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LeuTech®

Technetium Tc 99m Anti-CD15 Antibody

BLA # 99-1407

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate transaminase
BMI	body mass index
bpm	beats per minute
BUN	blood urea nitrogen
CI	confidence interval
CT	computed tomography
EDTA	ethylenediaminetetraacetic acid
FN	false negative
FP	false positive
FPF	false positive fraction
HAMA	human anti-mouse antibody
Hct	hematocrit
Hgb	hemoglobin
HMPAO	hexylmethylpropylene amine oxime
ID	injected dose
LAO	left anterior oblique
LDH	lactate dehydrogenase
LFOV	large field-of-view
LL	lower limit
LR	likelihood ratio
mm Hg	millimeters of mercury
MIRD	Medical Internal Radiation Dose
NOEL	no observed effect level
PID	pelvic inflammatory disease
PMN	polymorphonuclear neutrophil
PPV	positive predictive value
NSAID	non-steroidal anti-inflammatory drug
NPV	negative predictive value
RAO	right anterior oblique
RLQ	right lower quadrant
ROC	receiver operating characteristic
ROI	region-of-interest
SPECT	single photon emission computed tomography
SSEA-1	stage specific embryonic antigen-1
TN	true negative
TP	true positive
TPF	true positive fraction
UL	upper limit
WBC	white blood cell
WHO	World Health Organization

1. ORGANIZATION OF THE DOCUMENT

This document is organized into the following main sections to provide a brief but comprehensive overview of the efficacy and safety results from clinical studies conducted with Technetium Tc 99m LeuTech® for the diagnosis and ruling out of appendicitis in patients with equivocal signs and symptoms:

- Section 2: ***Introduction and Overview*** provides background material regarding the diagnosis of equivocal appendicitis, a discussion of the scientific rationale for the development of LeuTech® and an overview of the clinical development program for Tc 99m LeuTech®.
- Section 3: ***Chemistry and Formulation*** includes a brief description of the contents of the LeuTech® kit and a description of the reconstitution procedure.
- Section 4: ***Tabular Summary of Clinical Studies*** summarizes the design features of all studies conducted with Tc 99m LeuTech®.
- Section 5: ***Clinical Pharmacology and Radiation Dosimetry*** summarizes the results of the Phase 1 clinical pharmacology study (Study 97-002) conducted to evaluate the biodistribution and radiation dosimetry of Tc 99m LeuTech®. Results from literature reports are also summarized in this section.
- Section 6: ***Efficacy*** provides an overview of the efficacy data, which includes results from a pivotal Phase 3 study (Study 98-004) and a supporting Phase 2 study (Study 97-003). Details of study design and statistical methods are included, along with a presentation of the efficacy results from the individual studies and pooled data from both studies.
- Section 7: ***Safety*** presents a summary of safety data, which include occurrence of adverse events, changes in clinical laboratory measurements, changes in vital signs and assessment of human anti-mouse antibody (HAMA) response.
- Section 8: ***Non-Clinical Studies*** summarizes the preclinical pharmacology and toxicology studies that were conducted to evaluate the safety of LeuTech®.
- Section 9: ***Summary and Conclusions*** presents a final overview of the data presented to demonstrate efficacy and safety of Tc 99m LeuTech® for the diagnosis and ruling out of appendicitis in patients with equivocal signs and symptoms.
- Section 10: ***References*** provides literature citations for all sections.
- Section 11: ***Labeling (Package Insert)*** provides the proposed LeuTech® package insert that was submitted in the BLA.

2. INTRODUCTION AND OVERVIEW

2.1 THE PRODUCT AND ITS PROPOSED INDICATION

LeuTech® is a kit for the preparation of Technetium Tc 99m Anti-CD15 Antibody Injection (Tc 99m LeuTech®). The murine monoclonal IgM antibody in LeuTech® is produced by the hybrid hybridoma cell line RB5. Tc 99m LeuTech® recognizes CD15 antigens that are expressed on human polymorphonuclear neutrophils (PMNs). The radiolabeled antibody binds *in vivo* to PMNs that localize in the inflamed appendix.

The proposed indication for Tc 99m LeuTech® is as follows:

"Scintigraphy with Technetium Tc 99m Anti-CD15 Antibody is indicated for the diagnosis of appendicitis in patients with equivocal signs and symptoms. It is useful to rule out appendicitis in patients presenting with equivocal diagnostic evidence."

2.2 DIAGNOSIS OF EQUIVOCAL APPENDICITIS

Appendicitis is often diagnosed easily by its characteristic signs and symptoms that either appear by the time the patient presents to the emergency room, or that develop during a few hours after hospital admission for observation (Condon, 1986). However, Poole (1988) reported that as many as 55% of cases of appendicitis present without classic signs and symptoms. Additionally, symptoms may remain atypical and signs may not become unequivocally positive during any reasonable observation interval. In these cases, the disease progresses from acute appendicitis (a stage of the disease with relatively low morbidity) to perforated appendicitis, a stage of the disease with many-fold greater rates of morbidity and death (Hauswald *et al.*, 1976; Cooperman, 1983). Perforation of the appendix is associated with an increase in the rates of wound infection (from < 1% to 35% in one series (Scher & Coil, 1980) and from 1.4% to 6.4% in another (Hale *et al.*, 1997). Increased rates of urinary tract retention and infection (from 0.8% to 1.8%) and intraperitoneal abscess formation (from 0.3% to 0.8%) have also been reported in cases of perforation (Hale *et al.*, 1997). Perforation is also associated with prolonged hospitalization (from 3.4 days to 7.2 days in one series (Hale *et al.*, 1997), and from 4.9 days to 11.3 days in another) (Scher & Coil, 1980). Patients at either end of the age spectrum are especially at risk for such disease progression to occur, with a perforation rate of 38% in young children and 49% in the elderly (Scher & Coil, 1980). This is compared to a rate of 18% in patients between these two higher-risk age groups (Hale *et al.*, 1997). Children present a diagnostic challenge because of atypical presentation (Rothrock *et al.*, 1991) and the inability to articulate symptoms or their anatomic location (Hale *et al.*, 1997). Up to 28% of pediatric patients ultimately diagnosed as having appendicitis had been previously evaluated and misdiagnosed (Rothrock *et al.*, 1991). Likewise, the elderly, as a result of multiple infirmities and a more variable presentation of

the disease, present a more complicated differential diagnosis and, at the same time, are at greater liability for exaggerated morbidity and risk of mortality relative to the younger adult population (Lewis *et al.*, 1975; Owens & Hamit, 1978).

In order to counter this threat, some surgeons act preemptively by operating on patients with equivocally positive signs or in the presence of atypical but suggestive symptoms. Surgery that is performed to avoid the risk of perforated appendix is often an error of commission. Unnecessary surgery results in pain, additional costs, and the medical and surgical complications associated with surgery.

It is also well documented that unnecessary surgery in the face of equivocal appendicitis occurs especially in women of childbearing age. Diseases that prevail in reproductive-age female patients complicate the differential diagnosis. For example, urinary tract infections, pelvic inflammatory disease and ovarian abnormalities masquerade as appendicitis (Rothrock *et al.*, 1995; Nakhgevany & Clark, 1986). This leads to the danger that, while subjecting the woman to the unnecessary risk of general abdominal surgery, it deflects attention from, and treatment of, the primary underlying disease. The morbidity associated with negative appendectomy, although low, is not insubstantial and has given rise to concern among surgeons (Blind & Dahlgren, 1986; Lau *et al.*, 1984). In most series reported in the literature, as many as one-third to one-half of all appendectomies in women of childbearing age remove a normal appendix (Lewis *et al.*, 1975; Nakhgevany & Clark, 1986; Gough *et al.*, 1983).

Many individuals in the medical community have resigned themselves to this diagnostic dilemma. It is a balancing act between two alternatives, each with a risk of an untoward outcome (Velanovich & Satava, 1992; Sternbach & Rosen, 1995). The surgeon can wait to see whether equivocal symptoms will progress to a more definitive diagnosis, but at the risk that the disease will progress to perforated appendicitis with the associated increase in morbidity and mortality. Alternatively, the staff can act aggressively, sending the patient to surgery with the knowledge that, in nearly a quarter of the cases, the surgery will yield a negative finding (Berry & Malt, 1984). Hospital quality assurance boards, in judgement of a surgeon's performance, accept a 12% to 30% negative appendectomy rate in the management of this disease (Pearl *et al.*, 1995; Lewis *et al.*, 1975). Hospitals with large female populations or pediatric services tend to have higher negative laparotomy rates and the misdiagnosis of acute appendicitis has been one of the most common problems in the emergency room (Brewer *et al.*, 1965).

There have been advances in anatomically based medical imaging technology in the past decades, namely, the advent of high-resolution ultrasonography (Jeffrey *et al.*, 1987; Adams *et al.*, 1988) and of spiral (helical) x-ray computed tomography (CT) (Rao *et al.*, 1997a; Rao *et al.*, 1997b; Funaki *et al.*, 1998).

Some of these diagnostic tools, despite their shortcomings in sensitivity for acute appendicitis (Wade *et al.*, 1993), have found a restricted role in the management of equivocal appendicitis in certain subgroups. For example, ultrasonography is commonly used for diagnostic work-up of suspected acute appendicitis in pregnant patients, in whom concern over radiation exposure is paramount. However, these morphologically based technologies suffer from their inability to detect appendicitis until the disease has progressed so that grossly anatomically evident changes have already taken place (Schwerk *et al.*, 1989; Puylaert *et al.*, 1987). Additionally, CT requires administration of large volumes of contrast media with associated discomfort and risk.

Another pertinent technology that uses a mechanism of diagnostic action based on physiologic function has recently become available (Evetts *et al.*, 1994; Rypins *et al.*, 1997). This product, technetium Tc 99m exametazine (HMPAO, or hexamethylpropylene amine oxime), localizes infectious processes by the principle of white blood cell (WBC) labeling. Although promising, this method involves withdrawing 25 cc – 50 cc of the patient's blood, transport of the patient's blood to a remote site for manipulation, radiolabeling of the WBCs and, finally, transport of the radiolabeled WBC preparation back to the patient for re-infusion. The involved process poses significant risks of re-administration errors and/or contamination that could result in iatrogenic transmission of AIDS and other infections. These risks have hampered practical application of this technique. The time it takes to complete the labeling procedure minimizes any advantages associated with its diagnostic mechanism of action.
Tc 99m LeuTech® overcomes the disadvantages that hinder acceptance of Tc 99m HMPAO labeled WBC technology while maintaining its functional mechanistic advantage. Tc 99m LeuTech® involves no phlebotomy, no risk of cross-contamination of patients' blood and infectious disease transmission, and it is procedurally rapid and yields a timely result.

2.3 SCIENTIFIC RATIONALE

Technetium Tc 99m anti-CD15 antibody (Tc 99m LeuTech®) has high affinity and specificity for PMNs. The carrier molecule, anti-CD15 antibody, is directed against the carbohydrate moiety 3-fucosyl-N-acetyl-lactosamine contained in CD15 antigens that are expressed on human PMNs and eosinophils (Solter & Knowles, 1978; Macher & Beckstead, 1990). The CD15 antigen is not expressed on other major cellular blood components, such as lymphocytes, platelets or red blood cells (Solter & Knowles, 1978). The CD15 antigen is abundant on the surface of each circulating PMN, with approximately 5.1×10^5 antigenic sites per circulating PMN (Thakur *et al.*, 1988). Tc 99m LeuTech® has very high affinity for the CD15 receptor, with a dissociation constant of 10^{-11} *M* (Thakur *et al.*, 1988).

Following intravenous injection, Tc 99m LeuTech® is rapidly taken up in infectious sites. Although Tc 99m LeuTech® is an intact IgM antibody, blood

clearance is sufficiently rapid to reduce blood pool background, resulting in high target-to-background ratios. However, the blood clearance is slow enough to permit the antibody to bind to circulating PMNs to a significant extent.

2.4 CLINICAL DEVELOPMENT PROGRAM

The Tc 99m LeuTech® clinical development program was designed to assess the diagnostic accuracy of this radiopharmaceutical in appendicitis, not in the easily diagnosed cases, but in those cases where signs and symptoms are equivocal. The clinical program included, among others, the three groups in which equivocal appendicitis is most difficult to diagnose and carries the greatest threat of morbidity and mortality. Specifically, enrollment was open to children (24% of the total patient enrollment in the pivotal Phase 3 trial was in the pediatric age group, 5 years – 17 years), female patients of childbearing age (41% of total in the pivotal Phase 3 trial) and the elderly (5% of the total in the pivotal Phase 3 trial). The program was designed not only to determine how accurate Tc 99m LeuTech® is for the diagnosis of appendicitis, but also whether it can provide diagnostic information in a timely manner, because time is critical in the successful management of this rapidly progressing disease.

Palatin Technologies, Inc. (Palatin) conducted a total of four studies under two INDs that were summarized in the BLA. Under the first (BB-IND 7358) was a series of three studies, beginning with a Phase 1 study and progressing to the controlled Phase 2 and Phase 3 studies that were conducted in pursuit of the equivocal appendicitis indication. Under the second (BB-IND 7996), a Phase 2 study was conducted in pursuit of an indication not sought in the initial application (osteomyelitis). Also performed under BB-IND 7996 was a Phase 1 study to evaluate HAMA response following two injections of LeuTech®. This study was not included in the BLA, but the results were summarized in the 120-Day Safety Update. Brief summaries of these clinical studies are provided below:

- Study 97–002 was a Phase 1 study to evaluate safety, including radiation dosimetry, biodistribution and excretion of Tc 99m LeuTech® in normal, healthy volunteers. A total of 10 volunteers of both sexes were enrolled at a single site.

- Study 97–003 was a Phase 2 study to establish initial evidence of diagnostic efficacy for Tc 99m LeuTech® in the target population of patients with equivocal signs and symptoms of acute appendicitis. A total of 56 patients, of both sexes, ages nine years and older, were enrolled at two hospitals in the U.S.

- Study 98–004 was a Phase 3 pivotal study for safety and effectiveness of Tc 99m LeuTech® in the target population of patients with equivocal signs

and symptoms of acute appendicitis. A total of 203 patients, of both sexes, ages five years and older, were enrolled at 10 hospitals throughout the U.S.

- Study 98–005 was a Phase 2 study conducted to gain initial experience with Tc 99m LeuTech® in the diagnosis of osteomyelitis, an indication not sought in the initial application. Eight patients were enrolled as of March 8, 1999 and were included in the BLA submission. An additional 16 patients were enrolled before the study was terminated and were included in the 120-Day Safety Update to the BLA.

- Study 99-001 was a Phase 1 study conducted to evaluate HAMA response following two injections of non-radioactive LeuTech® (reconstituted with decayed Tc 99m). This study was not included in the original BLA filing; however, the results were summarized in the 120-Day Safety Update. A total of 30 adult volunteers of both sexes were enrolled at a single site.

The BLA also included descriptions of studies conducted by investigators in the U.S. under BB-IND 2995 sponsored by Prof. Mathew Thakur of Thomas Jefferson University in Philadelphia, Pennsylvania. (For ease of reference, these studies were assigned study numbers in the same format as for those sponsored by Palatin.)

- Study 97–001 was a Phase 1 study of the potential of the antibody to elicit a HAMA response in normal healthy volunteers. Thirty adult volunteers of both sexes were enrolled at a single hospital.

- Study 95–001 was a Phase 1/2 study conducted to explore the potential diagnostic applications of the radiolabeled antibody for a variety of infectious and other inflammatory processes. A total of 69 adult patients of both sexes were enrolled and included in the BLA.

Finally, description of one other study that has been reported in the peer-reviewed medical literature (Gratz *et al.*, 1998) was included for completeness. The study was conducted in Germany with antibody kits manufactured in Italy by Sorin Biomedica from antibody supplied from the United States. A total of 17 adult patients with infectious disease were evaluated.

3. CHEMISTRY AND FORMULATION

LeuTech®, Kit for the preparation of Technetium Tc 99m anti-CD15 Antibody, is a lyophilized, sterile, non-pyrogenic, unit dose formulation of a murine IgM monoclonal antibody intended for intravenous administration after reconstitution and radiolabeling with Sodium Pertechnetate Tc 99m Injection, USP. It is supplied as a kit consisting of a lyophilized, unit dose vial intended for reconstitution and instant radiolabeling with Sodium Pertechnetate Tc 99m Injection and commercially available 500 mg/mL Ascorbic Acid Injection, USP. The contents of the lyophilized vial are described below:

Component	Amount per vial
Partially Reduced RB5 IgM (anti-CD15) Antibody	0.25 mg
Maltose, monohydrate	12.5 mg
Succinic Acid, ACS	0.221 mg
Sodium Potassium Tartrate, tetrahydrate, USP	0.522 mg
Glycine, USP	28 µg
Disodium EDTA, dihydrate, ACS	9.3 µg
Stannous Tartrate	54 µg

At the time of clinical use, Technetium Tc 99m anti-CD15 Antibody is prepared by reconstituting the LeuTech® lyophilized vial with 0.2 mL to 0.35 mL of Sodium Pertechnetate Tc 99m Injection containing 20 mCi to 40 mCi (740 MBq to 1480 MBq) of Technetium Tc 99m and incubating the reconstituted vial at 37°C for thirty (30) minutes. After the reconstituted vial is incubated for thirty (30) minutes, a sufficient volume of 500 mg/mL Ascorbic Acid Injection is added to the preparation in the vial to bring the final preparation volume to 1.0 mL.

The lyophilized LeuTech® kit should be stored at 2°C to 8°C. After radiolabeling with Technetium Tc 99m and addition of Ascorbic Acid Injection, USP, it should be kept at room temperature (15°C to 25°C) and used within six (6) hours.

A complete description of the LeuTech® kit and the radiolabeling procedure is provided in the proposed Package Insert (Section 11).

4. TABULAR SUMMARY OF CLINICAL STUDIES

At the time of the BLA filing, the clinical development program for Tc 99m LeuTech® included eight clinical trials. Palatin Technologies, Inc. conducted five of the eight studies under two INDs: three studies under BB-IND 7358 in pursuit of the appendicitis indication sought in the current application and a single study under BB-IND 7996, in pursuit of an osteomyelitis indication not sought in this initial application. Also performed under BB-IND 7996 was a Phase 1 study to evaluate HAMA response following repeat injections. This study was not included in the original BLA filing, but results were summarized in the 120-Day Safety Update. Two other studies were conducted under an Investigators's IND, BB-IND 2995, and the seventh study, which was conducted in Germany, was reported in the peer-reviewed medical literature.

A summary of these eight studies is provided in Table 4.0-1.

Table 4.0-1 Table of Clinical Studies.

Protocol No. BB-IND No. Location Investigators	Completion Status (Starting Date)	Study Design, Study Population	Antibody Dose	Number of Subjects Entered	Age Range Years (Mean)	No. Male/Female Black/White/ Other
Phase 1 Studies						
97-001 **BB-IND 2995** **U.S.** Marcus, C.	Completed (05/08/97)	Phase 1, open-label to evaluate HAMA response; Normal volunteers	125 µg	30	19.3-59.7 (37.7)	12/18 2/17/11
97-002 **BB-IND 7358** **U.S.** Mozley, P. D.	Completed (11/22/97)	Phase 1, open-label to evaluate drug biodistribution; Normal volunteers	67.5 µg – 127.5 µg	10	20.9-46.2 (30.2)	4/6 2/7/1
Controlled Clinical Studies for Equivocal Appendicitis						
97-003 **BB-IND 7358** **U.S.** Kipper, S. Kramer, E.	Completed (11/17/97)	Phase 2, open-label, within-patient comparative with final institutional diagnosis; Patients with equivocal appendicitis	87.5 µg – 143.7 µg	56	9.1-77.5 (29.3)	25/31 3/22/31
98-004 **BB-IND 7358** **U.S.** Multicenter	Completed (09/14/98)	Phase 3, open-label, within-patient comparative with final institutional diagnosis; Patients with equivocal appendicitis	32.5 µg – 250 µg	203	5.2-85.9 (30.5)	82/121 16/149/38

Table 4.0-1 Table of Clinical Studies (continued).

Protocol No. BB-IND No. Location Investigators	Completion Status (Starting Date)	Study Design, Study Population	Antibody Dose	Number of Subjects Entered	Age Range Years (Mean)	No. Male/Female Black/White/ Other
Other Studies and Information						
98-005 **BB-IND 7996** **U.S.** Multicenter	Completed (01/22/99)	Phase 2, open-label, within-patient comparative; Patients with suspected osteomyelitis	125 µg	24[a]	48.0-91.4 (69.8)	9/15 1/23/0
99-001[b] **BB-IND 7996** **U.S.** Line, B.	Completed (09/22/99)	Phase 1, open-label to evaluate HAMA response in normal volunteers	125 µg	30	20.9-57.6 (33.8)	15/15 2/25/3
95-001 **BB-IND 2995** **U.S.** Marcus, C. Kipper, S.	Completed (10/04/90)	Phase 1/ 2, open-label, within-patient comparative; Patients with suspected infectious processes	62.5 µg – 209.9 µg[c]	69	9.0–67.0 (34.0)	46/23 13/8/32 Race not specified for 16 patients
Gratz-Becker **Germany** Gratz, S. Becker, W.	Completed (Unknown)	Phase 1, open-label, within-patient crossover; Patients with proven infectious foci	100 µg	17	22.0-75.0 (38.8)	9/8 Race not specified

[a] Data from 8 patients were included in the BLA; additional 16 patients were summarized in the 120-Day Safety Update to the BLA.
[b] Data from this study were not included in the BLA but were summarized in the 120-Day Safety Update.
[c] Antibody dose was not specified for 30 patients.

5. CLINICAL PHARMACOLOGY AND RADIATION DOSIMETRY

5.1 INTRODUCTION

One Phase 1 study was sponsored by Palatin Technologies to assess the safety, biodistribution and radiation dosimetry of Tc 99m LeuTech®. In addition, two Phase 1/2 studies of the anti-CD15 antibody were reported in the scientific and medical literature and are summarized here for completeness. The two studies were conducted by academic investigators independent of Palatin's IND and used formulations that differ from the LeuTech® formulation contained in the BLA.

5.2 SUMMARY OF PHASE 1 STUDY

5.2.1 Study Design

Study 97-002 was conducted by Dr. P. David Mozley at the Hospital of the University of Pennsylvania in Philadelphia, Pennsylvania. The objectives of the study were: 1) to evaluate the safety of a single injection of Tc 99m LeuTech® in normal volunteers; and 2) to assess the biodistribution and excretion of technetium-99m in normal volunteers after a single intravenous administration of Tc 99m LeuTech®.

5.2.2 Methods

Ten normal adult volunteers (6 females and 4 males) participated in the study. Subjects were 20 years to 46 years of age (mean = 30 years) and had a medical history and current physical examination indicating good health. Subjects received a single intravenous injection of 0.27 mL – 0.51 mL Tc 99m LeuTech® (mean = 0.37 mL) containing 8.9 mCi – 10.7 mCi (mean = 9.53 mCi) of technetium-99m and 67.5 μg – 127.5 μg of antibody (mean = 92.0 μg). Safety was assessed by monitoring vital signs, clinical laboratory test results and adverse events. Vital signs were monitored pre-injection, 5 and 30 minutes and 1, 2, 4 and 18 – 24 hours post-injection. White blood cell (WBC) counts and relative WBC differential counts also were determined at 3, 5, 10, 15, 30 and 45 minutes post-injection. Volunteers were observed for adverse events for the first hour post-injection, and again at 4 hours and 18 – 24 hours post-injection.

Conjugate anterior/posterior dynamic images of the thorax and abdomen were acquired for the first five minutes post-injection and successive 10-minute whole-body conjugate scans were acquired over the first hour post-injection. Thereafter, conjugate anterior-posterior whole-body images were acquired at approximately 1, 2, 4, 6, 8, 22, 26 and 30 hours following injection. Blood samples were collected at 3, 5, 10, 15, 30 and 45 minutes and at 1, 2, 4, 8 and 18 – 24 hours. Blood samples were assayed for technetium-99m concentration. Blood cell binding of radioactivity was determined at 30 and 60 minutes post-

injection. Urine samples (quantitative collections fractionated over approximately 30 hours) were assayed for technetium-99m, % injected dose in each sample was calculated and cumulative urinary excretion was determined. Biodistribution data from whole-body images and urinary-excretion results were used for estimating absorbed radiation doses utilizing Medical Internal Radiation Dose (MIRD) schema.

5.2.3 Results

Decay-corrected radioactivity from whole-body disappeared rapidly for the first 4 to 8 hours, in concert with excretion into urine, and then declined more gradually through the remainder of the imaging sessions. By approximately 30 hours after injection, about 40% – 60% of the injected dose (ID) remained in whole-body images. The time course of disappearance of decay-corrected radioactivity from blood was estimated by a bi-exponential equation fitted to the injected dose per liter blood data using nonlinear regression analysis. Decay-corrected technetium-99m disappeared from blood with mean distribution and elimination half-life values of 0.29 hours and 8.1 hours, respectively.

Blood cell binding analyses demonstrated that a mean of 19% of the circulating radioactivity was associated with WBCs at 30 minutes and 25% at 60 minutes post-injection. The degree to which radioactivity was associated with WBCs was directly proportional to the pre-injection PMN absolute differential count.

Quantitative urine collection was performed for 25.8 hours to 32.5 hours. Urine collection was limited to approximately 30 hours due to the relatively short physical half-life (6.02 hours) of the technetium-99m. The mean cumulative injected radioactive dose recovered in the urine was 38.0% (range 32.3% to 46.4%).

Recovery of radioactivity was calculated on the basis of whole-body image quantitative results and cumulative urinary excretion of radioactivity. Recovery averaged 95% of the injected dose at 24 hours post-injection. The major organ that took up radioactivity was the liver, with a peak content of 45% – 50% ID at about 40 minutes post-injection. This declined slowly to 25% – 40% ID by 24 hours post-injection. Spleen radioactivity reached its peak of 5% – 12% ID within the first hour after injection and declined to half that value by 24 hours. Left kidney radioactivity reached its peak content at about the same time as that in the spleen, but with a more gradual decline. Skull bone marrow generally contained its greatest amount of radioactivity at the earliest imaging session and declined thereafter. Content of radioactivity in regions of interest (ROIs) drawn over the heart followed a rapid decline, probably reflecting disappearance of the tracer from the blood pool. Although the lungs contained substantial radioactivity at early times after injection, this, too, appears to have reflected blood pool activity as the activity in lungs ROIs declined in parallel with that in ROIs drawn over the heart.

No adverse events and no clinically significant changes in vital signs were reported during the study. A transient decrease in WBC count was noted within 3 to 5 minutes post-injection and returned to pre-injection values within one hour. The transient decrease in WBC count correlated with hepatosplenic uptake of radioactivity and was not considered clinically significant by the investigator. Other minor statistically significant changes in some hematology, clinical chemistry and urinalysis parameters were noted; however, the magnitude of these changes was small and no clinically significant changes in these parameters were noted.

