NCT03427619

Protocol (May 2014) and Statistical Analysis Plan (7 April 2021)

A Phase 2, Multicenter, Open Label Study to Evaluate the Efficacy and Safety of OK-432 Immunotherapy in Individuals With Lymphatic Malformations

OK432 Investigators
Standard Operating Procedure and Protocol
(May 2014)

Study Objective

- To provide OK-432 immunotherapy to patients with macrocystic lymphatic malformations (LM) or mixed LM (>50% macrocystic)

Use of OK432

- The use of OK-432 immunotherapy in this clinical trial has been approved by the FDA (BB-IND# 5266).
- The manufacturer (Chugai Biopharmaceutical, Inc.) provides the drug to the PI at no cost.
- Both preparation (changing labeling from Japanese to English) and distribution are coordinated by the Pharmacy Department of the University of Iowa Hospitals and Clinics.

Administration

- OK-432 immunotherapy is administered at a concentration of 0.01-0.05 mg/ml (0.1 mg of OK-432 dissolved in 10 ml normal saline or 0.1 mg of OK-432 dissolved in 5ml normal saline)
- The total injected dose will not exceed 0.2 mg at any injection session
- Dose is not based on lesion size

Inclusion Criteria

- To be eligible to receive OK432 immunotherapy
    - Patients must be ages 6 months to 17 years
    - Patients must have a macrocystic LM
    - Patients may have had surgical treatment for their LM
    - Patients must have an imaging study to confirm the diagnosis of a macrocystic or mixed LM
        - An MR is preferred over a CT scan (an ultrasound may be used between injections if warranted, however an MR or CT should be done pre and post treatment)

Exclusion Criteria

- Patients may be ineligible to receive OK432 immunotherapy
- Exclusion criteria are
    - Penicillin allergy
    - Women who are pregnant or nursing
    - Patients who present with a temperature of 100.5°F or greater
    - Patients with mixed hemangioma-lymphangioma lesions
    - Patients with a history OR a family history of rheumatic heart disease or post-streptococcal glomerulonephritis
    - Patients with hemodynamic instability and respiratory failure
    - Patients with a history OR a family history of obsessive-compulsive, tic disorders, or PANDA (pediatric autoimmune neuro-psychiatric disorder associated with streptococcal infections)
    - Patients who demonstrate abnormalities in the history, physical examination or laboratory analysis which may indicate significant hepatic, hematologic, or renal disease
    - Patients who are not in "good general health" (including patients with congenital disorders, chronic diseases, immunologic dysfunction, transplant recipients)

Measurement of LM

- Patients must have macrocystic or mixed (>50% macrocystic) LMs
- The diagnosis of a LM must be based on clinical presentation **and** radiographic evaluation
- The distinction between macro- versus microcystic disease will be determined by radiographic examination
    - Either magnetic resonance imaging (MRI) or computed tomography (CT) must be completed
        - MR is preferred
- Macrocysts are defined as cystic spaces $\geq$ 2.0 ml (2 cm x 1 cm x 1 cm)

- Dimensions must be measured from axial and coronal imaging studies and volumes must be calculated as the product of the orthogonal dimensions
- Patients with mixed LMs (macro- and microcystic disease) can be included if the macrocystic component comprises at least 50% of the total disease burden
- LMs must be staged based on Table 1

Table 1. LM Clinical Staging Classification (Smith et al., 2009)

Clinical Stage	Anatomic Area
I	Unilateral infrahyoid
II	Unilateral suprahyoid
III	Unilateral infra and suprahyoid
IV	Bilateral suprahyoid
V	Bilateral infra and suprahyoid
VI	Bilateral infrahyoid
+/- RP	Retropharyngeal involvement
+/- M	Mediastinal involvement

Pre-Treatment Evaluation

- The pre-treatment evaluation should include
  - A complete history and physical exam
  - Laboratory analysis (CBC with differential, ESR, ASO titer, serum creatinine, chemistry panel including liver function tests)
  - Pregnancy test in females of child-bearing age
  - Medical photography
  - MRI and/or CT
- The concurrent evaluation should include
  - A focused history and physical exam
  - Laboratory analysis if greater than 3 months from the pre-treatment examination

Administration Scheme

- A 4-dose injection series of OK-432 immunotherapy should be planned
- The maximum dose of OK-432 immunotherapy per patient at each injection session should be 0.2 mg (see Use of OK432, above)
  - The maximum dose does NOT have to be used
- Treatments should be spaced approximately 6-12 weeks apart

OK-432 Administration

- **Institutional Review Board approval must be obtained by each participating site**
- Informed consent must be obtained on each patient
- All treatments must be performed under appropriate conditions (general or local anesthesia) using sterile technique in the appropriate facility
- An appropriately gauged needle (25-gauge, occasionally larger) will be introduced into the cyst(s)
- Fluid will be removed by aspiration
  - If necessary, needle placement can be confirmed by ultrasonography
- OK-432 is injected at the appropriate concentration

