CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-191

Clinical Pharmacology and Biopharmaceutics Review

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-191	Submission Date(s): 2/12/02, 4/5/02
Brand Name	Imagent™
Generic Name	AF0150; perflexane-phospholipid microbubbles
Reviewer	Young Moon Choi, Ph.D.
Team Leader	Young Moon Choi, Ph.D. (Acting)
Secondary reviewer	John Hunt, Deputy Director (DPE-2)
OCPB Division	DPE-2
ORM division	HFD-160 (Division of Medical Imaging and Radiopharmaceutical Products)
Sponsor	Alliance Pharmacetuical Corp., 3040 Science Park Rd, San Diego, CA
Relevant IND(s)	IND [redacted]
Submission Type; Code	Responses to 2/6/2002 approvable action letter
Formulation; Strength(s)	A kit containing a vial of 200 mg of Imagent powder, 20 ml of sterile water for intravenous injection; Each vial of Imagent powder contains 9.2 mg 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC); 75 mg hydroxyethyl starch; 2.1 mg poloxamer 188; 75 mg sodium chloride; and 36 mg sodium phosphate buffer in a vial filled with a mixture of 17 % v/v perflexane vapor in nitrogen.
Indication (Proposed by the sponsor)	Indicated for use in patients with suboptimal echocardiograms to opacify the left ventricle (LV), which enhances the delineation of the LV endocardial borders
Dose (Proposed by the sponsor)	The recommended dose is 0.00625 ml/kg (0.125 mg/kg) administered as a single intravenous bolus over a period of not less than 10 seconds and immediately followed by a saline flush. Imagent must be used within 30 minutes of reconstitution. Discard any unused portion.

1 Executive Summary

The current submission is the sponsor's responses to the Clinical Pharmacology and Biopharmacetuics comments contained in the 2/6/2002 approvable letter:
"The submission lacks data in the Pharmacokinetics (PK) of Imagent in pulmonary impaired patients."

In response to the above comments, the sponsor submitted
(1) a study report titled " Effect of minute ventilation on perfluorohexane elimination after intravenous administration of AF0150 in healthy, anesthetized and ventilated beagle dogs. " and
(2) an assessment of this study

The report was reviewed by the Pharm./Tox reviewer (Dr. Jin Chen). It was concluded that this study provided only limited information to address the issues about effects of pulmonary impairment on PK of PFH gas. (Refer to the Dr. Chen's review)

A teleconference was held with the sponsor on 4/1/2002 to discuss the evaluation of the above results and whether a PK study of Imagent in pulmonary impaired patients was needed. The Agency pointed out multiple deficiencies of the study (Refer to the minutes of teleconference), and recommended a Phase 4 commitment for a PK study in a dog pulmonary embolism model. Also, it was indicated that the results of the Phase 4 dog study will determine if a clinical study is necessary to address the effects of Imagent in compromised pulmonary subjects. The sponsor accepted the Agency's recommendations.

1.1 Recommendation

The Office of Clinical Pharmacology and Biopharmaceutics, Division of Pharmaceutical Evaluation II has reviewed the responses submitted on 2/12/2002 and 4/5/2002 to the approvable letter dated 2/6/2002. From a clinical pharmacology and biopharmaceutics perspective, the data is not acceptable. Therefore, based on an additional animal study as a Phase 4 commitment, it will be determined if a pharmacokinetic study in compromised pulmonary subjects is needed. From a clinical pharmacology and biopharmaceutics perspective, a labeling change is recommended (Refer to the following section of "Labeling recommendation")

1.2 Phase IV Commitments

At this moment, no Phase 4 commitment is recommended for a study in human subjects with compromised pulmonary function. However, based on the Phase 4 dog study findings, a clinical study (as a Phase 4 commitment) may be needed to address the effects of Imavist in compromised pulmonary subjects.

