VIII. CLINICAL DATA SUMMARY AND RESULTS OF STATISTICAL ANALYSES

A. CLINICAL PHARMACOLOGY STUDIES

1. Tabular Presentation of Clinical Pharmacology Studies

The clinical pharmacology of intravenous AF0150 was assessed in three Alliance-sponsored studies which included a Phase 1 ADME study (IMUS-012-USA), a Phase 1 safety and dose-tolerance study (IMUS-001-USA), and a dose-ranging study (IMUS-018-USA). A listing of these studies, including design features, is included in **Table 43.**

Table 43 Clinical Pharmacology Studies

Protocol #, Investigators, Publications	Completion Status (Starting Date)	Location Product Fill Size	Full Report Data Listings	CRFs Included*	Study Design Study Population	Treatment Doses	Number Entered Each Treatment	Age Range (Mean)	% M/F C/B/A/O	Duration of Drug Treatment
ADME Study										
IMUS-012-USA	Complete (12 May 99)	USA 200 mg	Vol. 083 pg. 002	none	Open-label, single-dose pharmacokinetic study in normal adult volunteers	**AF0150** Bolus: 4.0 mg/kg	**13**	23-55 (35)	54/46 31/54/0/15	~40 sec (25 mL/min)
Early Dose-Tolerance Study										
IMUS-001-USA	Complete (27 Mar 96)	USA 200 mg	Vol. 086 pg. 088	none	Single-blind, dose-ranging, placebo-controlled study in normal adult volunteers	**AF0150** Bolus:	**44**	18-45 (26)	44/56 98/2/0/0	~10 sec bolus ~10 min inf
						0.125 mg/kg	12			
						0.5 mg/kg	12			
						2.0 mg/kg	4			
						4.0 mg/kg	12			
						Infusion: 4.0 mg/kg	4			
						Placebo Bolus: 0.2 mL 0.9% NaCl/kg	**20**			
Dose-Ranging Study										
IMUS-018-USA	Complete (11 Aug 98)	US 200 mg	Vol. 089 pg. 146	none	Open-label, dose titration study in subjects with left ventricular dysfunction	**AF0150** Bolus: 0.125, 0.25, and 0.5 mg/kg	**18**	44-87 (67)	72/28 72/11/0/17	~10 sec/dose with a 10-min interval between doses

*Case report forms (CRFs) are provided only for subjects with serious AEs, those withdrawn from the study due to AEs, or for subjects who died.
C/B/A/O: Caucasian/Black/Asian/Other
inf: infusion

2. Summary of the Design and Results of Each Study

a. *IMUS-012-USA: An Open-Label, Single-Dose Study to Assess the Pharmacokinetic Parameters and Rate of Elimination of Perfluorohexane After a 4-mg/kg Bolus Intravenous Injection of AF0150 in Healthy Adult Volunteers*

Objectives: This study was designed to evaluate the pharmacokinetic parameters, blood and pulmonary clearance, of PFH after bolus intravenous injection of 4 mg/kg AF0150 in healthy adult volunteers.

Study Design/Population: IMUS-012-USA was an open-label, Phase 1 study conducted at one site in the United States. Thirteen male and female, healthy, nonsmoking, adult volunteers ranging in age from 23 to 55 years (mean age, 35 years) were enrolled in the study from May 12, 1999 to June 20, 1999. The mean weight of the subjects was 75.1 ± 15.8 kg (range, 49 to 96 kg) and the mean height was 172 ± 8.6 cm (range, 160 to 188 cm). All 13 subjects enrolled received a bolus intravenous injection of 4 mg constituted powder/kg AF0150. Subjects served as their own control for safety data comparison pre- and posttreatment.

Methodology: The study was conducted in two phases, a *pilot phase* that included 2 subjects and a *pivotal phase* that included 11 subjects. The *pilot phase* was conducted to check the logistics of the study procedures and to verify all aspects of collection and analysis. The *pivotal phase* began after the data and procedures of the *pilot phase* had been evaluated. Per a protocol amendment dated June 14, 1999, three subjects (Subjects 109, 110, 112) were re-enrolled in the study and repeated the dosing process. An additional female subject (Subject 113), who had not been previously exposed to AF0150, was also enrolled. This amendment was implemented to rectify an apparent technical difficulty that occurred on the day Subjects 110, 111, and 112 were dosed. Technical problems were suspected because all subjects on this day exhibited PFH expiration profiles that were unexpected and markedly different from the profiles exhibited by the first 9 (7 males and 2 females) subjects enrolled. Subject 111 did not return for a second dosing. Since data from the first dosing were considered suspect, summary tables are presented excluding Subject 111.

Each subject received 4 mg/kg AF0150 (0.2 mL/kg) at a rate not to exceed 25 mL/min. A saline flush of 30 mL was administered immediately following completion of the bolus injection of AF0150. Expired air and blood samples were collected at baseline (predosing) and at designated times after dosing for determination of PFH levels using a validated [redacted] assay (see **Section 6.I.E** for a description of analytical procedures). Safety was assessed through 24 hours postdosing and was based on evaluation of adverse events (AEs), clinical laboratory tests (hematology, blood chemistry, and urinalysis),

vital signs, oxyhemoglobin saturation (SaO_2), 12-lead electrocardiograms (ECGs), neurologic evaluations including cranial nerve examinations and mental status testing, and continuous cardiac monitoring.

AF0150 was supplied in a 200-mg vial and was prepared by constituting with 10 mL SWFI to a final concentration of 20 mg/mL.

Pharmacokinetic Analysis: PFH's model-independent pharmacokinetic variables (AUC_{0-24}, $AUC_{0-\infty}$, T_{max}, C_{max}, $t_{½}$, MRT_{last}, Cl_{sys}, Cl_{lung}, $\%PFH_{0\rightarrow 3hr}$ and $\%PFH_{0-\infty}$) were calculated using WinNonlin® Version 2.1 Professional.

Pharmacokinetic Results: The blood and expired air pharmacokinetics of PFH were analyzed as specified in the protocol and the results are tabulated below in **Table 44**:

Data are presented for 12 subjects, excluding Subject 111, who experienced technical difficulties on the day of dosing; the data for this subject are assumed to be compromised.

PFH was readily distributed to the lung following the AF0150 intravenous administration. The decline of PFH in both blood and expired air appears to follow first-order kinetics. Approximately 75% of PFH was eliminated through expired air within 3 hours after AF0150 administration, and approximately 87% was eliminated within 24 hours. A mean MRT_{last} value of 2.7 hours in blood was indicative of the rapid elimination of the drug from blood. There were no statistical differences between male and female subjects in the rate and extent of PFH exposure in blood as well as systemic and lung clearances. There were statistically significant differences in the extent of exposure and terminal half-life of PFH from expired air between the genders; however, the impact of these differences may not be clinically significant.

Table 44 Mean ± SD and Coefficient of Variance (%) of PFH in Blood and Air Pharmacokinetic Parameters*

	Blood	Air
AUC_{0-24hr} (ng*hr/mL)	3.7 ± 1.4 (38.9)	3.3 ± 0.7 (21.2)
$AUC_{0-\infty}$ (ng*hr/mL)	4.2 ± 2.2 (52.4)	3.4 ± 1.4 (20.9)
T_{max}† (min)	2.0	1.5
C_{max} (ng/mL)	28.0 ± 28.6 (102.2)	27.8 ± 8.3 (29.7)
$t_{½}$ (hrs)	5.3 ± 6.1 (114.7)	9.0 ± 5.0 (55.1)
MRT_{last} (hrs)	2.7 ± 3.6 (133.5)	1.6 ± 0.5 (32.3)
Cl (L/hr)	716.3 ± 735.3 (102.6)	603.7 ± 93.9 (15.6)
%PFH (0-3hr)	N/A	74.6 ± 17.6 (23.6)
%PFH (0-∞)	N/A	87.2 ± 19.3 (22.1)

* AUC = Area under the time-concentration curve T_{max} = Time of maximal concentration
C_{max} = Maximal concentration $t_{1/2}$ = Half life MRT = Mean residence time
Cl = Clearance
† T_{max} = Median T_{max}; all other data are mean ± SD.
N/A = not applicable.
Data reference: Section 11.4.2, Tables 11.4.2.1:2 and 11.4.2.2:2 in the IMUS-012-USA clinical study report. (A copy of the report is provided in **Section 6.IV.**)

Safety Results: There were no deaths or serious AEs associated with this study. Treatment-emergent AEs were reported by 2 (15.4%) of the 13 subjects enrolled. The reported AEs were one incidence each of headache and nasal pain. All AEs were mild, transient, and considered to be unrelated to the study medication. No subject required treatment with concomitant medication. There were no clinically significant abnormalities reported in clinical laboratory tests (hematology, blood chemistry, and urinalysis), vital signs, SaO_2, 12-lead ECGs, neurologic evaluations including cranial nerve examinations and mental status testing, and continuous cardiac monitoring.

Conclusions: AF0150 intravenous administration of 4 mg/kg was safe and well tolerated by adult volunteers participating in this study. PFH was readily distributed into expired air following the AF0150 intravenous administration. PFH was primarily eliminated via expired air with an MRT in air of 1.6 hours and in blood of 2.7 hours. Approximately 75% of PFH was eliminated through expired air within 3 hours after dosing and 87% within 24 hours. Gender differences observed in terminal elimination are not likely to be of clinical significance.

The full IMUS-012-USA clinical study report is provided in **Section 8.XV**.

b. IMUS-001-USA: A Single-Blind, Dose-Ranging, Placebo-Controlled, Safety, and Contrast Enhancement Study in Normal Volunteers Receiving AF0150 Administered by Intravenous Injection

Objectives: The primary objective of this study was to investigate the safety of intravenously administered AF0150 at 4 dose levels in normal volunteers. In addition, visual clearance of AF0150 was to be assessed on two-dimensional (2-D) gray-scale ultrasound in a subset of subjects. Potential efficacy of AF0150 was to be assessed by measuring the extent and duration of contrast enhancement on 2-D gray-scale and color Doppler ultrasound images of various vascular and anatomic structures.

Study Design/Population: IMUS-001-USA was a single-blind, placebo-controlled, dose-ranging, Phase 1 study conducted at a single center in the United States. Sixty-four male and female normal volunteers ranging in age from 18 to 45 years (mean age, 26.0 years) were enrolled in the study from March 27, 1996 to May 29, 1996. Assessment of the subject's health condition was based on medical history, laboratory assessments, and physical examination. Forty-four subjects received AF0150 and 20 subjects received placebo (0.9% sodium chloride) by intravenous injection.

Methodology: The study was conducted in three separate stages with regard to imaging specifications. Subjects enrolled in *Stage 1* of the protocol underwent ultrasound evaluations of visual clearance of AF0150. In *Stages 2* and *3*, contrast imaging of cardiac and abdominal regions, respectively, was assessed.

In *Stage 1*, subjects (n=24) were randomized to receive a bolus injection of either AF0150 at 1 of 4 dose levels (0.125, 0.5, 2.0, or 4.0 mg/kg) or placebo (saline; 0.2 mL/kg) over approximately 10 seconds. Subjects were randomized into each AF0150 dose level based on an escalating-dose design. Dosing progressed to each subsequent level following review of available safety data through Day 3 of the study. Within each AF0150 dose level, subjects were randomly assigned to receive either AF0150 (n=4) or placebo (n=2).