Routine Post-Injection Safety Monitoring in the Post-Operative Setting

- Following OK-432 injection, the patient should be **observed in the recovery room (RR)/hospital** for approximately ~4 hours
- Vital signs should be taken per RR routine
- In the absence of symptoms that necessitate admission (including but not limited to sepsis, shock, airway compromise, seizures), the patient can be discharged
- At discharge, parents/caregivers should be given contact information in the event of questions or an emergency

Post-treatment Evaluation

- An assessment form with a return envelope, will be given to the patient/family to fill out 3-5 days and 2-3 weeks after each injection series
- 1-6 months after the fourth or last injection series
  - A complete history and physical exam
  - Laboratory analysis at local lab (CBC with differential, ESR, ASO titer, serum creatinine, chemistry panel including liver function tests)
  - Medical photography
  - MRI and/or CT

Response to Therapy

- MRI and or CT is used to assess response to therapy
- Response to therapy is graded as percent improvement in increments of 10%
  - Complete (90-100% reduction in volume)
  - Substantial (60-89% reduction in volume)
  - Incomplete (20-59% reduction in volume)
  - None (0-19% reduction in volume)
- Expected responses to therapy are shown in Table 2

Table 2. Complete or Substantial Clinical Response (Smith et al., 2009)

Group*	Referred for Treatment		Appropriate Lesions	
ITG	62/91	(68.1%)	62/72	(86.1%)
DTG	15/26	(57.7%)	15/20	(75.0%)
OLG	23/34	(67.6%)	22/27	(81.5%)
Total	100/151	(66.2%)	99/119	(83.1%)

* ITG, immediate treatment group; DTG, delayed treatment group; OLG, open label group

Expected Drug Effects

- No serious hematologic, renal, hepatic or cardiac adverse side effects have been noted on analysis of pre-treatment, concurrent and post-treatment serum chemistries, urinalyses, and electrocardiograms
- Two markers of inflammation – platelet count and ESR – have been note to be elevated following the first OK432 injection session
  - Levels for both markers, dropped during the study period
  - The safety profile is consistent with experience reported in Japan using OK-432 as a sclerotherapy agent and/or as a systemic immunostimulant in over 30,000 patients
- The inflammatory response that is typical of OK-432 is temperature, pain, edema, erythema, fatigue and decreased appetite
  - These side effects peak within the first few days after an injection session and resolve to pre-treatment levels over the ensuing two weeks (see Figures 1 and 2)
- Most patients require only ibuprofen and/or acetaminophen during this period

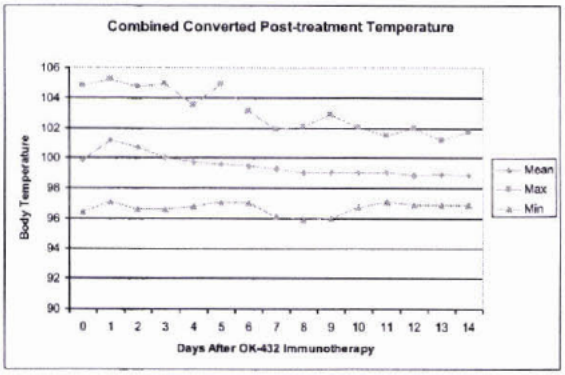

Figure 1. Body temperature – mean, maximum, and minimum values – as recorded by patient families in post-treatment diaries for two week periods following OK-432 immunotherapy [n=132].

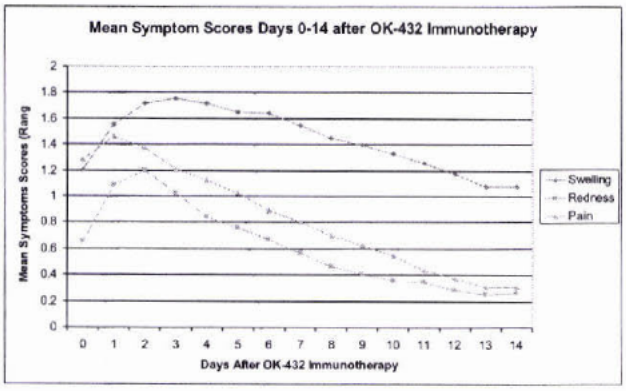

Figure 2. Mean symptom scores from post-treatment diaries for swelling, redness and pain for two week periods following OK-432 immunotherapy (0, none; 1, mild; 2, moderate; 3, severe; responses from 304 injections).

Adverse Events

- An adverse event is defined as any deviation from the expected response to OK-432 immunotherapy
- The **expected response** includes fever and chills for approximately 3-7 days after the injection, an inflammatory response approximately 1-5 days following the injection, and pain/discomfort at the injection site (see Figures 1 and 2)
- Examples of **adverse events** include sepsis, shock, airway compromise, seizures, cardiac disease, joint pain or inflammation, or hematuria
    - However, any finding other than those expected should be considered an "adverse event".