1.3 Labeling recommendation for the "Gender" subsection of the Clinical Pharmacology section of the labeling.

Gender

15 pages redacted from this section of the approval package consisted of draft labeling

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Young-Moon Choi
5/14/02 03:26:18 PM
BIOPHARMACEUTICS

John P. Hunt
5/14/02 03:38:00 PM
BIOPHARMACEUTICS

Clinical Pharmacology and Biopharmaceutics Review

NDA:	**21-191**
Type of Submission:	Amendment to Pending Application: Response to Approvable Letter
Generic Name:	**AFO150**
Other Name(s):	Perflexane-phospholipid microbubbles
Formulation; **Prescription Status;** **Strength** **Route of Administration**	A kit containing a vial of 200 mg of powder, 20 ml of sterile water for injection RX 200 mg intravenous
Brand Name:	**Imavist™**
Sponsor:	Alliance Pharmaceutical Corp., 3040 Science Park Rd, San Diego, CA
Submission Date(s):	Date of Submission: 8/16/01 Stamp Date: 8/20/01
Review Date:	11/21/01
Reviewers:	David J. Lee, Ph.D.

The current submission contains the responses to the Clinical Pharmacology and Biopharmaceutics comments contained in the 8/14/00 approvable letter. The Applicant indicated that the approach that they have taken in responding to each question was based on discussions with the Agency at the 11/3/00 Clinical meeting.

RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation II (OCPB/DPE-2) has reviewed the responses to the approvable letter submitted for NDA # 21-191 on 8/16/01.

Overall, the Applicant's submitted information is adequate, and therefore, the Applicant's responses are acceptable.

However, the Applicant did not submit any pharmacokinetic information in pulmonary impaired subjects. Rather than submitting the response to the request, the Applicant stated that they have conducted an animal study (to assess the effects of minute ventilation on perflexane elimination following intravenous administration of Imavist in healthy, anesthetized, and ventilated beagle dogs), and they will submit the results when available.

It is recommended that the request of the human pharmacokinetic information be re-assessed when the data from the dog study submitted to the Agency and examined for acceptability.

/S/

David J. Lee, Ph.D. — Date
Clinical Pharmacologist
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

/S/

John Hunt, Deputy Director — Date
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

REVIEW OF THE CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICAL INFORMATION

The Agency's questions conveyed to the Applicant are in **BOLD**.

A. PERFLEXANE (PFH) GAS PROTEIN BINDING INFORMATION.

FDA Comment: **The application lacks PFH gas plasma protein binding and distribution information. In order to resolve this deficiency, submit data (such as in vitro data) on PFH binding to proteins.**

Response: The Applicant stated that they designed and conducted an in vitro study to evaluate the solubility of perflexane in aqueous albumin as an approach to evaluating perflexane protein binding. The Applicant stated that the solubility of PFH in albumin solutions was very low (approx. 10 ppm) and was comparable to its solubility in water. The Applicant submitted the study report (Appendix 1).

REVIEWER'S COMMENT: *The study report is acceptable and overall response adequate.*

B. PHARMACOKINETICS OF INTACT MICROSPHERES

FDA Comment: **The application lacks data to describe the metabolism of the intact PFH filled microspheres. Intact microsphere pharmacokinetic data should be provided, if feasible, to complete the proposed dosage assessment. We acknowledge that an analytical method(s) or sufficient modification of the existing analytical method has not been identified. Efforts to develop such a method should be continued. If these efforts are unsuccessful your attempts to develop such a method should be fully documented.**

Response: The Applicant stated there are currently no analytical methods available to evaluate the fate of the intact microbubble (as discussed and agreed upon with the Agency at the November 2, 2000 Clinical meeting, and as presented in the pre-meeting briefing document submitted October 18, 2000). Additionally the Applicant stated that they will continue to investigate potential quantitative analytical methods to evaluate *in vivo* the fate of the intact bubble post-approval.

REVIEWER'S COMMENT: *The Applicant's response is acceptable.*

FDA Comment: **In addition, we request in vitro information on microsphere fragility. This should include information on the population of microspheres, the rate and time of disappearance, duration of microsphere detection, percent aggregation or coalescence rate, etc.**

Response: The Applicant stated that to address the issue of microbubble fragility, and thus persistence, they integrated information from both *in vivo and in vitro* studies. The Applicant stated the following (Volume 8, pages 5 and 6):

"All intact *Imavist* microbubbles will gradually shrink over a time span of minutes when introduced into the circulation due to exposure to hydrostatic (arterial) pressure and gas tension forces acting across the microbubble phospholipid shell. Evidence for microbubble shrinkage and the rate of microbubble disappearance has come from direct visualization of individual microbubbles in the microcirculation[2] and from bubble size-distribution measurements of microbubble populations in an *in vitro* system.

