, patients
20 who have received the placebo will be offered the study treatment regimen (30 days of
21 intravenous Rocephin, 2 grams per day, followed by 60 days of oral doxycycline, 200mg
22 per day) through their regular health care insurance coverage. If coverage for treatment is
23 denied by the patient's regular health care insurance, or if a patient does not have health
24 insurance, the costs of such treatment will be paid through the contract supporting these
25 studies.

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