Major Adverse Events

- Major adverse events have been reported when OK432 is used for LM
- The most serious adverse event directly attributable to OK-432 injection is **airway obstruction**
    - This potential complication should be anticipated in **all LMs that encroach on the upper airway**
    - Post-injection swelling can cause further airway compromise
        - Pre-treatment tracheotomy should be considered in these cases
        - Because OK-432 immunotherapy results in an inflammatory response, it is possible that post-injection infections are underestimated
        - Oral antibiotics can be used for inflammatory responses
- **All ADVERSE EVENTS SHOULD BE REPORTED IMMEDIATELY TO [redacted] AND [redacted].**

Reporting Adverse Events

- When reporting the adverse event, the event must be described in detail and the adverse event form must be completed (attached)
- The event should be reported within 24 hours, so outcome may NOT be known at the time the event is

reported

- Based on the nature of the event, it is possible that the treatment protocol we are currently using may be modified or discontinued
    - Alternatively, no changes may be made.
    - This decision will be made based on the nature of the event and in consultation with the FDA.
- All study centers will be notified of the nature of adverse events as they occur however confidentiality will be maintained in this reporting

Withholding Further OK-432 Immunotherapy

- Patients may experience **specific responses that will mandate temporarily or permanently withholding further injections of OK-432** for that subject
    - A serious adverse reaction, probably, or definitely related to treatment with OK-432
    - Signs of post-streptococcal glomerulonephritis (hematuria, proteinuria) with no alternative etiology established
- In the event of such reactions, the affected subject should receive **NO MORE INJECTIONS**
    - The event should be reported immediately to [redacted]
    - These persons will report the event to the FDA
    - A written safety report also will be submitted to the IND within 15 calendar days of receipt of this information
- **Any life-threatening or fatal events must be reported to the FDA as soon as possible and NO LATER THAN seven calendar days after receipt of this information**

Withdrawing from the Study

- Any patient can withdraw from the study at any time

Statistical Analysis Plan

Protocol OK-432-003-OPEN
Protara Therapeutics, Inc.

A MULTICENTER, OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF OK-432 IMMUNOTHERAPY IN INDIVIDUALS WITH LYMPHATIC MALFORMATIONS

Phase 2 Expansion

Protocol Version Dated : MAY2014

Prepared 

Version: Final

Date: April 7, 2021

Statistical Analysis Plan

Protocol OK-432-003-OPEN
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A MULTICENTER, OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF OK-432 IMMUNOTHERAPY IN INDIVIDUALS WITH LYMPHATIC MALFORMATIONS

Phase 2 Expansion

Protocol Version Dated : MAY2014

This Statistical Analysis Plan has been reviewed and approved by:

[redacted]

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LIST OF ABBREVIATION

AE	Adverse event
ATC	Anatomic therapeutic chemistry
BMI	Body mass index
CRF	Case report form
CI	Confidence interval
CT	Computed tomography
ECG	Electrocardiogram
ICF	Informed consent form
IND	Investigational new drug
LM	Lymphatic malformations
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intent-to-treat
MRI	Magnetic resonance imaging
PE	Physical examination
PT	Preferred term
SAE	Serious adverse events
SAP	Statistical analysis plan
SAS	Statistical analysis software
SD	Standard deviation
SE	Standard error
SOC	System organ class
TESAE	Treatment-emergent serious adverse events
WHO-DD	World Health Organization Drug Dictionary

1 INTRODUCTION

1.1 Study Background

The Sponsor is developing its proposed TARA-002 injection product for intracystic administration for the treatment of lymphatic malformations (LMs) in pediatric and adult subjects. TARA-002 is a lyophilized biological preparation for injection containing attenuated cells of Streptococcus pyogenes (A group, type 3) Su strain treated with benzylpenicillin. The characteristics of TARA-002 are intended to be comparable to OK-432 (manufactured by Chugai Pharmaceuticals) which is currently approved in Japan and Taiwan for use as treatment for LMs. Therefore, nonclinical and clinical studies with OK-432 support the development of TARA-002.

In the US, an Investigational New Drug application (IND) was opened by [redacted] at the University of Iowa to evaluate the potential safety and efficacy of OK-432 in the treatment of LMs. Under this IND, OK-432 was compassionately used for the treatment of LMs in pediatric and adult subjects as an alternative to surgery. After the conclusion of the Phase 2 randomized study (OK-432-002-RAND), all new subjects who presented with an LM and were eligible for treatment were treated under an open-label protocol for continued access to OK-432. The multicenter, open-label study (OK-432-003-OPEN) enrolled patients between September 2005 and November 2017.

1.2 Retrospective Analysis of OK-432-003-OPEN

This document provides a detailed description of the statistical methods and procedures to be implemented during the retrospective analysis of the multicenter, open-label study to evaluate the efficacy and safety of OK-432 immunotherapy in individuals with lymphatic malformations. This SAP includes the full dataset of subjects enrolled in study OK-432-003-OPEN between September 2005 and November 2017.