Stages 2 and *3* were identical in dosing and randomization scheme. Subjects (n=18/stage) were randomized to receive a bolus injection of either AF0150 at 1 of 3 dose levels (0.125, 0.5, or 4.0 mg/kg; n=4/dose) or placebo (0.2 mL/kg; n=2/dose) over approximately 10 seconds. After completion of dosing in these subjects, 2 additional subjects were enrolled in each stage to receive a 4.0-mg/kg dose of AF0150 as an infusion over a period of approximately 10 minutes. Subjects who received 4.0 mg/kg AF0150 as an infusion were not randomized. AF0150 was supplied in a 200-mg vial and was prepared by

constituting with 10 mL SWFI to a final concentration of 20 mg/mL. Placebo used was commercially available sodium chloride injection, USP.

Safety was assessed through Day 7 and was based on evaluation of AEs, vital signs, clinical laboratory tests (hematology, coagulation, blood chemistry, and urinalysis), SaO_2 by pulse oximetry, respiratory status (respiratory rate and expired carbon dioxide [CO_2] levels), and ECG changes using both 12-lead and ambulatory monitoring (Holter monitor). In addition, complement (C3, C3a, C4, and CH_{50}) activation and TNF-α release were measured as markers of inflammatory response.

Efficacy Results: This Phase 1 study was conducted to assess primarily safety and preliminary efficacy of AF0150. The preliminary qualitative assessment of efficacy from the IMUS-001-USA study (discussed at the End-of-Phase 2 Meeting) indicated that visual opacification of LV was observed at all doses (0.125 to 4.0 mg/kg). The preliminary assessment from IMUS-001-USA also led Alliance to conclude that for cardiac function, the lowest dose used in the trial, 0.125 mg/kg, would provide good imaging views with a reasonable duration of imaging time following a minimal period of attenuation. The data were not formally analyzed in the clinical study report and contrary to protocol plan, videodensity measurements were not reported. The study was conducted in normal volunteers and, therefore, the images obtained were not considered representative of the images to be expected in the target patient population (i.e., patients with suboptimal echocardiograms).

Safety Results: Ten (23%) of 44 AF0150-treated and 6 (30%) of 20 placebo-treated subjects experienced at least one AE. All AEs reported in AF0150-treated subjects were considered mild to moderate in intensity and nonserious by the investigator. Three events (i.e., taste perversion [0.125-mg/kg bolus], and dizziness, and nausea [4.0-mg/kg infusion]) were assessed as possibly related to AF0150. Only 2 AF0150-treated subjects required treatment for their AEs. These events, fever and headache, were each reported in 1 subject and were treated with acetaminophen or ibuprofen.

The most commonly reported AE was headache, which was noted in 4 (9%) of 44 AF0150-treated subjects and 1 (5%) of 20 placebo-treated subjects. The only other AE that was reported in more than one subject was taste perversion. This was noted in 1 (2%) of 44 AF0150-treated subjects and in 2 (10%) of 20 placebo-treated subjects. The remaining AEs were reported in 1 subject each and included hiccup, fever, conjunctivitis, vasodilatation, pain, dizziness, and nausea for AF0150-treated subjects and parosmia, postural hypotension, diarrhea, and dry skin for placebo-treated subjects.

No clinically significant changes in values were observed in standard tests for hematology, coagulation, blood chemistry, complement (C3, C3a, C4, and CH_{50}) levels, and urinalysis. There was also no evidence of systemic release

of TNF-α. Furthermore, AF0150 was not associated with clinically significant changes in vital signs, respiratory function, SaO_2, or ECG findings.

Conclusions: AF0150 was well tolerated at all the doses used in this study. The AEs observed were predominantly mild in intensity and considered unrelated to the study drug. In addition, all the events were transient and most resolved without treatment. No trends or clinically significant changes in clinical laboratory measurements, vital signs, respiratory function, SaO_2, and ECG findings were observed during the study.

On the basis of these data, it can be concluded that AF0150, administered intravenously at doses up to 4.0 mg/kg, either as a bolus or infusion, was well tolerated in this sample population of male and female normal volunteers.

The full IMUS-001-USA clinical study report is provided in **Section 8.XV**.

c. ***IMUS-018-USA: An Open-Label Dose-Titration Study of 3 Doses of AF0150 in the Echocardiographic Assessment of Patients with Left Ventricular Dysfunction***

Objectives: The objectives of this study were to evaluate the efficacy and safety of three doses of AF0150. The primary objective was to assess the ability of AF0150 to opacify the LV cavity in fundamental continuous and gated imaging modes. Secondary objectives included assessment of the duration of attenuation and the duration of useful contrast enhancement of the LV using fundamental continuous imaging, and assessment of safety.

Study Design/Population: IMUS-018-USA was an open-label, dose-ranging, Phase 2 study. The study was conducted at two sites in the United States. Eighteen male and female subjects ranging in age from 44 to 87 years (mean age, 67.3 years) with LV dysfunction (i.e., EF of 20% to 40%) were enrolled in the study from August 11, 1998 to October 15, 1998. All 18 subjects enrolled received AF0150.

Methodology: Each subject received three intravenous bolus doses of AF0150 in the following sequence: 0.125, 0.25, and 0.5 mg/kg. Each bolus dose was injected over approximately 10 seconds with a 10-minute interval between each dose, and following the last dose, during which resting 2-D contrast echocardiography and safety assessments were performed.

Efficacy was compared among doses and was assessed based on quantitative (videodensitometry) and qualitative (blinded review) data. Videodensitometry was conducted for LV opacification and duration of useful contrast enhancement. The reviewer was blinded to subject and dose. Safety was assessed up to 24 hours after administration of the initial dose and was based on evaluation of AEs, clinical laboratory tests (hematology, coagulation, blood chemistry, and urinalysis parameters), vital signs, and ECGs. Subjects

served as their own control for safety data comparison pre- and posttreatment. AF0150 was supplied in vials containing 200 mg and was prepared by constituting with 10 mL SWFI to a final concentration of 20 mg/mL.

Statistical Analysis: Differences among doses were tested using mixed effects analysis of variance (ANOVA). A significance level of 0.01 was used to define statistical significance.

Efficacy Results: LV opacification: Analysis of quantitative LV opacification data across all views demonstrated that LV opacification increased significantly ($P \leq 0.0078$) with increasing AF0150 dose. In addition, when the effect of dose was analyzed for each view, a statistically significant increase in LV opacification was observed from the 0.125- to 0.5-mg/kg dose groups for the apical 2-chamber view ($P=0.0007$) and apical long-axis view ($P=0.0003$).

Mean qualitative data showed that, for continuous and gated imaging modes, mean LV opacification was between moderate (score of 2) and complete (score of 3) for all doses and views and the mean score was already in the range of approximately 2.4 to 2.7 across views for the low dose (0.125 mg/kg) out of a possible maximum score of 3.0. Mean LV opacification increased with increasing AF0150 dose (with the exception of the apical 4-chamber view in continuous mode), however, mean values did not increase considerably at doses above 0.125 mg/kg. Additionally, analysis of qualitative data by dose across all views showed no statistically significant increases in LV opacification in continuous mode and a statistically significant increase in LV opacification only from the low to mid (0.125- to 0.25-mg/kg; $P=0.0033$) and low to high (0.125- to 0.5-mg/kg; $P=0.0001$) dose groups in gated mode.

Duration of attenuation: A statistically significant ($P=0.0001$) dose-dependent increase in duration of attenuation was observed with each increasing dose level. Mean duration of attenuation was approximately 0.4, 1.0, and 1.6 minutes in the 0.125-, 0.25-, and 0.5-mg/kg dose groups, respectively.

Duration of useful contrast enhancement: Mean duration of useful contrast enhancement increased in a dose-dependent manner. Mean duration of useful contrast enhancement was approximately 1.3, 2.5, and 2.8 minutes in the 0.125, 0.25, and 0.5-mg/kg dose groups, respectively, when assessed quantitatively, and approximately 1.2, 2.2, and 2.9 minutes in the 0.125, 0.25, and 0.5-mg/kg dose groups, respectively, when assessed qualitatively. This increase was statistically significant from the 0.125- to 0.25-mg/kg ($P=0.0013$) and the 0.125- to 0.5-mg/kg ($P=0.0001$) dose groups for the qualitative assessment only. These findings suggest that it may be beneficial to use a higher AF0150 dose for patients with EF $\leq 40\%$ if a longer duration for contrast imaging is needed.

Safety Results: AF0150 was well tolerated in this study with no serious or severe AEs reported. AEs were reported in 2 (11%) of 18 subjects and consisted of one moderate event each of hypokalemia and increased lactic dehydrogenase. Both of these events were considered by the investigator as possibly/probably related to study drug and resolved without treatment. No trends or clinically significant changes in other clinical laboratory measurements, including results of standard tests of hematology, coagulation, blood chemistry, and urinalysis were observed during the study. Furthermore, AF0150 was not associated with clinically significant changes in vital signs and ECGs.

Conclusions: These results show that intravenous administration of AF0150, at sequential doses of 0.125, 0.25, and 0.5 mg/kg, was well tolerated in this study. AF0150 at a dose of 0.125 mg/kg sufficiently opacified the LV cavity in fundamental continuous and gated imaging modes in patients with LV dysfunction (i.e., EF 20% to 40%) and little improvement in opacification was derived from using higher AF0150 doses (i.e., 0.25 and 0.5 mg/kg). Additionally, a dose-dependent increase in both duration of attenuation and duration of useful contrast enhancement was observed with increasing AF0150 dose.

The full IMUS-018-USA clinical study report is provided in **Section 8.XV**.

3. Conclusions from Clinical Pharmacology Studies

The results of the three clinical pharmacology studies, two in healthy volunteers and one in patients with LV dysfunction (i.e., low EF), supported the selection of an AF0105 dose of 0.125 mg/kg for full evaluation of safety and efficacy in Phase 3 studies. In addition, the results of pharmacokinetic evaluations of AF0150 demonstrated a profile in humans similar to that observed in animals, with an early clearance of PFH via expired air. MRT was 1.6 hours in air and 2.7 hours in blood; approximately 75% of the PFH was eliminated within 3 hours.

B. Overview of Clinical Studies

The initial IND, submitted on February 16, 1996, outlined the Phase 1 and Phase 2 clinical development plans for AF0150 as intravenous ultrasound agent with echocardiographic and radiologic applications.

The results from the Phase 1 and Phase 2 programs were discussed with FDA at the End of Phase 2 Meeting on November 13, 1997. The objectives of the meeting were to present the results and conclusions from the Phase 1 and Phase 2 studies, to update the clinical development plan for all indications, and to reach agreement with FDA on the design of the Phase 3 studies to support a cardiac function indication.

The early safety and preliminary efficacy results from the IMUS-001-USA and IMUS-002-USA studies supported the continued development of AF0150 for the

improved assessment of endocardial border delineation (EBD) and cardiac function in patients with suboptimal echocardiograms. The clinical development plan for AF0150 as an intravenous ultrasound contrast agent was updated and presented to the FDA at the End of Phase 2 Meeting. Only the cardiac function program will be discussed in this section as it is the subject of the proposed indication for AF0150 in this NDA.