5.3 RADIATION ABSORBED DOSE ESTIMATES

Radiation absorbed doses were estimated from the biodistribution in accordance with MIRD schema, with gastrointestinal kinetics modeled in accordance with the International Commission on Radiological Protection. For estimation of radiation dose, hepatobiliary excretion was taken as a secondary route of elimination, accounting for up to 20% ID, a conservative value from a radiation safety perspective, not inconsistent with that estimated above from net abdominal radioactivity. Four organs were estimated to receive mean absorbed radiation doses higher than 0.10 rad/mCi: spleen, 0.23 rad/mCi; kidneys, 0.19 rad/mCi; liver, 0.18 rad/mCi; and urinary bladder wall, 0.12 rad/mCi. The marrow dose was modest (0.038 rad/mCi). The mean effective dose equivalent was 0.068 rem/mCi. Tables summarizing radiation absorbed doses in adults and children are provided in the proposed LeuTech® package insert (Section 11). Estimates of radiation absorbed dose are well within acceptable ranges in the nuclear medicine community.

5.4 LITERATURE REPORTS

Thakur *et al.* (1996) determined quantitative organ distribution in nine of 24 patients exhibiting clinical evidence of ongoing inflammatory processes. A single injection of 100 μg anti-CD15 antibody labeled with 10 mCi – 20 mCi of technetium-99m was administered intravenously. This report summarized patients enrolled in Study 95-001.

The lack of radioactivity in the thyroid gland and gastrointestinal tract indicated that the *in vivo* stability of the agent was excellent. At three hours post-injection, splenic uptake (7.7% ID) and red marrow activity (14% ID) were lower than those of indium-labeled white blood cells, but liver uptake (49% ID) was at the upper limit of that found after indium-labeled white blood cell administration. Organ radioactivity uptake values for this laboratory preparation of technetium Tc 99m anti-CD15 antibody did not differ substantially from those measured in Study 97-002, described above. No adverse events were reported by any patients.

Gratz *et al.* (1998) evaluated technetium-99m anti-CD15 antibody in 17 patients with proven infectious foci. This study was conducted in Germany using nonradioactive reagent kits manufactured in Italy containing anti-CD15 antibody supplied from the United States. The formulation of the reagent was not identical to the formulation used in Palatin IND studies.

Patients received a single intravenous injection of anti-CD15 antibody radiolabeled with 15 mCi of technetium-99m. Biodistribution was evaluated in five patients. In four of the five patients, a transient, mild reduction in WBC count was noted at 15 minutes post-injection. Organ radioactivity uptake in this study was similar to that observed in Study 97-002, with approximately 40% ID in liver, 11% ID in spleen and 13% ID in bone marrow at one hour after injection. No adverse events were reported for any patient.

6. EFFICACY

A pivotal Phase 3 study (Study 98-004) and a supporting Phase 2 study (Study 97-003) were both conducted in patients presenting with equivocal signs and symptoms of appendicitis and provide the data to support the efficacy claims for Tc 99m LeuTech®. Both studies were very similar in design and objectives. The efficacy objective of Study 98-004 was to evaluate Tc 99m LeuTech® for its ability to diagnose acute appendicitis. The efficacy objective of Study 97-003 was to assess Tc 99m LeuTech® scintigraphy for its ability to diagnose acute appendicitis and other inflammatory causes of right lower quadrant abdominal pain. In both studies, final institutional diagnosis provided the "gold standard" for comparison, and blinded reader assessments provided the primary efficacy data.

This section provides an overview of the efficacy database, including details of study design and statistical methods employed. Following is a presentation of the efficacy results from the individual studies and the pooled results.

6.1 OVERVIEW OF EFFICACY DATABASE

6.1.1 Study Design

Study 98-004, the pivotal Phase 3 study, was a multicenter study conducted at ten U.S. sites in a total of 203 patients presenting with equivocal signs and symptoms of appendicitis. Study 97-003, the supporting Phase 2 study, was conducted at two U.S. sites in a total of 56 patients presenting with equivocal signs and symptoms of appendicitis. Because of the similarity in design of the two studies, details of the study protocol are provided together, and any differences in design between the studies are noted.

Patient Populations

Patients included in Study 98-004, the pivotal efficacy study, were ≥ 5 years old and presented with right lower quadrant pain (RLQ) and one or more signs or symptoms that were equivocal for acute appendicitis. These equivocal criteria, which were based on a scale developed by Alvarado (1986), were:

- Atypical history/symptoms, e.g.,
  - absence of periumbilical pain migrating to RLQ
  - no gradual onset of pain
  - no increasing intensity of pain over time
  - pain not aggravated by movement and coughing

- Atypical physical examination, e.g.,
  - absence of McBurney's point tenderness
  - absence of referred tenderness to RLQ with palpation in other quadrants
  - absence of abdominal muscular spasm with RLQ tenderness

- Fever less than 101° F

- WBC count less than 10,500/mm^3

Females who were pregnant or nursing, females with a diagnosis of pelvic inflammatory disease (PID), patients with a history of two or more hospital admissions for abdominal pain of unknown etiology in the past six months, and patients who had undergone computed tomography (CT) imaging procedures for work-up of the current episode of RLQ abdominal pain were excluded.

The inclusion/exclusion criteria in Study 97-003 were very similar. However, patients were to be 8 years of age and older, atypical history/symptoms and atypical examination were not explicitly defined (absence of McBurney's point tenderness was used as one possible component of an atypical examination), and "normal WBC count" was given as an example of the criterion "atypical lab results", rather than stating the specific WBC count of less than 10,500/mm^3.

Treatment Administered

In the pivotal efficacy study (98-004) and the supporting efficacy study (97-003), adult patients were to receive a single intravenous injection of approximately 10 mCi – 20 mCi Tc 99m LeuTech® containing 75 μg – 125 μg antibody. Doses for pediatric patients (5 years to 17 years of age) were to be scaled down on a per kilogram (kg) of body weight basis; doses were determined based on 0.21 mCi per kg of body weight, up to a maximal dose of 20 mCi.

Tc 99m LeuTech® Image Acquisition

In both the pivotal and supporting study, Tc 99m LeuTech® imaging of the lower abdomen was performed using a large field-of-view (LFOV) camera fitted with a low-energy, parallel-hole, high resolution collimator and photopeak set at 140 keV ± 10%. Dynamic image acquisition was collected using a 128 x 128 matrix or better. Planar images were collected using a 256 x 256 matrix. Imaging protocols were very similar for the two studies, and details are provided in Table 6.1-1.

Tc 99m LeuTech® Image Evaluation

Image evaluations on both studies were performed by the site investigators as well as by three blinded readers. The blinded reads were managed by an independent core laboratory. The blinded readers (different readers for each study) were

experienced nuclear medicine practitioners not otherwise participating in the studies. Each blinded reader independently evaluated all image sets, which were presented in a standard format on computer monitors, with only the randomization code number as identification. Prior to the first blinded read session for the pivotal efficacy study (98-004), the readers underwent a half-day training session.

In the pivotal efficacy study (98-004), the blinded readers were provided only with the criteria for equivocal presentation of appendicitis as defined in the protocol and with patient demographic information (age, sex, height, weight). They were not provided with individual patient profiles or outcomes. The blinded readers in the supporting efficacy study (97-003) were also provided with individual patients' presenting signs and symptoms.

Based on their assessment of a patient's Tc 99m LeuTech® scans, the readers (blinded readers and site investigators) on both studies were required to arrive at a diagnosis of "negative for infection" or "positive for infection", which they further categorized as acute appendicitis and/or other infection.

For Study 98-004, readers also evaluated image characteristics for scans with positive uptake. Uptake location, pattern and intensity, and the time the scan first became positive were recorded.

Final Institutional Diagnosis

In both Studies 98-004 and 97-003, the gold standard or 'true' diagnosis against which Tc 99m LeuTech® results were to be judged was provided by final institutional diagnosis. For the cases that underwent surgery, the surgical and pathology reports were the basis for the final diagnosis. In the event surgery was not performed, clinical follow-up was obtained (at two weeks for Study 98-004 and at one month follow-up for Study 97-003).

Intended Clinical Management and Estimates of Likelihood of Appendicitis

In both Studies 98-004 and 97-003, the surgeons who referred the patients for the study completed a questionnaire prior to and after the Tc 99m LeuTech® procedure, indicating their intended clinical management of the patient. Choices were "surgery", "admit for observation" and "send home". The post-scan questionnaire was to be completed after reviewing the results of the Tc 99m LeuTech® scan, but prior to treatment, and without information from any additional diagnostic tests.

For all patients in Study 98-004 and about half the patients (25 of 56) in Study 97-003, the surgeons also were asked to estimate the likelihood of appendicitis, using the following categories:

- almost definitely not appendicitis (0% – 19%)
- probably not appendicitis (20% – 39%)
- indeterminate appendicitis (40% – 59%)
- probably appendicitis (60% – 79%)
- almost definitely appendicitis (80% – 100%)

A summary of the design features of the two studies is presented in Table 6.1-1.

Table 6.1-1 Design Features of Studies Supportive of Efficacy.

Study	Truth	Tc 99m LeuTech® Imaging Times	Readers of Tc 99m LeuTech® Images	Efficacy Measures
98-004 (N = 203)	Final Institutional Diagnosis	Immediately post-injection to acquisition of 10, 4 min. dynamic image sets; then Static supine anterior, posterior, 20°-25° RAO and LAO planar images of lower abdomen; then Standing anterior image of lower abdomen.	3 Blinded Readers; Site Investigators (10 Sites)	• Blinded Readers' Image Evaluations Relative to Final Institutional Diagnosis • Site Investigators' Image Evaluations Relative to Final Institutional Diagnosis • Image Characteristics • Intended Clinical Management • Estimated Likelihood of Appendicitis
97-003 (N = 56)	Final Institutional Diagnosis	Immediately post-injection to acquisition of 10 (or 15), 4 min. dynamic image sets; then Static supine anterior, posterior, 20-25° RAO and LAO planar images of lower abdomen; then (Additional dynamic images if scans equivocal or negative), then Standing anterior image of lower abdomen.	3 Blinded Readers; Site Investigators (2 Sites)	• Blinded Readers' Image Evaluations Relative to Final Institutional Diagnosis • Site Investigators' Image Evaluations Relative to Final Institutional Diagnosis • Intended Clinical Management • Estimated Likelihood of Appendicitis (25 of the 56 patients)

Only patients who met the inclusion/exclusion criteria for the study protocol were considered eligible for efficacy evaluations. In addition, patients had to have completed the Tc 99m LeuTech® procedure according to protocol and have a diagnostic evaluation completed for final diagnosis. Table 6.1-2 provides a summary of the clinical trials database for the two studies supportive of efficacy. All patients were eligible for efficacy analysis in Study 97-003. In Study 98-004, three patients were ineligible for efficacy analysis (two patients with negative scans were lost to follow-up and one patient with a positive scan went to surgery before the protocol-specified minimum of 30 minutes imaging was completed). For this study (pivotal efficacy), evaluable-patient analyses and intent-to-treat analyses were performed for primary efficacy endpoints.

Table 6.1-2 Clinical Trials Database for Studies Supportive of Efficacy.

	STUDY		
NUMBER OF PATIENTS	**98-004**	**97-003**	**TOTAL**
Entered (Male/Female)	203 (82/121)[a]	56 25/31	259 (107/152)[a]
Received Study Drug (Male/Female)	203 (82/121)[a]	56 25/31	259 (107/152)[a]
Eligible for Efficacy Evaluations (Male/Female)	200 (79/121)[a]	56 25/31	259 (104/152)[a]
Dropped - Ineligible or Incomplete	3	0	3
Age (yr): Mean (Range)	30.5 (5.2 – 85.9)	29.3 (9.1 – 77.5)	30.2 (5.2 – 85.9)
Anti-CD15 IgM Antibody Dose (µg): Mean (Range)	120.5 (32.5 – 250.0)	124.6 (87.5 – 143.7)	121.4 (32.5 – 250.0)
Radioactive Dose (mCi): Mean (Range)	16.0 (4.2 – 33.0)	14.3 (8.1-19.5)	15.6 (4.2 – 33.0)

[a] Numbers of males and females were switched in similar table in BLA but are presented correctly here.

6.1.2 Statistical Methodology

Unless otherwise stated, the same methodology was applied to both the Phase 3 pivotal efficacy study (98-004) and the Phase 2 supporting efficacy study (97-003).

In all evaluations of Tc 99m LeuTech® images, results were judged relative to the "gold standard" (final institutional diagnosis) as truth. The primary efficacy analyses evaluated the performance of Tc 99m LeuTech® for diagnosis of acute appendicitis. In these analyses, patients classified as negative for acute appendicitis included patients with no infection and patients with a diagnosis of other infection. Each reader's results were classified into one of four categories as follows:

Final Institutional Diagnosis	Tc 99m LeuTech® Diagnosis	
	Negative for Acute Appendicitis[a]	Positive for Acute Appendicitis
Negative for Acute Appendicitis[a]	TN	FP
Positive for Acute Appendicitis	FN	TP

[a] Includes patients negative for infection or with other infection.
TN is true negative, FP is false positive, FN is false negative and TP is true positive.

Tc 99m LeuTech® results were evaluated for each of three blinded readers and for the study site investigators. In addition to the results based on the individual blinded readers, the **aggregate read** of the three readers' evaluations was derived for each patient as the majority result of the three readers' findings. For each reader (blinded reader or site investigator) and the aggregate read, the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) were calculated as follows:

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100\%$$

$$\text{Accuracy} = \frac{TP + TN}{TP + FN + TN + FP} \times 100\%$$

$$\text{PPV} = \frac{TP}{TP + FP} \times 100\%$$

$$\text{NPV} = \frac{TN}{TN + FN} \times 100\%$$

The binomial distribution was used to establish confidence intervals for all proportions, including accuracy, sensitivity, specificity, PPV and NPV. Planned analyses in the Phase 3 study (Study 98-004) called for 95% one-sided, lower-bound confidence intervals for each proportion. Two-sided 95% confidence intervals were specified for the Phase 2 study (Study 97-003).

In addition to the above diagnostic measures, the likelihood ratios, positive (LR+) and negative (LR–), were evaluated for the pivotal Phase 3 study. They were calculated as follows:

$$\text{Likelihood Ratio, Positive (LR+)} = \frac{\text{TP/FP}}{\text{(TP + FN)/(FP+TN)}},$$

$$\text{Likelihood Ratio Negative (LR–)} = \frac{\text{FN/TN}}{\text{(TP + FN)/(FP+TN)}}.$$

Kappa statistics and concordance rates were used to measure inter-observer agreement between pairs of blinded readers. A concordance rate was calculated as the proportion of patients for whom each pair of readers agree.

The potential impact of Tc 99m LeuTech® on clinical patient management was assessed by comparing the referring surgeons' intended clinical management course post-scan with their intended clinical management course pre-scan, in light of final patient outcomes. Management decisions were scored and tests of symmetry (Bowker's or McNemar's) were used to compare pre- and post-scan distributions.

For the pivotal efficacy study (Study 98-004), receiver operating characteristic (ROC) analysis was used to evaluate and compare pre-scan and post-scan diagnostic performance as measured by the referring surgeons' rating scores for the likelihood that the patient had appendicitis. For Study 97-003, ROC analysis could not be performed because of the sparseness of the data (only 25 of the 56 patients had likelihood estimates).

Where appropriate, data from the Phase 3 (Study 98-004) and Phase 2 (Study 97-003) studies were combined to obtain pooled estimates of diagnostic performance.

6.2 PIVOTAL EFFICACY RESULTS

A single Phase 3 study, Study 98-004, provided the pivotal efficacy data to support the claim that Tc 99m LeuTech® is effective for diagnosing and ruling out appendicitis in patients presenting with equivocal signs and symptoms.

6.2.1 Study Population

Ten sites enrolled 203 patients in Study 98-004; all sites were located in the United States.

Site	Investigator	Institution
A	Samuel Kipper, M.D.	Tri-City Medical Center Oceanside, California
B	Andrew Klonecke, M.D.	Kaiser Permanente Medical Center Sacramento, California

Site	Investigator	Institution
C	Alan Waxman, M.D.	Cedars-Sinai Medical Center Los Angeles, California
D	Robert McDonald, M.D.	Providence St. Vincent Medical Center Portland, Oregon
E	Charles Neal, M.D.	Memorial Medical Center Springfield, Illinois
F	Stephen Bunker, M.D.	California Pacific Medical Center San Francisco, California
G	Bruce Barron, M.D.	Univ. of Texas Health Science Center Houston, Texas
H	Frederick Weiland, M.D.	Sutter Roseville Medical Center Roseville, California
I	Christopher Palestro, M.D.	Long Island Jewish Medical Center New Hyde Park, New York
J	Bruce Line, M.D.	Albany Medical Center Hospital Albany, New York

The distribution of patients by site is provided in Table 6.2-1.

Table 6.2-1 Distribution of Patients Enrolled by Site, Study 98-004.

SITE	No. of Patients	% of Total
A	39 (1)*	19.2
B	19	9.4
C	7	3.5
D	23 (1)*	11.3
E	29 (1)*	14.3
F	3	1.5
G	11	5.4
H	36	17.7
I	8	3.9
J	28	13.8
Total	203 (3)*	

* Number ineligible for efficacy.

The protocol specified a maximum of 40 patients at a single site. Seven of the ten sites enrolled more than 10 patients; three sites enrolled between 20 and 30 patients and two sites enrolled more than 30 patients.

Three patients were ineligible for all efficacy evaluations, based on protocol specifications. Two patients were lost to follow-up and a third patient was taken to surgery before 30 minutes of Tc 99m LeuTech® imaging could be completed (a protocol violation).

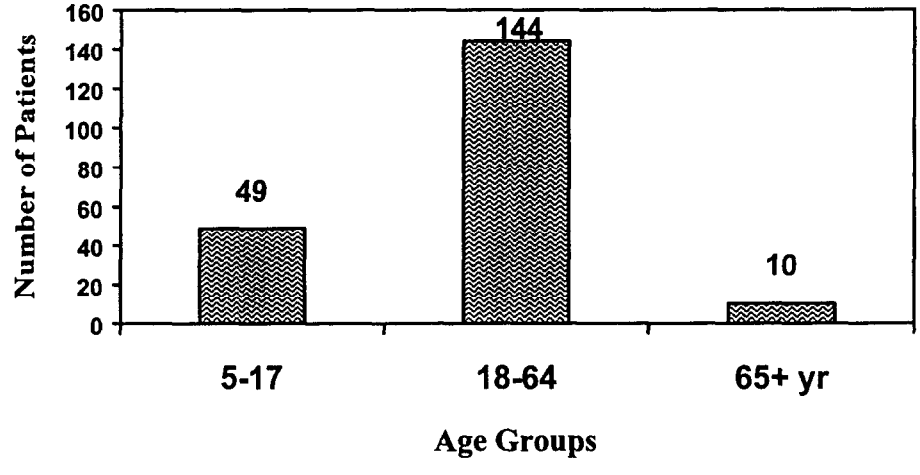
6.2.2 Demographic Characteristics

Summary statistics for age, weight, height and body mass index (BMI) (weight/height^2) are provided in Table 6.2-2. Figure 6.2-1 provides the age distribution of patients according to pediatric (5 years – 17 years), adult (18 years – 64 years) and geriatric (≥ 65 years) age groups.

Table 6.2-2 Summary Statistics for Age, Weight, Height and BMI, Study 98-004.

	N	MIN.	MAX.	MEAN	STD. DEV.
AGE (yr)	203	5.2	85.9	30.5	16.53
WEIGHT (kg)	203	21.4	127.3	69.2	20.82
HEIGHT (cm)	201	104.1	198.1	165.2	14.36
BMI	201	12.6	46.7	25.0	5.79

Figure 6.2-1 Distribution of Age Groups of Patients Receiving Tc 99m LeuTech®, Study 98-004.



Considering patients by age group, 49 patients (24%) were in the pediatric age group and 10 patients (5%) were geriatric. Of the pediatric group, 13 patients were younger than 10 years. One of the pediatric patients, aged 16 years, was lost to follow-up and was not evaluable for efficacy.

A frequency distribution for gender and race is presented in Table 6.2-3.

Table 6.2-3 Distribution of Gender and Race, Study 98-004.

		N	%
GENDER	Female	121	59.6
	Male	82	40.4
	TOTAL	203	
RACE	White (Caucasian)	149	73.4
	Hispanic	32	15.8
	Black	16	7.9
	Other	6	3.0
	TOTAL	203	

One hundred twenty-one patients (60%) were female and 82 were male (40%). Almost three-quarters of the patients (73%) were white and 32 (16%) were Hispanic.

6.2.3 Equivocal Signs and Symptoms of Appendicitis

Equivocal presentation was determined by the referring surgeon and included the presence of one or more of the following criteria:

Table 6.2-4 Distribution of Signs and Symptoms Comprising Equivocal Presentation, Study 98-004.

CRITERIA	N[a]	%
Atypical history/symptoms	148	72.9
Atypical physical examination	138	68.0
Fever less than 101° F	185	91.1
White blood cell (WBC) count less than 10,500/mm^3	115	56.7

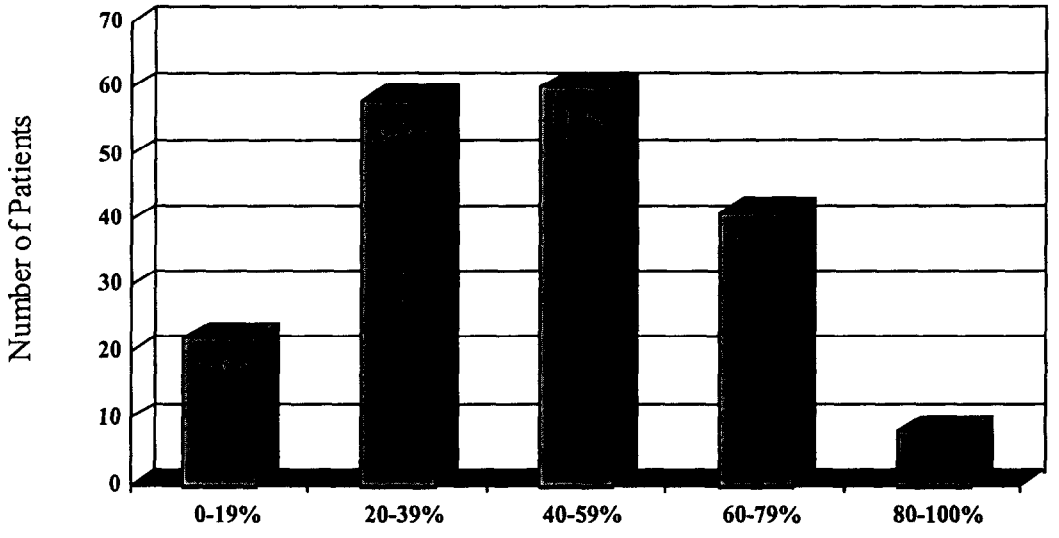
[a] Sum of the Ns exceeds the number of patients because some patients had more than one equivocal sign or symptom.

Most patients had more than one sign or symptom of equivocal presentation of appendicitis: 185 patients (91%) who presented with right lower quadrant pain had a fever of less than 101° F and 148 (73%) had an atypical history or symptoms. One hundred thirty-eight patients (68%) had atypical findings on physical examination and 115 (57%) had a normal WBC count (less than 10,500/mm^3).

To confirm that the patient population was, in fact, equivocal for the diagnosis of acute appendicitis, the surgeons' estimates of the likelihood of appendicitis were evaluated. (See Section 6.2.9 for a full discussion of the results of the surgeons' questionnaire data before and after the Tc 99m LeuTech® procedure.)
Figure 6.2-2 shows the distribution of patients in the following categories: almost definitely not appendicitis (0-19% likelihood), probably not appendicitis (20-39% likelihood), indeterminate appendicitis (40-59% likelihood), probably appendicitis

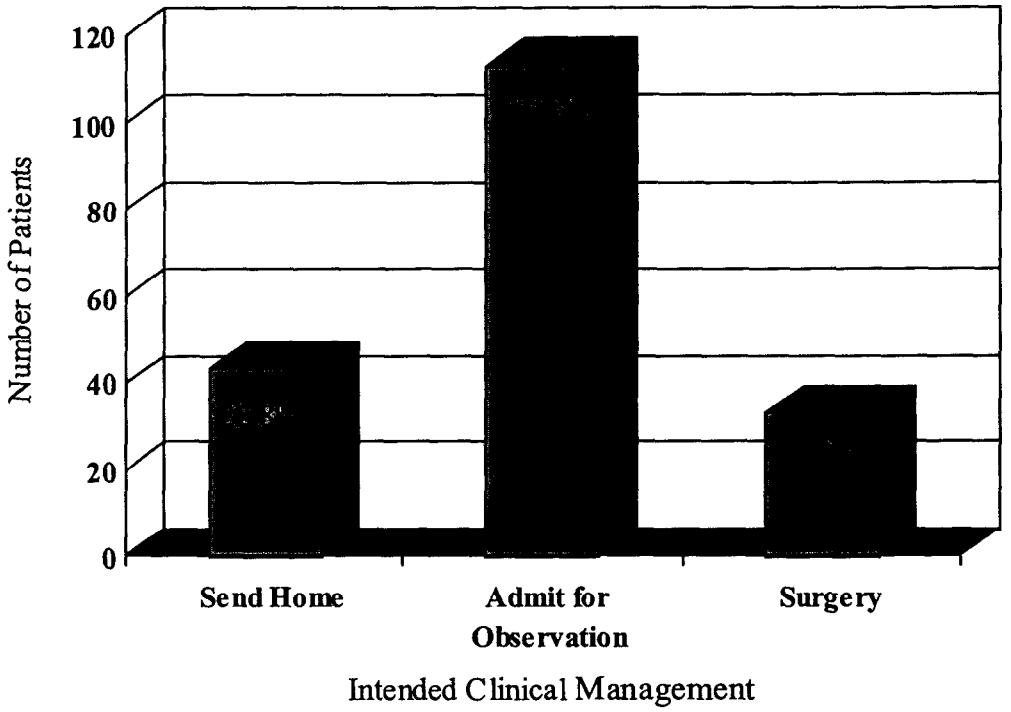
(60-79% likelihood) and almost definitely appendicitis (80-100% likelihood). These results clearly demonstrate that the patient population was equivocal for the diagnosis of appendicitis.