2 STUDY OBJECTIVES

The objective of this study is to provide immunostimulant OK-432 to subjects with macrocystic or mixed (>50% macrocystic) LMs and investigate the efficacy and safety of OK-432 as a treatment option in subjects with LMs.

2.1 Primary Efficacy Objective

To evaluate the rate of clinically successful outcomes of OK-432 immunotherapy in subjects with LMs.

2.2 Secondary Efficacy Objective

To further evaluate the response rate to OK-432 immunotherapy in subjects with LMs.

2.3 Safety Objective

To evaluate the safety and tolerability of OK-432 immunotherapy in subjects with LMs.

3 STUDY OVERVIEW

3.1 Study Design

Study OK-432-003-OPEN is a multicenter, open-label clinical study to evaluate the efficacy and safety of OK-432 immunotherapy in subjects with LMs. The study was conducted across 14 centers across the United States between September 2005 and November 2017. Most eligible subjects were between 6 months and 18 years of age with a LM that was macrocystic or mixed (>50% macrocystic). Classification was determined radiographically by computed tomography (CT) or magnetic resonance imaging (MRI) scans by measuring the volume of all cysts. Macrocystic LMs were defined as cystic spaces ≥ 2.0 ml (2 cm × 1 cm × 1cm). LM with both macrocystic and microcystic (multiple cysts, each less than 2 cc in volume) lesions were designated mixed LM.

Subjects received OK-432 immunotherapy shortly after enrollment. Each subject received a series of four-dose injections of OK-432 immunotherapy, unless a contraindication arose or a complete response was observed prior to completion of the injection series.

3.2 Study Procedures and Visit Structure

The maximum dose of OK-432 injected per subject at each session was 0.2 mg at a concentration of 0.01-0.05 mg/ml. This concentration was achieved by dissolving 0.1 mg vial of OK-432 in 10 ml of normal saline or 0.1 mg of OK-432 dissolved in 5ml normal saline. The dose was not based on lesion size.

Each injection session was performed by pediatric otolaryngologists, pediatric surgeons, or other surgical specialists with comparable expertise. Intracystic fluid was aspirated using a 25-gauge (occasionally larger) intravenous catheter. If necessary, needle placement was confirmed by ultrasonography. Following aspiration, OK-432 was injected into the cyst at the appropriate concentration. Following injection, subjects were observed in the recovery room/hospital for approximately ~4 hours. In the absence of symptoms that necessitate admission (including but not limited to sepsis, shock, airway compromise, seizures), subjects will be discharged.

A 4-dose injection series of OK-432, 6 to 12 weeks apart was planned for all subjects. If a contraindication existed, or a complete response was observed prior to completion of all 4 injections, subjects were treated with fewer than 4 injections. Subjects may have been treated with more than 4 injections if multiple LMs were treated or if there was a recurrence.

Study visits and procedures are summarized in the schedule of events in Table 1.

Table 1 Schedule of Events

Evaluation	Pre-Therapy	Treatment Period[5]					Post-treatment Period/Follow up		
	V1	V2; (1st Inj.)	V3; 2 weeks after First Inj.	V4; (2nd Inj.)	V5; (3rd Inj.)	V6; (4th Inj.)	V7; 1-6 months after Final Inj.	V8; 1 year after Final Inj. (Optional)	V9/Study Exit; 2 years after Final Inj. (Optional)
Informed Consent	x								
Complete History	x						x		
Focused History		x	x	x	x	x		x	x
Penicillin Allergy Testing	x								
Physical Examination	x	x	x	x	x	x	x	x	x
Clinical Staging[1]	x								x
Vital Signs		x	x	x	x	x	x	x	
Laboratory Analysis[7]	x						x		
Pregnancy Test[4]	x								
Medical Photography	x						x		
AE/SAE		x	x	x	x	x	x	x	x
MRI/CT[6]	x						x		
Assessment Questionnaire[2]		x		x	x	x			
Drug Administration		x		x	x	x			
Subject Diaries[3]		x		x	x	x			

V – Visit, Inj. – Injection

[1] Clinical staging based on modified de Serres classification.
[2] An assessment form with a return envelope was given to patient/family to fill out 3-5 days and 2-3 weeks after each injection series.
[3] Subjects documented daily temperature, pain, erythema, edema, and side effects for 14 days after each injection session.
[4] In females of child-bearing age.
[5] A 4-dose injection series was planned for all subjects spaced approximately 6-12 weeks apart. If a contraindication existed, or a complete response was observed prior to completion of all 4 injections, subjects were treated with fewer than 4 injections. Subjects may have been treated with more than 4 injections if multiple LMs were treated or if there was a recurrence.
[6] MRI/CT may have been performed at additional time points per Investigator's discretion.
[7] Laboratory analysis was conducted at concurrent evaluations if it was completed greater than 3 months from pre-treatment examination.