1. Highlights of Phase 3 Discussions

The IMUS-007-USA and IMUS-008-USA protocols were provided to FDA on December 9, 1997 (Serial Number 031) after the End of Phase 2 Meeting. A total of four telephone conferences were held with FDA prior to initiation of subject enrollment to discuss the development of the Phase 3 protocols. The telephone conferences were held on January 16, February 4, February 12, and March 4, 1998. The principal issues discussed were study endpoints, statistical analysis and methodology, blinded read, dosing, entry criteria, safety, and saline control. Based on discussions with FDA, the protocols for IMUS-007-USA and IMUS-008-USA were amended (Amendment 1) prior to initiation of subject enrollment and provided to FDA on March 19, 1998 (Serial Number 037). A summary of the principal issues discussed follows:

a. Study Endpoints

i. MULTIPLE ENDPOINTS

The study endpoints of EBD, EF, and SWM were considered achievable indications and, that depending on the trial design, it might be possible to obtain three indications.

The protocol endpoints were later clarified in Amendment 2 (submitted July 17, 1998, Serial Number 048) to reflect that the primary efficacy analyses for EBD and EF were to be based on the continuous imaging mode.

ii. ENDOCARDIAL BORDER DELINEATION

EBD scores were discussed and defined and resulted in following scoring system in **Table 45.**

Table 45. EBD Scoring System

Score Value	EBD Score Definitions
0	No delineation
1	Mild or fair delineation; inadequate to assess function
2	Moderate or good delineation; adequate to assess function
3	Excellent delineation; excellent demarcation of borders throughout the cardiac cycle
N	No view available for segment

iii. EJECTION FRACTION

The calculation of EF estimated by echocardiography was discussed. The modified Simpson's rule was considered the preferred method for calculation of EF.[74]

As described in the amended IMUS-007-USA and IMUS-008-USA protocols (Amendment 1) provided in Serial Number 037 (March 19, 1998), the number of EF classifications was expanded from four to six, and the descriptors (i.e., normal, mild, moderate, and severe) were eliminated because they did not provide clinically meaningful information for assessment of efficacy. The four EF classes originally chosen were based upon literature that suggested that the assessment of the extent of LV dysfunction could be classified by the system developed by Maseri.[75] Maseri classified LV EF into the following four classes:

Class 1	>50%
Class 2	40-49%
Class 3	25-39%
Class 4	<25%

Maseri's four classes were expanded to six based upon additional research[76] that indicated that patients surviving myocardial infarction could be risk-stratified using a 10% interval for EF. The six EF categories included:

>65%
55 - 65%
45 - 54%
35 - 44%
25 - 34%
<25 %

iv. SEGMENTAL WALL MOTION

Because the FDA and Alliance could not agree on a standard of evaluation, it was agreed that SWM would no longer be considered as a primary endpoint. During several discussions between FDA and Alliance, it was agreed that SWM would be a secondary endpoint.

b. Statistical Analysis Issues and Methodology

The Phase 3 statistical discussions focused on sample size and the appropriate statistical analyses and methods.

i. SAMPLE SIZE

FDA indicated that Alliance should be aware of the risk that the sample size may not be adequate because the dose and method of administration planned for the Phase 3 studies is different from the dose and method of administration evaluated in the Phase 2 study. Alliance acknowledged this to be an uncertainty that might jeopardize the efficacy evaluations in the Phase 3 studies.

ii. ENDPOINTS

Alliance agreed that the primary endpoints for the Phase 3 studies would be EBD and EF.

FDA and Alliance discussed specific cardiac medications that might affect the results of the EF determined by echocardiogram or RVG. Alliance agreed to carefully monitor the number of subjects that had a change in their cardiac medication during the study period and adjust the sample size as necessary. Adjustment of the sample size did not become necessary during the conduct of the Phase 3 studies.

c. Blinded Read

Alliance decided to provide for three independent blinded readers per study instead of two readers per study. The blinded read methodology also included an independent image selector for each Phase 3 study in addition to the blinded reviewers. An independent cardiologist with expertise in the interpretation of echocardiograms would pick the frames for the determination of EF instead of the blinded readers to ensure that each of the three readers evaluated the same frames. The image selector would review all baseline noncontrast and contrast views, and for each view, would select end-diastolic and end-systolic frames from a single cardiac cycle, which would be digitized and evaluated by the blinded reviewers. The selector would not be involved in any other aspect of the study, either as a site investigator, or as a blinded reader of any other study images (i.e., RVG).

FDA and Alliance discussed standardization of the images presented to the blinded reviewers. FDA proposed that imaging views be standardized (e.g., start all imaging at 30 seconds and then select the same time segment for all subjects). Alliance explained a number of variables that made this proposal difficult, such as individual patient responses to ultrasound and duration of contrast attenuation. Alliance provided a copy of the blinded read methodology to FDA in Amendment 1 (March 19, 1998, Serial Number 037).

d. Dosing

i. MULTIPLE DOSING

FDA and Alliance had several discussions concerning the addition of a second 0.125-mg/kg dose to the Phase 3 studies. Alliance proposed a second dose if the duration of contrast enhancement was not long enough to obtain the required images. After discussions of the complexities of analyses, FDA and Alliance concurred that the second dose should be eliminated from the study protocols.

ii. DOSE SELECTION

FDA reiterated that the dose selected for the Phase 3 studies, based on the normal population in the Phase 1 study, was an uncertainty that might jeopardize the efficacy results in the Phase 3 program. Alliance acknowledged the implications of using this dose. As a direct result, Alliance developed and conducted a dose-ranging study in subjects with cardiac dysfunction (EF between 20% and 40%) which would represent a cardiac population more severe than that studied in Phase 3. The results of this study (IMUS-018-USA) confirmed that the dose selected (i.e., 0.125 mg/kg) was appropriate. IMUS-018-USA is discussed in **Section 8.III**.

e. Entry Criteria

FDA requested clarification as to the entry criteria for determining cardiac disease in the Phase 3 studies. Alliance reiterated that all candidates had referral for an echocardiogram. Subjects were required to have suboptimal echocardiograms.

The time from suboptimal echocardiogram to study entry was specified in the protocols as 72 hours. If the echocardiogram was suboptimal at screening the subject was eligible for study entry. The baseline echocardiogram was performed within 1 hour of administration of study agent.

f. Safety

Additional safety assessments including vital signs and ECG monitoring at 15 and 30 minutes, 1 hour, and 24 hours postdosing were added. The protocols were also modified in Amendment 1 to include cardiac enzymes and ECG assessments if an AE of a cardiac nature occurred. The cardiac enzyme panel evaluation was specified as creatine kinase (CK) and CK-MB (the isoenzyme or MB fraction of CK).

g. Saline Control

FDA advised that, although it was better to have more comparator/placebo data, they would agree that 60 saline controls in one study (IMUS-007-USA) was sufficient if there were, in fact, very few AEs seen in the early studies with AF0150. Based on these discussions, the number of saline controls to be enrolled in IMUS-007-USA was increased from 60 to 80 subjects.

2. Pre-NDA Meeting

Based on the results from the Phase 3 program, a Pre-NDA Meeting was held on July 29, 1999. The objective of the meeting was to reach agreement with FDA on the format and content of the NDA to support the cardiac function indication. A brief summary of the principal issues discussed (subset analyses, microbubble size and total number of bubbles injected, and efficacy analyses) follows.

a. Subset analyses by:

- age (i.e., <65 years, 65 to 80 years, and >80 years,
- cardiac disease severity, and
- pulmonary disease severity.

b. Microbubble size and total number of bubbles injected

- include an upper limit of bubble size,
- include information regarding the relationship of bubble size and count to risk, and
- include dose by number of bubbles, bubbles/kg, and bubbles/body surface area (BSA) in summary tables.

In addition, FDA expressed concern about the potential for pulmonary microembolism with the microbubble contrast agents, as FDA does not know if these effects are product or class specific. FDA recommended that Alliance conduct a study in a nonclinical model of chronic pulmonary hypertension if such a model exists. Alliance also agreed to perform a microscopic, microvascular study in an appropriate animal model. Design of these nonclinical studies was not required for filing of the NDA and it was agreed that discussion of the design of these nonclinical studies could take place after filing.

c. Efficacy analyses

- include all Phase 3 efficacy data in the Integrated Summary of Effectiveness Data (ISE) for consideration in labeling, including all endpoints (primary and secondary) even those not achieved,
- include blinded reader methodology;

- analyze EF categories by the raw percent values (±5% was acceptable);
- justify the EF ranges;
- exclude patients with missing RVG data from the EF analysis; and
- include information on duration of contrast enhancement.

Alliance accepted all of FDA's recommendations and agreed to their incorporation into the NDA, with the exception of the additional nonclinical studies as discussed above.

C. CONTROLLED CLINICAL STUDIES

1. Tabular Presentation of Controlled Clinical Studies

Alliance-sponsored two well controlled Phase 3 clinical studies to evaluate AF0150 in the echocardiographic assessment of left ventricular function in patients with suboptimal noncontrast images. IMUS-007-USA was conducted with a concurrent placebo (saline) control and IMUS-008-USA was conducted with no concurrent placebo control. These studies, including design features, are listed in **Table 46**.

Table 46 Controlled Clinical Studies

Protocol #, Investigators, Publications	Completion Status (Starting Date)	Location Product Fill Size	Full Report Data Listings	CRFs Included*	Study Design Study Population	Treatment Doses	Number Entered Each Treatment	Age Range (Mean)	% M/F C/B/A/O	Duration of Drug Treatment
IMUS-007-USA	Complete (31 Mar 98)	USA 200 mg	Vol. 095 pg. 168	none	Placebo-controlled, single-blind, multicenter, paired-comparison study in subjects with suboptimal noncontrast echocardiographic images	**AF0150** Bolus: 0.125 mg/kg	**213**	22-83 (56)	63/37 85/11/1/3	~10 sec
						Placebo Bolus: 0.125 mL 0.9% NaCl/kg	**81**	24-86 (56)	80/20 79/10/1/10	~10 sec
IMUS-008-USA	Complete (31 Mar 98)	USA 200 mg	Vol. 112 pg 172	none	Open-label, multicenter, paired-comparison study in subjects with suboptimal noncontrast echocardiographic images	**AF0150** Bolus: 0.125 mg/kg	**232**	30-85 (62)	68/32 82/15/1/2	~10 sec

*Case report forms (CRFs) are provided only for subjects with serious AEs, those withdrawn from the study due to AEs, or for subjects who died.
C/B/A/O: Caucasian/Black/Asian/Other
inf: infusion

2. Summary of the Design and Results of Each Study

a. *IMUS-007-USA: A Multicenter, Saline-Controlled Study of AF0150 in the Echocardiographic Assessment of Left Ventricular Function in Patients with Suboptimal Noncontrast Images*

Objectives: The primary objective of this study was to evaluate the effectiveness of AF0150 in improving the assessment of cardiac function, as measured by the multiple primary endpoints, EBD and EF. Secondary objectives included assessment of SWM and safety.