In a recently completed microcirculation study **using direct visualization with an optical resolution** of the system of approximately 3 μm,[2] by 15 minutes after a high-dose intraarterial *Imavist* injection, there

was no evidence of any microbubbles in skeletal muscle microcirculation (in normal and hyperlipidemic rats).

At time points before 5 minutes postinjection, however, **there was documented a transient lodging of occasional single microbubbles of a diameter slightly larger than the capillary each was traversing.** These two temporal pieces of data taken together suggest that the larger microbubbles (e.g., those that initially lodge) shrink at a rate such that they are < 3 μm by 15 minutes postinjection (in the absence of ultrasound power). Further in this study, there was no evidence of aggregated or coalesced microbubbles in microvessels at any time point after *Imavist* injection in normal rats as well as in hyperlipidemic rats that had markedly elevated cholesterol and triglyceride levels.

Using an *in vitro* system designed to simulate the *in vivo* vascular system, *Imavist* microbubbles circulating in an albumin solution were continuously exposed to various clinically relevant ultrasound power levels and serial measurements of bubble-size distribution were made for up to 6 minutes.[3] **Although the *in vitro* system was unable to adequately mimic typical vascular pressures, there was negligible change in total microbubble count over 6 minutes when low ultrasound mechanical indices (<0.2) were used. High ultrasound mechanical indices (0.5 to 1.7)** accelerated this otherwise slow dissolution of microbubbles **with a reduction of total microbubble count by approximately 50% after 4-6 minutes of exposure.** Importantly, the data showed that **microbubbles only dissolve** (bubble counts decrease across the range of microbubble sizes) and **there was no growth or generation of microbubbles of any size.**[3]

It can be stated that the flexible lipid shell of the *Imavist* microbubble is resilient. This statement is supported by studies designed to expose the microbubbles to pressure extremes. In a hypertensive animal model designed to maximize intravascular pressures, the efficacy signal (echocardiography image, carotid artery Doppler response) was maintained (i.e., it was not different in magnitude and persistence as compared to non-hypertensive controls).[4] In a study where high pressure (>250 mm Hg for 2 minutes) was applied to the microbubbles within the syringe prior to injection, upon administration the efficacy signal was again maintained.[5]

Additionally, the flexible microbubble exhibits elastic material behavior with the ability to change shape by deforming and/or shrinking to move through capillaries. In the previously referenced microcirculation study,[2] with direct visualization of microbubbles moving through the microcirculation (15-20 individual microbubbles), **there was never a case when a lodged microbubble appeared to burst as if the membrane was ruptured and deflated of its interior gas. There were, however, several examples of microbubbles deforming to move through smaller vessels, demonstrating the elasticity of the DMPC monolayer membrane.** In summary, various pieces of data support that the *Imavist* microbubble is resilient enough to withstand physical forces while circulating with persistence on the order of minutes during diagnostic imaging, yet flexible enough to resonate and move through the capillaries in the microcirculation."

REVIEWER'S COMMENT: *The Applicant's response is acceptable. The study reports (Appendex 2) are adequate and acceptable.*

C. PHARMACOKINETICS OF 1,2-DIMYRISTOLY-SN-GLYCERO-3-PHOSPHOCHOLINE (DMPC), THE MICROSPHERE SHELL

FDA Comment: The application lacks sufficient data to characterize the lipid components of the microspheres. Specifically, supportive information regarding DMPC metabolism should be submitted.

Response: The Applicant stated that an in vitro study demonstrated that the metabolism of DMPC (a semi-snythetic phospholipid) follows the same pathway as that of egg yolk L-alpha phosphatidylcholine (a naturally occurring phospholipid) when exposed to enzymatic degradation by phospholipase D. The Applicant submitted the study report.

REVIEWER'S COMMENT: *The Applicant's reponse is acceptable.*

D. PFH GAS ASSAY INFORMATION

FDA Comment: The application lacks sufficient information to complete the PFH gas assay assessment.

Based upon the application, it appears that PFH gas assay blood samples were analyzed at three different starting days, 6/29, 7/6, and 7/12. In order to clarify the analysis, the follow assay information should be submitted.