4 STUDY ENDPOINTS

4.1 Primary Efficacy Endpoint(s)

The primary efficacy endpoint is the proportion of subjects with clinical success defined as having either a complete or a substantial response to therapy at 1-6 months post-therapy as assessed by imaging (MRI and/or CT). The post-therapy imaging was done at 1-6 months after the fourth or last injection series (if fewer than four). If subjects were treated with more than 4 injections, post-therapy response for the primary analysis was evaluated 1-6 months after the 4th injection.

Response to therapy will be graded as percentage reduction in LM volume from pre-treatment to post-treatment as assessed by imaging. Table 2 describes the criteria for assessment of clinical response.

Table 2 Response Assessment Criteria

Complete Response	90%–100% reduction in volume
Substantial Response	60%– 89% reduction in volume
Intermediate Response	20%–59% reduction in volume
No Response	<20% reduction in volume

4.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints include:

1. The proportion of subjects who demonstrated complete, substantial, intermediate, or no response 1-6 months post-therapy.
2. The proportion of subjects whose investigator-evaluated overall response was recorded as “Clinical Improvement” or “No Change”. The evaluation was performed visually at each injection series and follow up visits from second injection series onwards.
3. The change and percent change from baseline in lesion volume from the imaging data, where the baseline volume is defined as the pre-therapy volume.

4.3 Safety Endpoints

The safety endpoints include:

- Reactions to OK-432 including:

  - Subject study diaries documenting daily temperature, pain, erythema, edema, and side effects for 14 days after each injection session.
  - Reactions reported through post-treatment questionnaire Case Report Form (CRF).
  - Investigator-evaluated clinical reactions to OK-432 reported through post-treatment Clinical Response CRF.
- Incidence of serious adverse events.
- Clinically significant changes in laboratory values (chemistry, hematology, serology and urinalysis).
- Physical exam.
- Vital Signs.

5 ANALYSIS POPULATIONS

5.1 Enrolled Population

The Enrolled population includes all subjects that have been enrolled in the study. All subjects not designated as Screen Failures will be considered enrolled.

5.2 Safety Population

The Safety population includes all Enrolled subjects that have been treated with at least one injection of OK-432.

5.3 Modified Intent-to-Treat Population

The Modified Intent-to-Treat (mITT) population includes all Enrolled subjects that have been treated with at least one injection of OK-432 and have either post-therapy imaging assessment data or at least one investigator assessment for overall response throughout the study. Since the open-label study was conducted so that subjects may have continued access to OK-432 on a compassionate use basis and response data was not well documented for everyone, the modified Intent-to-Treat population (mITT) population is defined to better assess the response rate based on the observed data.

6 STUDY SUBJECTS

6.1 Subject Disposition

Subject disposition information, including the counts and percentages of subjects who complete or discontinue from the study, as well as reasons of discontinuation, will be summarized for the Enrolled population.

6.2 Demographic and Baseline Characteristics

Subject demographics will be summarized for the Safety and mITT populations. The summary will include age, sex, race, ethnicity, clinical stage, laterality, lymphatic malformation type, whether the subject had prior surgery, and weight, height, and BMI at baseline. Age groups will be summarized by the following categories:

- < 6 months
- 6 months to <18 years
- 6 months to <2 years
- 2 to <6 years
- 6 to <12 years
- 12 to <18 years
- 18 to < 65 years
- ≥ 65 years

6.3 Concomitant Medications

Concomitant medications include all medications taken during the conduct of the study (from pre-treatment through post-treatment period). New concomitant medications include all medications started after receiving the first OK-432 treatment.

The World Health Organization Drug Dictionary (WHO-DD) will be used to categorize verbatim descriptions of non-study medications into the Anatomic Therapeutic Chemistry (ATC) classification system. Each verbatim name will be classified by anatomical main group (ATC level 1), therapeutic subgroup (ATC level 2), pharmacological subgroup (ATC level 3), chemical subgroup (ATC level 4), preferred term, and trade name (if appropriate).

Numbers and percentages of subjects with concomitant and new concomitant medications will be summarized by ATC classification (ATC level 2 and level 4) for the Safety population.

6.4 Medical and Surgical History

Medical and surgical history and prior treatments will be summarized for all subjects in the Safety population. Subject level data will be presented in data listings.

6.5 Protocol Deviations and Violations

No traditional protocol deviation data was collected in this study.

6.6 Study Drug Administration and Extent of Exposure

Study drug administration and dosing information will be presented in a listing. The extent of study medication exposure will be summarized for the Safety Population. The summary will include total dose administered and number of injection sites during each injection session along with the number of injections and sum of doses taken during the full treatment period.

7 STATISTICAL METHODS OF ANALYSIS

7.1 General Considerations

Efficacy endpoints will be summarized for the mITT population. Descriptive statistics as well as data listings will be provided. Safety analysis will be conducted on the Safety population. The summaries of the categorical data will be calculated based on the number of subjects with non-missing response data.

Continuous variables will be summarized by frequency, mean, standard deviation, standard error, median, minimum, and maximum, and categorical variables will be summarized by frequency and percentage in corresponding categories.