Study Design/Population: IMUS-007-USA was a randomized, multicenter, single blind, paired-comparison, Phase 3 study conducted at 16 sites in the United States. Two hundred ninety-four male and female subjects ranging in age from 22 to 86 years (mean age, 56 years) with suboptimal echocardiograms were enrolled from March 31, 1998, to January 17, 1999. The first 161 subjects were randomized to receive either saline or AF0150 in a 1:1 ratio. The remaining subjects received AF0150. Subjects with suboptimal echocardiograms were defined as those in whom at least 2 segments, but not more than 9 of 12 segments, were not visualized in the apical 4-chamber and apical 2-chamber views. At least one segment was to be visualized in each chamber for the evaluation of EF. Subjects with an abnormal sinus rhythm, EF <20%, or New York Heart Association Class IV were excluded. Subjects randomized to AF0150 received 0.125 mg/kg AF0150 intravenously as a bolus over approximately 10 seconds. Subjects randomized to saline received 0.9% Sodium Chloride Injection USP, in a volume equivalent to the AF0150 dose. AF0150 was prepared by constitution of 200-mg dry powder with 10 mL SWFI to a final concentration of 20 mg/mL. Subjects served as their own control for efficacy and safety data comparison pre- and posttreatment.

Methodology: Fundamental continuous echocardiography was performed before and after administration of AF0150. All subjects underwent a gated radionuclide ventriculography (RVG) within 48 hours of study treatment to evaluate EF. The gated-RVG was used as the standard for EF evaluation. Image evaluations were conducted by three independent blinded reviewers and were assessed for EBD, EF, and SWM. For EBD and SWM, each segment in the apical 4-chamber, 2-chamber, and long-axis was scored, using a standardized methodology, for both noncontrast and contrast images. Total scores for both noncontrast and contrast, and change scores, noncontrast to contrast, were computed for each subject. EF values obtained from noncontrast and contrast echocardiograms were compared with the value obtained from the gated-RVG to evaluate concordance. Statistically significant study results were to be found for at least 2 of the 3 blinded readers to declare efficacy. Safety was assessed through 24 hours following study treatment and was based on evaluation of AEs, clinical laboratory tests (hematology, blood chemistry, and urinalysis), vital signs, SaO_2 (by pulse

oximetry), ECGs, and mental status (using the Mini-Mental Status Examination [MMSE][77]).

Efficacy Results: The trial provided statistical evidence that AF0150 was effective in improving visualization of EBD; all three reviewers evaluated AF0150 as significantly improving the visualization of the segments of the LV endocardium (P=0.001). More endocardial segments were visualized for the assessment of SWM after administration of AF0150. EF determination was not improved with the use of contrast when compared to gated-RVG. Total EBD score using continuous mode with missing data imputed as no change are included for each reader in **Table 47**. EF using continuous mode with missing data imputed as no change are included for echocardiogram and RVG agreement for each reader in **Table 48**.

Table 47 Total Endocardial Border Delineation Score* Using Fundamental Continuous Mode with Missing Data Imputed as No Change

	Change From Baseline		
Statistic	**Reader 1**	**Reader 2**	**Reader 3**
N	206	206	206
Mean	10.5	4.9	9.3
Std. Deviation	8.0	6.0	7.5
Minimum		[redacted]	
Median	11	4	10
Maximum		[redacted]	
P value†	0.001	0.001	0.001

Data reference: Section 14.2.1, Table 2.0.0a in the IMUS-007-USA clinical study report. (A copy of the report is located in **Section 8.XV**.)
*EBD was scored as 0= no delineation; 1 = mild or fair delineation; is not adequate to assess function, 2 = moderate or good delineation; good enough to assess function, 3 = excellent delineation; excellent demarcation of segments, 9 = no view available.
n = number of subjects (Efficacy Population, N=206).
†Analysis of variance.

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Table 48 EF Using Fundamental Continuous Mode: Noncontrast and Contrast Echocardiogram and Radionuclide Ventriculography Agreement* With Missing Data Imputed as No Change

Baseline Noncontrast ECHO and RVG		Contrast ECHO and RVG					
		Reader 1		Reader 2		Reader 3	
		Agree	Disagree	Agree	Disagree	Agree	Disagree
Agree	n (%)	29 (15.2)	45 (23.6)	28 (14.7)	47 (24.6)	31 (16.2)	43 (22.5)
Disagree	n (%)	31 (16.2)	86 (45.0)	27 (14.1)	89 (46.6)	28 (14.7)	89 (46.6)
	P value†	0.108		0.020		0.075	

Data reference: Section 14.2.1, Tables 2.39.0a and 2.39.0b in the IMUS-007-USA clinical study report. (A copy of the report is located in **Section 8.XV.**)
*There is agreement if ECHO (echocardiogram) and RVG EF results are assigned the same EF class and disagreement if ECHO and RVG EF results are assigned different EF classes. EF was categorized into one of the following classes: >65%, 55-65%, 40-54%, 35-44%, 25-34%, and <25%.
n = number of subjects. Percentages are based on the total number of subjects in the Efficacy Population (N=206).
†McNemar's Test.

Safety Results: AF0150 appeared to be well tolerated in this study. There were no deaths, serious or severe AEs, or premature withdrawals due to AEs reported. The overall incidence of AEs was low and similar in the saline-treated (4 subjects; 5%) and AF0150-treated (16 subjects; 8%) groups (see **Table 49**). The majority of AEs were mild in severity. The most frequently reported AE was hypertension (saline, no subjects, AF0150, 4 subjects [2%]); however, 2 of the subjects who experienced hypertension had elevated blood pressure at baseline. There was no temporal relationship between AE onset and time of study drug administration. The incidence of AEs reported as possibly/probably-related to study medication was similar in the saline (2 subjects; 3%) and AF0150 (7 subjects; 3%) groups. Clinical laboratory assessments, vital signs, ECGs, SaO_2, and mental status were stable relative to baseline after study drug administration and no clinically meaningful differences were detected between the saline-treated and AF0150-treated groups.

Table 49 IMUS-007-USA: Number and Percentage of Subjects with Treatment-Emergent Adverse Events by Body System and Severity

BODY SYSTEM Preferred Term	AF0150 (n=213)			Control (n=81)		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Total Number of Treatment-Emergent AEs	18	2	0	5	0	0
Total Number of Subjects with a Treatment-Emergent AE	16 (8%)	2 (1%)	0	4 (5%)	0	0
BODY AS A WHOLE						
Asthenia	1 (0.5%)	0	0	0	0	0
Headache	2 (1%)	0	0	1 (1%)	0	0
Pain	1 (0.5%)	0	0	0	0	0
CARDIOVASCULAR SYSTEM						
Hypertension	4 (2%)	0	0	0	0	0
Hypotension	2 (1%)	0	0	0	0	0
Supraventricular Tachycardia	1 (0.5%)	0	0	0	0	0
Tachycardia	1 (0.5%)	0	0	0	0	0
DIGESTIVE SYSTEM						
Diarrhea	2 (1%)	0	0	0	0	0
Nausea	1 (0.5%)	1 (0.5%)	0	0	0	0
METABOLIC & NUTRITIONAL DISORDERS						
Bilirubinemia	0	0	0	1 (1%)	0	0
Creatine Phosphokinase Increased	0	1 (0.5%)	0	0	0	0
Hyperglycemia	0	0	0	1 (1%)	0	0
Lactic Dehydrogenase Increased	0	0	0	1 (1%)	0	0
NERVOUS SYSTEM						
Dizziness	2 (1%)	0	0	0	0	0
SPECIAL SENSES						
Taste Perversion	1 (0.5%)	0	0	0	0	0
UROGENITAL SYSTEM						
Dysuria	0	0	0	1 (1%)	0	0

Data reference: Section 14.3.1, Table 3.1.1 in the IMUS-007-USA clinical study report. (A copy of the report is located in **Section 8.XV**.)
0 = No AEs were reported.

Conclusions: The results of this study demonstrated that AF0150 statistically significantly improves EBD. AF0150 had no effect on the determination of EF when RVG was used as the comparative standard. In addition, AF0150 statistically significantly improved the number of segments visualized for SWM with minimal risk to subjects.

The full IMUS-007-USA clinical study report is provided in **Section 8.XV**.

b. IMUS-008-USA: A Multicenter, Open-Label Study of AF0150 in the Echocardiographic Assessment of Left Ventricular Function in Patients with Suboptimal Noncontrast Images

Objectives: The primary objective of this study was to evaluate the effectiveness of AF0150 in improving the assessment of cardiac function, as

measured by the multiple primary endpoints, EBD and EF. Secondary objectives included assessment of SWM and safety.

Study Design/Population: IMUS-008-USA was a multicenter, paired-comparison, Phase 3 study conducted at 11 sites in the United States. Two hundred thirty-two male and female subjects ranging in age from 30 to 85 years (mean age, 62.6 years) with suboptimal echocardiograms were enrolled from March 31, 1998 to January 7, 1999. Subjects with suboptimal echocardiograms were defined as those in whom at least 2 segments, but not more than 9 of 12 segments, were not visualized in the apical 4-chamber and apical 2-chamber views. At least one segment was to be visualized in each chamber for the evaluation of EF. Subjects with an abnormal sinus rhythm, EF <20%, or New York Heart Association Class IV were excluded. No comparative agents, placebo, or separate control subjects were to be used in this study. All subjects received 0.125 mg/kg AF0150 intravenously as a bolus over approximately 10 seconds. AF0150 was prepared by constitution of 200 mg dry powder with 10 mL SWFI to a final concentration of 20 mg/mL. Subjects served as their own control for efficacy and safety data comparison pre- and posttreatment.

Methodology: Fundamental continuous echocardiography was performed before and after administration of AF0150. All subjects underwent a gated radionuclide ventriculogram (RVG) within 48 hours of study treatment to evaluate EF. The gated-RVG was utilized as the standard for EF evaluation. A subset of subjects underwent further evaluations of EF and SWM with MRI. Image evaluations were conducted by three independent blinded reviewers and were scored for EBD, EF, and SWM. For EBD and SWM, each segment in the apical 4-chamber, 2-chamber, and long-axis was scored, using a standardized methodology, for noncontrast and contrast images. Total scores for both noncontrast and contrast, and change scores, from noncontrast to contrast, were computed for each subject. EF values obtained from noncontrast and contrast echocardiograms were compared with the value obtained from gated-RVG to evaluate concordance. Statistically significant study results were to be found for at least 2 of the 3 blinded readers to declare efficacy. Safety was assessed through 24 hours following study treatment and was based on evaluation of AEs, clinical laboratory tests (hematology, serum chemistry, coagulation, and urinalysis), vital signs, ECGs, SaO_2 (by pulse oximetry), and mental status (using MMSE[77]).

Efficacy Results: Administration of AF0150 statistically significantly improved EBD. AF0150 did not improve the determination of EF when RVG was used as the comparative standard. AF0150 statistically significantly improved the number of segments visualized for SWM. A statistically significantly higher percentage of segments on the contrast echocardiogram agreed with MRI than on the noncontrast echocardiogram. Total EBD score using continuous mode with missing data imputed as no change are included for each reader in **Table 50**. EF using continuous mode with missing data

imputed as no change are included for echocardiogram and RVG agreement for each reader in **Table 51**.

Table 50 Total Endocardial Border Delineation Score* Using Fundamental Continuous Mode With Missing Data Imputed as No Change

	Change From Baseline		
Statistic	**Reader 1**	**Reader 2**	**Reader 3**
N	203	203	203
Mean	10.8	8.9	8.6
Std. Deviation	8.7	6.9	4.9
Minimum			
Median	11	9	9
Maximum			
P value†	0.001	0.001	0.001

Data reference: Section 14.2.1, Table 2.0.0a in the IMUS-008-USA clinical study report. (A copy of the report is located in **Section 8.XV**.)
*EBD was scored as 0 = no delineation; 1 = mild or fair delineation; not adequate to assess function, 2 = moderate or good delineation; good enough to assess function, 3 = excellent delineation; excellent demarcation of segments, 9 = no view available.
n = number of subjects (Efficacy Population, N=203).
†Analysis of variance.