Figure 6.2-2 Distribution of Surgeons' Estimates of Likelihood of Appendicitis Prior to Tc 99m LeuTech® Imaging, Study 98-004



The surgeons were also asked to specify their intended clinical management plan prior to the Tc 99m LeuTech® study. Figure 6.2-3 shows the distribution of patients in the three categories: send home, admit for observation, surgery. The surgeons planned to admit 113 patients (59.8%) for observation, which again confirms the predominance of an equivocal diagnosis in the patient population.

Figure 6.2-3 Distribution of Intended Clinical Management Plan Prior to Tc 99m LeuTech® Imaging, Study 98-004



6.2.4 Concomitant Medications

Investigators were asked to record all concomitant medications (prescription drugs and over-the-counter medications) used by patients from 24 hours prior to the Tc 99m LeuTech® study throughout the Tc 99m LeuTech® procedure. Medications were coded according to the World Health Organization (WHO) drug dictionary. The Level 3 Medication Classification was used to group medications and usage was summarized according to these classes. Seventy-five percent (75%) of patients were taking at least one concomitant medication. Opioids (such as morphine and meperidine hydrochloride) were the single most common medication. Thirty-two patients (16%) were taking antibiotics. Table 6.2-5 presents a distribution of usage for antibiotics and the Level 3 classes for which usage was in 10% or more of patients.

Table 6.2-5 Distribution of Concomitant Medication Usage, Study 98-004.

MEDICATION CLASS[a]	NUMBER OF PATIENTS	%
Opioids	49	24.1
Other Analgesics and Antipyretics	37	18.2
Antibiotics[b]	32	15.8
Psychotherapeutics	32	15.8
Non-steroidal anti-inflammatory drugs (NSAIDs)	25	12.3
Anesthetics	24	11.8
Any Medication	153	75.4
None	50	24.6

[a] WHO Level 3 Classification.
[b] All Level 3 medications that are types of antibiotics were included in this category.

6.2.5 Dosing

Summary statistics for the injected dose are provided in Table 6.2-6.

Table 6.2-6 Summary Statistics for Tc 99m LeuTech® Dosing, Study 98-004.

	N	MIN.	MAX.	MEAN	STD. DEV.
Injected Volume (mL)	203	0.1	1.0	0.5	0.12
Injected Radioactivity (mCi)	203	4.2	33.0	16.0	4.13
Injected Antibody (μg)	203	32.5	250.0	120.5	29.03

Injections of Tc 99m LeuTech® contained, on the average, 120.5 μg antibody (32.5 μg – 250 μg) and 16 mCi radioactivity (4.2 mCi – 33 mCi). At the beginning of the study, two sites (Sites C and G) administered 0.8 mL to 1.0 mL to a total of eight patients, rather than the target volume of 0.3 mL to 0.5 mL (noted as a protocol violation). As a result, those patients received antibody doses ranging from 200 μg to 250 μg, exceeding the target range of 75 μg to 125 μg.

6.2.6 Imaging

All 203 patients had dynamic planar imaging performed and 198 patients had static planar imaging performed as part of their primary imaging series. Seventy-three patients had additional imaging performed, including nine patients with SPECT imaging. All images were available to the site investigators, but the SPECT images were not included with the blind-read image sets. Summary statistics for the durations of the primary dynamic and static imaging series are provided in Table 6.2-7.

Table 6.2-7 Duration (Minutes) of Dynamic and Static Planar Imaging Series, Study 98-004.

	MIN.	MAX.	MEAN	STD. DEV.
Dynamic (N = 203)	24	58	40.2	3.01
Static (N = 198)	7	128	30.8	16.67

The durations of the primary dynamic series ranged from 24 minutes to 58 minutes, with a mean of 40.2 minutes. The patient with 24 minutes of dynamic imaging (Patient E-09) had no additional imaging and was considered not evaluable for efficacy per protocol specification (< 30 minutes of imaging). The duration of the primary static planar series ranged from 7 minutes to 128 minutes, with a mean of 30.8 minutes. The median duration of total elapsed time for imaging, including additional imaging, was 86 minutes.

The primary criterion for a positive scan for appendicitis was abnormal, persistent Tc 99m LeuTech® accumulation within the right lower abdominal quadrant. An illustration of the "appendicitis zone" and representative Tc 99m LeuTech® images with associated case histories are provided in Appendix A.

6.2.7 Blinded Reader Evaluations

Primary Efficacy Endpoints

The primary efficacy endpoints for the study were the blind-read sensitivity and specificity of Tc 99m LeuTech® for the diagnosis of appendicitis. The "gold standard" or true diagnosis was provided by a patient's final institutional diagnosis. The distribution of final institutional diagnosis for the 200 patients evaluable for efficacy is provided in Table 6.2-8. Fifty-nine (59) patients (29.5%) had a final diagnosis of acute appendicitis (3 of these patients also were diagnosed with other infections). Twenty-three (23) patients were diagnosed exclusively with other infections, and 118 patients were diagnosed with no infection, resulting in 141 patients (70.5%) negative for appendicitis.

Table 6.2-8 Distribution of Final Institutional Diagnosis for Evaluable Patients, Study 98-004.

FINAL INSTITUTIONAL DIAGNOSIS	N	%
Acute Appendicitis[a]	59	29.5
No Appendicitis	141	70.5
Other Infection	23	11.5
No Infection	118	59.0
Total	200	

[a] Three patients with acute appendicitis also had another infection.

For each reader, agreement with the final institutional diagnosis of appendicitis/no appendicitis was categorized as TP, TN, FP, FN. The primary efficacy variables with their one-sided, 95% confidence intervals, are presented by blinded reader, and for the aggregate read (uses the majority result of the three readers' findings) in Table 6.2-9.

Table 6.2-9 Sensitivity and Specificity of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Evaluable Patients, Study 98-004.

EVALUATION	$N_{(+)}$	TP	Sensitivity	95% LL of CI[a]
Blinded Reader 1	59	48	81.4	70.8
Blinded Reader 2	59	39	66.1	54.6
Blinded Reader 3	59	45	76.3	65.2
Aggregate	59	44	74.6	63.4
EVALUATION	**$N_{(-)}$**	**TN**	**Specificity**	**95% LL of CI[a]**
Blinded Reader 1	141	124	87.9	82.3
Blinded Reader 2	141	127	90.1	84.7
Blinded Reader 3	141	133	94.3	89.8
Aggregate	141	131	92.9	88.1

[a] One-sided, 95% lower limit (LL) of confidence interval (CI).
$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.

Secondary Efficacy Endpoints

Secondary efficacy endpoints for blinded readers evaluations included accuracy, positive predictive value (PPV) and negative predictive value (NPV) for diagnosis of appendicitis. The results for these endpoints are presented in Table 6.2-10.

Table 6.2-10 Accuracy, PPV and NPV of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Evaluable Patients, Study 98-004.

EVALUATION	N_T	TP + TN	Accuracy	95% LL of CI[a]
Blinded Reader 1	200	172	86.0	81.2
Blinded Reader 2	200	166	83.0	77.9
Blinded Reader 3	200	178	89.0	84.5
Aggregate	200	175	87.5	82.9
EVALUATION	**TP + FP**	**TP**	**PPV**	**95% LL of CI[a]**
Blinded Reader 1	65	48	73.8	63.2
Blinded Reader 2	53	39	73.6	61.7
Blinded Reader 3	53	45	84.9	74.1
Aggregate	54	44	81.5	70.3
EVALUATION	**TN + FN**	**TN**	**NPV**	**95% LL of CI[a]**
Blinded Reader 1	135	124	91.9	86.7
Blinded Reader 2	147	127	86.4	80.7
Blinded Reader 3	147	133	90.5	85.3
Aggregate	146	131	89.7	84.5

[a] One-sided, 95% lower limit (LL) of confidence interval (CI).
N_T is the number of patients whose images were evaluated by the reader.

In addition, the positive and negative likelihood ratios, LR(+)and LR(–), were calculated. The likelihood ratios can be interpreted as odds ratios of post-test to pre-test odds that a patient has acute appendicitis, with the pre-test odds provided by the ratio of positive to negative patients in the presenting patient sample. The positive likelihood ratio, LR(+), is the odds ratio given a positive test result, and the negative likelihood ratio, LR(–), is the odds ratio given a negative test result. LR(+) and LR(–) are presented in Table 6.2-11, along with their 95% two-sided confidence intervals.

Table 6.2-11 Likelihood Ratios of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Evaluable Patients, Study 98-004.

EVALUATION	LR(+)	95% Conf. Int.	LR(–)	95% Conf. Int.
Blinded Reader 1	6.75	4.25 – 10.71	0.21	0.12 – 0.36
Blinded Reader 2	6.66	3.92 – 11.31	0.38	0.26 – 0.54
Blinded Reader 3	13.44	6.76 – 26.75	0.25	0.16 – 0.40
Aggregate	10.52	5.68 – 19.46	0.27	0.18 – 0.43

With a positive test result for appendicitis, the odds that a patient has appendicitis increased by a factor of 7 to 13 times the pre-test odds. For a negative test result, the odds decreased by a factor of 0.21 to 0.38.

Intent-to-Treat Patients

An assessment of the primary efficacy endpoints (blinded readers' sensitivity and specificity for diagnosing acute appendicitis) was performed for all patients enrolled in the trial, including the three patients unevaluable for efficacy. For the two patients lost to follow-up, the worst-case outcome was assumed for each reader's diagnosis and they were categorized as FP or FN depending on the reader's Tc 99m LeuTech® result. The results are presented in Table 6.2-12.

Table 6.2-12 Sensitivity and Specificity of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Intent-To-Treat Patients, Study 98-004.

EVALUATION	$N_{(+)}$	TP	Sensitivity	95% LL of CI[a]
Blinded Reader 1	61	48	78.7	68.0
Blinded Reader 2	61	39	63.9	52.6
Blinded Reader 3	61	45	73.8	62.8
Aggregate	61	44	72.1	61.0
EVALUATION	**$N_{(-)}$**	**TN**	**Specificity**	**95% LL of CI[a]**
Blinded Reader 1	142	124	87.3	81.6
Blinded Reader 2	142	127	89.4	84.0
Blinded Reader 3	142	133	93.7	89.0
Aggregate	142	131	92.3	87.3

[a] One-sided, 95% lower limit (LL) of confidence interval (CI).
$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.

Agreement Among Blinded Readers

Agreement between pairs of blinded readers was assessed with the kappa statistic and the concordance rate (the rate of agreement between readers) for the evaluable patients. Agreement was based on whether the patient diagnosis agreed (TP or TN), or did not agree (FN or FP), with the final institutional diagnosis. Results are presented in Table 6.2-13.

Table 6.2-13 Measures of Inter-Reader Agreement for Diagnosis of Appendicitis, Evaluable Patients, Study 98-004.

Pairs of Blinded Readers	Concordance Rate (95% Confidence Interval)	Kappa Statistic (95% Confidence Interval)
1, 2	0.88 (0.82 – 0.92)	0.54 (0.38 – 0.70)
1, 3	0.90 (0.84 – 0.93)	0.54 (0.37 – 0.72)
2, 3	0.89 (0.84 – 0.93)	0.55 (0.38 – 0.71)

Reader-to-reader agreement was good for all pairs of readers, with concordance rates of 0.88 to 0.90 and kappa statistics of 0.54 and 0.55.

6.2.8 Site Investigator Evaluations

The Tc 99m LeuTech® images for each patient were also evaluated by the investigators at the study sites. The first two patients enrolled in the study, at each site other than the lead site (Site A), constituted the training cases for the site investigators and were not included in the site investigator's efficacy evaluations.

Sensitivity, specificity, accuracy, PPV and NPV of investigators' evaluations relative to final institutional diagnoses for appendicitis/no appendicitis are presented in Table 6.2-14.

Table 6.2-14 Sensitivity, Specificity, Accuracy, PPV and NPV of Site Investigators' Evaluations for Diagnosis of Appendicitis, Evaluable Patients, Study 98-004.

ENDPOINT	ESTIMATE	95% LL of CI[a]
Sensitivity	90.7 (49/54)	81.0
Specificity	85.9 (110/128)	79.7
Accuracy	87.4 (159/182)	82.4
PPV	73.1 (49/67)	62.7
NPV	95.7 (110/115)	90.8

[a] One-sided 95% lower limit (LL) of confidence interval (CI).

Overall accuracy was about the same as blind-read accuracy. However, the site investigators read the images for higher sensitivity and correspondingly lower specificity.

6.2.9 Intended Clinical Management and Estimated Likelihood of Appendicitis

The questionnaire completed by surgeons prior to and after the Tc 99m LeuTech® procedure included entries for intended clinical management, estimates of likelihood of appendicitis and other diagnostic tests planned. Frequency distributions of intended clinical management prior to and following review of the Tc 99m LeuTech® studies, relative to final institutional diagnosis, are presented in Figure 6.2-2. Ten patients for whom the pre- and post-Tc 99m LeuTech® estimates were completed by different surgeons are not included in this figure. An additional patient was excluded because the pre-study questionnaire was actually completed following Tc 99m LeuTech® imaging.

Figure 6.2-2 Distribution of Intended Clinical Management Decisions Pre- and Post-Tc 99m LeuTech® Images, Evaluable Patients, Study 98-004.

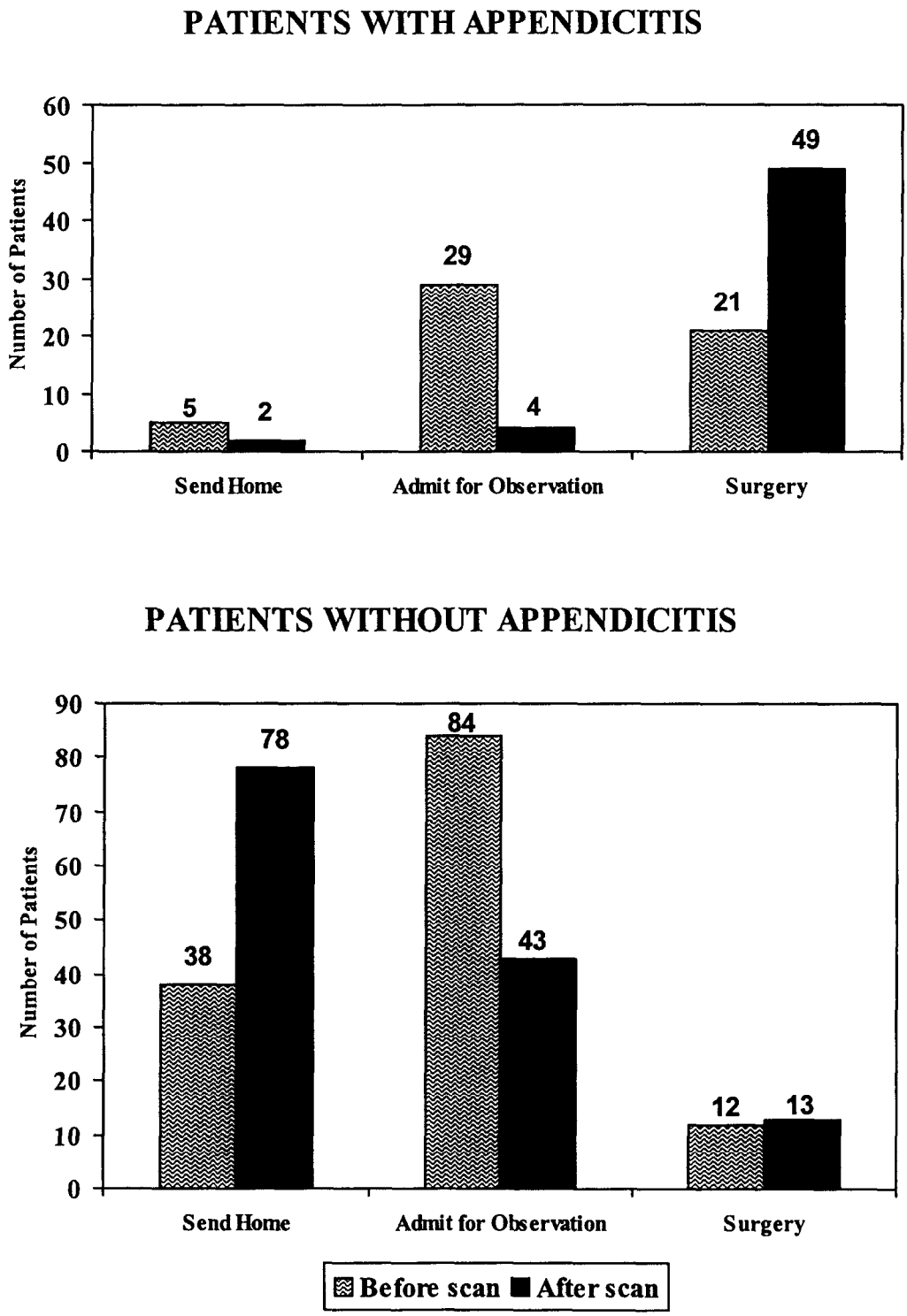


Table 6.2-15 presents shifts in intended clinical management, e.g., from "admit for observation" prior to review of Tc 99m LeuTech® images to "send home" following review of Tc 99m LeuTech® images.

Table 6.2-15 Shifts in Intended Clinical Management Pre- and Post-Tc 99m LeuTech® Imaging, Evaluable Patients, Study 98-004.

	Post-Tc 99m LeuTech®			
Pre-Tc 99m LeuTech®	Send Home	Admit for Observation	Surgery	**Pre-Total**
Final Diagnosis = Acute Appendicitis				
Send Home	2	0	3	**5**
Admit for Observation	0	4	25	**29**
Surgery	0	0	21	**21**
Post-Total	**2**	**4**	**49**	**55**
Final Diagnosis = No Acute Appendicitis				
Send Home	34	2	2	**38**
Admit for Observation	39	39	6	**84**
Surgery	5	2	5	**12**
Post-Total	**78**	**43**	**13**	**134**

For purposes of analysis, the management decisions were scored 0, 1 or 2, representing worst to best management decisions as follows:

Acute Appendicitis
- Send Home — Score = 0
- Admit for Observation — Score = 1
- Surgery — Score = 2

No Acute Appendicitis
- Send Home — Score = 2
- Admit for Observation — Score = 1
- Surgery — Score = 0

Bowker's test of symmetry was used to compare pre- and post-scan distributions of management scores. The difference between pre- and post-scan score distributions was statistically significant ($p < 0.0001$), with many more shifts following the Tc 99m LeuTech® study in the direction of correct management versus shifts in the other direction. For example, prior to the Tc 99m LeuTech® study, 29 patients whose final institutional diagnosis was acute appendicitis were to be admitted for observation. Following review of the Tc 99m LeuTech® images, 25 of those 29 patients would have been sent appropriately to surgery, if the Tc 99m LeuTech® images had been used in diagnosis. No patients with acute appendicitis shifted from surgery pre-scan to admit for observation or send home

post-scan. Similarly, 39 patients whose final institutional diagnosis was negative for acute appendicitis, and who were to be admitted for observation prior to review of the Tc 99m LeuTech®, would have been sent home appropriately on the basis of the Tc 99m LeuTech® study.

The distributions of the surgeon's estimated likelihood of appendicitis before and after evaluating the Tc 99m LeuTech® images, according to final institutional diagnosis, are presented in Table 6.2-16.

Table 6.2-16 Distribution of Estimates of Likelihood of Appendicitis Pre- and Post-Tc 99m LeuTech® Imaging, Study 98-004.

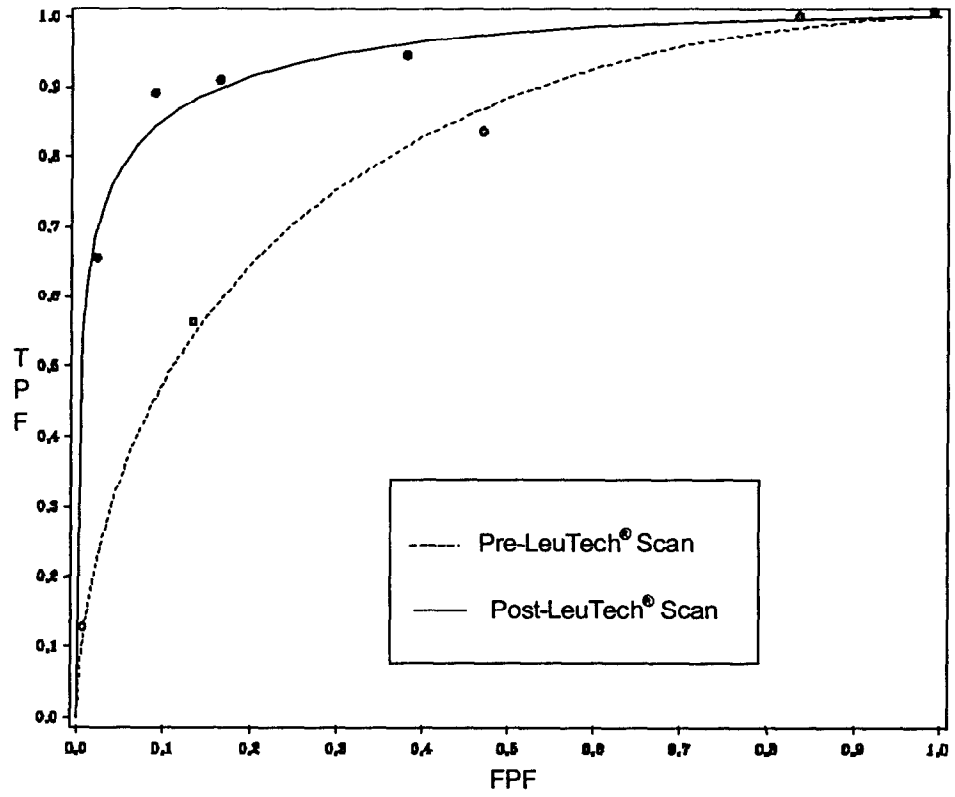
	Post-Tc 99m LeuTech® Study					
Pre-Tc 99m LeuTech® Study	0 – 19%	20 – 39%	40 – 59%	60 – 79%	80 – 100%	**Pre-Total**
Final Diagnosis = Acute Appendicitis						
0 – 19%	0	0	0	0	0	**0**
20 – 39%	3	1	1	1	3	**9**
40 – 59%	0	0	0	5	10	**15**
60 – 79%	0	1	0	7	16	**24**
80 – 100%	0	0	0	0	7	**7**
Post-Total	**3**	**2**	**1**	**13**	**36**	**55**
Final Diagnosis = No Acute Appendicitis						
0 – 19%	20	1	0	1	0	**22**
20 – 39%	33	13	2	1	0	**49**
40 – 59%	24	10	7	4	0	**45**
60 – 79%	6	5	1	2	3	**17**
80 – 100%	0	0	0	1	0	**1**
Post-Total	**83**	**29**	**10**	**9**	**3**	**134**

* 0 - 19% = Almost definitely not appendicitis; 20 - 39% = Probably not appendicitis; 40 - 59% = Indeterminate appendicitis; 60 - 79% = Probably appendicitis; 80 - 100% = Almost definitely appendicitis.

ROC curves depict the diagnostic accuracy of a test as a function of the threshold set for the test to be designated as positive. In this case, the test is the surgeon's estimate of the likelihood of appendicitis. If the threshold for positivity is set low (i.e., 0-19% likelihood), then all the patients with appendicitis will be identified: true positive fraction (TPF) = sensitivity = 1.0. However, all the patients without appendicitis will be identified as well: false positive fraction (FPF) = 1.0, specificity = 0. Higher thresholds will reduce the FPF, but will reduce the TPF as well. The more accurate a test is, the lower will be the FPF for any TPF. Receiver operating characteristic (ROC) analysis was performed to compare the ROC curves based on estimated likelihoods pre- and post-Tc 99m LeuTech®.

Figure 6.2-3 presents the estimated ROC curves. The difference between the diagnostic performance pre- and post-scan was tested with the univariate z-score test, which compares the areas under the respective curves. Table 6.2-17 provides a table of actual and estimated operating points on the fitted ROC curves.

Figure 6.2-3 ROC Curves Based on Surgeons' Estimated Likelihood of Appendicitis, Pre- and Post-Tc 99m LeuTech®, Study 98-004.



TPF = Sensitivity FPF = 1 - Specificity

Table 6.2-17 Actual and Predicted Operating Points of ROC Curve for Estimates of Likelihood of Appendicitis, Evaluable Patients, Study 98-004.

Likelihood Threshold for Positive	Final Diagnosis Negative for Appendicitis $N_{(-)} = 134$			Final Diagnosis Positive for Appendicitis $N_{(+)} = 55$		
	Number FP	Actual FPF	Predicted FPF	Number TP	Actual TPF	Predicted TPF
Prior to Tc 99m LeuTech® Scan						
0 – 19%	134	1.000	1.000	55	1.000	1.000
20 – 39%	112	0.836	0.841	55	1.000	0.985
40 – 59%	63	0.470	0.457	46	0.836	0.863
60 – 79%	18	0.134	0.140	31	0.564	0.556
80 – 100%	1	0.007	0.008	7	0.127	0.124
After Tc 99m LeuTech® Scan						
0 – 19%	134	1.000	1.000	55	1.000	1.000
20 – 39%	51	0.381	0.379	52	0.945	0.961
40 – 59%	22	0.164	0.173	50	0.909	0.903
60 – 79%	12	0.090	0.103	49	0.891	0.855
80 – 100%	3	0.022	0.015	36	0.655	0.655

$N_{(+)}$ is total number of patients with a final institutional diagnosis of "acute appendicitis".
$N_{(-)}$ is total number of patients with a final institutional diagnosis of "no acute appendicitis".

Figure 6.2-3 clearly shows that the surgeons' estimates of the likelihood of appendicitis were more accurate after the LeuTech® scans. The post-Tc 99m LeuTech® ROC curve shows a lower FPF for every value of TPF. For example, the post-scan curve indicates that a likelihood threshold of 60-79% will be associated with a TPF (sensitivity) of 86% and a FPF (1–specificity) of 10% (corresponding to observed values of 89% and 10%). A comparable TPF of 85% on the pre-scan curve requires a likelihood threshold of 40-59%, but this is associated with a FPF of 46%. The difference between the two ROC curves (assessed from the area under the curves) is statistically significant in favor of better diagnostic accuracy after the Tc 99m LeuTech® scan, $p < 0.0001$.

6.2.10 Image Characteristics

Following the diagnostic evaluation of the combined image sets, readers were to assess, for positive cases, the location, uptake pattern, intensity, time post-injection at which the scan was first positive, whether or not the abnormal uptake was present during the entire imaging sequence, and whether or not there was positional change of areas of uptake during the imaging session. Frequency distributions of these characteristics, and distribution of the time at which scans became positive for patients with a final institutional diagnosis of appendicitis, are presented in Tables 6.2-18 and 6.2-19 (limited to those patients with a final diagnosis of appendicitis). The cumulative distribution of time at which Tc 99m

LeuTech® scans first became positive also is presented in Figure 6.2-4, based on the site investigators' findings.