1. **Standard curves and quality control runs from days 7/6 and 7/12.**

Response: The Applicant stated the following:

"As indicated in the final report for 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 AFO150 in healthy adult volunteers,* a single 18-point calibration curve was run on 6/29/99 (refer to NDA 21-191, Section 6, Vol.-Page 035-083 to 035-084; a copy of these pages is provided in Appendix IV.H). Accuracy and precision measurements were conducted every 20 runs over the course of the study, from 6/30/99 to 7/16/99. Chromatography system stability was evaluated using one concentration ([redacted] on the calibration curve and using one of the daily accuracy and precision measurements of the same concentration. The 18-day duration of assays was considered a continuous run, i.e., the system was not shut down nor used for any other assays. Therefore, no additional standard curves or quality control runs were necessary."

REVIEWER'S COMMENT: *The Applicant's response is acceptable. It should be noted that the Applicant used only one quality control concentration to evaluate the chromatography system. Generally three quality control concentrations (usually low, medium, and high clinical plasma concentrations observed) are used to evaluate the assay system.*

FDA Comment: 2. **Profiles from blood assay (as in the expired air samples)**

Response: The Applicant stated the following:

"As agreed at the November 3, 2000 Clinical meeting with FDA, representative chromatograms are provided for blood and expired air. Chromatograms for blood and expired air samples from subjects 101, 102, and 103 (referred to as subjects 1, 2, and 3, respectively, in the raw data) are provided in Appendix IV.I and IV.J, respectively. All chromatograms are labeled with analysis file number, subject number (i.e., 1, 2, 3), sampling interval, and volume sampled. The listings for blood and expired air samples from these three subjects were provided in the original NDA 21-191 submission (Vol-Page 035-094 to 035-096, and 035-189 to 035-191, respectively)."

REVIEWER'S COMMENT: *The Applicant's response is acceptable.*

FDA Comment: 3. **For expired air samples, submit a complete table of calculated PFH expired air concentration data, as in Vol. 35, p. 206.**

Response: The Applicant stated the following:

"A table listing the calculated perflexane expired air concentration data is provided in **Appendix IV.K. Appendix** IV.K includes the subject number, sampling interval, peak heights of PFH and the internal standard [redacted] obtained from the chromatograms, and provides the calculated mean PFH concentration (ng/mL). The mean concentration per sampling interval data (pre-dose through 90 minutes post-dose) were presented in the IMUS-012-USA study report in NDA 21-191 (Section 6.IV, IMUS-012-USA Table 6.1.9.2:1, Vol.-Page 035-016)."

REVIEWER'S COMMENT: *The Applicant's response is acceptable.*

E. PHARMACOKINETICS OF INTACT MICROSPHERES/PFH GAS IN PULMONARY IMPAIRED SUBJECTS

FDA Comment: Please submit pharmacokinetic information from pulmonary impaired subjects/patients with impaired pulmonary function.

Response: The Applicant stated the following:

"As discussed with FDA at the November 3, 2000 clinical meeting, Alliance has designed and conducted a study to assess the effects of minute ventilation on perflexane (PFH) elimination following intravenous administration of Imavist in healthy, anesthetized, and ventilated beagle dogs. The protocol for this study is provided in Appendix IV.G. Alliance commits to provide the results and final report for this study when available; either during the review process or post-approval, depending on the availability."

REVIEWER'S COMMENT: *The Applicant's response is not acceptable since they did not submit any human data; however, the data obtained from this study may provide additional information. Generally the pharmacokinetic information in the pulmonary impaired population is needed, and, thus, the Agency requested the needed information in the past. At this time, it is recommended that this issue should be revisited when the Applicant submits the data from this study.*

APPENDIX

APPENDIX 1

PFH GAS PROTEIN BINDING INFORMATION

INTRODUCTION

This study was conducted to provide information on possible binding of perflexane (perfluorohexane) by serum proteins. Because of the low water solubility and high vapor pressure of perflexane, and also because of its lack of structural features that would be expected to lead to significant protein binding, it was predicted that the degree of binding would be small.

The analytical needs associated with this project were best met by using a [redacted] and by applying headspace [redacted] to determine the solubility of perflexane in a set of aqueous solutions including aqueous protein. Albumin, USP was selected as the model for serum protein.