Table 51 Ejection Fraction Using Fundamental Continuous Mode: Noncontrast and Contrast Echocardiogram and Radionuclide Ventriculography Agreement* With Missing Data Imputed as No Change

Baseline Noncontrast ECHO and RVG		**Contrast ECHO and RVG**					
		Reader 1		**Reader 2**		**Reader 3**	
		Agree	**Disagree**	**Agree**	**Disagree**	**Agree**	**Disagree**
Agree	**n (%)**	21 (11.2%)	42 (22.3%)	22 (11.7%)	40 (21.3%)	22 (11.7%)	38 (20.2%)
Disagree	**n (%)**	45 (23.9%)	80 (42.6%)	48 (25.5%)	78 (41.5%)	46 (24.5%)	82 (43.6%)
	***P* value†**	0.748		0.394		0.383	

Data reference: Section 14.2.1, Tables 2.39.0a and 2.39.0b in the IMUS-008-USA clinical study report. (A copy of the report is located in **Section 8.XV**.)
*There is agreement if ECHO and RVG EF results are assigned the same EF class and disagreement if ECHO and RVG EF results are assigned different EF classes. EF was categorized into one of the following classes: >65%, 55-65%, 40-54%, 35-44%, 25-34%, or <25%.
N = number of subjects in the Efficacy Population (N=203).
n = number of subjects. Percentages are based on the total number of subjects in the Efficacy Population.
†McNemar's Test.

Safety Results: AF0150 was well tolerated in this study. No deaths, serious or severe AEs were reported. The overall incidence of AEs was low (29 subjects; 13%) and there was no temporal relationship between AE onset and AF0150 administration. The majority of AEs were mild in severity (see **Table 52**). The most frequently reported AE was headache (6 subjects;

2.6%). No clinically significant changes in clinical laboratory assessments, vital signs, ECGs, or mental status were observed after AF0150 administration.

Table 52. IMUS-008-USA: Number and Percentage of Subjects with Treatment-Emergent Adverse Events by Body System and Severity

BODY SYSTEM Preferred Term	AF0150 (n=232)		
	Mild	Moderate	Severe
Total Number of Treatment-Emergent AEs	41	4	0
Total Number of Subjects with a Treatment-Emergent AE	31 (13.4%)	3 (1.3%)	0
BODY AS A WHOLE			
Abdominal Pain	2 (1%)	0	0
Asthenia	3 (1.3%)	0	0
Chest Pain	0	1 (0.4%)	0
Chills	0	1 (0.4%)	0
Headache	6 (2.6%)	0	0
Injection Site Reaction	1 (0.4%)	0	0
CARDIOVASCULAR SYSTEM			
Electrocardiogram Abnormal	2 (0.9%)	0	0
Hypertension	0	1 (0.4%)	0
T Inverted	1 (0.4%)	0	0
Vasodilatation	2 (0.9%)	0	0
DIGESTIVE SYSTEM			
Diarrhea	2 (0.9%)	0	0
Dyspepsia	1 (0.4%)	0	0
Nausea	3 (1.3%)	1 (0.4%)	0
Tongue Disorder	1 (0.4%)	0	0
HEMIC & LYMPHATIC SYSTEM			
Fibrinogen Increased	1 (0.4%)	0	0
Leukocytosis	2 (0.9%)	0	0
Thrombocytopenia	1 (0.4%)	0	0
METABOLIC & NUTRITIONAL DISORDERS			
Creatine Phosphokinase Increased	2 (0.9%)	0	0
Hyperglycemia	1 (0.4%)	0	0
MUSCULOSKELETAL SYSTEM			
Myalgia	1 (0.4%)	0	0
NERVOUS SYSTEM			
Paresthesia	1 (0.4%)	0	0
SPECIAL SENSES			
Taste Perversion	2 (0.9%)	0	0
UROGENITAL			
Albuminuria	1 (0.4%)	0	0

Data reference: Section 14.3.1, Table 3.1.1 in the IMUS-008-USA clinical study report.
(A copy of the report is located in **Section 8.XV.**).
0 = No AEs were reported.

Conclusions: The results of this study demonstrated that AF0150 statistically significantly improved EBD. AF0150 had no effect on the determination of EF when gated RVG was used as the comparative standard. Further, AF0150 statistically significantly improved the number of segments visualized for SWM, and the percentage of segments that agreed with MRI for SWM with minimal risk to the subject.

The full IMUS-008-USA clinical study report is provided in **Section 8.XV**.

3. Conclusions from Controlled Clinical Studies

The two Phase 3 studies (IMUS-007-USA and IMUS-008-USA) provide substantial evidence that AF0150, at a dose of 0.125-mg/kg, provides a clear delineation of the endocardial border and an evaluation of segmental wall motion in subjects with known or suspected cardiovascular disease and suboptimal echocardiograms. The studies demonstrated a statistically significant difference between observations with and without the use of contrast in a clinical population who would most likely benefit from improved visualization of endocardial borders. This interpretation is consistent with the general scientific demand for reproducible results that not only have statistical significance, but also clinical significance and relevance in medical practice.

In both Phase 3 studies, the primary objective was achieved: demonstration that administration of a single bolus of 0.125 mg/kg AF0150 significantly improved overall EBD in the fundamental continuous imaging mode which improved the ability of the readers to assess function. AF0150 administration also resulted in a statistically significant difference in the delineation of segments in all imaging views evaluated (apical 4-chamber, apical 2-chamber and apical long-axis) and for each of the 16 individual segments visualized in these imaging views. Furthermore, the Phase 3 studies demonstrated that, for subjects with known or suspected cardiovascular disease and suboptimal echocardiograms imaged in the fundamental gated mode, delineation was also statistically significantly improved. This finding is important because imaging in gated mode does not provide the additional benefit of motion to segmental visualization (i.e., no wall movement) that continuous imaging provides. Despite this limitation, visualization of the segments that were suboptimal at entry was significantly improved with the use of contrast when using the gated mode; AF0150 provided a clear, clinically relevant, delineation of the endocardial border.

Improvement in determination of EF comparing contrast echocardiography to baseline noncontrast echocardiography could not be demonstrated in these studies when using RVG as the comparative standard. IMUS-007-USA results demonstrated that EF from contrast echocardiograms in continuous or gated mode showed no greater agreement with RVG than EF from baseline noncontrast echocardiograms. Conversely, in IMUS-008-USA, contrast echocardiography using gated images (and not in continuous mode) demonstrated a trend towards improving determination of EF. In these studies, the correlation coefficients for

the baseline echocardiogram EF and RVG EF and contrast echocardiogram EF and RVG EF were approximately 0.5 for all readers. This finding was similar to published results[78] of a large study that have shown two commonly used "gold standard" techniques for the measurement of EF often disagree. In that study, the correlation coefficient between the RVG and contrast left ventriculography was r = 0.42. In light of this recent finding, it is not surprising that EF measured by echocardiography did not demonstrate a better correlation with EF measured by RVG. If an alternative method to assess EF is warranted, as the authors of the articles suggest, echocardiography clearly is an alternative technique that provides an estimate of EF that is less invasive, more widely available, and considerably less expensive than either RVG or contrast left ventriculography.

Another important component in the clinical evaluation of LV function is assessment of SWM. Supporting the conclusions seen in the Phase 3 studies that EBD is significantly enhanced, visualization of segments during assessment of SWM in continuous mode was significantly improved for both studies. In IMUS-008-USA, the study used MRI, which is considered a clinically meaningful method to assess SWM, as a comparative standard. The percentage of segments with SWM scores that agreed with MRI was significantly higher for the contrast echocardiogram than for the baseline noncontrast echocardiogram.

Thus, the results of the Phase 3 studies have demonstrated a statistically significant difference between observations with and without the use of AF0150 in subjects with suboptimal echocardiograms. This finding is consistent with the general scientific demand for reproducible results that not only have statistical significance, but also clinical significance and relevance in medical practice. AF0150 provides a clear delineation of the endocardial borders and an improved ability to evaluate and assess segmental wall motion. Contrast echocardiography with AF0150 provides valuable, clinically relevant information for the evaluation of subjects with known or suspected cardiovascular disease and suboptimal echocardiograms.

D. UNCONTROLLED CLINICAL STUDIES

No uncontrolled clinical studies pertinent to this application were conducted.

E. OTHER STUDIES AND INFORMATION

1. Tabular Presentation of Other Studies and Information

Two Alliance-sponsored clinical studies, IMUS-002-USA and IMUS-003-USA, are reported here to provide additional safety information on AF0150 at doses up to 4.0 mg/kg. These studies were conducted to evaluate AF0150 for uses other than that claimed in this application. IMUS-002-USA was conducted to evaluate the ability of AF0150 to assess left ventricular function and myocardial perfusion following Q-wave myocardial infarction. IMUS-003-USA was conducted to evaluate the potential of AF0150 for contrast ultrasound assessment of focal

lesions of the kidney and liver. These studies, including design features, are listed in **Table 53**.

Table 53 Other Clinical Studies

Protocol #, Investigators, Publications	Completion Status (Starting Date)	Location Product Fill Size	Full Report Data Listings	CRFs Included*	Study Design Study Population	Treatment Doses	Number Entered Each Treatment	Age Range (Mean)	% M/F C/B/A/O	Duration of Drug Treatment
IMUS-002-USA	Complete (7 Sept 96)	US 200 mg	Vol. 125 pg. 037	Section 11, Vol. 200	Open-label, multicenter study in subjects with Q-wave myocardial infarction	**AF0150** Bolus & Infusion: 0.25 mg/kg bolus & up to 80 mg titrated infusion	**41**	39-83 (61)	83/17 5/32/3/2	~30 sec bolus ~10 min inf
IMUS-003-USA	Complete (26 Nov 96)	US 100 & 200 mg	Vol. 130 pg. 078	none	Open-label, paired comparison, multicenter study in subjects with known focal lesions in the liver or kidney	**AF0150** Bolus: 40 mg + max of 4 10-20 mg	**47** 4	30-76 (55)	66/34 72/19/4/4	~30 sec bolus ~4 min inf
						Infusion: 80 mg infusion	2			
						Bolus & Infusion: up to 1.0 mg/kg bolus + up to 160 mg inf	41			

*CRFs are provided only for deaths, other serious AEs, or withdrawals for AEs.
C/B/A/O: Caucasian/Black/Asian/Other
inf: infusion

2. Summary of the Design and Results of Each Study

a. IMUS-002-USA: Safety, Dosing, and Efficacy Study of AF0150 in the Contrast-Echocardiographic Assessment of Left Ventricular Function and Myocardial Perfusion in Patients Following Q-Wave Myocardial Infarct:

Objectives: The primary objective of this study was to assess the clinical safety of and dosing strategy for AF0150 in patients undergoing contrast echocardiography following myocardial infarction. The secondary objectives included pilot assessments of the extent to which the contrast echocardiographic determination of resting LV function is improved compared to noncontrast echocardiography and that of myocardial perfusion is consistent with nuclear perfusion scintigraphy.