Table 6.2-18 Distribution of Image Characteristics of Abnormal Uptake in Scans of Evaluable Patients with a Final Diagnosis of Appendicitis, Study 98-004.

CHARACTERISTIC	Blinded Reader 1 N	Blinded Reader 2 N	Blinded Reader 3 N	Site Investigators N
LOCATION OF ABNORMAL UPTAKE				
Appendicitis Zone	48	41	45	49
Other	1	2	0	2
UPTAKE PATTERN				
Focal	44	32	24	28
Multifocal (in one anatomic area)	1	2	3	2
Linear	16	5	16	19
Diffuse (crossing abdominal quadrant)	4	2	1	3
Other	2	4	1	0
INTENSITY OF ABNORMAL UPTAKE				
Low	12	17	9	6
Moderate	21	21	16	22
High	16	5	20	23
ABNORMAL UPTAKE ALWAYS PRESENT?				
Yes	48	19	43	39
No	1	24	2	11
POSITIONAL CHANGE IN ABNORMAL UPTAKE?[a]				
Yes	10	9	14	8
No	39	34	31	42

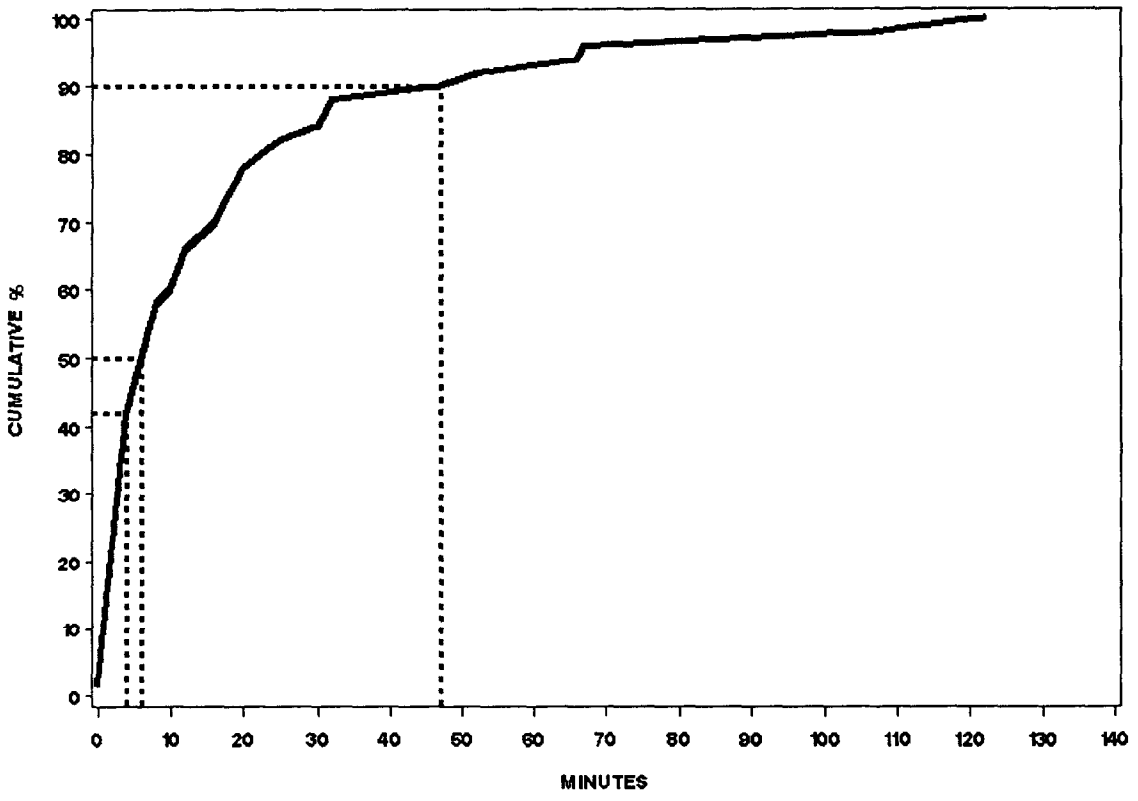
[a] Readers were asked to indicate whether or not the abnormal uptake was in a different position for standing images than for supine images.

Table 6.2-19 Distribution of Times at which Tc 99m LeuTech® Scans First Became Positive, Evaluable Patients with a Final Diagnosis of Appendicitis, Study 98-004.

EVALUATION	TIME INTERVAL OF SCAN, POST-INJECTION				TOTAL
	0 to 5 min. N (%)	6 to 30 min. N (%)[a]	31 to 60 min. N (%)[a]	> 60 min. N (%)[a]	
Blinded Reader 1	18 (36.7)	20 (77.6)	8 (93.9)	3 (100)	49
Blinded Reader 2	7 (16.3)	27 (79.1)	7 (95.3)	2 (100)	43
Blinded Reader 3	7 (15.6)	23 (66.7)	14 (97.8)	1 (100)	45
Investigators	23 (46.0)	19 (84.0)	4 (92.0)	4 (100)	50

[a] Percentages are cumulative over time.

Figure 6.2-4 Time At Which Tc 99m LeuTech® Scans First Became Positive (Cumulative Percentages), Study 98-004.



For the site investigators, 46% of scans for patients with a final institutional diagnosis of appendicitis were deemed positive within the first five minutes post-injection; by 30 minutes post-injection, 84% of images were positive and by 60 minutes, over 90% of images were positive.

6.3 SUPPORTIVE EFFICACY RESULTS

A single Phase 2 study, Study 97-003, provided efficacy data supportive of the claim that Tc 99m LeuTech® is effective for the diagnosis and ruling out of appendicitis in patients presenting with equivocal signs and symptoms.

6.3.1 Study Population

Fifty-six patients were enrolled at two sites in the United States.

Site	Investigator	Institution
A	Samuel Kipper, M.D.	Tri-City Medical Center Oceanside, California
B	Elissa Kramer, M.D.	New York University Medical Center New York, New York

All patients completed the study and were evaluable for efficacy (49 at Site A and 7 at Site B).

6.3.2 Demographic Characteristics

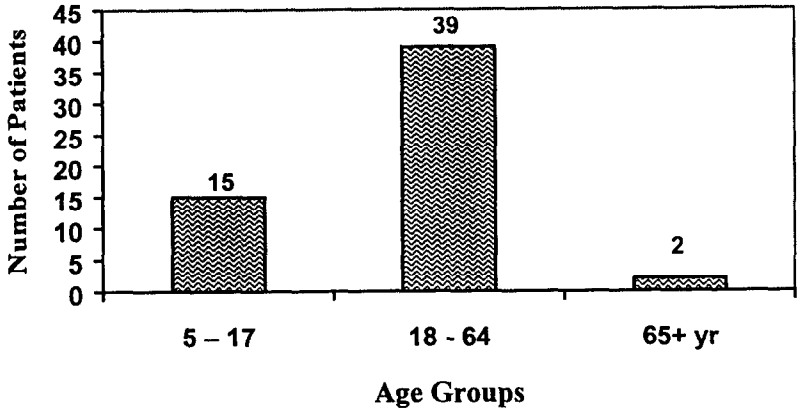
Summary statistics for age, weight and height are shown in Table 6.3-1.

Table 6.3-1 Summary Statistics for Age, Weight and Height, Study 97-003.

	N	MIN.	MAX.	MEAN	STD. DEV.
AGE (yr)	56	9.1	77.5	29.3	14.2
WEIGHT (kg)	56	29.5	104.5	65.9	17.9
HEIGHT (cm)	56	121.9	193.0	162.8	14.4

Figure 6.3-1 shows the distribution of patients' ages according to pediatric (5 years to 17 years), adult (18 years to 64 years) and geriatric (≥ 65 years) groups of patients. Of the 15 pediatric patients, 2 patients were < 10 years (both 9 years old).

Figure 6.3-1 Distribution of Age Groups of Patients Receiving Tc 99m LeuTech®, Study 97-003.



Frequency distributions for gender and race are shown in Table 6.3-2.

Table 6.3-2 Distribution of Gender and Race, Study 97-003.

		N	%
GENDER	Female	31	55.4
	Male	25	44.6
	TOTAL	56	
RACE	White	22	39.3
	Hispanic	28	50.0
	Black	3	5.4
	Other	3	5.4
	TOTAL	56	

Forty-five percent (45%) of patients were male and 55% were female. Fifty percent (50%) of patients were Hispanic and 39% were white.

6.3.3 Equivocal Signs and Symptoms of Appendicitis

Frequency distributions of the signs and symptoms that were used as criteria for equivocal presentation of appendicitis are presented in Table 6.3-3.

Table 6.3-3 Distribution of Signs and Symptoms Comprising Equivocal Presentation, Study 97-003.

CRITERIA	PATIENTS	
	N[a]	%
Atypical history and/or symptoms	34	60.7
Atypical physical examination	18	32.1
Fever less than 101° F	44	78.6
Atypical lab results (i.e., normal WBC count)	21	37.5

[a] Sum of the Ns exceeds the number of patients because some patients had more than one equivocal sign or symptom.

More than three-quarters of patients (78.6 %) who presented with right lower quadrant pain had a fever of less than 101° F and 60.7% had an atypical history or symptoms. Almost one-third (32.1%) had atypical findings on physical examination and 37.5% had a normal WBC count.

6.3.4 Concomitant Medications

Concomitant medications (prescription drugs and over-the-counter medications) taken within 24 hours of the Tc 99m LeuTech® study are summarized in Table 6.3-4. WHO Level 3 Medication Classifications were used to categorize concomitant medications. Thirty-three patients (58.9%) had taken one or more medications in the 24 hours prior to the Tc 99m LeuTech® study.

Table 6.3-4 Distribution of Concomitant Medication Usage, Study 97-003.

MEDICATION CLASS[a]	NUMBER OF PATIENTS	%
Other Analgesics and Antipyretics	9	16.1
Antibiotics[b]	5	8.9
Non-steroidal anti-inflammatory drugs (NSAIDs)	3	5.4
Intestinal Absorbents	3	5.3
Any Medication	33	58.9
None	23	41.1

[a] WHO Level 3 Classification.

[b] All Level 3 medications that are types of antibiotics were included in this category.

6.3.5 Dosing and Imaging

Summary statistics for the Tc 99m LeuTech® injection data are presented in Table 6.3-5.

Table 6.3-5 Summary Statistics for Tc 99m LeuTech® Dosing, Study 97-003.

	N	MIN.	MAX.	MEAN	STD. DEV.
Injected Volume (mL)	56	0.35	0.50	0.49	0.03
Injected Radioactivity (mCi)	56	8.14	19.50	14.32	2.82
Injected Antibody (μg)	56	87.50	143.68	124.55	8.30

Dynamic imaging was performed in all 56 patients and planar imaging was performed in 52 patients as part of the first (primary) imaging series. Thirty-eight patients underwent imaging beyond the first planar series. Summary statistics for imaging durations are presented in Table 6.3-6.

Table 6.3-6 Duration (Min) of Dynamic and Static Planar Imaging Series, Study 97-003.

	MIN.	MAX.	MEAN	STD. DEV.
First Imaging Series				
First Dynamic, (N = 56)	30	60	44.5	8.5
First Static, (N = 52)	5	54	21.4	11.2
Second Imaging Series				
Second Dynamic, (N = 38)	12	33	30.3	4.9
Second Static, (N = 35)	12	45	22.4	9.8

The duration of the first dynamic series ranged from 30 minutes to 60 minutes, with a mean of 44.5 minutes. The duration of the first static planar series, performed on 52 patients, ranged from 5 minutes to 54 minutes, with a mean of 21 minutes. For the subset of patients having a second series of images, durations averaged 30 minutes and 22 minutes for dynamic and static planar images, respectively. No patients underwent SPECT imaging.

6.3.6 Blinded Reader Evaluations

Primary and Secondary Efficacy Endpoints

The primary efficacy endpoints for the study were the blind-read accuracy of Tc 99m LeuTech® for the diagnosis of appendicitis. Secondary endpoints included the corresponding sensitivity, specificity, PPV and NPV. The "gold standard" diagnosis was provided by a patient's final institutional diagnosis, and the distribution of these diagnoses is presented in Table 6.3-7. Fifty percent

(50%) of patients had a final diagnosis of acute appendicitis. Seven patients who did not have appendicitis were diagnosed with some type of other infection

Table 6.3-7 Distribution of Final Institutional Diagnosis, Study 97-003.

FINAL INSTITUTIONAL DIAGNOSIS	N	%
Acute Appendicitis	28	50.0
No Appendicitis	28	50.0
Other Infection	7	12.5
No Infection	21	37.5
Total	56	

Estimates of the primary and secondary efficacy endpoints are presented in Table 6.3-8. Their associated two-sided 95% confidence intervals (LL = lower limit, UL = upper limit) also are presented.

Table 6.3-8 Accuracy, Sensitivity, Specificity, PPV and NPV of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Study 97-003.

				95% Confidence Int.	
EVALUATION	**N_T**	**TP + TN**	**Accuracy**	**LL**	**UL**
Blinded Reader 1	56	41	73.2	59.5	83.3
Blinded Reader 2	56	46	82.1	69.2	90.1
Blinded Reader 3	56	41	73.2	59.5	83.3
Aggregate	56	44	78.6	65.2	87.5
EVALUATION	**$N_{(+)}$**	**TP**	**Sensitivity**	**LL**	**UL**
Blinded Reader 1	28	26	92.9	75.0	96.5
Blinded Reader 2	28	23	82.1	62.4	91.8
Blinded Reader 3	28	23	82.1	62.4	91.8
Aggregate	28	25	89.3	70.6	95.4
EVALUATION	**$N_{(-)}$**	**TN**	**Specificity**	**LL**	**UL**
Blinded Reader 1	28	15	53.6	34.2	70.9
Blinded Reader 2	28	23	82.1	62.4	91.8
Blinded Reader 3	28	18	64.3	44.1	79.5
Aggregate	28	19	67.9	47.6	82.2
EVALUATION	**TP + FP**	**TP**	**PPV**	**LL**	**UL**
Blinded Reader 1	39	26	66.7	49.7	79.7
Blinded Reader 2	28	23	82.1	62.4	91.8
Blinded Reader 3	33	23	69.7	51.1	82.8
Aggregate	34	25	73.5	55.3	85.5
EVALUATION	**TN + FN**	**TN**	**NPV**	**LL**	**UL**
Blinded Reader 1	17	15	88.2	62.3	94.5
Blinded Reader 2	28	23	82.1	62.4	91.8
Blinded Reader 3	23	18	78.3	55.8	89.9
Aggregate	22	19	86.4	64.0	94.1

N_T is the number of patients whose images were evaluated by the reader.
$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.

Agreement Among Blinded Readers

Measures of inter-reader agreement were calculated for each pair of blinded readers for the diagnosis of appendicitis/no appendicitis. These measures are presented in Table 6.3-9.

Table 6.3-9 Measures of Inter-Reader Agreement for Diagnosis of Appendicitis, Study 97-003.

Pairs of Blinded Readers	Concordance Rate (95% Confidence Interval)	Kappa Statistic (95% Confidence Interval)
1, 2	0.80 (0.67 – 0.89)	0.44 (0.17 – 0.71)
1, 3	0.79 (0.65 – 0.87)	0.45 (0.19 – 0.72)
2, 3	0.77 (0.63 – 0.86)	0.34 (0.06 – 0.62)

Reader-to-reader agreement was moderate but consistent for all pairs of readers, with concordance rates of 0.77 to 0.80 and kappa statistics of 0.34 to 0.45.

6.3.7 Site Investigator Evaluations

The investigators at the study sites evaluated Tc 99m LeuTech® images. Accuracy, sensitivity, specificity, PPV and NPV relative to final institutional diagnoses of appendicitis/no appendicitis are presented in Table 6.3-10.

Table 6.3-10 Accuracy, Sensitivity, Specificity, PPV and NPV of Site Investigators' Evaluations for Diagnosis of Appendicitis, Study 97-003.

ENDPOINT	ESTIMATE	95% CONF. INT.
Accuracy	87.5 (49/56)	75.3 – 93.8
Sensitivity	96.4 (27/28)	79.8 – 96.6
Specificity	78.6 (22/28)	58.5 – 89.6
PPV	81.8 (27/33)	63.9 – 91.2
NPV	95.7 (22/23)	76.0 – 96.0

6.3.8 Intended Clinical Management and Estimated Likelihood of Appendicitis

Frequency distributions of referring surgeons' intended clinical management prior to and following review of the Tc 99m LeuTech® studies are presented in Figure 6.3-2. Table 6.3-11 presents the shifts in intended clinical management from pre- to post-scan review.

Figure 6.3-2 Distribution of Intended Clinical Management Decisions, Pre- and Post-Tc 99m LeuTech® Images, Evaluable Patients, Study 97-003.

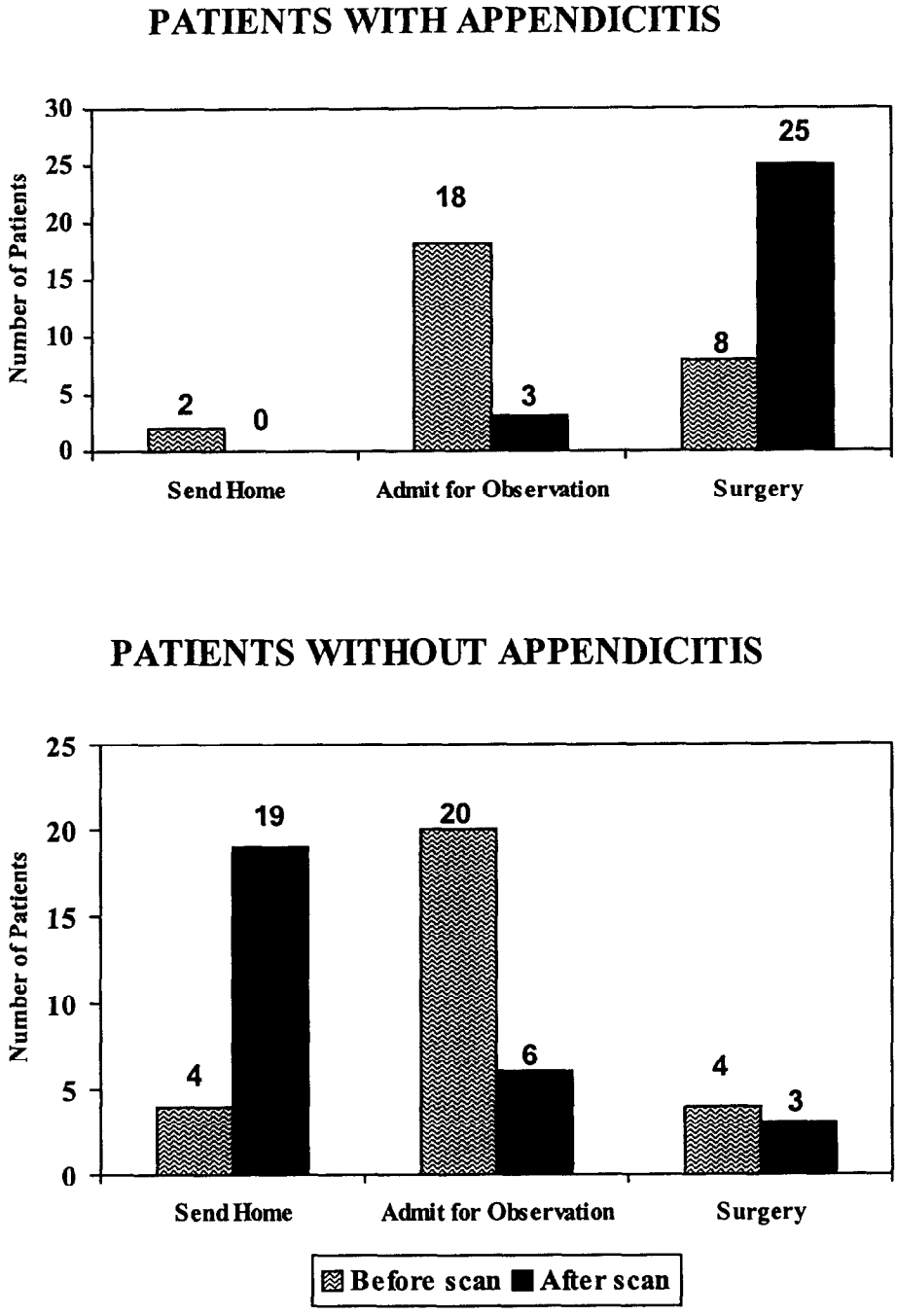


Table 6.3-11 Shifts in Intended Clinical Management Pre- and Post-Tc 99m LeuTech® Imaging, Study 97-003.

Pre-Tc 99m LeuTech®	Post-Tc 99m LeuTech®			
	Send Home	Admit for Observation	Surgery	Pre-Total
Final Diagnosis = Acute Appendicitis				
Send Home	0	0	2	**2**
Admit for Observation	0	3	15	**18**
Surgery	0	0	8	**8**
Post-Total	**0**	**3**	**25**	**28**
Final Diagnosis = No Acute Appendicitis				
Send Home	4	0	0	**4**
Admit for Observation	13	6	1	**20**
Surgery	2	0	2	**4**
Post-Total	**19**	**6**	**3**	**28**

McNemar's test was used to compare the pre- and post-scan proportions of correct decisions. The optimal or "correct" treatment decision for an acute appendicitis patient is "surgery", and the correct treatment decision for a patient without appendicitis is "send home". "Admit for observation", while the standard of care if a definitive diagnosis cannot be made, is not optimal and was defined as incorrect for the purpose of the analysis. There were 44 (78.6%) correct management decisions following the Tc 99m LeuTech® study versus 12 (21.4%) correct management decisions pre-study, and the difference in proportions was highly significant ($p < 0.001$). Seventeen additional patients with a final diagnosis of acute appendicitis would have been sent to surgery, and 15 additional patients without appendicitis would have been sent home.

The distribution of estimated likelihood of appendicitis scores prior to and after review of the Tc 99m LeuTech® scans is provided in Table 6.3-12.

Table 6.4-4 Summary Statistics for Tc 99m LeuTech® Dosing, Studies 98-004 and 97-003.

	Age Group	N	MIN.	MAX.	MEAN	STD. DEV.
Injected Volume (mL)	5-17 yr	64	0.1	0.5	0.4	0.12
	≥ 18 yr	195	0.3	1.0	0.5	0.08
	All ages	259	0.1	1.0	0.5	0.10
Injected Radioactivity (mCi)	5-17 yr	64	4.2	19.9	12.8	4.34
	≥ 18 yr	195	7.8	33.0	16.5	3.32
	All ages	259	4.2	33.0	15.6	3.93
Injected Antibody (μg)	5-17 yr	64	32.5	143.7	101.3	30.46
	≥ 18 yr	195	82.5	250.0	128.0	20.55
	All ages	259	32.5	250.0	121.4	26.03
Injected Antibody (μg/kg)	5-17 yr	64	0.8	3.9	2.0	0.58
	≥ 18 yr	195	0.8	4.2	1.8	0.53
	All ages	259	0.8	4.2	1.9	0.55

Across all ages, patients received a radioactive dose between 4.2 mCi and 33 mCi, and a dose of antibody between 32.5 μg and 250 μg; corresponding mean doses were 15.6 mCi and 121.4 μg. Pediatric patients received between 4.2 mCi and 19.9 mCi (mean 12.8 mCi); their antibody dose ranged from 32.5 μg to 143.7 μg (mean 101.3 μg). On a per kilogram basis, antibody dose for pediatric and adult patients were comparable (means 1.9 μg/kg and 1.8 μg/kg, respectively).

6.4.4 Efficacy Results

The distribution of patients' final institutional diagnoses both for acute appendicitis and for infection (inclusive of other infections and appendicitis) is presented in Table 6.4-5. It is limited to the 256 patients who were evaluable for efficacy from the combined studies (three patients in the Phase 3 study, 98-004, were not evaluable).

Table 6.4-5 Distribution of Final Institutional Diagnosis for Evaluable Patients, Studies 98-004 and 97-003.

FINAL INSTITUTIONAL DIAGNOSIS	N	%
Acute Appendicitis[a]	87	34.0
No Appendicitis	169	66.0
Other Infection	30	11.7
No Infection	139	54.3
Total	256	

[a] Three patients with acute appendicitis also had another infection.

Thirty-four percent (34%) of the patients from the combined studies had a final institutional diagnosis positive for acute appendicitis.

Pooled estimates of sensitivity, specificity, accuracy, NPV and PPV for the diagnosis of acute appendicitis are provided in Table 6.4-6. The total number of patients included in investigators' evaluations is 238; training cases (18 patients from the Phase 3 study, 98-004) are excluded.

Table 6.4-6 Sensitivity, Specificity, Accuracy, PPV and NPV of Blinded Readers' Evaluations for Diagnosis of Appendicitis, Studies 98-004 and 97-003.

EVALUATION	$N_{(+)}$	TP	Sensitivity	95% LL of CI
Blind-Read Aggregate[a]	87	69	81.7	73.3
Site Investigators	82	76	92.7	85.7
EVALUATION	**$N_{(-)}$**	**TN**	**Specificity**	**95% LL of CI**
Blind-Read Aggregate	169	150	91.5	86.9
Site Investigators	156	132	84.6	78.9
EVALUATION	**N_T**	**TP**	**Accuracy**	**95% LL of CI**
Blind-Read Aggregate	256	219	86.1	82.0
Site Investigators	238	208	87.4	83.2
EVALUATION	**TP +FP**	**TP**	**PPV**	**95% LL of CI**
Blind-Read Aggregate	88	69	78.9	70.3
Site Investigators	100	76	76.0	67.8
EVALUATION	**TN + FN**	**TN**	**NPV**	**95% LL of CI**
Blind-Read Aggregate	168	150	89.4	84.5
Site Investigators	138	132	95.7	91.4

[a] Blind-read aggregate estimates based on weighted 98-004 and 97-003 estimates.
$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.
N_T is the total number of patients.

Sensitivity and specificity for the blind-read aggregate results were 81.7% and 91.5%, respectively. For the site investigators, who had access to each patient's clinical information, sensitivity was higher, 92.7%, and specificity was correspondingly lower, 84.6%. Accuracies for blind-read aggregate and site investigators were close, 86.1% and 87.4%, respectively, as were PPVs, 78.9% and 76.0%, respectively. NPV was higher for the site investigators at 95.7% versus 89.4% for the blind-read aggregate.

Evaluation of efficacy of Tc 99m LeuTech® was based solely on the presence or absence of acute appendicitis. Tc 99m LeuTech® scans for patients with other infections also demonstrated uptake, which contributed to a higher false-positive incidence in both the Phase 2 and Phase 3 studies. Some of the patients who were

classified as false-positive for appendicitis actually had other conditions that required surgery.