The study was carried out at the by [redacted]

METHODS

A full report of the experimental details and results is attached as **Appendix A.**[1] Briefly, excess liquid perflexane was equilibrated with five different aqueous solutions at 37°C. The aqueous solutions were as follows: (1) distilled water, (2) phosphate buffered saline (PBS), (3) 1% Albumin, USP in PBS, (4) 5% Albumin, USP in PBS, and (5) 10% Albumin, USP in PBS. The perflexane-aqueous mixtures were centrifuged and samples of the clear aqueous phase were removed for analysis. Quadruplicate samples were sealed into headspace vials and analyzed for perflexane by [redacted]

RESULTS

The solubilities of perflexane in the aqueous solutions are listed in **Table I.** **Figure 1** is a plot of the solubility data; the points are the data given in **Table I** and the solid line represent the least squares linear fit.

Table I. Solubility of Perflexane in Aqueous Solutions

Solution	Perflexane Solubility (ppm)
Distilled water	8.3 ± 0.17
PBS	0.3 ± 0.3
1% Albumin, USP	8.6 ± 1.1
5% Albumin, USP	9.5 ± 1.8
10% Albumin, USP	12.2 ± 2.1

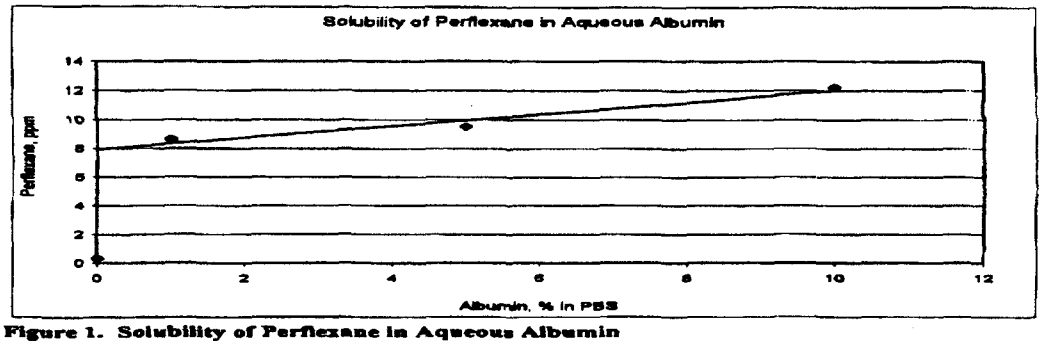


Figure 1. Solubility of Perflexane in Aqueous Albumin

DISCUSSION

The observed solubility of perflexane in water at 37°C is reasonable for this non-polar, non-hydrogen bonding molecule. The solubility drops by more than an order of magnitude when the ionic strength is increased by adding the salts that comprise PBS. This is an example of the "salting out" effect, which essentially makes the solvent water molecules less able to accept the very dissimilar perflexane molecules. Adding albumin to the PBS increases the solubility of perflexane. For 1% albumin in PBS, the perflexane solubility is essentially restored to what it was in distilled water. The solubility increments in the 5% and 10%

albumin - PBS solvents are rather small; a 10-fold increase in the albumin concentration increases the solubility by only about 40%.

The data allow calculation of the mole ratio of albumin to dissolved perflexane. The molecular weight of perflexane is 338, and that of albumin is 66000. For example, a liter of 1% albumin saturated with perflexane contains 10 grams of albumin (0.15 mmole/L) and 8.6 mg of perflexane (0.025 mmole/L). For this solution at saturation only 1 in 6 albumins binds a perflexane. The apparent binding efficacy of albumin decreases with increasing albumin concentration: in the 10% solution there are 42 albumin molecules per dissolved perflexane. The inconsistency of these results suggests that more than one mechanism may be involved, and that in addition to binding some perflexane, the presence of albumin may counteract the "salting out" effect noted earlier.

Possibly the best measure of the binding of perflexane by albumin is the slope of the least squares line, which is 0.4. (Figure 1) This slope describes protein binding over the range from 1% to 10% albumin at the physiological conditions set by PBS. The slope of 0.4 ppm perflexane/1% albumin corresponds to 1 perflexane per 128 albumin molecules.

CONCLUSION

The results suggest that the solubility of perflexane is very low, indicating a low affinity for protein binding.

REVIEWER'S COMMENT: The [redacted] analysis report (Reference #1) appears to be adequate; calibration or standard curve and [redacted] profiles look appropriate.