Study Design/Population: IMUS-002-USA was an open-label, Phase 2 study conducted at 7 centers in the United States. Forty-two male and female subjects in stable recovery from a first Q-wave myocardial infarction who had been referred for a nuclear perfusion study prior to hospital discharge were enrolled in the study from September 7, 1996 to March 12, 1997. Forty-one subjects received AF0150 (1 subject was not treated) and ranged in age from 39 to 83 years (mean age, 61.0 years).

Methodology: The study was conducted in two stages: a *pilot stage* and an *open stage*. The *pilot stage* included subjects (n=10) with suspected myocardial perfusion defect encompassing 20% or more of the LV as inferred from the results of prestudy ECG, cardiac isoenzyme levels, and/or conventional 2-D echocardiography. Imaging data were reviewed to determine the need to adjust dosing or ultrasound data collection strategy in the subsequent stage of the study. The *open stage* included subjects (n=32) meeting the same inclusion and exclusion criteria, but without regard for the presence or extent of any existing myocardial perfusion abnormality.

All subjects were to receive a bolus injection of 0.25 mg/kg AF0150 administered over approximately 30 seconds followed by an infusion of up to 80 mg AF0150 over approximately 10 minutes. AF0150 was supplied in a 200-mg vial and was prepared by constituting with 10 mL SWFI to a final concentration of 20 mg/mL.

Fundamental echocardiography was performed prior to and following administration of AF0150 using both apical and parasternal views. The images were read by three blinded readers (readers were blinded to subject and imaging period [i.e., noncontrast or contrast images]). For EF determination, it was not possible to completely blind the readers to subject and imaging period. Interobserver and intraobserver variability was determined for SWM. Visualization of the presence of any myocardial perfusion abnormality in noncontrast and contrast echocardiograms was

evaluated in the context of a resting nuclear perfusion scintigram employing single photon emission computed tomography (SPECT) imaging with ^{99m}Tc sestamibi by a single blinded reader.

Safety was assessed through Day 7 and was based on evaluation of AEs, vital signs, clinical laboratory tests (hematology, coagulation, blood chemistry, and urinalysis), and ECG.

AF0150 Dosing: The 41 AF0150-treated subjects received a mean total dose (bolus + infusion) of 1.29 ± 0.20 mg/kg (range of 0.95 to 1.94 mg/kg).

Efficacy Results: Data analysis was limited to images obtained following infusion of AF0150. Acceptable images (those of good technical quality and availability of views) were provided for 20 subjects for EBD, 19 subjects for SWM, 16 subjects for EF, and 20 subjects for nuclear perfusion studies.

Administration of AF0150 increased the number of ventricular segments visualized and degree of visualization for EBD (P=0.048) and the differences between noncontrast and contrast echocardiography did not vary significantly among readers. These positive effects of AF0150 on EBD are particularly noteworthy because the protocol did not require suboptimal echocardiograms at baseline for inclusion into the study, and despite this, a positive effect of AF0150 on EBD was observed.

For determination of EF, no statistically significant difference between noncontrast and contrast echocardiograms was noted. This is not surprising because the baseline echocardiograms were of good quality and it would be difficult to show a positive effect with such a small sample size (n=16). For most of the ventricular segments analyzed for SWM, there was poor agreement among readers for both noncontrast and contrast echocardiography with AF0150, and no clinical reference standard for SWM was used. Use of AF0150 as a contrast agent for echocardiographic evaluation of myocardial perfusion in postmyocardial infarction subjects showed agreement with SPECT as the standard in 70% of subjects.

Safety Results: Seventeen (42%) of the 41 AF0150-treated subjects experienced one or more treatment-emergent AEs. A total of 46 AEs were reported. Most of the AEs involved the cardiovascular system (11 of 41 subjects; 27%), of which atrial fibrillation and hypotension were most commonly reported (3 of 41 subjects each; 7%) followed by hypertension (2 of 41 subjects; 5%). Four subjects had a total of 8 serious treatment-emergent AEs reported during the study of which one (myocardial infarction) resulted in death. None of the serious AEs was considered related to the study drug. The investigators considered the serious AEs to be related to the underlying coronary artery disease or other cardiac disease and recent myocardial infarction in these subjects. (Narratives of the one death and other serious AEs are provided in **Section 8.VIII.Q**). Clinical laboratory values and

vital signs indicated no trend associated with AF0150 administration and there were no electrocardiographic abnormalities noted in any of the postcontrast ECG tracings that could reasonably be attributed to AF0150 administration.

Conclusion: Overall, the study provided preliminary evidence of efficacy for use of AF0150 as a contrast agent. Based on the results of this study, it was concluded that the intended population for Phase 3 would be subjects with suboptimal echocardiograms and that the efficacy of AF0150 to enhance the assessment of cardiac function in this patient population will be even more positive. In addition to the efficacy data, AF0150 appeared to be safe and well tolerated in the doses used in this study population.

The IMUS-002-USA clinical study report is provided in **Section 8.XV**.

b. IMUS-003-USA: A Safety, Dosing, and Efficacy Study of AF0150 for Contrast-Ultrasound Assessment of Focal Lesions of the Liver or Kidney in Patients with CT- or MRI-Confirmed Abnormalities

Objectives: The primary objective of this study was to evaluate the safety and dosing of AF0150 for the assessment of patients with known focal lesions of the liver or kidney. Secondary objectives were to evaluate the efficacy of AF0150 for the characterization of liver or kidney lesions and their vascular flow patterns using fundamental gray-scale, color-Doppler, and second-harmonic ultrasound imaging techniques.

Study Design/Population: IMUS-003-USA was an open-label, multicenter, paired-comparison, Phase 2 study conducted at six sites in the United States. Forty-seven male and female subjects ranging in age from 30 to 76 years (mean age, 54.9 years) with liver or kidney lesions confirmed by CT or MRI were enrolled in the study from November 26, 1996 to February 26, 1998. All 47 subjects enrolled received AF0150. Subjects served as their own control for safety and efficacy data comparison pretreatment and posttreatment.

Methodology: The study was conducted in two stages, a *pilot stage* followed by an *open stage*. The *pilot stage* was conducted to enable the investigator to define instrumentation settings and AF0150 dosing parameters for visualizing lesions during the administration of AF0150. A total of 22 subjects were enrolled in the *pilot stage*. The first 6 subjects were randomized to receive either a series of bolus doses of AF0150, 40 mg followed by a maximum of four 10- to 20-mg doses (n=4), or a continuous infusion at a dose of 80 mg AF0150 administered over 4 minutes (n=2). Review of the sonograms from the first 6 subjects revealed that visualization of tissue parenchyma was equivocal following AF0150 administration. As a result, the dosing strategy was changed and randomization was discontinued so that the remaining 16 subjects received both a single bolus dose of up to 1.0 mg/kg AF0150 over 30 seconds followed by an infusion of up to 160 mg AF0150, with the rate titrated to effect.

In the *open stage*, 25 subjects received a single bolus followed by an infusion as described above (i.e., single bolus dose of up to 1.0 mg/kg AF0150 over 30 seconds followed by an infusion of up to 160 mg AF0150, with the rate titrated to effect). Continuous fundamental gray-scale ultrasound imaging of the liver or kidney was performed upon dosing. Efficacy was based on data collected during the *open stage*.

Safety was assessed through Day 3 and was based on evaluation of AEs, clinical laboratory tests (hematology, blood chemistry, and urinalysis), vital signs, SaO_2, mental status testing, and headache characterization. AF0150 was supplied in vials containing 100 mg powder for the *pilot stage* and 200-mg powder for the *open stage*. Vials containing 100 mg powder were constituted with 10 mL NaCl (0.45%) to a final concentration of 10 mg/mL. Vials containing 200 mg powder were constituted with 10 mL SWFI to a final concentration of 20 mg/mL.

AF0150 Dosing: Overall, the mean dose received by all subjects was 210.2 ± 61.0 mg AF0150. The total AF0150 dose based on body weight ranged from 0.4 to 1.0 mg/kg for subjects who received AF0150 either as a bolus or infusion, and for subjects who received AF0150 as both a bolus and infusion, the dose ranged from 1.6 to 3.8 mg/kg (*pilot stage*) and 2.1 to 4.1[2] mg/kg (*open stage*).

Efficacy Results: Visualization of kidney and liver lesion vascularity was observed postcontrast using fundamental gray-scale imaging. Results obtained from the randomized image review (i.e., the reader was blinded to subject, time, method of AF0150 administration, and mode of imaging) demonstrated that AF0150 administration enhanced visualization of lesion vascularity. Lesion vascularity was not visualized in any subject precontrast. After AF0150 administration, vascularity was visualized in 10 (50%) of 20 subjects after bolus administration and in 7 (33%) of 21 subjects after infusion administration. Similarly, in the side-by-side comparison of precontrast and postcontrast images, there was an improved ability to assess lesion vascularity in 11 (55%) of 20 subjects after bolus administration of AF0150 and in 8 (38%) of 21 subjects after infusion administration of AF0150. Side-by-side comparison of post-AF0150 bolus and post-AF0150 infusion images indicated that more diagnostic information was obtained postbolus (8 subjects) than postinfusion (2 subjects) with fundamental gray-scale imaging. Post-AF0150 bolus imaging provided more information specifically with regard to visualization of lesion borders (7 of 8 subjects) and lesion visualization (5 of 8 subjects).

Safety Results: AF0150 appeared to be well tolerated in this study. There were no serious or severe AEs reported and the AEs reported were predominantly mild in severity. Twenty-five subjects (53%) experienced at

[2] Only one subject received 4.1 mg/kg due to the dosing strategy (the infusion was based on mg and not mg/kg).

least one treatment-emergent AE during the study. The most commonly reported treatment-emergent AEs (those reported in more than 1 subject) included headache (5 of 47 subjects; 11%), chest pain and nausea (3 of 47 subjects each; 6%), and diarrhea and flatulence (2 of 47 subjects each; 4%). AEs considered related to the study drug included headache (in 1 of the 5 subjects who reported this event), flatulence (in 1 of 2 subjects who reported this event), and fever (in the 1 subject who had this event). All the remaining AEs were reported in one subject each: injection site hypersensitivity, abnormal liver function tests, bilirubinemia, hyperglycemia, hyperlipemia, hypophosphatemia, increased LDH, increased serum glutamic oxaloacetic transaminase (SGOT), dry mouth, and hypoxia.

Mean values for clinical laboratory parameters changed little over the course of the study. Abnormal laboratory values appeared to be due to the subject's underlying medical condition and no trends associated with AF0150 administration were observed. In addition, vital signs, SaO_2, and mental status appeared to be stable and within acceptable ranges, following AF0150 administration. No temporal relationship to onset of headache and administration of AF0150 was observed.

Safety Conclusions: The results of this study demonstrated that AF0150 was well tolerated at a maximum dose of 1 mg/kg as a bolus followed by 160 mg as an infusion (up to a maximum dose of 4.1 mg/kg) in male and female patients with CT- or MRI-confirmed focal lesions of the liver or kidney.

The IMUS-003-USA clinical study report is provided in **Section 8.XV**.

3. Conclusions from Other Studies and Information

The results from IMUS-002-USA and IMUS-003-USA indicated that AF0150 was well tolerated. Safety information from these studies is included in **Section 8.VIII**.