The likelihood ratios, LR(+) and LR(−), for diagnosing acute appendicitis by Tc 99m LeuTech®, and their 95% confidence intervals, are provided in Table 6.4-7.

Table 6.4-7 Likelihood Ratios for Diagnosis of Appendicitis, Studies 98-004 and 97-003[a].

EVALUATION	LR(+)	95% Confidence Interval	LR(−)	95% Confidence Interval
Blind-Read Aggregate	5.03	3.34 – 7.60	0.25	0.17 – 0.38
Site Investigators	6.02	4.15 – 8.75	0.09	0.04 – 0.19

[a] Estimates based on weighted 98-004 and 97-003 estimates.

Given a test result positive for acute appendicitis, the odds that a patient had appendicitis increased by a factor of 5 for the blind-read aggregate and by a factor of 6 for the site investigators. A negative test result reduced the odds that a patient had appendicitis by a factor of 0.25 for the blind-read aggregate and by a factor of 0.09 for the site investigators.

6.4.5 Intended Clinical Management and Estimated Likelihood of Appendicitis

Patient management questionnaires were completed by the referring surgeons pre- and post-Tc 99m LeuTech® scans in both the Phase 2 and Phase 3 studies. The combined results for evaluable patients (56 patients and 189 patients in Phase 2 and 3, respectively) are presented in Figure 6.4-2.

Figure 6.4-2 Distribution of Intended Clinical Management Decisions, Pre- and Post-Tc 99m LeuTech® Images, Evaluable Patients, Studies 98-004 and 97-003.

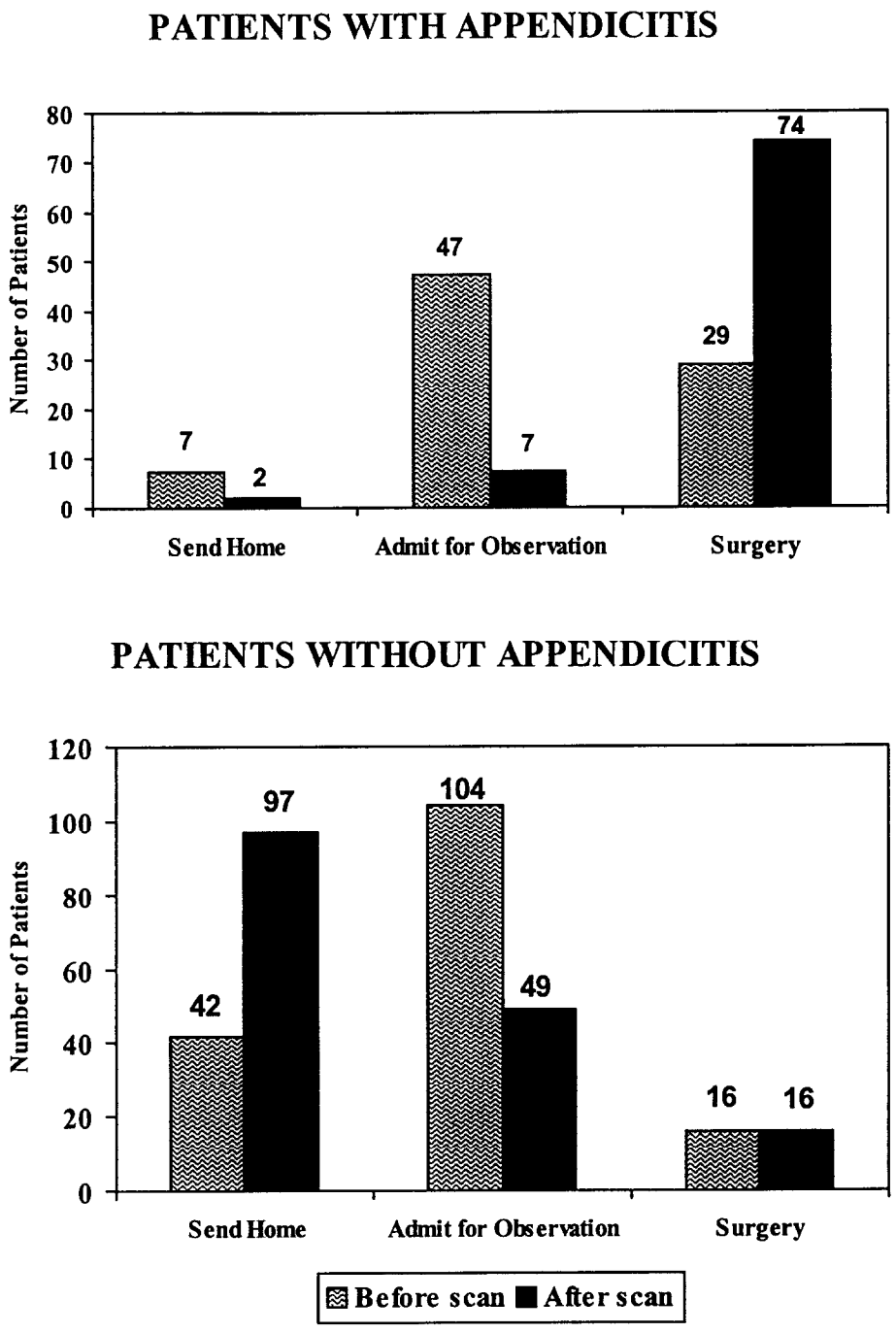


Table 6.4-8 presents the shifts in intended clinical management decisions following evaluation of the Tc 99m LeuTech® study.

Table 6.4-8 Shifts in Intended Clinical Management Pre- and Post-Tc 99m LeuTech® Imaging, Studies 98-004 and 97-003.

Pre-Tc 99m LeuTech®	Post-Tc 99m LeuTech®			
	Send Home	Admit for Observation	Surgery	Pre-Total
Final Diagnosis = Acute Appendicitis				
Send Home	2	0	5	7
Admit for Observation	0	7	40	47
Surgery	0	0	29	29
Post-Total	**2**	**7**	**74**	**83**
Final Diagnosis = No Acute Appendicitis				
Send Home	38	2	2	42
Admit for Observation	52	45	7	104
Surgery	7	2	7	16
Post-Total	**97**	**49**	**16**	**162**

The intended management decisions were scored 0, 1 or 2, representing worst to best management decisions as follows:

Acute Appendicitis
- Send Home — Score = 0
- Admit for Observation — Score = 1
- Surgery — Score = 2

No Acute Appendicitis
- Send Home — Score = 2
- Admit for Observation — Score = 1
- Surgery — Score = 0

The difference between pre- and post-scan score distributions was highly statistically significant ($p < 0.0001$, Bowker's test of symmetry), with the vast majority of the shifts post-study in the direction of correct management versus shifts in the other direction. For the 83 patients with a final diagnosis of acute appendicitis, 74 patients (89%) would have been sent to surgery post-scan compared to 29 patients (35%) pre-scan; intended management shifts to surgery post-scan included 5 patients that would have been sent home pre-scan and 40 that would have been admitted for observation. For the 162 patients with a final diagnosis of no acute appendicitis, 97 would have been sent home post-scan compared to 42 pre-scan; intended management shifts to send home included 52

patients who would have been admitted for observation and 7 patients who would have been sent to surgery.

The distributions of the surgeon's estimated likelihood of appendicitis before and after evaluating the Tc 99m LeuTech® images, according to final institutional diagnosis, are presented in Table 6.4-9.

Table 6.4-9 Distribution of Estimates of Likelihood of Appendicitis Pre- and Post-Tc 99m LeuTech® Imaging, Studies 98-004 and 97-003.[a]

	Post-Tc 99m LeuTech® Study					
Pre-Tc 99m LeuTech® Study	0 – 19%	20 – 39%	40 – 59%	60 – 79%	80 – 100%	**Pre-Total**
Final Diagnosis = Acute Appendicitis						
0 – 19%	0	0	0	0	0	**0**
20 – 39%	3	1	1	2	6	**13**
40 – 59%	0	0	0	5	11	**16**
60 – 79%	0	1	0	9	20	**30**
80 – 100%	0	0	0	0	8	**8**
Post-Total	**3**	**2**	**1**	**16**	**45**	**67**
Final Diagnosis = No Acute Appendicitis						
0 – 19%	21	1	0	1	0	**23**
20 – 39%	35	13	2	1	0	**51**
40 – 59%	28	10	9	6	0	**53**
60 – 79%	7	5	1	3	3	**19**
80 – 100%	0	0	0	1	0	**1**
Post-Total	**91**	**29**	**12**	**12**	**3**	**147**

[a] 0 - 19% = Almost definitely not appendicitis; 20 - 39% = Probably not appendicitis; 40 - 59% = Indeterminate appendicitis; 60 - 79% = Probably appendicitis; 80 - 100% = Almost definitely appendicitis.

6.4.6 Comparison of Age Subgroups

Sixty-three (63) of the 256 evaluable patients were pediatric patients between the ages of 5 years and 17 years. Fifteen of them were younger than 10 years. Sensitivity and specificity were compared for the pediatric (5 years – 17 years), adult (18 years – 64 years) and geriatric (≥ 65 years) subgroups and it was established that Tc 99m LeuTech® performed equally well across all three age groups. The results are provided in Table 6.4-10.

Table 6.4-10 Comparison of Age Subgroups: 5 – 17, 18 – 64, ≥ 65 Years, Studies 98-004 and 97-003.

SENSITIVITY											
	5 – 17 yr.			18 – 64 yr.			≥ 65 yr.				Sig.
	$N_{(+)}$	TP	Sens.	$N_{(+)}$	TP	Sens.	$N_{(+)}$	TP	Sens.[b]	χ^2	Prob.
Blind-Read Aggregate[a]	20	17	72.7	61	46	76.1	6	6	100.0	2.155	0.340
Site Investigators	19	18	94.7	57	53	93.0	6	5	83.3	0.829	0.660
SPECIFICITY											
	5 – 17 yr.			18 – 64 yr.			≥ 65 yr.				Sig.
	$N_{(-)}$	TN	Spec.	$N_{(-)}$	TN	Spec	$N_{(-)}$	TN	Spec.[c]	χ^2	Prob.
Blind-Read Aggregate	43	40	94.0	120	105	91.1	6	5	83.3	1.549	0.460
Site Investigators	41	37	90.2	109	90	82.6	6	5	83.3	1.246	0.536

$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.
[a] Blind-read aggregate estimates based on weighted 98-004 and 97-003 estimates.
[b] Unweighted average based on sensitivities of 2/2 and 4/4 for Phase 2 and 3, respectively.
[c] Result based on Phase 3 study, 98-004, only; no Phase 2 patients in this age subgroup had a negative final institutional diagnosis.

No statistically significant differences were found between the age subgroups.

Pediatric efficacy data were examined further for patients 5 years to 9 years of age and patients 10 years to 17 years of age. Table 6.4-11 presents efficacy for the two subgroups.

Table 6.4-11 Sensitivity, Specificity, Accuracy, PPV and NPV for Pediatric Subgroups, Studies 98-004 and 97-003.

	5 – 9 yr			10 – 17 yr		
	$N_{(+)}$	TP	Sensitivity	$N_{(+)}$	TP	Sensitivity
Blind-Read Aggregate[a]	7	6	80.0	13	11	66.7
Site Investigators	7	7	100.0	12	11	91.7
	$N_{(-)}$	TN	Specificity	$N_{(-)}$	TN	Specificity
Blind-Read Aggregate	8	8	100.0	35	32	92.3
Site Investigators	7	7	100.0	34	30	88.2
	N_T	TN+TP	Accuracy	N_T	TN+TP	Accuracy
Blind-Read Aggregate	15	14	92.3	48	43	89.9
Site Investigators	14	14	100.0	46	41	89.1
	TP+FP	TP	PPV	TP+FP	TP	PPV
Blind-Read Aggregate	6	6	100.0	14	11	81.9
Site Investigators	7	7	100.0	15	11	73.3
	TN+FN	TN	NPV	TN+FN	TN	NPV
Blind-Read Aggregate	9	8	88.9	34	32	93.1
Site Investigators	7	7	100.0	31	30	96.8

$N_{(+)}$ is the number of patients positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients negative for acute appendicitis by final institutional diagnosis.
[a] Blind-read aggregate estimates based on weighted 98-004 and 97-003 estimates.

Based on limited numbers of patients, it appears that the diagnostic performance of Tc 99m LeuTech® is comparable for the two pediatric age groups for both the aggregate blind-read data and the site investigators' results. It is notable that, for the younger age group (5 – 9 years) of 15 patients, site investigators achieved 100% accuracy.

Intended patient management was also examined for pediatric patients. Shift tables displaying changes in referring surgeons' intended clinical management following findings based on the Tc 99m LeuTech® procedure, are provided in Table 6.4-12.

Table 6.4-12 Shifts in Intended Clinical Management Pre- and Post-Tc 99m LeuTech® Imaging for Pediatric Subgroups, Studies 98-004 and 97-003.

FINAL DIAGNOSIS = ACUTE APPENDICITIS				
	Post-Tc 99m LeuTech®			
Pre-Tc 99m LeuTech®	Send Home	Admit for Observation	Surgery	**Pre-Total**
5 – 9 YR				
Send Home	0	0	1	**1**
Admit for Observation	0	0	3	**3**
Surgery	0	0	2	**2**
Post-Total	**0**	**0**	**6**	**6**
10 – 17 YR				
Send Home	1	0	0	**1**
Admit for Observation	0	0	8	**8**
Surgery	0	0	3	**3**
Post-Total	**1**	**0**	**11**	**12**
FINAL DIAGNOSIS = NO ACUTE APPENDICITIS				
	Post-Tc 99m LeuTech®			
Pre-Tc 99m LeuTech®	Send Home	Admit for Observation	Surgery	**Pre-Total**
5 – 9 YR				
Send Home	3	0	0	**3**
Admit for Observation	1	4	0	**5**
Surgery	0	0	0	**0**
Post-Total	**4**	**4**	**0**	**8**
10 – 17 YR				
Send Home	11	0	1	**12**
Admit for Observation	12	7	2	**21**
Surgery	1	0	0	**1**
Post-Total	**24**	**7**	**3**	**34**

Of the 29 shifts in intended management following the Tc 99m LeuTech® study, 26 were in the direction of correct management ("surgery" for acute appendicitis patients and "send home" for patients without appendicitis) versus shifts in the other direction. Among pediatric patients with acute appendicitis, all six 5 – 9 year-olds would have been sent to surgery post-scan versus two patients pre-scan, and 11 of the twelve 10 – 17 year-olds would have been sent to surgery post-scan versus three pre-scan. Among the pediatric patients without acute appendicitis, one additional 5 – 9 year-old and 13 additional 10 – 17 year-olds would have been

sent home. The three shifts in intended management in the wrong direction occurred for three 10 – 17 year old patients without appendicitis. Post-scan, all three would have been sent to surgery incorrectly.

6.4.7 Comparison of Drug Interaction Subgroups

Potential drug-drug interactions were evaluated for two classes of drugs, antibiotics and NSAIDs. Efficacy endpoints from the combined Phase 2 and 3 studies were compared for patients who were taking the class of medication concomitantly with patient who were not taking those medications concomitantly. Results are summarized in Table 6.4-13 for the antibiotic use subgroups.

Table 6.4-13 Comparison of Antibiotic Use Subgroups, Evaluable Patients, Studies 98-004 and 97-003.

SENSITIVITY								
	ANTIBIOTICS			NO ANTIBIOTICS			χ^2	Sig.
	$N_{(+)}$	TP	Sensitivity	$N_{(+)}$	TP	Sensitivity		Prob.
Blind-Read Aggregate[a]	12	11	88.9	75	58	79.8	1.492	0.222
Site Investigators	12	11	91.7	70	65	92.9	0.005	0.944
SPECIFICITY								
	ANTIBIOTICS			NO ANTIBIOTICS			χ^2	Sig.
	$N_{(-)}$	TN	Specificity	$N_{(-)}$	TN	Specificity		Prob.
Blind-Read Aggregate[a]	24	19	80.2	145	131	93.8	4.734	0.029
Site Investigators	22	15	68.2	134	117	87.3	5.938	0.014

$N_{(+)}$ is the number of patients diagnosed as positive for acute appendicitis by final institutional diagnosis
$N_{(-)}$ is the number of patients diagnosed as negative for acute appendicitis by final institutional diagnosis.
[a] Blind-read aggregate estimates based on weighted 98-004 and 97-003 estimates.

While sensitivities for subgroups according to antibiotic usage were comparable, specificities were significantly higher for patients not taking antibiotics, both for the aggregate blind-read results (13% higher) and for the site investigators (19% higher). The prevalence of other infections was three times higher for the patients taking antibiotics, whereas the prevalence of appendicitis was the same.

Final Diagnosis	Antibiotics	No Antibiotics
Acute Appendicitis	12 (33%)	75 (34%)
Other Infection	10 (28%)	20 (9%)
Negative	14 (39%)	125 (57%)

The rate of false-positive findings for appendicitis was higher among patients who had other infections: 43% of other infections (13 cases) were read as false positive

for appendicitis by the blind-read aggregate, while 31% of other infections (9 cases) were read as false positive for appendicitis by the site investigators. This compares with 4% of negative patients being read as false positive by the blind-read aggregate and 12% of negative patients being read as false positive by the site investigators. False positive findings for appendicitis among patients with other infections were similar whether patients were taking antibiotics or not. Thus, differences in specificities are not a result of a drug interaction, per se, but rather the result of the higher prevalence of other infections among patients being treated with antibiotics.

Results are summarized in Table 6.4-14 for the NSAID use subgroups.

Table 6.4-14 Comparison of NSAID Use Subgroups, Evaluable Patients, Studies 98-004 and 97-003.

SENSITIVITY								
	NSAIDs			NO NSAIDs			χ^2	Sig. Prob.
	$N_{(+)}$	TP	**Sensitivity**	$N_{(+)}$	TP	**Sensitivity**		
Blind-Read Aggregate[a]	8	6	71.4	79	63	81.9	0.010	0.921
Site Investigators	8	8	100.0	74	68	91.9	0.841	0.359
SPECIFICITY								
	NSAIDs			NO NSAIDs			χ^2	Sig. Prob.
	$N_{(-)}$	TN	**Specificity**	$N_{(-)}$	TN	**Specificity**		
Blind-Read Aggregate[a]	20	18	93.4	149	132	91.2	0.004	0.947
Site Investigators	19	17	89.5	137	115	83.9	0.317	0.574

$N_{(+)}$ is the number of patients diagnosed as positive for acute appendicitis by final institutional diagnosis.
$N_{(-)}$ is the number of patients diagnosed as negative for acute appendicitis by final institutional diagnosis.
[a] Blind-read aggregate estimates based on weighted 98-004 and 97-003 estimates.

Sensitivity and specificity were comparable for patients taking NSAIDs concomitantly with the Tc 99m LeuTech® study compared to those who were not.

6.5 EFFICACY CONCLUSIONS

Appendicitis is often diagnosed easily by its characteristic symptoms and pathognomic signs that either appear by the time the patient presents to the emergency room, or develop during a few hours after hospital admission for observation. However, diagnosing acute appendicitis can be very difficult in cases of appendicitis presenting without classic signs and symptoms. Symptoms may remain atypical and signs may not become unequivocally positive during any reasonable observation interval. In these cases, the disease progresses from acute appendicitis (a stage of the disease with relatively low morbidity) to perforated appendicitis, with greater risk of morbidity and death.

High sensitivity is important to confidently rule out disease and forgo surgery without incurring risk of the disease progressing to the more dangerous stage of perforated appendicitis, i.e., reduce the incidence of false-negative diagnoses. In the Phase 3 pivotal study, sensitivity of Tc 99m LeuTech® in three blinded readers' evaluations was 66%, 76% and 81%, with aggregate results of 75%. When scans were evaluated by the unblinded site investigators, sensitivity was 91%. Evidence supportive of efficacy was also provided by the Phase 2 study in 56 additional patients presenting with equivocal signs and symptoms of appendicitis. When results were pooled with the Phase 3 study for a total of 256 evaluable patients (87 patients, 34%, with a final institutional diagnosis of acute appendicitis and 169 patients, 66%, with a final institutional diagnosis of no appendicitis), pooled blind-read aggregate sensitivity was 82%, and pooled site investigators' sensitivity was 93%.

Tc 99m LeuTech® was associated with consistently high negative predictive value (NPV). For the Phase 3 study, it ranged from 86% to 92% with an aggregate result of 90% for blinded readers, and was 96% for site investigators. For pooled Phase 2 and 3 studies, the aggregate blind-read NPV was 89% and the site investigators' NPV was 96%. The prevalence of acute appendicitis in the Phase 3 study sample of patients with equivocal signs and symptoms was 30% (59 of 200 patients); it was slightly higher for the combined Phase 2 and 3 studies, 34% (87 of 256 patients). These levels emulate the disease prevalence one would expect to see in this patient population as a whole. Consequently, the sample estimates of NPV are representative estimates of the population values. For many practicing physicians, high NPV is of critical importance. It provides them with the confidence to discharge patients appropriately from the ER, avoiding either unnecessary time in the hospital for observation or, in some cases, inappropriate surgery.

High specificity is important in the effort to avoid unnecessarily sending the patient to surgery, i.e., reduce the incidence of false-positive diagnoses. In the pivotal study, Tc 99m LeuTech® displayed specificities of 90%, 94% and 88% by blinded readers, with aggregate results of 93%. Site investigators obtained a specificity of 86%. In the pooled Phase 2 and 3 studies, Tc 99m LeuTech® had a specificity of 92% among blinded readers, and 85% for site investigators.

The overall performance of Tc 99m LeuTech® in the Phase 3 study as measured by accuracy was similar for blinded readers and site investigators, 83%, 86% and 89% for blinded readers, and 87% for site investigators. This indicates the consistency of performance among blinded readers and investigators, and suggests that differences between blinded readers' and site investigators' sensitivity and specificity can be attributed to the different readers' confidence thresholds for calling a scan positive for acute appendicitis. Pooled (Phase 2 and Phase 3) accuracy for aggregate blind-read and site investigators was close, 86% and 87%, again suggestive of the consistency of performance for blinded readers and site investigators. Evaluation of the efficacy of Tc 99m LeuTech® was based

solely on the presence or absence of acute appendicitis. Tc 99m LeuTech® scans for patients with other infections also demonstrated uptake, which contributed to a higher incidence of patients who were classified as false-positive for appendicitis, some of whom had other conditions requiring surgery.

The efficacy of Tc 99m LeuTech® was also demonstrated by the likelihood ratios associated with image evaluations. Based on the Phase 3 results, positive likelihood ratios indicate that with a positive test result, the odds that a patient has appendicitis increase 7 to 13 times the pre-test odds for blinded readers, and 6 times the pre-test odds for site investigators. Negative likelihood ratios indicate that with a negative test result, the odds of appendicitis are reduced by a factor of 0.21 to 0.38 for blinded readers and 0.11 for investigators. Pooled Phase 2 and 3 results were similar.

There was strong evidence in both the Phase 3 and 2 studies to support the potential for Tc 99m LeuTech® to significantly impact the clinical management of the patients. This clinical management benefit was based on a comparison of referring surgeons' intended management decisions that were made before and after review of the Tc 99m LeuTech® scans. Both studies demonstrated statistically significant shifts in intended management post-scan versus pre-scan, with the vast majority of shifts in management occurring in the direction of improved or correct decision post-scan. Based on the combined studies, of 83 patients with a final diagnosis of acute appendicitis, 74 patients (89%) would have been sent to surgery post-scan as compared to 29 patients (35%) pre-scan; shifts to "surgery" post-scan included 5 patients that would have been sent home pre-scan and 40 patients that would have been admitted for observation pre-scan. Of 162 patients with a final diagnosis of no acute appendicitis for whom there were intended management decisions, 97 patients (60%) would have been sent home post-scan as compared to 42 patients (26%) pre-scan; shifts to "send home" post-scan included 52 patients who would have been admitted for observation pre-scan and 7 patients who would have been sent to surgery pre-scan.

In the Phase 3 study, there was good inter-observer agreement among the three blinded readers. Kappa statistics for pairs of readers were consistently greater than 0.5 and concordance rates were high, 88% to 90%. Agreement among readers for the Phase 2 study was also moderate to good, though slightly lower than Phase 3. Kappa statistics ranged form 0.34 to 0.45 and concordance rates were 77% to 80%. The good agreement among readers suggest that scans from Tc 99m LeuTech® scintigraphy are uniformly easy to read.

Scintigraphic abdominal imaging with Tc 99m LeuTech® required only standard imaging techniques, which could be completed in a relatively short period of time. Only conventional imaging equipment, not SPECT, was required. In the Phase 3 study, where time to first positive image was recorded, more than two-thirds of patients had positive uptake in the first 30 minutes and more than 90% of patients within 60 minutes.

Tc 99m LeuTech® appeared to perform equally well in all age groups (pediatric, adult and geriatric patients). Based on the subgroup of 63 pediatric patients (ages 5 to 17 years) from the combined Phase 2 and 3 studies, sensitivity and specificity were comparable to the estimates for adults. Sensitivity was 73% and 95% for the aggregate blinded read and site investigators, respectively, with corresponding specificities of 94% and 90%. Efficacy was also examined for patients 5 years to 9 years (15 patients) and 10 years to 17 years (48 patients). Performance was similar in the two groups. It was notable that for the 15 patients who were 5 years to 9 years of age, the site investigators' diagnoses were 100% accurate. Intended patient management decisions were compared pre- and post-review of the scans and, of 29 shifts in decisions post-scan, 26 were in the direction of correct management ("surgery" for acute appendicitis and "send home" for negative patients). Among the pediatric patients with acute appendicitis (six 5 to 9 year-olds and twelve 10 to 17 year-olds), all but one patient (a 10 to 17 year-old) would have been sent to surgery post-scan as compared to five patients pre-scan. For the patients without acute appendicitis, an additional 14 of 42 pediatric patients would have been sent home post-scan as compared to pre-scan.

Overall, Tc 99m LeuTech® has been shown to be an effective diagnostic agent for diagnosing and ruling out appendicitis in adult, geriatric and pediatric patients presenting with equivocal signs and symptoms. Tc 99m LeuTech® scintigraphic imaging can be performed quickly and without the need for special equipment or techniques. It is effective, easy to use and provides rapid results. Tc 99m LeuTech® will be a valuable addition to the diagnostic options available for patients with suspected appendicitis.

7. SAFETY

Safety was assessed in terms of the occurrence of adverse events, changes in clinical laboratory parameters (chemistry, hematology, and urinalysis), changes in vital sign parameters and assessment of HAMA response.

This section presents a cumulative summary of the safety data submitted in the original BLA filing and that submitted in the 120-Day Safety Update dated March 27, 2000.