APPENDIX 2

PHARMACOKINETICS OF INTACT MICROSPHERES

Reference #2 used in the text Volume 8, page 5:

TITLE: Effects of Intra-Arterial Injection of AF0150 on Microhemodynamics in the Cremaster Muscle Model of Normal and Hyperlipidemic Rats

Introduction: AF0150 is an ultrasound contrast agent being developed by Alliance Pharmaceutical Corp. to enhance tissue echogenicity for the diagnostic assessment of organ perfusion, function, and pathophysiology (e.g., in echocardiography and radiology). AF0150 is comprised of perfluorohexane-filled flexible lipid shell microbubbles with a volume median diameter of 6 μm and a fraction (<0.64%) of microbubbles with diameter >10 μm. Since larger microbubbles are the same size dimension as capillary diameters, this study was undertaken to examine whether a "first pass" transit of a high dose of AF0150 microbubbles via intra-arterial (IA) injection would cause hemodynamic alterations in the microcirculation. This examination was carried out in the cremaster (skeletal) muscle of normal rats, and additionally in hyperlipidemic rats that display certain metabolic and hemodynamic characteristics of human cardiovascular disease (e.g., elevated cholesterol and triglyceride levels, hypertension). In some cases, acute inflammation of the muscle was induced to replicate features of microvascular dysfunction associated with cardiovascular disease.

Purpose: The purpose of this study was to assess microcirculatory flow dynamics in the cremaster muscle model, with and without induced acute inflammation, after an IA injection of a high dose of AF0150 in normal and hyperlipidemic rats.

Methods: Forty-eight (48) anesthetized, room-air free breathing male rats (normal Wistar and genetic hybrid hyperlipidemic) were prepared for systemic blood pressure measurements and bolus administration of AF0150 (40 mg/kg; 320x planned clinical dose for echocardiography [0.125 mg/kg]) and saline (vehicle, 2 mL/kg) via a carotid artery catheter advanced into the aorta. High-speed camera imaging was used to visualize individual AF0150 microbubbles traversing the cremaster muscle microcirculation (1000x). *(continued)*

Methods (continued): intravital microscopy of the muscle was performed to assess the diameter and velocity changes in arterioles and venules and the number of perfused capillaries in 2 fields of view. intravital microscopy was used for off-line analysis of the velocity of red blood cells (RBCs, obtained from 13 donor normal rats) in a subset of these capillaries. Additionally, the muscle was scanned at various times after AF0150 injection for the presence of microbubbles that had lodged, adhered, aggregated, and coalesced (1360x). Acute inflammation of the muscle was induced with topical application of platelet-activating factor (10^{-8} M) in experiments examining capillary flow dynamics. At the completion of some experiments, stiff 10-μm polystyrene microparticles were injected IA to demonstrate that the cremaster muscle could be plugged. Statistical analyses were performed on the data with $p<0.05$ considered as statistically significant.

Results: Hyperlipidemic rats had statistically significantly elevated cholesterol, triglyceride, and glucose levels as compared to normal rats. IA administration of AF0150 and saline caused no statistically significant changes in mean arterial pressure and heart rate over the 10-minute observation period after injection in normal (n=27) and hyperlipidemic (n=21) rats. High-speed camera imaging for 8 seconds at the end of AF0150 injection in normal (n=6) and hyperlipidemic (n=5) rats showed individual microbubbles of various sizes ≥ 3 μm (minimum optical resolution) with a distinct optical appearance moving freely with the capillary RBC flow (unless lodged in a capillary). In separate experiments in both animal types (n=2 each), scanning of the muscle in the first 5 minutes after AF0150 injection revealed a low incidence of transient lodging of microbubbles in capillaries. There were no notable statistically significant changes with AF0150 over time and compared to saline in arteriolar and venular diameters and velocities in normal rats (n=6). Moreover, there were no statistically significant effects of AF0150 over time and compared to saline in the number of perfused capillaries and capillary RBC velocity, with and without induced acute inflammation of the muscle, in normal and hyperlipidemic rats (n=6-8/group). Scanning of 33 to 50% of the muscle starting at 15 minutes after AF0150 injection revealed no lodged, adhered, aggregated, or coalesced microbubbles in the microvessels. The cremaster muscle model was demonstrated to be a suitable model to determine whether AF0150 microbubbles become lodged in capillaries since the capillaries could be plugged by stiff 10-μm polystyrene microparticles.