F. Safety Summary - General Safety Conclusions

1. Extent of Exposure

Table 54displays the number of subjects who received AF0150 or Saline in each study. AF0150 subjects received AF0150 by IV administration either as a single 0.125-mg/kg dose (*AF0150 0.125-mg/kg Single Dose*), other single doses (*AF0150 Other Single Doses*), or as multiple doses – either multiple bolus doses or a bolus and an infusion (*AF0150 Multiple Doses*). Bolus doses were administered over approximately 10 seconds and infusions were given over approximately 4 to 10 minutes.

Table 55 AF0150 Dose Received in All Studies Based on Dosing Regimen

Dose	Statistics	AF0150 Dose Group			
		0.125-mg/kg Single Dose (N=457)	Other Single Doses* (N=48)	Multiple Doses** (N=103)	All Doses (N=608)
Mg	n	457	47	103	607
	Mean	11.1	191.7	157.2	49.9
	SD	2.5	128.2	88.2	84.9
	Range		[redacted]		
mg/kg	n	457	47	103	607
	Mean	0.125	2.6	2.1	0.6
	SD	0.001	1.6	1.4	1.2
	Range		[redacted]		
mg/m^2	n	457	47	100	604
	Mean	5.5	102.8	83.5	26.0
	SD	0.7	65.7	51.1	45.7
	Range		[redacted]		
mL/kg	n	457	47	103	607
	Mean	0.006	0.13	0.13	0.04
	SD	0.000	0.08	0.10	0.07
	Range		[redacted]		
Microbubbles† (10^8)	n	457	47	103	607
	Mean	5.4	97.7	93.8	27.6
	SD	1.2	60.3	68.3	50.6
	Range		[redacted]		
Microbubbles† (10^8) /kg	n	457	47	103	607
	Mean	0.06	1.3	1.2	0.4
	SD	0.00	0.8	1.0	0.7
	Range		[redacted]		
Microbubbles† (10^8) $/m^2$	n	457	47	100	604
	Mean	2.7	52.2	50.2	14.4
	SD	0.3	30.8	38.1	27.2
	Range		[redacted]		

Data reference: **Section 8.XIII.A, Table 1.1.0.**
*One subject in IMUS-003-USA (04-001) did not have weight reported; all doses calculated in this table were from the mg/kg dose.
**1 subject in IMUS-012-USA received two 4.1-mg/kg doses with approximately a 3-week interval between doses.
†Based on AF0150 release specification of 9.8×10^8 microbubbles/mL (9QAM700 r07).

2. Adverse Reactions

a. All Studies

In all clinical studies with AF0150, 608 subjects received AF0150 and 101 subjects received Saline. Doses of AF0150 administered in these studies ranged from approximately 0.125 mg/kg to 4.0 mg/kg[3]. The AEs observed in AF0150 subjects represent an acceptable AE profile that does not differ

[3] One (1) healthy volunteer in IMUS-012-USA received two 4.1-mg/kg doses with an approximate 3-week interval between doses.

substantially from the profile observed with the administration of Saline. AEs that occurred in ≤1% of the population are similar to events that are expected to occur in subjects with cardiovascular disease.

b. Phase 3 Studies

The Phase 3 program comprised two studies, IMUS-007-USA, and IMUS-008-USA. Forty-eight (10.8%) of 445 subjects who received AF0150 (0.125 mg/kg) reported AEs and 5 (6.2%) of 81 subjects who received Saline reported AEs. There were no serious AEs reported in either study. Headache was the most frequently reported AE in AF0150 subjects (8 subjects, 1.8%), followed by hypertension (5 subjects, 1.1%) and nausea (5 subjects, 1.1%). Among Saline subjects, one subject each (1.2%) reported headache, hypertension, bilirubinemia, hyperglycemia, LDH increased, and dysuria. Thus, the administration of 0.125 mg/kg of AF0150 is associated with minimal risk and that risk is similar to the Saline control.

In the Phase 3 Studies, a slightly increased incidence of subjects reporting AEs was observed in subsets of concurrent diseases and concurrent medications. Since most of these events were mild in severity, this does not pose a serious risk to these subjects. These results might suggest that administration of AF0150 in subjects with more severe disease (i.e., recent myocardial infarction or focal lesions of the liver or kidney), and more underlying conditions, there may be a slightly higher incidence of AEs. However, first, the overall incidence of AEs in the subjects with concurrent diseases of hypercholesterolemia, hyperlipidemia, hypertension, CAD were similar to the AE profile of those subjects receiving HMG CoA inhibitors, ACE inhibitors, beta-blocking agents, and platelet-aggregation inhibitors (excluding heparin). Secondly, the overall AE profile of those receiving HMG CoA inhibitors, ACE inhibitors, beta-blocking agents, and platelet-aggregation inhibitors is similar to the respective concomitant medications. Thus, these medications are suspected to be the main contributor to the slightly higher incidence in the subsets rather than the administration of AF0150.

The Phase 3 Studies were chosen for analyses of population subsets because there was a substantial sample size with consistent coding of information across studies and sites. In addition, these studies were conducted with the PCD dose (0.125 mg/kg), and one of the studies had a Saline control. To evaluate the types of AEs observed in these subsets, the effect of demographics, diagnosis, and concomitant medications were evaluated. Because of the uncertainty regarding the potential of intravenously administered microbubbles to cause adverse effects that may be related to bubble size, for example, microemboli in the pulmonary or cerebral circulation, certain evaluations of the data from the Phase 3 Studies were performed to assess this possibility. For the Phase 3 Studies, a review of the clinical laboratory tests, ECG evaluations, SaO_2 measurements, vital signs

measurements, and mental status examinations in these subsets, indicated that there were no safety findings that differed from those occurring in the Saline group. The observed AEs were likely due to the underlying disease condition of the subject. Furthermore, there were no safety findings that suggested an increased risk associated with administration of AF0150 to subjects with compromised pulmonary function or compromised cardiac function. The AE profile of subjects with COPD did not appear to differ from the AE profile observed in all the AF0150 subjects in Phase 3 Studies. In fact, a notable exception was the lack of respiratory system AEs that might have been expected if there were any potential concern with the administration of microbubbles.

*c. **Phase 1 Studies***

In the Phase 1 Studies, the incidence of AEs in the normal volunteer population appeared to be higher than that observed in AF0150 *All Doses* group. However, the incidences in these normal volunteers were no greater than in the Saline group In addition, the AEs observed in AF0150 subjects did not differ substantially from the AE profile observed with the administration of Saline.

It is concluded that the potential risks of IV administration of AF0150 in all doses studied will be minimal.

3. Deaths, Dropouts Due to AEs, and Other Potentially Serious AEs

Among all studies, 8 serious AEs, including one resulting in death, were reported in 4 subjects (narratives of these events are provided in **Section 8.VIII.Q**).

Table 56 presents the dropout profile for subjects in all the completed studies sorted by treatment group and reason for discontinuation.

Table 56 Incidence of Dropouts and Reason in All Studies

Reasons for Discontinuation from Study	Treatment Group				
	AF0150 Dose Group				
	0.125-mg/kg Single Dose (N=457) n (%)	Other Single Doses (N=48) n (%)	Multiple Doses (N=103) n (%)	All Doses (N=608) n (%)	Saline (N=101) n (%)
All subjects discontinued from study	8 (1.7%)	1 (2.1%)	7 (6.8%)	16 (2.7%)	2 (2.0%)
Adverse event	0	0	1 (1.0%)	1 (0.2%)	0
Withdrawal of consent	0	0	1 (1.0%)	1 (0.2%)	0
Lost to follow-up	1 (0.2%)	1 (2.1%)	2 (1.9%)	4 (0.7%)	0
Other	7 (1.5%)	0	3 (2.9%)	10 (1.6%)	2 (2.0%)

Data reference: **Section 8.XIII.A, Tables 6.1, 6.2, and 6.3.**
0 = No subject was discontinued in that Treatment Group for that reason.

A similar percentage of all subjects were discontinued from study for both the AF0150 and the Saline groups. Only one subject was discontinued from study for an Adverse Event (Subject 05-010 from IMUS-002-USA). A complete narrative of this subject is found in **Section 8.VIII.Q**. **Table 57** lists the subjects who experienced serious AEs.

Table 57. Number of Serious AEs Reported in All Studies*

Subject ID	Age (yr) Gender	Serious Adverse Event	Day of Event	Severity	Outcome	Relationship to AF0150
01-010	83 Male	Chest Pain	Day 0 (3.5 hrs**)	Severe	Resolved	Not Related†
		Atrial Fibrillation		Severe	Resolved	Not Related†
		Heart Failure		Severe	Resolved	Not Related†
		Dyspnea		Severe	Resolved	Not Related†
05-006	68 Male	Cardiogenic Shock	Day 1	Severe	Resolved	Not Related†
		Hypotension		Moderate	Resolved	Not Related†‡
05-009	72 Male	Heart Arrest	Day 1	Severe	Resolved	Not Related†
05-010	66 Female	Myocardial infarction§	Day 2	Severe	Death§	Not Related†

Reference: Section 16.2.7, Listing 22 of the IMUS-002-USA clinical study report (a copy of the report is located in **Section 8.XV**).
*Serious AEs were reported only in IMUS-002-USA.
**After start of dosing.
†Related to current illness/disease.
‡During the course of the event, causality was changed to "related to new concurrent illness".
§The SAE was reported as "myocardial infarction" in the subject's CRF. However, the cause of death was reported as "myocardial rupture following myocardial infarction."

The events all occurred in one clinical study (IMUS-002-USA), a study conducted in patients who had recently experienced a myocardial infarction. There were no

other deaths or serious AEs reported in any other study including the incomplete studies (IMUS-005-USA, IMUS-013-USA, and IMUS-014-USA). Serious AEs reported included chest pain, atrial fibrillation, heart failure, dyspnea, cardiogenic shock, heart arrest, and myocardial infarct (all reported as severe) and 1 moderate event of hypotension. None of the serious AEs was considered by the investigator to be related to study drug. One serious and severe event, myocardial rupture following myocardial infarct (Subject 05-010), resulted in death. The death was considered by the investigator not to be related to study drug. All other serious and severe AEs resolved on the day of onset or the following day, all required therapy, and all were considered by the investigator to be related to current illness/disease. The serious and moderate event of hypotension resolved 4 days after onset with therapy. This event was initially considered by the investigator to be related to the subject's current illness/disease and later (time and date unknown) considered that event to be related to a new illness.

4. Clinical Laboratory Data

Generally, no clinically relevant abnormalities or trends were noted in the analyses of hematology, coagulation, blood chemistry, and urine parameters in AF0150 and Saline subjects. Additional analyses performed on subsets of the population did not show evidence or trends that might represent drug-related or dose-related AEs or interactions.

For hematology and coagulation, two subjects (0.2%) with normal baseline hematocrit and monocytes, 12 subjects (0.3%) with normal baseline PT/PTT, and seven subjects (1%) with normal baseline fibrinogen levels showed shifts to PCS values post-AF0150 dosing. None of these shifts were considered by the investigators to be related to administration of AF0150.

For blood chemistry, 11 subjects (2%) had abnormal baseline liver (n=4) and kidney (n=7) function tests that shifted to PCS values post-AF0150 dosing. The investigators considered these shifts to be related to the preexisting medical conditions or disease(s) of the subjects.