Minimum and maximum values will be rounded to the precision of the original value. Means and medians will be rounded to one decimal place greater than the precision of the original value. SDs, SEs, and 95% confidence intervals (CIs) will be rounded to two decimal places greater than the precision of the original value. Percentages for summarizing categorical data will be rounded to the nearest whole number.

7.1.1 Hypothesis Testing

There is no pre-specified formal hypothesis of efficacy in the protocol.

7.1.2 Defining the Study Baseline

In general, the baseline value for each variable is defined as the value recorded at the last visit on or before the first OK-432 injection is administered.

7.1.3 Deriving Response Data

Response categories as defined in Section 4 will be derived based on change in legion volume from pre-treatment to post-treatment, if applicable. Orthogonal measurements of LM size on CT or MRI findings will be used to calculate volume/size. Volume will be derived from length × width × height if all the three values are available. If any of the length, width, or height data are missing, the volume value as reported will be used to derive the response. If volume data is not available but there is collected response data, then collected response data will be used.

7.1.4 Handling of Multiple Observations

For multiple observations for the same endpoints that are collected multiple times on the same day and time, the sample that was deemed the re-test will be used for data analysis. For multiple records of subject diary and questionnaire data, the highest severity or temperature will be used to conservatively capture safety data.

Only data from the scheduled visits will be used for tables, but all visits, including unscheduled visits will be presented in listings.

For observations from multiple radiologists, the average of the two values will be used for analysis. For multilocular volume measurements (i.e. results from multiple sides of the same LM), the values will be added together for analysis.

7.1.5 Visit Mapping due to Change in Protocol

Initially, the Phase 2 expansion open-label study enrolled subjects from September 2005 using similar visit structures post-treatment as the Phase 2 randomized study (post-treatment visits occurred at ~2 weeks, 24-26 weeks, 50-52 weeks and 2 years after treatment). The post-treatment efficacy assessment was performed at ~2 weeks after treatment. However, the protocol was amended in January 2011, which expanded the visit window from ~2 weeks post-treatment to 1-6 months post-treatment in order to allow flexibility with visits after completion of the injection series. Additionally, any visits after the 1-6 months post-treatment was made optional.

For the purpose of analysis, the ~ 2 weeks post-treatment and the 24-26 weeks post-treatment visit will both be mapped to the 1-6 months post-treatment for patients who were enrolled prior to the protocol amendment. For these subjects who may have multiple records of visits at the 1-6 months post-treatment, the first record corresponding to the ~ 2 weeks post-treatment will be used in table summaries. Both records will be presented in the listings with associated visit dates.

7.1.6 Handling of Missing or Partial Dates

In cases of incomplete dates for AEs, the missing component(s) will be assumed as the most conservative value(s) possible. For example, the imputation rule is to conservatively capture AEs with missing start dates as treatment-emergent AEs (TEAEs). Actual data values, as they appear in the clinical database, will be shown in the data listings. Rules for partial dates are described in Table 3.

Table 3 Rules for Missing or Partial Dates

Parameter	Missing	Additional Conditions	Imputation
Start date for AEs	D only	M and Y same as M and Y of first injection	Date of first injection

Parameter	Missing	Additional Conditions	Imputation
		M and/or Y not same as M and Y of first injection	First day of non-missing month
	D and M	Y same as Y of first injection	Date of first injection
		Y not same as Y of first injection	Use Jan 1 of non-missing year
	M, D and Y	None – date completely missing	Date of first injection
Stop date for AEs	D only		Last day of non-missing month
	D and M		Use Dec 31 of non-missing year
	M, D and Y	Deceased	Date of death
		Not deceased	Date of the end of study participation

Notes: D=Day, M=Month, Y=Year

Start and stop dates were not collected for non-study medications and will not be imputed. Determinations for new concomitant medications will be based on the collected visit date.

7.1.7 Handling of Missing Efficacy Data

In general, no imputation of missing efficacy data will be performed. The efficacy data will be presented as observed.

7.2 Efficacy Analyses

7.2.1 Analyses of Primary Endpoint(s)

The proportion of subjects with clinical success (a complete or substantial response) at 1-6 months post-therapy as assessed by imaging will be summarized for the mITT population.

7.2.1.1 Sensitivity Analysis

The primary analysis will also be repeated for a subgroup of the mITT population by excluding subjects with missing informed consent documentation. Note that the informed consent form (ICF) documentation for some Enrolled subjects were not retrievable from study sites for this legacy study. Therefore, this sensitivity analysis will be performed to evaluate the robustness of the efficacy results on subjects that were enrolled to the study with available ICF documentation.

7.2.2 Analyses of Secondary Endpoints

7.2.2.1 Response Rate Post-Therapy as Assessed by Imaging

The proportion of subjects who demonstrated a complete (90%–100% reduction in volume), substantial (60%– 89% reduction in volume), intermediate (20%–59% reduction in volume), or no (<20% reduction in volume) response 1-6 months post-therapy as assessed by imaging will be summarized for the mITT population.