The safety data are summarized according to the following three groupings:

1. All studies of Tc 99m LeuTech® (eight studies – see Table 4.0-1)
2. All studies conducted under Palatin IND involving a single injection (Studies 97-002, 97-003, 98-004 and 98-005)
3. All studies conducted under a Palatin IND involving repeat injection (Study 99-001)

7.1 EXTENT OF EXPOSURE

The extent of exposure of subjects to Tc 99m LeuTech® is provided in Table 7.1-1. It provides the number of subjects exposed across all lots and developmental formulations, along with summary statistics for demographic and dosing data. Across all studies, a total of 439 subjects were exposed to Tc 99m LeuTech®. Two-hundred ninety-three (293) subjects were exposed in studies conducted under a Palatin IND involving a single injection, and 30 subjects were exposed in a study conducted under a Palatin IND involving two injections. All subjects received a single injection of Tc 99m LeuTech® with one exception. In a Phase 1 study to investigate HAMA response in normal volunteers following repeat injection, each subject received two injections of decayed Tc 99m LeuTech® (Study 99-001, 30 subjects). In a Phase 1 study to investigate HAMA response in normal volunteers, subjects received a single dose of decayed Tc 99m LeuTech® injection (Study 97-001, 30 subjects).

Table 7.1-1 Extent of Exposure.

Number Of Subjects	All Subjects in All Studies	All Subjects In Palatin IND Studies	
		Single Injection	Multiple Injection
Entered (Male/Female)	439 202/237	293 120/173	30 15/15
Received Study Drug (Male/Female)	439 202/237	293 120/173	30 15/15
Eligible for Safety Evaluations (Male/Female)	439 202/237	293 120/173	30 15/15
Age (yr.): Mean (Range)	34.1 (5.2-91.4)	33.5 (5.2-91.4)	33.8 (20.9-57.6)
Anti-CD15 IgM Antibody Dose (μg): Mean (Range)	120.1 (N=409)[a,b] (32.5–250.0)	120.7 (32.5–250.0)	125[a]
Anti-CD15 IgM Antibody Dose (μg/kg): Mean (Range)	1.8 (N=387)[b,c] (0.6-4.2)	1.8 (N=292)[c] (0.8-4.2)	1.7 (0.8-2.6)
Radioactive Dose (mCi): Mean (Range)	14.5 (N=373)[d] (1.1–33.0)	15.4 (4.2–33.0)	-----

[a] Only the first injection for subjects in Study 99-001 included; each subject in that study also received a second injection of 125 μg.
[b] Antibody dose was not recorded for 30 patients in Study 95-001.
[c] Body weight was not recorded for all patients injected.
[d] Radioactive dose was not recorded for 6 patients in Study 95-001; 60 subjects (30 in Study 97-001 and 30 in Study 99-001) received decayed Tc 99m LeuTech®.

7.2 DEMOGRAPHIC CHARACTERISTICS OF SAFETY POPULATION

Table 7.2-1 presents summary statistics for demographic data (as available) in each of the three groupings of studies.

Table 7.2-1 Demographic Summary.

		All Subjects in All Studies	All Subjects in Palatin IND Studies	
			Single Injection	Multiple Injection
Age (Years)	N	439	293	30
	Minimum	5.2	5.2	20.9
	Maximum	91.4	91.4	57.6
	Median	31.0	29.0	31.2
	Mean	34.1	33.5	33.8
	SD	17.0	19.0	10.9
Age Category N (%)	5-9	16 (3.6)	15 (5.1)	0 (0.0)
	10–17	50 (11.4)	49 (16.7)	0 (0.0)
	18-64	343 (78.1)	202 (68.9)	30 (100.0)
	≥ 65	30 (6.8)	27 (9.2)	0 (0.0)
Gender N (%)	Female	237 (54.0)	173 (59.0)	15 (50.0)
	Male	202 (46.0)	120 (41.0)	15 (50.0)
Race N (%)	White	251 (57.2)	201 (68.6)	25 (83.3)
	Black	39 (8.9)	22 (7.5)	2 (6.7)
	Hispanic	94 (21.4)	60 (20.5)	0 (0.0)
	Other	22 (5.0)	10 (3.4)	3 (10.0)
	Not specified	33 (7.5)	0	0
Height (cm)	N	350	290	30
	Minimum	104.1	104.1	152.4
	Maximum	198.1	198.1	190.5
	Median	167.6	167.6	174.0
	Mean	165.8	165.1	172.2
	SD	13.6	14.0	9.9
Weight (kg)	N	410	292	30
	Minimum	21.4	21.4	48.2
	Maximum	170.0	139.5	161.4
	Median	68.5	68.2	73.6
	Mean	70.8	69.8	77.3
	SD	21.0	20.4	21.6
Body Mass Index (BMI)	N	350	290	30
	Minimum	12.6	12.6	19.2
	Maximum	49.6	46.7	49.6
	Median	24.3	24.4	24.4
	Mean	25.4	25.3	25.9
	SD	5.7	5.7	6.2

Subjects enrolled in the clinical studies ranged in age from 5.2 years to 91.4 years, with an average age of 34.1 years. Sixteen (3.6%) subjects were between 5 years and 9 years old, 50 (11.4%) were between 10 years and 17 years, 343 (78.1%) were between 18 years and 64 years, and 30 (6.8%) were 65 or older. Two hundred thirty-seven (54%) were female and 202 (46%) were male. There were 251 (57.2%) whites, 39 (8.9%) blacks, 94 (21.4%) Hispanics, and 22 (5%) "Other"; race was not specified for 33 subjects. Height ranged from 104.1 cm to 198.1 cm, with an average of 165.8 cm. Weight ranged from 21.4 kg to 170.0 kg, with an average of 70.8 kg. BMI ranged from 12.6 to 49.6, with an average of 25.4. The three subject groupings were similar with respect to mean age, height, weight and BMI, and with respect to gender. Unlike the other two groupings, most of the multiple injection subjects were white, and none were younger than 18 years or older than 64 years.

7.3 CONCOMITANT MEDICATIONS

Medications were coded according to the WHO drug dictionary, and the Level 3 Medication Classification was used to group medications. A summary of concomitant medication data according to Level 3 classes or appropriate grouping of Level 3 classes is presented in Table 7.3-1.

Table 7.3-1 Concomitant Medications Received by Subjects.

MEDICATION CLASS	All Subjects in All Studies[a] (N=353) N (%)	All Subjects in Palatin IND Studies	
		Single Injection (N=293) N (%)	Multiple Injection (N=30) N (%)
Antibiotics	54 (15.3)	51 (17.4)	3 (10.0)
NSAIDs	52 (14.7)	33 (11.3)	16 (53.3)
Opioids	58 (16.4)	56 (19.1)	2 (6.7)
Other analgesics and antipyretics	77 (21.8)	56 (19.1)	17 (56.7)
Psychotherapeutic	47 (13.3)	44 (15.0)	3 (10.0)

[a] Not including Studies 95-001 and Gratz-Becker

Including all subjects with medication data available, 54 (15.3%) received antibiotics and 52 (14.7%) received NSAIDs. Opioids were received by 58 (16.4%) subjects and 77 (21.8%) subjects were taking other analgesics and antipyretics. Psychotherapeutic medications were received by 47 (13.3%) subjects.

7.4 ADVERSE EVENTS

Adverse event (AE) data were collected for all subjects in all studies. Assessments were performed at various time-points following injection of Tc 99m LeuTech®. Data were coded according to the COSTART dictionary of terms.

7.4.1 Deaths, Discontinuations for Adverse Events, and Serious Adverse Events

No deaths, serious AEs or any other significant AE occurred in any subject enrolled in any of the clinical trials included in this summary. No subjects discontinued participation due to AEs.

7.4.2 Adverse Event Summary

A total of 39 adverse events were reported in 30 of 439 subjects (6.8%). Adverse events are summarized by COSTART preferred term and body system in Table 7.4-1.

Table 7.4-1 Summary Of Adverse Events By COSTART Term And Body System.

Body System	COSTART Term	All Subjects in All Studies (Total Subjects=439) N (%)	All Subjects in Palatin IND Studies	
			Single Injection (Total Subjects=293) N (%)	Multiple Injection (Total Subjects=30) N (%)
All Systems	ANY EVENT	30 (6.8)	21 (7.2)	7 (23.3)
Body as a Whole	ANY EVENT	10 (2.3)	6 (2.0)	2 (6.7)
	HEADACHE	2 (0.5)	2 (0.7)	0 (0.0)
	PAIN INJECT SITE	2 (0.5)	1 (0.3)	0 (0.0)
	ASTHENIA	1 (0.2)	1 (0.3)	0 (0.0)
	INJECT SITE REACT	1 (0.2)	1 (0.3)	0 (0.0)
	MALAISE	1 (0.2)	0 (0.0)	1 (3.3)
	PAIN	2 (0.5)	1 (0.3)	1 (3.3)
	PAIN ABDO	1 (0.2)	0 (0.0)	0 (0.0)
	PAIN CHEST	1 (0.2)	1 (0.3)	0 (0.0)
Cardiovascular	ANY EVENT	13 (3.0)	12 (4.1)	1 (3.3)
	VASODILAT	11 (2.5)	10 (3.4)	1 (3.3)
	SYNCOPE	2 (0.5)	2 (0.7)	0 (0.0)
Digestive	ANY EVENT	1 (0.2)	1 (0.3)	0 (0.0)
	DIARRHEA	1 (0.2)	1 (0.3)	0 (0.0)
Hemic and Lymphatic	ANY EVENT	1 (0.2)	0 (0.0)	1 (3.3)
	ECCHYMOSIS	1 (0.2)	0 (0.0)	1 (3.3)
Musculoskeletal	ANY EVENT	1 (0.2)	0 (0.0)	1 (3.3)
	JOINT DISORDER	1 (0.2)	0 (0.0)	1 (3.3)
Nervous	ANY EVENT	5 (1.1)	3 (1.0)	2 (6.7)
	DIZZINESS	2 (0.5)	2 (0.7)	0 (0.0)
	PARESTHESIA	3 (0.7)	1 (0.3)	2 (6.7)
Respiratory	ANY EVENT	7 (1.6)	5 (1.7)	2 (6.7)
	DYSPNEA	4 (0.9)	4 (1.4)	0 (0.0)
	PHARYNGITIS	2 (0.5)	0 (0.0)	2 (6.7)
	RHINITIS	1 (0.2)	1 (0.3)	0 (0.0)
Total Number of Events		39	28	9

The most frequently reported AE was vasodilatation, experienced by 11 (2.5%) subjects. Dyspnea was reported by 4 (0.9%) subjects and paresthesia was reported by 3 (0.7%) subjects. Headache, pain, pain at injection site, syncope, pharyngitis and dizziness were reported in 2 (0.5%) subjects each. Asthenia, injection site reaction, malaise, abdominal pain, chest pain, diarrhea, ecchymosis,

joint disorder and rhinitis were reported in 1 (0.2%) subject each. The greater incidence of AEs in the multiple injection subjects may have been due to the longer AE surveillance period (7 weeks) in Study 99-001.

Severity of AEs was classified as mild, moderate or severe. Thirty-one (31) events were mild in severity, 6 were moderate, 1 (injection site pain) was specified as moderate-severe, and 1 (abdominal pain) was not specified. The moderate or severe events are summarized by COSTART term and body system in Table 7.4-2.

Table 7.4-2 Summary of Moderate or Severe Adverse Events by COSTART Term and Body System.

Body System	COSTART	All Subjects in All Studies (Total Subjects=439) N (%)	All Subjects in Palatin IND Studies	
			Single Injection (Total Subjects = 293) N (%)	Multiple Injections (Total Subjects = 30) N (%)
All Systems	ANY EVENT	7 (1.6)	5 (1.7)	1 (3.3)
Body as a Whole	ASTHENIA	1 (0.2)	1 (0.3)	0 (0.0)
	HEADACHE	2 (0.5)	2 (0.7)	0 (0.0)
	PAIN	1 (0.2)	1 (0.3)	0 (0.0)
	PAIN INJECT SITE	1 (0.2)	0 (0.0)	0 (0.0)
Cardiovascular	VASODILAT	1 (0.2)	1 (0.3)	0 (0.0)
Nervous	PARESTHESIA	1 (0.2)	0 (0.0)	1 (3.3)
Total Number of Events		7	5	1

Of the 39 adverse events, 20 in 14 subjects were considered possibly or probably related to study drug (relationship was not specified for 2 events). They are summarized in Table 7.4-3.

Table 7.4-3 Summary of Adverse Events Considered Possibly or Probably Related To Study Drug by COSTART Term and Body System.

Body System	COSTART	All Subjects in All Studies (Total Subjects=439) N (%)	All Subjects in Palatin IND Studies	
			Single Injection (Total Subjects = 293) N (%)	Multiple Injections (Total Subjects = 30) N (%)
All Systems	ANY EVENT	14 (3.2)	12 (4.1)	2 (6.7)
Body as a Whole	HEADACHE	1 (0.2)	1 (0.3)	0 (0.0)
	INJECT SITE REACT	1 (0.2)	1 (0.3)	0 (0.0)
	PAIN CHEST	1 (0.2)	1 (0.3)	0 (0.0)
	PAIN INJECT SITE	1 (0.2)	1 (0.3)	0 (0.0)
Cardiovascular	VASODILAT	11 (2.5)	10 (3.4)	1 (3.3)
Hemic and Lymphatic	ECCHYMOSIS	1 (0.2)	0 (0.0)	1 (3.3)
Nervous	DIZZINESS	1 (0.2)	1 (0.3)	0 (0.0)
	PARESTHESIA	1 (0.2)	1 (0.3)	0 (0.0)
Respiratory	DYSPNEA	2 (0.5)	2 (0.7)	0 (0.0)
Total Number of Events		20	18	2

7.5 CLINICAL LABORATORY ASSESSMENTS

Clinical laboratory measurements were obtained in 4 clinical trials. Study 97-002 (clinical pharmacology; 10 normal volunteers) measured hematology, chemistry and urinalysis parameters pre-injection and at 1 hour, 4 hours and 18-24 hours post-injection. In addition, white blood cell (WBC) counts and differential assessments were performed at 3, 5, 10, 15, 30 and 45 minutes post-injection. Study 98-004 (Phase 3 appendicitis; 203 patients) and Study 98-005 (Phase 2 osteomyelitis; 24 patients) measured hematology and chemistry parameters pre-injection and at 2 hours post-injection. Study 99-001 (Phase 1 repeat-injection HAMA; 30 normal volunteers) measured hematology and chemistry parameters immediately prior to each injection and at 7 days and 28 days after the second injection.

7.5.1 Clinical Laboratory Parameters: Clinically Significant Changes from Baseline

In each of the studies, study investigators were to evaluate post-injection changes in clinical laboratory parameters from baseline for clinical significance. Any clinically significant changes were to be assessed for relationship to Tc 99m LeuTech®.

Clinically significant changes from baseline are presented, by subject, in Table 7.5-1.

Table 7.5-1 Clinically Significant Laboratory Parameter Changes From Baseline, All Subjects with Laboratory Assessments.

Study Number, Site and Subject Number	Parameter (Normal Range)	Baseline Value	Post-injection Value	Time Post-Injection (hh:mm)	Attributability
97-002, Subject I-03	LDH (313-618 U/L)	556	1084	01:10	Lab Error
98-004, Patient D-21	AST (11-39 U/L)	61	154	31:26	Disease
	ALT (6-42 U/L)	37	97	31:26	Disease
98-004, Patient H-10	Hgb (12.0-16.0 g/dL)	14.7	11.2	12:27	Disease
	Hct (38.0%-47.0%)	42.8	32.6	12:27	Disease
99-001, Subject 25	LDH (90-225 U/L)	182	1496	167:33[a]	Study Drug
	AST (5-45 U/L)	31	132	167:33[a]	Study Drug

[a]Time following second injection; second injection was 21 days after first injection

A total of 7 clinically significant changes in laboratory parameters were reported in 4 of 242 subjects (1.7%) enrolled in the studies. The change in LDH in Subject I-03 (Study 97-002) was determined to be a laboratory error. The changes in AST and ALT in Patient D-21 (Study 98-004) and in hemoglobin (Hgb) and hematocrit (Hct) in Patient H-10 (Study 98-004) were attributed to disease with no follow-up required. The only subject who experienced clinically significant changes in laboratory parameters possibly attributed to Tc 99m LeuTech® was Subject 25 in Study 99-001. This subject experienced an elevation in AST and LDH one week after the second injection. Values for both measurements returned to normal range at 4 weeks following the second injection without any treatment (AST = 29 U/L and LDH = 201 U/L). The investigator noted that this elevation in liver enzymes was unexpected and clinical and laboratory follow-up revealed no cause. Therefore, he was unable to exclude the possibility of relationship to the injection of LeuTech®.

7.5.2 Clinical Laboratory Parameters: Range Shifts

Clinical laboratory measurements post-injection were categorized according to each laboratory's normal range as low (less than the lower limit), normal (within

the normal range), or high (greater than the upper limit). In Study 98-004 (Phase 3 Appendicitis) and Study 98-005 (Phase 2 Osteomyelitis), laboratory measurements were performed 2 hours post-injection. Bowker's or McNemar's test was used to test the pre- versus post-injection distributions (shift tables) for symmetry. A summary of the statistically significant findings is provided in Table 7.5-2.

Table 7.5-2 Statistically Significant Shifts in Laboratory Parameters at 2 Hours, All Patients in Studies 98-004 and 98-005.

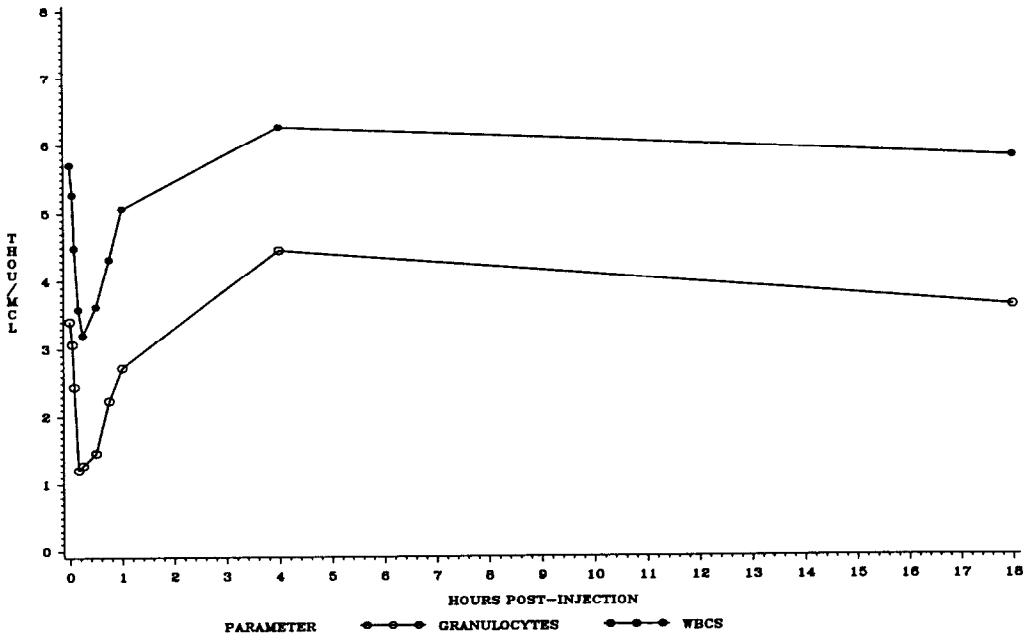
Parameter	Number of Patients with Shift[a]	Shift Upward		Shift Downward		
		Low to Normal	Normal to High	Normal to Low	High to Normal	High to Low
Hematology						
WBCs	32 (N=184)	2	5	5	20	0
Neutrophils	36 (N=144)	5	3	6	21	1
Hemoglobin	27 (N=184)	6	1	20	0	0
Clinical Chemistry						
Total Protein	27 (N=171)	2	0	16	8	1
BUN	18 (N=188)	2	0	13	3	0

[a] N is total number of patients in shift table, i.e., patients with both baseline and 2-hour measurements.

For each parameter, there was a significantly higher incidence of downward shifts post-injection. Some changes in laboratory parameters may have been due to increases in hydration from intravenous fluids given in a hospital emergency room or diagnostic imaging area. Overall, the clinical laboratory parameter changes were small and none of the changes were considered to be clinically significant. No change, other than those of WBCs and neutrophils, was thought to represent a treatment-related trend, and changes for WBCs and neutrophils were of a small magnitude.

Similar downward shifts in WBCs and granulocytes were observed for the Phase 1 study in 10 normal volunteers (Study 97-002) during the first 30 minutes post- injection. Upward shifts in the relative percent of lymphocytes and monocytes were also noted in this time period, and could be attributed to the reduction in absolute granulocyte count. There were fewer shifts at 1 hour and by 4 hours, there was no evidence of downward shifts. These shifts corresponded to the transient decrease in WBC count and percent granulocytes (Figure 7.5-1) and appeared to be related to hepatosplenic sequestration of the granulocytes. The investigator did not consider these transient shifts clinically meaningful. No other shifts in hematology parameters were statistically significant.

Figure 7.5-1 Mean Changes in WBC and Granulocyte Counts as a Function of Time Following Tc 99m LeuTech® Administration (N = 10, Study 97-002)



7.6 HUMAN ANTI-MOUSE ANTIBODY (HAMA) RESPONSE

7.6.1 HAMA Response Following Single Injection

Under BB-IND 2995, HAMA response was evaluated in Study 97-001 (clinical pharmacology, 30 normal volunteers) and in four patients in Study 95-001. Under Palatin BB-IND 7358, HAMA response was evaluated in 20 patients enrolled in Study 98-004 (Phase 3 appendicitis). Blood samples were obtained prior to injection of Tc 99m LeuTech® and at 3 weeks to 4 weeks post-injection. Subjects in Studies 97-001 and 95-001 were re-tested at 3 months to 4 months for HAMA response. Patients in Study 98-004 who had a positive response at 3 weeks to 4 weeks were to be re-tested for HAMA response at 12 weeks to 16 weeks post-injection. Table 7.6-1 summarizes the distribution of post-injection HAMA response.

Table 7.6-1 Distribution of Positive HAMA Response Following Single Injection.

Study	3 – 4 Weeks	12 – 16 Weeks
	Proportion Positive (%)	Proportion Positive (%)
Study 97-001	0/30 (0.0)	0/30 (0.0)
Study 98-004[a]	0/20 (0.0)	–
Study 95–001	0/4 (0.0)	0/4 (0.0)
All	0/54 (0.0)	0/34 (0.0)

[a] Patients who had a positive response at 3-4 weeks were to be re-tested for HAMA response at 12-16 weeks post-injection; however, none had to be retested.

No subjects had a positive HAMA response at any time-point tested.

7.6.2 HAMA Response Following Multiple Injections

Under BB-IND 7996, HAMA response was evaluated in Study 99-001 (30 normal volunteers). This study was an evaluation of HAMA response following two injections of LeuTech® three weeks apart. The results from this study were not included in the original BLA filing, but they were provided in the 120-Day Safety Update. A baseline control serum sample was collected from each subject, followed by the first LeuTech® injection. Approximately 3 weeks later, another serum sample was collected followed by the second LeuTech® injection. One week and 4 weeks after the second LeuTech® injection, additional serum samples were collected from each subject. Any serum sample collected post- LeuTech® administration having a HAMA titer greater than or equal to 4X the baseline value (prior to the first LeuTech® administration) for the same subject was considered positive (Greenman *et al.*, 1991). Five of the 30 subjects exhibited positive HAMA responses at one or more post-injection times and they are presented below in Table 7.6-2.

Table 7.6-2 Subjects Having Positive HAMA Response at One or More Post-Injection Times (HAMA Values in ng/mL), Study 99-001.

Subject	Baseline	Pre-Dose 2	Follow-up 1	Follow-up 2
08	5	8	14	31*
23	7	8	13	228*
27	7	17	140*	270*
28	5	20*	24*	10
30	7	12	35*	450*

* HAMA titer post-LeuTech® ≥ 4X baseline value (positive HAMA response)

Two of the five subjects had marginal responses (Subjects 08 and 28) and three subjects had moderate responses (Subjects 23, 27 and 30). None of the responses were considered strong (greater than 1000 ng/mL).

7.7 VITAL SIGNS

7.7.1 Changes in Vital Signs from Baseline

Vital signs were measured in six of the clinical trials. The vital signs were assessed at varying time points depending on the study, but in most cases they were measured immediately pre-injection and at 5 minutes, 30 minutes and 1 hour post-injection. A summary of mean changes at these time-points is provided in Table 7.7-1.

Table 7.7-1 Summary of Mean Changes in Vital Signs.

	All Subjects[a] (N)	All Subjects in Palatin IND Studies	
		Single Injection (N)	Multiple Injection[c] (N)
Systolic Blood Pressure (mm Hg)			
Immediately Pre-Injection	121.2 (N=384)	121.2 (N=293)	118.4 (N=30)
Change @ 5 Min Post[b]	–0.8* (N=378)	–0.9* (N=292)	–0.2 (N=30)
30 Min Post	–0.4 (N=381)	–0.1 (N=291)	–0.4 (N=30)
1 Hr Post	0.1 (N=370)	0.5 (N=283)	0.6 (N=30)
Diastolic Blood Pressure (mm Hg)			
Immediately Pre-Injection	72.5 (N=384)	71.6 (N=293)	76.2 (N=30)
Change @ 5 Min Post[b]	–1.1* (N=378)	–1.2* (N=292)	–0.5 (N=30)
30 Min Post	–1.0* (N=381)	–0.9* (N=291)	–1.3 (N=30)
1 Hr Post	–0.8* (N=370)	–0.7* (N=283)	–0.9 (N=30)
Pulse Rate (bpm)			
Immediately Pre-Injection	78.7 (N=383)	78.6 (N=292)	68.5 (N=30)
Change @ 5 Min Post[b]	0.2 (N=377)	0.1 (N=291)	0.6 (N=30)
30 Min Post	–0.5 (N=380)	–0.3 (N=290)	–0.4 (N=30)
1 Hr Post	–1.3* (N=369)	–1.4* (N=282)	0.6 (N=30)
Body Temperature (Oral °C)			
Immediately Pre-Injection	37.1 (N=372)	37.1 (N=282)	36.9 (N=30)
Change @ 5 Min Post[b]	0.0 (N=365)	0.0 (N=281)	0.1 (N=30)
30 Min Post	0.0 (N=369)	0.0 (N=280)	0.0 (N=30)
1 Hr Post	0.0 (N=357)	0.0 (N=271)	0.0 (N=30)

[a] Vital signs not collected for subjects in Study 97-001 and not available for patients in the Gratz-Becker study.
[b] Includes change at end of injection for Study 95-001.
[c] Includes data following first injection only.
* Statistically significant change ($p < .05$) according to Wilcoxon's signed-rank test.