Fifteen subjects (2.5%) had normal baseline cardiac enzymes/isoenzyme levels and two subjects (0.3%) had normal glucose or calcium levels that shifted to PCS values post-AF0150 dosing. None of these shifts were considered related to administration of AF0150. In addition, the elevation of in cardiac enzymes and isoenzyme is not unusual in the population enrolled in the studies.

Six subjects with normal baseline urine specific gravity and pH showed shifts to PCS values post-AF015 dosing that were considered by the investigators to be not clinically relevant.

The potential for AF0150 to activate complement and TNF-α was also studied in 64 normal healthy volunteers. Results showed an increase in C3a levels post-AF0150 dosing that was not associated with any clinically significant

changes in vital signs and laboratory tests measured. In addition, there was no indication of a systemic release of TNF-α since the concentrations were below or close to detection limit of the assay.

Based on the results of clinical laboratory evaluation, it can be concluded that AF0150 is relatively safe and well tolerated at the doses used in the studies.

5. Summary of Other Safety Assessments

a. ECG

A comprehensive review of the changes from baseline observed in PR, QRS, QT, and QTc intervals and heart did not reveal any consistent changes that could be related to AF0150 administration. Those ECG abnormalities reported as PCS or AEs were most likely due to underlying cardiovascular disease.

b. SaO2

A comprehensive review of the changes from baseline observed in SaO_2 measurements did not reveal any consistent changes that could be related to AF0150 administration even in subjects with pulmonary disease.

c. Vital Signs

Vital signs including blood pressure, heart rate, respiratory rate and temperature were evaluated in all studies. All these parameters showed minor fluctuations at different time points. However the small number of cases and the fact that there is no consistent pattern in these fluctuations are suggestive of a coincidental finding. Therefore, it is concluded that the influence of AF0150 on vital signs is minimal.

d. Mini Mental Examination

A comprehensive review of the changes from baseline observed in the MMSE scores did not reveal any consistent changes that could be related to AF0150 administration even in subjects with pulmonary disease.

6. Drug Abuse

No potential for abuse or overuse has been reported with AF0150. In addition, the critical ingredients in AF0150 (PFH and DMPC) are not pharmacologically nor structurally related to any drug known to have abuse potential.

Literature searches on the Internet, and online databases (*Medline*, *Embase*, *Biosis*, *Toxline*, *Toxlit*, *Scisearch*, *Caplus*, *Caold*) did not reveal any reports or evidence of abuse or overuse of marketed contrast imaging products, *Albunex*®

and *Optison*®, or any of the perfluorocarbons currently being developed for contrast enhancement.

7. Overdosage

The recommended clinical dose of AF0150 is 0.125 mg/kg administered as an IV bolus over 10 seconds. Clinical studies have been conducted at doses of up to 4.0 mg/kg either as a single dose or as multiple doses in normal volunteers and in patients. These studies provide high-dose and multiple-dose experience with AF0150.

Three studies, IMUS-001-USA, IMUS-012-USA, and IMUS-003-USA, provide high-dose experience with AF0150 (up to 4.0 mg/kg) in both normal volunteers and patients. Three studies, IMUS-002-USA, IMUS-003-USA, and IMUS-018-USA provide multiple-dose experience with AF0150.

AF0150 was well tolerated at all the doses used in these studies. The treatment-emergent AEs observed were predominantly mild to moderate in intensity and considered unrelated to the study drug. In addition, all the events were transient and most resolved without treatment. No dose-response effect was observed. No trends or clinically significant changes in clinical laboratory measurements, vital signs, respiratory function, SaO_2, and ECG findings were reported during the studies.

A series of nonclinical studies was conducted to evaluate the safety of exaggerated doses of AF0150. This series of studies included single-dose toxicity studies in mice, rats and dogs, and repeated-dose toxicity studies in rats, and dogs. In the single-dose toxicity studies, AF0150 was administered intravenously to rats and mice at doses up to 1600 mg/kg (12800 × PCD) and to dogs at doses up to 400 mg/kg (3200 × PCD). Repeated-dose toxicity studies were conducted to assess the toxicity of daily intravenous administration of AF0150 for at least 28 days in rats at doses of 50, 200, or 400 mg/kg/day (400-3200 × PCD) and in dogs at doses of 25, 50, or 100 mg/kg/day (200-800 × PCD). There were no deaths or indications of toxicity reported at the doses administered in either the single-dose or repeated-dose studies and none of the observed AF0150-related findings appeared to adversely affect the health of the animals.

Based on the safety data from the above clinical studies, it can be concluded that AF0150 has a wide margin of safety. Doses up to 32 × PCD of 0.125 mg/kg have been well tolerated when given as single doses (IMUS-001-USA and IMUS-012-USA) and as multiple doses (IMUS-002-USA, IMUS-003-USA, and IMUS-018-USA) in normal volunteers and patients. In addition, because of the medical supervision required to constitute and administer AF0150, it is highly unlikely that AF0150 would be abused or overdosed.

Since AF0150 is eliminated primarily in expired air, in the unlikely event that overdose occurs, maintenance of an adequate airway is essential.

8. Safety Conclusions

The clinical safety of AF0150 was evaluated by assessing reports of AEs, clinical laboratory tests, including hematology, coagulation, blood chemistry, and urinalysis, ECG evaluations, arterial oxyhemoglobin saturation (SaO_2) measurements, vital signs, and mental status examinations. In general, the conclusions from these evaluations are that the potential risks associated with AF0150 at a dose of 0.125 mg/kg in the target population (e.g., patients with suboptimal echocardiograms) are minimal. Furthermore, in a broader range of patients with more severe disease (i.e., post-myocardial infarction or cancer), AF0150 was shown to be safe at doses up to 4.0 mg/kg.

In clinical studies, a total of 608 subjects received doses of AF0150 that ranged from 0.125 to 4.0 mg/kg and 101 subjects received Saline as the control agent. Of the 608 AF0150-treated subjects, 457 (75%) received the PCD of 0.125 mg/kg.

Among the 608 AF0150-treated subjects in all studies, 17.1% experienced adverse events (AEs) as compared to 10.9% in the Saline-treated (control) group. The incidences of AEs reported for greater than 1% of subjects are presented in **Table 58**.

Table 58 Incidence of AEs Reported in >1% of Subjects in All Studies by Treatment Group

Body System Preferred Term	Treatment Group AF0150 (N=608) n (%)	 Saline (N=101) n (%)
Any	104 (17.1%)	11 (10.9%)
Body as a Whole	42 (6.9%)	2 (2.0%)
Headache	19 (3.1%)	2 (2.0%)
Cardiovascular	29 (4.8%)	2 (2.0%)
Hypertension	7 (1.2%)	1 (1.0%)
Digestive	25 (4.1%)	1 (1.0%)
Diarrhea	7 (1.2%)	1 (1.0%)
Nausea	10 (1.6%)	0
Special Senses	6 (1.0%)	3 (3.0%)
Taste perversion	4 (0.7%)	2 (2.0%)

Data reference: **Section 8.XIII.A, Tables 3.1.1 and 3.2.1.**
0 = No subject in this treatment group reported an AE for this body system category.

This represents an acceptable AE profile that does not differ substantially from that of the Saline control.

In the Phase 3 pivotal studies, 48 of 445 (10.8%) AF0150-treated subjects reported AEs while 5 of 81 (6.2%) Saline-treated subjects reported AEs. Headache was the most frequently reported AE in AF0150-treated subjects (1.8%), followed by hypertension (1.1%) and nausea (1.1%). Among Saline-treated subjects, one subject each (1.2%) reported headache, hypertension, bilirubinemia, hyperglycemia, and dysuria. The incidence rates for AEs in the Phase 3 studies are less than the rates that were observed in the population of all subjects who received AF0150. These studies represent the risk of administration of the recommended dose of 0.125 mg/kg to the target population (i.e., subjects with suboptimal echocardiograms). There were no serious AEs reported in the Phase 3 pivotal studies.

Serious AEs were reviewed for all studies. A total of eight serious AEs, including one death, were reported. These events all occurred in one Phase 2 study conducted in subjects who had recently experienced a myocardial infarction. None of the serious AEs reported was considered by the investigator to be related to study drug, but were considered to be related to the patient's current illness. The investigator considered the death not related to study drug. There were no other deaths or serious AEs reported in any other study.

Because the FDA has expressed concern regarding the potential for intravenously administered microbubbles to have adverse effects that may be related to bubble size, for example, microemboli in the pulmonary or cerebral circulation, certain evaluations of the data from the Phase 3 studies were performed. Specifically, the data were examined for evidence of respiratory AEs, and the AE profiles of patients with pulmonary disease (e.g., COPD) or cardiac disease (i.e., EF <50%) were reviewed. To examine the potential for cerebral effects, the results of MMSE were assessed.

There were no Phase 3 subjects in either treatment group who experienced any AE in the Respiratory Body System category. Respiratory AEs were only observed in the two Phase 2 studies that enrolled subjects with recent acute myocardial infarction or who were cancer patients. The investigators concluded that these AEs were related to the subjects' current disease.

Ninety-seven AF0150-treated subjects (22%) in the Phase 3 studies had COPD. The AE profile (i.e., types of AEs observed) for these subjects was similar to those seen among all AF0150-treated subjects. While the AE incidence rate for AF0150-treated COPD subjects (17.5%) was slightly higher than that for all AF0150-treated subjects (10.8%), this difference did not suggest an increased risk in the COPD population. No subject in the Phase 3 studies reported an adverse event within the Respiratory body system.

Eighty-eight subjects (19.8%) in the Phase 3 studies had cardiac disease (i.e., EF <50%). Fourteen (15.9%) of 88 subjects with EF <50% and 27 (9.3%) of 291 subjects with EF ≥50% who received AF0150 reported AEs. The AE profile (i.e., types of AEs observed) in the subjects with an EF <50% was similar to the

AE profile observed in subjects with an EF ≥50% and to that in all AF0150-treated subjects (10.8%). The slightly higher incidence rate seen in subjects with cardiac disease did not suggest an increased risk in this population.

Clinical laboratory tests, including hematology, coagulation, blood chemistry, and urinalysis were evaluated in all studies. A comprehensive review of these parameters did not reveal any risks.

ECG evaluations including assessments of PR, QRS, and QTc intervals were conducted in the Phase 3 studies before and after the administration of AF0150. A comprehensive review of these parameters did not reveal any risks.

SaO_2 measurements did not reveal any risks even in subjects with compromised pulmonary function.

Vital signs including blood pressure, heart rate, respiration rate, and temperature were evaluated in all studies. A comprehensive review of these parameters did not reveal any risks.

Mental status was evaluated in the Phase 3 studies by administering a MMSE before and after the administration of AF0150. There were no indications of any postdosing impairment in mental status.

Thus, a review of AEs, clinical laboratory tests, ECG evaluations, SaO_2 measurements, vital signs measurements, and mental status examinations indicated that there were no safety findings that differed from those occurring in the control population. The observed AEs were likely due to the underlying disease condition of the subject. Furthermore, there were no safety findings that suggested there was an increased risk associated with administering AF0150 microbubbles to subjects with pulmonary disease or cardiac disease. Therefore, it is concluded that the potential risks of IV administration of AF0150 in all doses studied will be minimal.

COMBINED WITH EFFICACY SUMMARY
SEE TAB B-8