To explore how the post-therapy response rates vary in the population by various covariates, the categories of response rates in subjects will also be summarized by the following covariates:

- Age groups as defined in Section 6.2,
- Sex,
- Race (White/Non-White),
- Prior Surgery (Yes/No),
- LM type (macrocystic, mixed, microcystic),
- Clinical stage (clinical stage tables will include clinical stage modifier data for mediasinal involvement and retropharyngeal involvement),
- Number of injections received.

The primary endpoint, proportion of clinical success, will also be summarized by the above covariates.

7.2.2.2 Investigator-evaluated Overall Response

The proportion of subjects who ever demonstrate a Clinical Improvement and No Change as determined by an investigator judgement throughout the conduct of the study will be summarized for the mITT population. Investigator-evaluated Overall response (either Clinical Improvement or No Change) will also be summarized by visit (including an overall row that pooled data from all visits) for the mITT population. A similar summary will be produced for overall response as determined by investigator judgement by the covariates as outlined in Section 7.2.2.1 for the mITT population.

In addition, the proportion of subjects who recorded "Clinical Improvement" on the Post-therapy Clinical Response CRF collected from the second injection visit onwards will be pooled and summarized ≥ 6 months post-treatment. Only subjects who received 4 injections or less will be included in this summary to assess persistence of response on the same LM. The subjects who were treated with more than 4 injections could have had multiple LMs treated so excluding them ensures persistence of response is measured for the same LM in a subject. For the subgroup of subjects with or without clinical success 1-6 months post-treatment, ≥ 6 months post-treatment will be summarized for the mITT population.

7.2.2.3 Reduction in Volume Post-Therapy

The baseline lesion volume, post-therapy volume, change from baseline, and percent change from baseline will be summarized numerically for the mITT population. Additionally, pre- and post-therapy volume will be presented in a listing for all subjects.

7.2.3 Time to Event Analyses for Clinical Improvement

The time from receiving the first OK-432 to observing the first record of "Clinical Improvement" on the Post-therapy Clinical Response CRF during the treatment period and 1-6 months post-treatment evaluation will be computed for each subject in the mITT population. If subjects did not record "Clinical Improvement" during those specific treatment periods, subjects will be censored at the last date available among the investigator assessment date, vital signs, injection date, or termination date within the specific period, whichever is the latest. Additionally, subjects who had more than 4 injections will be censored at the fourth injection series if they have not recorded Clinical Improvement. The Kaplan-Meier estimates and the 95% CIs of the survival functions of the time to observe 'Clinical Improvement' will be calculated.

7.2.4 Other Analysis

For subjects with ≥6 months of post treatment follow up, clinical outcome at study completion such as no recurrence, microcystic residue/residual cyst, recurrence, surgery, and no response/change will be summarized for the mITT population.

7.3 Safety Analyses

7.3.1 Adverse Events

Adverse Events (AE) for this study was defined as any deviation from the expected response to OK-432. The expected response includes fever and chills usually beginning six hours after OK-432 injection and later subsiding spontaneously. The pain and fever are temporary and usually subside with the use of medications such as Tylenol. The typical local reactions to the initial OK-432 injection is redness and swelling. This usually evolves over a period of week, leaving a superficial scar at the site of injection, which last for several weeks.

Details of collection and reporting of Reactions to OK-432 is provide in Section 7.3.1.1. At the time of the trial, only serious AEs were collected per protocol. Refer to Section 7.3.1.2 for serious AEs data analysis.

7.3.1.1 Reactions to OK-432

The collection of reactions data in this assessment included an evaluation of commonly occurring local and systemic reactions including measurement of body temperature in the form of Subject Diaries, Post-treatment Questionnaires, and Post-therapy Clinical Response in the CRF. Subject Diaries were completed from the day of injection up to 14 days post injection and were assessed by visit, time point, and severity (for local reactions and side effects). Post-treatment Questionnaires were completed 24 hours post injection, 3–5 days post injection, and 2–3 weeks post injection via a clinic visit or safety phone call, and were assessed by visit, time point, and severity (for local reactions). Post-therapy Clinical

Response CRFs were completed from the second injection onwards on the day of injection and included an assessment of local and systemic reactions.

Additionally, the Post-therapy Clinical Response CRF included an assessment of Hematuria to assess post-streptococcal glomerulonephritis and an assessment of Jones Criteria for the diagnosis of an initial attack of Rheumatic Fever.