Statistically significant decreases in systolic and diastolic blood pressures were noted following injection, and a statistically significant decrease in pulse rate was

noted at 1 hour post-injection. However, the changes were small and not clinically significant.

7.7.2 Clinically Significant Changes in Vital Signs

For studies under Palatin INDs, clinical significance of changes in vital signs was defined in the protocols as any change (increase or decrease) that met any of the following criteria:

- systolic blood pressure > 35 mm Hg
- diastolic blood pressure > 25 mm Hg
- pulse rate > 20 beats per minute

In addition, any other changes the investigator considered clinically significant were noted. Any clinically significant change was assessed by the investigator for its possible relationship to the study drug.

The criteria defining clinically significant changes in vital signs also were applied retrospectively to the vital sign data for Study 95-001 (BB-IND 2995) to identify clinically significant changes.

Across all studies, 20 subjects experienced protocol-defined clinically significant changes in vital signs from baseline values. The changes in 18 subjects were not related to Tc 99m LeuTech® in the opinion of the investigators. Relationship of changes to Tc 99m LeuTech® in 2 subjects in Study 95-001, noted on retrospective review of vital sign data, was not specified. The frequency of clinically significant changes from baseline is summarized in Table 7.7-2.

Table 7.7-2 Incidence of Clinically Significant Changes in Vital Signs.

Type of Vital Sign Change Post-Injection	All Subjects in All Studies[a] (N = 383) N (%)	All Subjects in Palatin IND Studies	
		Single Injection (N = 292 Subjects) N (%)	Multiple Injection (N = 30 Subjects) N (%)
Heart Rate Decrease >20 beats/min	5 (1.3)	4 (1.4)	0 (0.0)
Heart Rate Increase >20 beats/min	7 (1.8)	5 (1.7)	2 (6.7)
BP Decrease Systolic > 35 mm Hg Diastolic > 25 mm Hg	3 (0.8)	2 (0.7)	0 (0.0)
BP Increase Systolic > 35 mm Hg Diastolic > 25 mm Hg	5 (1.3)	5 (1.7)	0 (0.0)

[a] Vital signs were not collected for subjects in Study 97-001 and were not available for patients in the Gratz-Becker study

Of 383 subjects with vital sign data both pre- and post-injection, protocol-defined changes were observed as follows: 5 (1.3%) experienced a significant decrease in heart rate and 7 (1.8%) experienced a significant increase in heart rate; 3 subjects (0.8%) experienced a significant decrease in blood pressure and 5 subjects (1.3%) experienced a significant increase in blood pressure. The incidence of clinically significant vital signs changes was similar for all three subject-groups.

7.8 COMPARISON OF AGE SUBGROUPS

Incidence of adverse events and clinically significant changes from baseline for clinical laboratory parameters and vital signs were summarized for the following age subgroups: 5-9 years, 10-17 years, 18-64 years and ≥ 65 years. Results are summarized in Tables 7.8-1, 7.8-2 and 7.8-3, below.

Table 7.8-1 Proportion of Subjects Reporting One or More Adverse Events, by Age Group.

	All Subjects in All Studies Proportion (%)	All Subjects in Palatin IND Studies	
		Single Injection Proportion (%)	Multiple Injection Proportion (%)
5-9 Years	1/16 (6.3)	1/15 (6.7)	0 (0.0)
10-17 Years	0/50 (0.0)	0/49 (0.0)	0 (0.0)
18-64 Years	29/343 (8.5)	20/202 (9.9)	7/30 (23.3)
≥ 65 Years	0/30 (0.0)	0/27 (0.0)	0 (0.0)

Overall, 30 of 439 subjects (6.8%) reported one or more AEs. One AE was reported in the age group of 5-9 years (1/16; 6.3%). Twenty-nine subjects in the age group of 18-64 reported one or more AEs (29/343; 8.5%). No AEs were reported in the age group of 10-17 years (0/50; 0%), nor in subjects 65 years of age or older (0/19; 0%).

Table 7.8-2 Proportion of Subjects Having a Clinically Significant Change from Baseline in One or More Laboratory Parameters, by Age Group.

	Subjects in Palatin IND Studies	
	Single Injection Proportion (%)	Multiple Injection Proportion (%)
5-9 Years	0/11 (0.0)	0/0 (0.0)
10-17 Years	0/36 (0.0)	0/0 (0.0)
18-64 Years	3/146 (2.1)	1/30 (3.3)
≥ 65 Years	0/19 (0.0)	0/0 (0.0)

Only 4 of 242 subjects (1.7%) had a clinically significant change from baseline in laboratory parameters and all subjects were between the ages of 18-64 years. The laboratory parameter change in one subject was attributed to laboratory error, and the changes in two subjects were attributed to disease. The only subject who experienced clinically significant changes in laboratory parameters possibly

attributed to Tc 99m LeuTech® was in the multiple-injection HAMA study. This subject experienced elevations in AST and LDH one week after the second injection. Both values returned to normal at 4 weeks after the second injection without treatment.

Table 7.8-3 Proportion of Subjects Having a Clinically Significant Change from Baseline in One or More Vital Signs, By Age Group.

	All Subjects in All Studies Proportion (%)	All Subjects in Palatin IND Studies	
		Single Injection Proportion (%)	Multiple Injection Proportion (%)
5-9 Years	1/16 (6.3)	0/15 (0.0)	0/0 (0.0)
10-17 Years	3/49 (6.1)	3/49 (6.1)	0/0 (0.0)
18-64 Years	13/290 (4.5)	10/201 (5.0)	2/30 (6.7)
≥ 65 Years	3/28 (10.7)	3/27 (11.1)	0/0 (0.0)

Twenty of 383 subjects (5.2%) reported one or more clinically significant changes from baseline in vital signs. Across all studies, changes were reported for 1 subject in the age group of 5-9 years (1/16; 6.3%) and for 3 subjects in the 10-17 year age group (3/49; 6.1%). Thirteen subjects in the 18-64 year age group (13/290; 4.5%) and three subjects in the 65 years of age or older group (3/28; 10.7%) reported one or more clinically significant changes. The percentage of clinically significant changes reported was similar for all three age groups. No change in vital signs was thought to represent an age-related trend.

7.9 SAFETY CONCLUSIONS

Safety data were summarized for 439 subjects enrolled and dosed in the 8 trials included in the BLA filing and the 120-Day Safety Update. Subjects ranged in age from 5.2 years to 91.4 years, with an average age of 34.1 years. Sixteen (16; 3.6%) subjects were between 5 years and 9 years old, 50 (11.4%) were between 10 years and 17 years old, 343 (78.1%) were between 18 years and 64 years, and 30 (6.8%) were 65 years or older. Two hundred thirty-seven (237; 54.0%) were female and 202 (46.0%) were male. There were 251 (57.2%) whites, 39 (8.9%) blacks, 94 (21.4%) Hispanics, and 19 (5.0%) "other".

Of the 439 subjects, 293 subjects participated in studies conducted under a Palatin IND that involved a single injection and 30 subjects participated in a Palatin IND study that involved two injections.

All subjects received a single injection of Tc 99m LeuTech®, except for the subjects enrolled in Study 99-001, who received two injections of decayed Tc 99m LeuTech® to evaluate HAMA response. Subjects enrolled in Study 97-001 received a single injection of decayed Tc 99m LeuTech® to evaluate HAMA response. The average antibody dose was 120.1 μg (range 32.5 μg to 250 μg). Excluding the subjects who received decayed Tc 99m LeuTech® in

studies 97-001 and 99-001, the radioactive dose ranged from 1.1 mCi to 33.0 mCi, with a mean of 14.5 mCi.

No deaths, serious AEs or any other significant AE occurred in any subject enrolled in any of the clinical trials included in this document. No subjects discontinued participation due to AEs.

Thirty subjects (of 439; 6.8%) reported a total of 39 AEs. Fourteen subjects (3.2% of 439 subjects) experienced a total of 20 AEs that were considered possibly or probably related to the study drug. The most frequently reported AE was vasodilatation, experienced by 11 (2.5%) subjects. Dyspnea was reported by 4 (0.9%) subjects and paresthesia was reported by 3 (0.7%) subjects. Headache, pain, pain at injection site, syncope, pharyngitis and dizziness were reported in 2 (0.5%) subjects each. Asthenia, injection site reaction, malaise, abdominal pain, chest pain, diarrhea, ecchymosis, joint disorder and rhinitis were reported in 1 (0.2%) subject each. All 11 cases of vasodilatation were considered possibly or probably related to the study drug, as were 2 cases of dyspnea, and single cases of dizziness, headache, chest pain, paresthesia, injection site pain, injection site reaction and ecchymosis. One event (injection site pain) was classified as moderate-severe and six events were classified as moderate. The remaining events were classified as mild, with the exception of one event (abdominal pain) for which severity was not specified.

Clinical laboratory parameters were measured and evaluated for changes from baseline in four studies: a Phase 1 study (97-002), the Phase 3 study (98-004), a Phase 2 study for diagnosis of osteomyelitis (98-005) and the Phase 1 repeat injection HAMA study (99-001). A total of 7 clinically significant changes in laboratory parameters were reported in 4 of the 242 subjects (1.7%). The change in one subject (LDH) was determined to be a laboratory error and changes in two subjects (AST and ALT in one subject, hemoglobin and hematocrit in one subject) were attributed to disease. The only clinically significant changes in laboratory parameters that were possibly attributed to LeuTech® were elevations in AST and LDH in one subject after a second injection. These elevated liver enzymes returned to baseline without treatment; clinical and laboratory follow-up revealed no cause, so the investigator was unable to exclude the possibility of relationship to the injection of LeuTech®.

For Studies 98-004 and 98-005 combined, statistically significant shifts downward in laboratory parameters were noted at 2 hours post-injection for WBC, neutrophils, hemoglobin, total protein and BUN. The highest incidence of shifts occurred for neutrophils and WBCs. Twenty-five of 184 (13.6%) and 28 of 133 (21.2%) patients demonstrated a negative shift in WBCs and neutrophils, respectively. In Study 97-002, a Phase 1 study in 10 normal volunteers, WBC and granulocyte count were measured frequently during the first hour and at 4 hours and 18-24 hours post-injection. Both WBC and granulocyte counts demonstrated transient decreases after Tc 99m LeuTech® administration, which

had fully recovered by 4 hours. This phenomenon was thought to be related to transient sequestration of labeled cells in the liver and spleen.

The shifts in the other laboratory parameters (hemoglobin, total protein and BUN) were not believed to be related to study drug. They may have been due to increases in hydration from intravenous fluids given in a hospital emergency room.

None of the 54 subjects having HAMA assays before and after a single injection of LeuTech® had a positive HAMA response at any time point. Five of the 30 subjects who received two injections of LeuTech® exhibited positive HAMA responses. Two of the five subjects had marginal responses and three had moderate responses. None of the responses were considered strong (greater than 1000 ng/mL).

Mean changes in vital signs were evaluated at 5 minutes, 30 minutes and 1 hour post injection. Statistically significant decreases in systolic and diastolic blood pressures were noted following injection, and a statistically significant decrease in pulse rate was noted at 1 hour post-injection. However, the changes were small and not clinically significant.

Clinically significant changes in vital signs were defined *a priori* for blood pressure and heart rate: change (increase or decrease) > 35 mm Hg for systolic blood pressure, > 25 mm Hg for diastolic blood pressure, and > 20 beats/min for heart rate. There was a low incidence of clinically significant changes for each vital sign parameter (< 2% of subjects), and both positive and negative changes were observed. None of the investigators thought the changes noted in vital signs were related to Tc 99m LeuTech®.

In summary, Tc 99m LeuTech® has been shown to be well-tolerated and safe in all populations, including the pediatric, adult and geriatric populations, for use according to the proposed indication.

8. NON-CLINICAL STUDIES

8.1 INTRODUCTION

The murine IgM monoclonal antibody that forms the basis for LeuTech® was originally raised against stage specific embryonic antigen-1 (SSEA-1) and is produced by a hybrid hybridoma cell line (RB5). The antibody is produced in serum-free media and purified in a multi-step process yielding virus-free IgM of not less than 95% purity. In humans, the CD15 antigen on human PMNs corresponds to SSEA-1 found in mouse embryos. Knowles *et al.* (1982) found that the anti-SSEA-1 antibody binds specifically to human neutrophils. The antibody reacts strongly with the CD15 antigen found on human neutrophils. In *ex vivo* human cells and tissues, Technetium Tc 99m anti-CD15 Antibody recognizes the carbohydrate moiety 3-fucosyl-N-acetyl-lactosamine that defines the CD15 family of antigens also known as SSEA-1, Lewis-X antigen, or X-haptens (Solter *et al.*, 1978; Gooi *et al.*, 1981; Stocks *et al.*, 1990). The antibody also reacts with various glycolipids and glycoproteins expressed preferentially on activated neutrophils.

The CD15 antigen is densely expressed on the surface of human neutrophils with approximately 5.1×10^5 to 7.0×10^5 antigenic sites per neutrophil. The affinity constant (Kd) of anti-CD15 (anti-SSEA-1) for neutrophils has been reported in the literature to be in the range of 1.6×10^{-11} M to 1.6×10^{-12} M (Thakur *et al.*, 1988; Gratz *et al.*, 1998). The affinity of anti-CD15 (anti-SSEA-1) for neutrophils, in combination with their high membrane concentrations of CD15 antigen, results in a high specificity of Tc 99m anti-CD15 for localized sites of infection.

However, no significant binding of anti-CD15 to the circulating neutrophils of commonly studied laboratory animals, including three species of monkeys, has been observed. Binding to rat, cat, guinea pig, dog, rabbit, sheep and pig neutrophils has been reported in the literature to be less than 3% (Thakur *et al.*, 1988). As a result, animal models for systematic evaluation of pharmacologic and toxicologic properties of the drug substance and/or drug product do not exist. Consequently, the most pertinent information on the mechanisms of action of Tc 99m anti-CD15, its safety, and its distribution and elimination, arise from non-clinical *in vitro* studies, from limited acute and descriptive non-clinical *in vivo* studies, and from clinical studies.

Results from sponsor-conducted *in vitro* human tissue cross reactivity studies, as well as those reported in the literature, demonstrate a wide histochemical distribution of CD15 antigenic determinants in most major organ systems and many different tumor cells. However, the *in vitro* histochemical results do not correlate consistently with *in vivo* immunologic reactivity since the large IgM molecules have limited volumes of distribution, relatively short *in vivo* half-lives, and very high specificity for neutrophils. This characteristic difference between

Tc 99m anti-CD15 *in vitro* histochemical reactivity and its clinically apparent *in vivo* immunoreactivity has been demonstrated in clinical studies.

This section presents results from preclinical studies using anti-CD15 obtained from both mouse ascites and serum-free murine RB5 cell culture. Characteristics of the antibody and its antigenic determinants are described by reference to reports from the literature. Please note that the murine IgM monoclonal antibody is referred to variously as either anti-CD15 or anti-SSEA-1 antibody in the following discussion.

8.2 NON-CLINICAL PHARMACOLOGY

8.2.1 Characteristics of Cell Binding

In a study using In-111 labeled anti-SSEA antibody obtained from ascites fluid and conjugated to diethylenetriaminepentaacetic acid, Thakur *et al.* (1988) determined by Scatchard Plot analysis that there are approximately 5.1×10^5 antigen sites per neutrophil and demonstrated high affinity of anti-CD15 for neutrophils (Kd = 1.6×10^{-11} M). Similarly, Gratz *et al.* (1998) reported a high affinity constant of anti-CD15 for neutrophils (Kd = 1.6×10^{-12} M) using Tc-99m labeled anti-SSEA-1 murine monoclonal antibody. In studies conducted by Rhodes *et al.* (1994) using formalin fixed human neutrophils and formalin fixed HL-60 cells, a similar Scatchard Plot analysis showed 7×10^5 antigen sites per neutrophil and 9.4×10^5 antigen sites per HL-60 cell. The affinity constant of the antibody for human neutrophils was 3×10^{-12} M in this study.

Anti-CD15 binding to blood cellular components occurs almost exclusively to human neutrophils. Thakur *et al.* (1988) reported less than 2% of anti-SSEA-1 associated with platelets. They also reported 30% binding of radiolabeled anti-CD15 to erythrocytes in whole blood, but Knowles *et al.* (1982) reported that, while SSEA-1 is a carbohydrate structurally related to the human blood group antigen 1 expressed on erythrocytes, SSEA-1 is found exclusively on human granulocytes. Fox *et al.* (1983) reported that red blood cells were not reactive for SSEA-1 in an *in vitro* immunochemical reaction. It is, therefore, likely that the observed binding to erythrocytes by Thakur *et al.* (1988) is an experimental artifact. Significant erythrocyte labeling was not observed when Tc 99m labeled anti-CD15 was injected into human volunteers, as demonstrated by isolated blood samples analyzed for blood cell labeling at 30 minutes and 1 hour after injection (Phase 1 Study 97-002).

Binding experiments were performed using technetium Tc 99m anti-CD15 antibody with varying amounts of HL-60 cells (Rhodes *et al.*, 1994), according to the method of Lindmo *et al.* (1984), to assess the immunoreactive fraction of anti-CD15. In this comparative study, the immunoreactive fraction of Tc 99m anti-CD15 with formalin fixed HL-60 cells and formalin fixed human neutrophils was 80% and 82%, respectively. This observation is of particular

significance since application of Tc 99m anti-CD15 for *in vivo* imaging is based on its binding to human neutrophils. The functional quality control cell binding assay for LeuTech® uses fixed HL-60 cells in place of human neutrophils because of the difficulty of reproducibility in obtaining the latter. This data confirms the logic of testing LeuTech® immunoreactivity using the better defined HL-60 cells available from ATCC.

Thakur *et al.* (1990) evaluated the functional performance of human neutrophils at various levels of antigenic saturation with anti-CD15. When an average of 10% of the available surface antigens were bound to anti-SSEA-1, the phagocytic ability and nylon wool adherence of human neutrophils was approximately 70% and 80% of the respective control cells (cells without labeled antibody). At 4% or lower antigenic saturation, no apparent changes in cell function were observed.

8.2.2 Cross-reactivity with Normal Human Tissues

A study was performed to evaluate the potential cross-reactivity of the test article, LeuTech® reconstituted with Tc-99m generator eluate and ascorbic acid, with cryosections of normal human tissues. The antibody was applied to a battery of human tissues at two concentrations, 1.0 and 10.0 µg/mL. The test article reacted strongly with the positive control, SSEA-1-expressing human leukemia HL-60 cells. The test article did not react with negative control SSEA-1-negative lymphoblastoid Raji cells.

The test article bound to the membrane and/or the cytoplasm of multiple cells in multiple human test tissues. The cell types reactive with LeuTech® included resident histiocytes, circulating monocytes and neutrophils, myelomonocytic progenitor cells, glial cells, perithelial cells, various mucosal, glandular and ductular epithelia, and rare mesothelial cells. Specifically, the test article reacted with the membrane and/or cytoplasm or resident histiocytes in the majority of tissues examined. LeuTech® also bound to specialized histiocytic or reticuloendothelial cells such as hepatic Kupffer cells, alveolar macrophages, splenic red pulp histiocytes and marginal zone macrophages in all donors examined. In addition, LeuTech® reacted with monocytes and/or neutrophils in blood smears, vessel lumens, bone marrow, and tonsil.

Variable binding of LeuTech® was observed to glial cells and neuropil at multiple levels of the central nervous system including cerebrum, cerebellum, spinal cord, and posterior pituitary. Within the central nervous system, binding occurred in both the glial cells surrounding the blood vessels and the glial cells associated with neurons. Binding of LeuTech® to neurons was never observed. LeuTech® did not bind to the supporting Schwann cells in any of the peripheral nerves examined.

LeuTech® reacted with perithelial cells in a variety of tissues; sometimes the immunoreactive cells were noted below the mesothelium or supporting fetal

capillaries. Specific binding to mucosal epithelium was observed in the esophagus, tonsil, stomach, small intestine, large intestine, ureter and urinary bladder.

LeuTech® reacted with exocrine and/or endocrine glandular epithelia in pancreas, salivary gland and anterior pituitary. Specific binding was also noted for proximal tubules and Henle's epithelium in the kidney, endometrial glands in the uterus, acinar epithelium in the prostate, ductular epithelium in mammary gland and epididymis adjacent to the testis. LeuTech® also bound to Hassall's corpuscles (stratified squamous epithelium in the medulla of the thymus) and to stromal fibers in the lens in the eyes. Occasional reactivity was also noted for the mesothelial lining cells for macrophages.

In conclusion, the pattern of binding demonstrated for LeuTech® in the study was expected based on the known tissue distribution of the SSEA-1/CD-15/ Lewis x/3-fucosyl-N-acetyl-lactosamine antigen. The binding of LeuTech® to various epithelial and mesothelial tissues and lens stroma may reflect a cross-reactivity with carbohydrate moieties present in epithelial and connective tissue mucins. A similar pattern of reactivity with human tissues and tumors was demonstrated by Fox *et al.* (1983).

8.2.3 Antibody Reactivity with Neutrophils from Laboratory Animals

The CD15 antigen is not expressed on the neutrophils of the usual laboratory animals and therefore, reactivity with circulating neutrophils of laboratory animals, including monkeys, has not been demonstrated. Thakur *et al.* (1988) evaluated the interaction of labeled anti-SSEA-1 antibody with neutrophils separated from blood of rats, cats, guinea pigs, dogs, rabbits, sheep, and pigs. The results indicated that no more than 3% of the radioactivity was PMN associated using neutrophils from the different animal species tested.

Additionally, in an *in vitro* study conducted with rhesus, cynomolgus and marmoset monkey neutrophils, the percentage Tc 99m anti-CD15 antibody binding to neutrophils was not significantly different from the negative 293 cell control (4.91%, 4.85% and 5.02%, respectively, for rhesus, cynomolgus and marmoset monkey neutrophils compared to 5.24% for the negative cell control). At the cell concentration studied, the HL-60 positive cell control was 83.7% bound.

8.2.4 Antibody Effect on PMN Function

Specific membrane antigen complexes are vitally important for adherence-related PMN functions. Characteristics of PMN function, mediated in part by a group of cell surface glycoproteins, include surface adherence, chemotaxis, and phagocytosis. Thakur *et al.* (1990) examined the effects of antigenic saturation on specific PMN functions, including adherence, chemotaxis, and phagocytosis, by the blockage, at various degrees, of lacto-N-fucopentoase PMN surface receptors with anti-SSEA-1 antibody. The results indicated that at a surface receptor saturation of 10%, the surface adherence was reduced to 80% and continued to decrease reaching nearly 55% at 50% saturation. Analogous to this, the ability of PMNs to opsonize both gram positive and gram negative organisms was also diminished in a dose-related fashion.

The ability of the neutrophil to migrate directionally toward a chemoattractant, *E. coli* broth, was not impaired at any degree of saturation studied of the surface receptors. It is possible that the lacto-N-fucopentoase receptors are not involved in the chemotactic property of the PMNs, or that during the 3 hour incubation period, new receptors were generated, reviving the PMN function to the normal level.

Given the surface receptor number per cell, and assuming the uniform interaction of the antibody molecules with each of the estimated 3×10^{10} circulating PMNs in adult humans, a human dose of 100 μg of antibody would only saturate 0.4% of the available receptors. This is well below the level at which any of the three important PMN functions are expected to be adversely affected.

8.3 TOXICOLOGY

The following table summarizes the *in vivo* safety studies performed with Technetium Tc 99m anti-CD15 antibody.

Table 8.3-1 Summary of Laboratory Safety Studies.

Species/ Strain	Group Size (M/F)	Duration/Route of Administration	Dose or Concentration	Multiple of Human Dose	Significant Results
Mouse/ CD-1	10/10	Acute Intravenous; 7-day observation	0, 25, 125, 250, 500 µg/kg	0, 10, 50, 100, 200‡	No toxicological or histopathologic effects; NOEL > 500 µg/kg
Rabbit/ New Zealand White	5/5	Acute Intravenous; 7-day observation	0, 25, 125, 250, 500 µg/kg	0, 10, 50, 100, 200‡	No toxicological or histopathologic effects; NOEL > 500 µg/kg
Mouse/ Albino Outbred	3/3	Acute Intravenous; 2-day observation	1µg/mouse	20†	No gross pharmacological effects and no gross abnormalities at necropsy
Rabbit/ New Zealand White	0/3	Acute Intravenous; 2-day observation	25 µg/rabbit	5†	No gross pharmacological effects and no gross abnormalities at necropsy

† Based upon a clinical dose of 100 µg antibody administered to a 70-kg adult.
‡ Based upon a clinical dose of 125 µg antibody administered to a 50-kg adult.

Acute toxicity studies were performed in mice and rabbits at 20 times and 5 times the human dose, respectively, on antibody obtained from ascites fluid. The toxicity test doses were based on a human dose of 100 µg antibody per 70 kg person. The three female rabbits and six mice (3 females and 3 males) observed at 48 hours after intravenous administration of the test antibody showed no gross pharmacologic effects and no gross abnormalities at necropsy attributable to the test material.

Additional single-dose, intravenous toxicity studies were performed in mice and rabbits using bioreactor produced anti-CD15 antibody prepared according to the formulation intended for commercial distribution. The LeuTech® kits used in these studies were reconstituted with decayed Tc 99m to simulate clinical use of the product. The dose levels used were 10, 50, 100 and 200 times the maximum anticipated human dose. The maximum human dose was based upon 125 µg of antibody being administered to a 50 kg patient (2.5 µg/kg). In mice, 10 males and 10 females were studied at each dose level and a control group was included. In rabbits, 5 males and 5 females were studied at each dose level and a control group was included. Test and control animals were observed for an in-life period of at least 7 days. Following the observation period, the animals were sacrificed and gross necropsies were performed. Tissues from the control group and high dose group animals were evaluated histologically.

The results showed that treatment of mice and rabbits with up to 200 times the maximum anticipated human dose of anti-CD15 antibody had no adverse effects on survival, clinical conditions, hematologic or serum biochemical parameters, nor gross or microscopic histology. The No Observable Effect Level (NOEL) dose in these studies was greater than 500 μg/kg.

8.4 CONCLUSIONS

The non-clinical pharmacology and toxicology studies provide the basis for assessing the non-clinical safety of LeuTech® and indicate that it has a safety profile that would support the proposed use of LeuTech® in humans.

9. SUMMARY AND CONCLUSIONS

LeuTech® displayed no evidence of potential or known serious side effects during its nonclinical and clinical development program. Tc 99m LeuTech® was found to be significantly and consistently effective in two clinical trials (one exploratory and one confirmatory).

In the confirmatory, pivotal clinical trial, the primary efficacy endpoints were sensitivity and specificity for appendicitis. Both were estimated from evaluation of medical images by a blinded panel of evaluators. Sensitivity ranged from 66% to 81% and specificity from 88% to 94%. This is excellent evidence of efficacy for a diagnostic test subjected to the more conservative blinded-read method of assessment.