The following OK-432 reaction summaries will be presented for the Safety population:

- Reactions reported through Subject Diary by reaction category, verbatim terms, visit/timepoint, severity (for local reactions and side effects).
- Temperature (collected via Subject Diary) will be summarized and total as (a) number and percent of subjects with at least 38°C (100.4°F), and (b) number and percent of subjects with at least 40°C (104°F). Fever will be defined as a body temperature of at least 38°C.
- Reactions reported through questionnaire (Post-Treatment Questionnaire CRF) by reaction category, verbatim terms, visit/timepoint, severity (for local reactions).
- Investigator-evaluated reactions (Clinical Response CRF) to OK-432 by reaction category, verbatim terms, visit.
- Jones Criteria for the Diagnosis of an Initial Attack of Rheumatic Fever (Clinical Response CRF) by major/minor manifestations, visit/timepoint; Assessment of Hematuria to assess post-streptococcal glomerulonephritis.

A listing will be provided for the information reported through the subject study diary, questionnaires, and clinical response CRFs.

Additionally, the reactions reported through Subject Diary, Post-Treatment Questionnaire CRF and Clinical Response CRF will be summarized for the subgroup of subjects with non-missing informed consent dates similar to the primary efficacy analysis.

Time to Event Analysis

The time from first pain to resolution and time from first fever to resolution will be computed for each subject at each injection. The first day of pain after a specific injection will be defined as the first diary report of pain on or after the injection day. The first day of fever and the time to fever resolution will be defined similarly using temperature collected in the diary data. If subjects did not record fever/pain resolution during the specified time period (i.e. within 14 days after injection), they will be censored at the last available date of diary report of fever/pain. The Kaplan-Meier estimates and the 95% confidence intervals of the survival functions of pain and fever will be calculated and plotted by injection.

7.3.1.2 Serious Adverse Events

AEs were referred to as "Serious Adverse Events" (SAEs) in the study CRF. For the purposes of the retrospective analysis, treatment-emergent serious adverse events (TESAEs) are defined as SAEs that occur after the first injection and within 35 days of the final injection. TESAEs will be summarized and listed for the Safety population and the subgroup of subjects with non-missing informed consent dates. The verbatim term used by investigators will be retrospectively mapped to preferred terms using the MedDRA dictionary. TESAEs, TESAEs that were determined by the investigator to be related to the study drug, and TESAEs leading to study discontinuation will be summarized by System Organ Class (SOC) and Preferred Term (PT).

7.3.2 Clinical Laboratory Evaluations

All hematology, chemistry, urinalysis and serology results will be summarized for the Safety population, including data from the scheduled assessments. Laboratory values collected from unscheduled visits will be provided in listings only. Laboratory assessments that are outside of normal ranges and/or with potential clinical significance will be considered an abnormal value and flagged in the listings. Note that due to lack of a central labs normal ranges are different across sites. Therefore, shift tables based on reference normal ranges will be used.

Baseline values, the values at post-baseline visits, and changes from the baseline values will be summarized descriptively for all laboratory parameters with numerical measures. Additionally, shift tables will be generated to summarize the normal and abnormal (abnormal high and abnormal low) status changes from baseline to post-baseline visits. The frequency and percentage of subjects with laboratory results above or below the normal range at each scheduled assessment or any time during the treatment will be summarized.

The following lab tests will be summarized:

Test Category		
Chemistry	**Hematology**	**Urinalysis**
• Alanine Aminotransferase • Albumin • Alkaline Phosphatase • Aspartate Aminotransferase • Bilirubin • C Reactive Protein • Calcium • Carbon Dioxide • Chloride • Creatinine • Globulin • Glucose • Potassium • Protein • Sodium • Urea Nitrogen	• Basophils • Eosinophils • Ery. Mean Corpuscular HGB Concentration • Ery. Mean Corpuscular Hemoglobin • Ery. Mean Corpuscular Volume • Erythrocyte Sedimentation Rate • Erythrocytes • Erythrocytes Distribution Width • Hematocrit • Hemoglobin • Leukocytes • Lymphocytes • Mean Platelet Volume • Monocytes • Neutrophils • Neutrophils Band Form • Platelets	• Bacteria • Bilirubin • Casts • Color • Crystals • Epithelial Cells • Erythrocytes • Glucose • Ketones • Leukocyte Esterase • Leukocytes • Mucus • Nitrite • Occult Blood • Protein • Specific Gravity • Turbidity • Urobilinogen • Volume • pH **Serology** • Streptolysin O Antibody

7.3.3 Electrocardiogram

Although Electrocardiogram (ECG) was not included in the amended protocol after January 2011, subjects enrolled prior to the protocol amendment underwent ECG measurements as part of an earlier version of the protocol as described in Section 7.1.5. Available ECG raw values and change from baseline will be summarized descriptively at each visit for heart rate, P wave axis, P wave duration, PR interval, QRS duration, QT dispersion, QT interval with Correction method, R wave axis and T wave axis. Subject level data will be provided in a listing.

7.3.4 Physical Examination

The results of physical examination including the focused physical exam will be provided in a listing.

7.3.5 Vital Signs

Vital signs (temperature, systolic and diastolic blood pressure, heart rate, and respiration rate) and weight, and its change from baseline, will be summarized descriptively.

8 STATISTICAL SOFTWARE

All analyses will be performed with SAS® version 9.4.