In the pivotal clinical trial, the sensitivity and specificity for clinical investigators evaluating the medical images prior to knowing the patient's final outcome (final diagnosis) were 91% and 86%, respectively. Of particular note was the consistency of test accuracy based on blinded readers (83% – 89%) with that of the unblinded investigators (87%). This strongly suggests that most of the diagnostic information used by the unblinded investigators resides within the scintigrams and not in ancillary clinical information. These findings of excellent diagnostic power in the pivotal trial corroborated the earlier exploratory trial.

Sixty-three patients in the combined pivotal and supporting efficacy studies were pediatric patients (ages 5 years to 17 years). Tc 99m LeuTech® performed equally well in this subgroup of patients. Additional exploration of efficacy in the 5 years to 9 years group (15 patients), the 10 years to 17 years group (48 patients), the 18 years to 64 years group (181 patients) the ≥ 65 years group (12 patients) confirmed the high levels of efficacy across all age groups.

Time to diagnosis is an important consideration in the management of patients with suspected acute appendicitis. Both the investigators and the blinded readers made the correct diagnostic evaluation within one hour in 90% of the patients with acute appendicitis. In addition, the procedure is simple to perform, requiring standard equipment commonly available at a community hospital, i.e., a gamma camera with standard collimation. SPECT is not required with Tc 99m LeuTech®, nor is any other special equipment required.

Evaluation of Tc 99m LeuTech® scintigrams is relatively easy, requiring only the skills commonly possessed by nuclear medicine physicians as a result of their training and experience with other radiopharmaceuticals. As in other uncomplicated diagnostic nuclear medicine procedures, a physician using Tc 99m LeuTech® judges positivity by asymmetry in the scintigram and persistence or continued accumulation of radioactivity in the affected region with time. The good agreement among blinded readers in the Phase 3 trial (they agreed in 88% to 90% of cases, with kappa statistics greater than 0.5) support this conclusion.

There was strong evidence in both the pivotal and supporting efficacy studies to support the potential for Tc 99m LeuTech® to significantly impact clinical management of the patients. Based on the referring surgeon's completion of questionnaires pre- and post-findings from the Tc 99m LeuTech® procedure, there was a marked improvement in their choice of intended management, "send home", "admit for observation" or "surgery". Of 83 patients with a final outcome of appendicitis in the combined studies, an additional 45 patients would have been sent to surgery post-scan. Of 162 patients without appendicitis, an additional 55 patients would have been sent home post-scan, avoiding unnecessary hospitalization or surgery.

To date, 439 human subjects, most suspected of having an infectious or inflammatory process such as acute appendicitis, have been exposed to the anti-CD15 antibody. A total of 293 subjects participated in studies conducted under a Palatin IND that involved a single injection and 30 subjects participated in a Palatin IND study that involved two injections. The dose of antibody ranged from 32.5 μg to 250 μg with a mean of 120.1 μg, which covers exposure to the antibody anticipated in marketed use. Both sexes were well represented (54% females and 46% males) and all age groups were represented.

Only 30 of the 439 subjects experienced an adverse event (39 adverse events in total) after Tc 99m LeuTech® injection. Under Palatin IND studies, 28 of the 323 subjects reported adverse events. No serious adverse events or deaths were reported.

Of the 39 adverse events, only seven events had a severity other than mild; a single event was classified as moderate-to-severe (pain at the injection site) and six were classified as moderate. Using COSTART definitions, the most frequent event was vasodilatation. Dyspnea was the only other adverse event type that had an incidence approaching 1% (0.9%). Although vasodilatation (flushing) is a common accompaniment of venipuncture, all of the cases of vasodilatation were classified by the investigators as possibly or probably related to Tc 99m LeuTech®. Two of 4 cases of dyspnea were also reported as possibly or probably related to Tc 99m LeuTech®.

As with all murine antisera, there is a potential that Tc 99m LeuTech® could elicit a human antimouse antibody (HAMA) response. This potential was evaluated in 54 patients and healthy volunteers following a single injection and no positive responses were noted. Five of the 30 subjects who received two injections of LeuTech® exhibited positive HAMA responses; however, none of the responses were considered strong (greater than 1000 ng/mL).

Tc 99m LeuTech® does not have any measurable effect on physiological function at the clinical dose. Vital signs were unchanged in closely monitored healthy volunteers during the Phase 1 studies. In Phase 2 and Phase 3 studies, no

medically important change from baseline values was noted for pulse, body temperature or blood pressure.

There were no medically important changes in clinical laboratory tests with the exception of a transient decrease in absolute white blood cell counts. These white blood cell count changes reverse within hours of administration and probably represent a temporary sequestration of the cells within the reticuloendothelial system (as observed in the early scintigrams). Similar transient changes in the circulating number of leukocytes have been reported with similar anti-granulocyte products and do not appear to present a safety issue (Locher *et al.*, 1997; Harris *et al.*, 1984).

Estimates of radiation absorbed doses fall well within acceptable range for technetium-99m radiopharmaceuticals. There was no unusual concentration or retention of radioactivity in any radiosensitive organ or tissue. Liver, spleen, kidneys and urinary bladder wall are the primary target organs, with absorbed doses of 0.18 rad/mCi, 0.23 rad/mCi, 0.19 rad/mCi and 0.12 rad/mCi, respectively.

Overall, Tc 99m LeuTech® has been shown to be a safe and effective diagnostic agent for diagnosing and ruling out appendicitis in adult, pediatric and geriatric patients presenting with equivocal signs and symptoms.

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11. LABELING (PACKAGE INSERT)

LeuTech™

Kit for the Preparation of Technetium Tc 99m Anti-CD15 Antibody Injection
Diagnostic—For intravenous administration

DESCRIPTION

LeuTech™ (Kit for the Preparation of Technetium Tc 99m Anti-CD15 Antibody Injection) contains Anti-CD15 Antibody, a partially reduced murine IgM monoclonal antibody, and all excipients needed to reconstitute and radiolabel with Sodium Pertechnetate Tc 99m Injection, USP. Technetium Tc 99m Anti-CD15 Antibody injection is administered intravenously as a diagnostic agent which is used for detection of infection of the appendix. Anti-CD15 Antibody[1] is directed against the carbohydrate moiety 3-fucosyl-*N*-acetyllactosamine that defines the cluster of differentiation 15 (CD15) family of antigens. Technetium Tc 99m Anti-CD15 Antibody is an *in vivo* diagnostic radiopharmaceutical that binds with high affinity to CD15 cell markers on polymorphonuclear neutrophils (PMNs).

The mouse monoclonal anti-CD15 antibody is produced in suspension culture of hybrid hybridoma cells. The IgM monoclonal antibody is purified in a series of steps designed to achieve specific viral inactivation or removal, and protein purification.

Each single-use LeuTech™ kit consists of two nonradioactive components: a vial containing nonpyrogenic sterile lyophilized Anti-CD15 Antibody and excipients, and an ampul of Ascorbic Acid Injection, 500 mg/mL, USP. The contents of the lyophilized reagent vial are: 0.25 mg Anti-CD15 Antibody, maltose monohydrate, succinic acid, sodium potassium tartrate tetrahydrate, USP, glycine, USP, disodium edetate dihydrate and 54 μg stannous tartrate. After constitution and radiolabeling with Sodium Pertechnetate Tc 99m Injection, the pH of the resulting single-dose solution is approximately 6.2.

Physical Characteristics

Technetium-99m decays by isomeric transition with a physical half-life of 6.02 hours. The photon that is useful for imaging studies is listed in Table 1.

Table 1. Principal radiation emission data for technetium-99m

Radiation	Mean Percent per Disintegration	Mean Energy (keV)
Gamma-2	89.07	140.5

External Radiation

The specific gamma-ray constant for technetium-99m is 5.4 $\mu C \cdot kg^{-1} \cdot MBq^{-1} \cdot h^{-1}$ (0.78 R/mCi·h) at 1 cm. The first half-value thickness of lead for technetium-99m is 0.017 cm. A range of values for the relative attenuation of the radiation emitted by this radionuclide that results from the interposition of various thicknesses of lead is shown in

Table 2. For example, the use of a 2.5-mm thickness of lead will decrease the external radiation exposure by a factor of 1,000.

Table 2. Radiation attenuation by lead shielding

Lead Shield Thickness (cm)	Coefficient of Attenuation
0.017	0.5
0.08	0.1
0.16	0.01
0.25	0.001
0.33	0.0001

To correct for physical decay of this radionuclide, the fractions that remain at selected time intervals after the time of calibration are shown in Table 3.

Table 3. Physical decay chart—technetium-99m half-life 6.02 hours

Hours	Fraction Remaining	Hours	Fraction Remaining
0*	1.00	7	0.45
1	0.89	8	0.40
2	0.79	9	0.36
3	0.71	10	0.32
4	0.63	11	0.28
5	0.56	12	0.25
6	0.50		

*Calibration Time

CLINICAL PHARMACOLOGY

General

Technetium Tc 99m Anti-CD15 Antibody radiolabels human white blood cells. *In vitro* human tissue cross-reactivity studies demonstrate that PMNs and monocytes are the only blood cellular components exhibiting the CD15 antigenic site.[2,3] Monocytes constitute approximately 5% of circulating leukocytes; therefore, most of the circulating blood cellular activity resides on PMNs. In blood cell fractions isolated from patients who had received Technetium Tc 99m Anti-CD15 Antibody, radioactivity was either on the PMNs or in plasma.[4] No significant radioactivity was associated with platelets, lymphocytes or red blood cells. In normal volunteers, radioactivity was associated with granulocytes (25%) or residing in plasma (72%) when measured one hour after injection. Anti-CD15 antibody has a very high affinity ($K_d = 1.6 \times 10^{-11}$ *M*) for the antigenic site on human PMNs and each human PMN has a high number (5.1×10^5) of binding sites per cell,[5] suggesting that the anti-CD15 binding to PMNs should be rapid and stable. In clinical diagnostic images, localization of Technetium Tc 99m Anti-CD15 Antibody at infection

sites was observed within minutes after injection, and localized radioactivity persists or intensifies over time.

Although human tissue cross-reactivity studies indicate the presence of CD15 antigenic sites on many human tissues, clinical diagnostic images do not exhibit high and widespread background radioactivity. There is essentially no background in the abdominal area over the primary imaging time interval (0–2 hours).

In normal volunteers, it was noted that the white blood cell count decreased transiently shortly after injection. This brief drop and rapid recovery were not clinically significant. *In vitro* experiments indicate that functional properties of human PMNs, including adherence, chemotaxis and phagocytosis, are not diminished when PMNs are exposed to 10 times the anti-CD15 antibody concentration expected to prevail in an adult after administration of a recommended clinical dose of Technetium Tc 99m Anti-CD15 Antibody (75–125 μg).[5]

Pharmacokinetics

In a study of 10 healthy volunteers, following intravenous injection of Technetium Tc 99m Anti-CD15 Antibody, blood concentrations of radioactivity decreased rapidly with an initial half-life of 0.3 hours and a second phase half-life of about eight hours. Whole-body scintigraphy at two hours post-injection in these healthy volunteers indicated that the organ with the highest uptake and retention was the liver, followed by the kidney, spleen and red marrow. At that time, liver contained 45–50% of the injected dose of radioactivity. Over the 26–33 hours after injection, 38% of the injected dose of radioactivity was recovered in urine.

CLINICAL STUDIES

In a multicenter, within-patient comparative clinical trial in 203 patients with equivocal signs and symptoms of appendicitis, the diagnostic performance of Technetium Tc 99m Anti-CD15 Antibody was compared to a final diagnosis based upon a surgical pathology report (in cases that proceeded to appendectomy) or upon two weeks of follow-up (in cases without surgical intervention). Disease prevalence in this study was 30%. Table 4 presents diagnostic results using planar imaging. Single-photon emission computed tomography (SPECT) was performed electively in nine of the 203 patients, and the results are not included in the table.

For investigators at the clinical sites, scintigraphy with Technetium Tc 99m Anti-CD15 Antibody had a sensitivity of 91% and a specificity of 86%. Accuracy was 87%. The positive predictive value and negative predictive value were 73% and 96%, respectively.

Based upon the aggregate evaluation by three independent blinded readers of the images presented in a randomized sequence, Technetium Tc 99m Anti-CD15 Antibody had a sensitivity of 75%, a specificity of 93% and an accuracy of 88% for a diagnosis of appendicitis. Blinded readers were provided with information regarding patient sex, age and body habitus, but not with any diagnostic information other than the scintigrams.

The positive predictive value and negative predictive value were 82% and 90%, respectively, for the aggregate blind read.

Table 4. Overall diagnostic outcome

Sensitivity			
	$N_{(+)}$	TP	Percent
Investigators	54	49	91
Blinded Reader 1	59	48	81
Blinded Reader 2	59	39	66
Blinded Reader 3	59	45	76
Blinded Aggregate	59	44	75

Specificity			
	$N_{(-)}$	TN	Percent
Investigators	128	110	86
Blinded Reader 1	141	124	88
Blinded Reader 2	141	127	90
Blinded Reader 3	141	133	94
Blinded Aggregate	141	131	93
Accuracy			
	N_T	TP + TN	Percent
Investigators	182	159	87
Blinded Reader 1	200	172	86
Blinded Reader 2	200	166	83
Blinded Reader 3	200	178	89
Blinded Aggregate	200	175	88

Values represent number of cases with true-positive (TP) or true-negative (TN) findings in the indicated numbers of patients with equivocal signs and symptoms of appendicitis subsequently found by pathology or follow-up to have the disease ($N_{(+)}$) or not to have the disease ($N_{(-)}$).
TP = true-positive cases TN = true-negative cases. FP = false-positive cases. N_T = total cases FN = false-negative cases.

The contents of LeuTech™ are intended only for use in the preparation of Technetium Tc 99m Anti-CD15 Antibody Injection and are not to be administered directly to the patient. Before preparation, the contents of the vial and ampul are not radioactive; however, after sodium pertechnetate Tc 99m injection is added, adequate shielding of the preparation must be maintained. Sodium Pertechnetate Tc 99m Injection that contains oxidizing agents is not suitable for preparation of Technetium Tc 99m Anti-CD15 Antibody Injection

Technetium Tc 99m Anti-CD15 Antibody Injection, like other radioactive medical products, must be handled with care and appropriate safety measures should be used to minimize radiation exposure to clinical personnel. Care should also be taken to minimize radiation exposure to the patient consistent with proper patient management.

Radiopharmaceuticals should be used by or under the control of physicians who are qualified by specific training and experience in the safe use and handling of radionuclides, and whose experience and training have been approved by the appropriate governmental agency authorized to license the use of radionuclides.

Information for patients

Murine monoclonal antibodies are foreign proteins and their administration can induce human antimouse antibodies (HAMA). To minimize radiation absorbed dose to the bladder, adequate hydration should be encouraged to permit frequent voiding during the first few hours after injection.

Human Antimouse Antibody (HAMA) Formation

HAMA development was evaluated in 50 patients and normal volunteers for up to 3–4 months after a single exposure to Anti-CD15 Antibody. HAMA formation was not detected in any of these subjects. In one patient, an existing (pretreatment) level of HAMA was not exacerbated by exposure to a single dose of Technetium Tc 99m Anti-CD15 Antibody; thus, in no patient did a single exposure to Technetium Tc 99m Anti-CD15 Antibody elicit new or additional HAMA. Multiple-exposure studies have not been conducted with Technetium Tc 99m Anti-CD15 Antibody in humans or in laboratory animals. While limited data exist concerning the clinical significance of HAMA, their presence may interfere with murine antibody based immunoassays, compromise the efficacy of diagnostic or therapeutic murine antibody-based agents, and increase the risk of adverse reactions. For these reasons, patients should be informed that the use of this product could affect the future use of other murine based products, and should be advised to discuss prior use of murine antibody based products.

Drug Interaction

Drug interactions were not noted in clinical studies in which Technetium Tc 99m Anti-CD15 Antibody was administered to patients receiving concomitant medication. Specific studies on drug interaction with Technetium Tc 99m Anti-CD15 Antibody have not been conducted. Patients in Phase 2 and Phase 3 clinical trials have received one or more of the following concomitant medications: opioid analgesic drugs (20%), other analgesic and antipyretic drugs (18%), antibiotic drugs (14%), psychotherapeutic drugs (14%) and

nonsteroidal anti-inflammatory drugs (NSAIDs, 11%), all commonly used in patients suspected to have appendicitis.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies have not been conducted to evaluate carcinogenic potential or effects on fertility.

Pregnancy

Pregnancy Category C. Animal reproduction studies have not been conducted with Technetium Tc 99m Anti-CD15 Antibody. It is also not known whether Technetium Tc 99m Anti-CD15 Antibody can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Technetium Tc 99m Anti-CD15 Antibody should be given to a pregnant woman only if clearly needed.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Technetium Tc 99m Anti-CD15 Antibody is administered to a nursing woman. Wherever possible, infant formula should be substituted for breast milk until the radioactivity has cleared from the body of the nursing woman.

Pediatric Use

In sponsored clinical studies of LeuTech™, 5.4 percent of subjects were 5–9 years old and 17.7 percent were 10–17 years old. No overall differences in safety or effectiveness were observed between these patients and subjects in other age brackets. Safety and effectiveness have not been established for patients under five years of age. See DOSAGE AND ADMINISTRATION for use in children five years old and over.

Geriatric Use

Of the 277 subjects in sponsored clinical studies of LeuTech™, 5.8 percent were 65 and over, while 1.4 percent were 75 and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients.

ADVERSE REACTIONS

Adverse events were evaluated in clinical studies of 277 patients and normal volunteers (114 male and 163 female) with a mean age of 31 years (5 to 85 years). The subjects received a mean antibody dose of 1.9 μg/kg and a mean radioactive dose of 0.23 mCi/kg.

No serious adverse events occurred after LeuTech™ administration.

Overall adverse events occurred in 21 of 277 (7.6%) patients and normal volunteers.

The most frequently reported adverse reactions to LeuTech™ were vasodilation, which was noted in 3.6% of patients and normal volunteers, and dyspnea noted in 1.4%. The

following events were less frequent and noted in less than 1% of patients:

Body as a Whole:	Headache, Asthenia, Injection Site Pain, Injection Site Reaction, Pain, Chest Pain
Cardiovascular:	Syncope
Digestive:	Diarrhea
Nervous:	Dizziness, Paresthesia
Respiratory:	Rhinitis

In some patients at two hours following administration of the agent, there were clinically insignificant decreases in white blood cell count, neutrophil relative differential count, hemoglobin, total protein or blood urea nitrogen. These changes were not associated with any clinical symptoms.

DRUG ABUSE AND DEPENDENCE

There is no indication that LeuTech™ has potential for drug abuse or dependence.

OVERDOSAGE

There is no experience with overdosage in clinical trials. The recommended clinical dose is 75–125 μg, and single doses higher than 250 μg have not been tested. The no-observable-effect level in mice or rabbits was at least 500 μg/kg intravenously.

DOSAGE AND ADMINISTRATION

Adults

For imaging, Technetium Tc 99m Anti-CD15 Antibody Injection is administered in a single dose with 10 to 20 mCi (370 to 740 MBq) of technetium-99m, corresponding to 75 to 125 μg of Anti-CD15 Antibody. Imaging may begin immediately following administration of the agent. Imaging of the patient with Technetium Tc 99m Anti-CD15 Antibody is by planar gamma scintigraphy. Planar imaging should be performed using a large field of view camera fitted with a low-energy, parallel-hole, high-resolution collimator. The camera should be positioned so that the lower edge of the liver is at the upper end of the field of view at the midline of the patient.

Dynamic image acquisition should begin at the time of injection over the lower abdomen and consist of 10 sequential four-minute images. Radioactivity excreted in urine flowing down a ureter may be distinguished from appendicitis by motion of the localized radioactivity, especially in a cine display. Following dynamic image acquisition, the patient should ambulate for approximately 10 to 15 minutes and void. Static planar images should then be collected, including supine anterior, posterior, 10–25 degree RAO and LAO views of the lower abdomen, followed by a standing anterior image of the lower abdomen. It is recommended that a total of one million counts be collected for the anterior supine image. All remaining images should be collected for the same duration of time required for the anterior supine image.

A positive finding is characterized by asymmetric uptake (a "hot spot") in the right lower abdominal quadrant that typically remains constant or increases in intensity with time. In the pivotal clinical trial, 46% of the true-positive cases of appendicitis displayed uptake of activity in the appendix within 5 minutes after injection, and more than 90% within one hour after injection. A patient showing a persistent or intensifying hot spot in the appendix zone before completion of the imaging sequence may be considered positive, and imaging may be terminated at that time.

LeuTech™ is not intended for direct administration to the patient without reconstitution and labeling with Sodium Pertechnetate Tc 99m Injection. Technetium Tc 99m Anti-CD15 Antibody is intended for a single intravenous administration through an intravenous access that has been demonstrated to be patent, e.g., butterfly, running IV line, or equivalent injection system to assure that no dose infiltration occurs. The injection line should be flushed with saline after the injection to assure administration of the total dose.

Children (five years old and over)

Technetium Tc 99m Anti-CD15 Antibody is administered in a single dose of 0.21 mCi/kg to a maximum of 20 mCi in total. Recommended imaging times and procedures are the same as for adults.

Dose adjustment has not been established in patients with renal insufficiency, in geriatric patients or in pediatric patients under five years of age.

Radiation Dosimetry

Based on human data, the absorbed radiation dose to an average human adult (70 kg) from an intravenous injection of the agent is listed in Table 5. The values are listed as rad/mCi and mGy/MBq and assume urinary bladder emptying at 4.8 hours. Radiation absorbed dose estimates for children are given in Table 6.

Table 5. Estimated absorbed radiation dose in adults

Target Organ	rad/mCi	mGy/MBq
Spleen	0.23	0.062
Kidneys	0.19	0.051
Liver	0.18	0.048
Urinary Bladder Wall	0.12	0.032
Heart	0.061	0.017
Gallbladder	0.056	0.015
Upper Large Intestine Wall	0.051	0.014
Adrenal Glands	0.044	0.012
Lungs	0.043	0.012
Thyroid Gland	0.042	0.011
Red Marrow	0.038	0.010
Lower Large Intestine Wall	0.034	0.0091
Bone Surface	0.031	0.0083
Brain	0.0052	0.0014
Testes / Ovaries	0.0039 / 0.019	0.0010 / 0.0052
Total Body	0.019	0.0050

Dose calculations were performed using the standard MIRD method (MIRD Pamphlet No. 1 rev., Soc. Nucl. Med., 1976). Effective dose equivalent was calculated in accordance with ICRP 53 (Ann. ICRP 18, 1-4, 1988) and gave a value of 0.018 mSv/MBq (0.068 rem/mCi).

Table 6. Estimated absorbed radiation dose for a five-year old child

Target Organ	rad/mCi	mGy/MBq
Spleen	0.70	0.19
Kidneys	0.43	0.11
Liver	0.41	0.11
Urinary Bladder Wall	0.27	0.072
Upper Large Intestine Wall	0.21	0.056
Thyroid Gland	0.19	0.052
Lower Large Intestine Wall	0.16	0.042
Heart	0.15	0.041
Gallbladder	0.13	0.036
Red Marrow	0.11	0.030
Lungs	0.11	0.028
Adrenal Glands	0.095	0.026
Bone Surface	0.085	0.023
Testes / Ovaries	0.019 / 0.059	0.0052 / 0.016
Brain	0.0075	0.0020
Total Body	0.049	0.013

Dose calculations were performed using the standard MIRD method based upon biodistribution studies conducted in adults. Effective dose equivalent was calculated in accordance with ICRP 53 and gave a value of 0.047 mSv/MBq (0.17 rem/mCi).

Instructions for the preparation of Technetium Tc 99m Anti-CD15 Antibody Injection

All transfers and needle penetrations of the vial stopper must use aseptic technique.

Wear waterproof gloves during the entire procedure and while withdrawing the patient dose from the LeuTech™ reaction vial.

Transfer Sodium Pertechnetate Tc 99m Injection with an adequately shielded, sterile syringe.

Adequate shielding should be maintained at all times until the preparation is administered to the patient, disposed of in an approved manner, or allowed to decay to background levels. A shielded, sterile syringe should be used to withdraw and inject the labeled preparation.

Examine the vial contents for particulates and discoloration prior to injection. The material should not be used if particulates or discoloration are observed.

1. Remove a LeuTech™ kit from refrigerated storage (2 to 8° C) and allow it to come to room temperature.

2. Swab the rubber stopper of the LeuTech™ reaction vial with an appropriate antiseptic and allow the stopper to dry.

3. Without the addition of air, aseptically add 20 to 40 mCi (740 to 1480 MBq) Sodium Pertechnetate Tc 99m Injection in 0.20 to 0.35 mL generator eluate. If Sodium Pertechnetate Tc 99m Injection must be diluted prior to kit reconstitution, only Sodium Chloride Injection (without preservatives) should be used. Gently swirl the vial until the lyophilized product is completely dissolved.

   Note: The amount of Sodium Pertechnetate Tc 99m Injection used to reconstitute the vial should be determined based on the desired radioactive dose and the estimated injection time.

4. Allow the reconstituted vial to stand at 37° C for 30 minutes. (Shorter incubation times may result in inadequate labeling.)

5. Aseptically add sufficient Ascorbic Acid Injection, USP (500 mg/mL) to make the final preparation volume up to 1.0 mL.

Note: Further dilution is not recommended.

6. Assay the product in a suitable calibrator and record the time, date of preparation and the activity of technetium Tc 99m Anti-CD15 Antibody onto the radioassay information label.

7. Each patient should receive a dose of 0.3 to 0.5 mL (75 to 125 μg antibody) of the reconstituted product after dilution to a final volume of 1.0 mL.

Storage

The lyophilized LeuTech™ kits should be stored at 2 to 8° C. After labeling with Sodium Pertechnetate Tc 99m Injection and addition of Ascorbic Acid Injection, the vial should be kept at room temperature (15 to 25° C) and used within six hours.

HOW SUPPLIED

Five single-use kits and one package insert are included in each package. Each single-use kit includes one reagent vial and one ampul of Ascorbic Acid Injection, 500 mg/mL, USP. The reagent vial contains a sterile, nonpyrogenic, lyophilized mixture of 0.25 mg Anti-CD15 Antibody, 12.5 mg maltose monohydrate, 0.221 mg succinic acid, 0.522 mg sodium potassium tartrate tetrahydrate, USP, 28 μg glycine, USP, 9.3 μg disodium edetate dihydrate and 54 μg stannous tartrate.

NDC XXXX-XXXX-XX

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1. The CD15 antigen is also known as Stage-specific Embryonic Antigen (SSEA-1). Much of the literature refers to the anti-CD15 antibody as anti-SSEA-1.
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