Conclusion: In this cremaster muscle model in normal and hyperlipidemic rats, there were no statistically significant effects of a high dose (40 mg/kg) of IA-injected AF0150 on the microcirculation even in the presence of acute muscle inflammation. Individual AF0150 microbubbles had a very low incidence of lodging in capillaries immediately after, and for the first few minutes after injection. This phenomenon was transient with no residual microbubbles in capillaries by 15 minutes after AF0150 injection. Additionally, there was no evidence that microbubbles adhered, aggregated, or coalesced in microvessels. Thus, AF0150 is safe for patients, including those with cardiovascular risk factors, from the perspective that no untoward alterations in the microcirculation were associated with its use.

ER-00-01

REVIEWER'S COMMENT ON REFERENCE #2:

The non-clinical animal study report appears to be acceptable and adequate. This study was also submitted in the Non-clinical section. Thus, the reader should be referred to the Pharm/Tox review.

Reference #3 used in the text Volume 8, page 6:

APPEARS THIS WAY ON ORIGINAL

Research and Development Technical Report

DIVISION: . **DEPARTMENT:**

REPORT NUMBER: RE-99-46 **REPORT DATE:** September 14, 1999

TITLE: Effect of Ultrasound Exposure on the Size Distribution of AF0150 Microbubbles Under *in vitro* Simulated Physiological Conditions

ABSTRACT: The purpose of this study was to measure the effect of clinically relevant ultrasound exposure on the size distribution of AF0150 microbubbles under *in vitro* conditions that simulated an *in vivo* vascular system. To measure the effect of sustained ultrasound power levels on the size distribution of AF0150 microbubbles *in vitro*, a model resembling the vascular system was built to simulate the key physical and rheological features of an artery or heart chamber with blood flowing through it. A 37°C, 6% bovine albumin solution was used to simulate blood. The albumin solution was circulated by means of a peristaltic pump through [redacted] tubing that was imaged with a clinical ultrasound instrument while the entire system was maintained at 37°C. The mean circulation time was set to 90 seconds, therefore, on average, all the suspended AF0150 microbubbles passed through the imaged region every 90 seconds, as would be the circulation rate for cardiac or major vessel imaging in a clinical patient. A longitudinal section of the tubing was imaged to maximize the ultrasound exposure time of a circulating microbubble. The chosen AF0150 dose (0.63 mL constituted AF0150 in 225 mL 6% albumin solution) was equivalent to a 4 mg/kg dose of AF0150 (the maximum clinical dose) in a 70 kg human with a 5-L blood volume. Bubble size distribution measurements (count and diameter) were made in separate runs on samples taken after 0, 2, 4 and 6 minutes of ultrasound exposure with ultrasound mechanical index (MI) settings of 0.0, 0.2, 0.5, 1.0 and 1.7. These settings span the range of the [redacted] machine that was used in this study. Initially, while the 6% albumin solution was pumped through the system at 37°C, the transducer (4.0 MHz) was in frozen mode (shut off) and positioned 5 cm from the far inside wall of the silicone tubing. The 0.63-mL sample of constituted AF0150 was injected into the 6% albumin solution and allowed to circulate for 90 seconds before continuous exposure to ultrasound power (transducer turned on). Aliquots of 0.5 mL were removed at the designated time points and immediately diluted into 10 mL of purified water and presented for analysis for bubble size distribution using a [redacted] system. The ultrasound-exposed albumin control sample had a large median diameter of 14.1 to 19.6 µm representing non-bubble background. The ultrasound-exposed samples containing constituted AF0150 changed very little in size (median diameter range of 5 to 8 µm), showing only a slight shift to smaller bubble diameters with the exception of the highest power samples. Total bubble counts for the ultrasound-exposed albumin control sample yielded nearly consistent background particle counts that were equal to approximately 20% of the initial AF0150 microbubble concentration. The unexposed (0.0 MI) and 0.2 MI AF0150 samples had relatively constant counts over the 6 minute exposure time. With a MI of 0.5, there was a reduction in bubble counts starting after 4 minutes of exposure. Mechanical indices of 1.0 and 1.7 produced more dramatic drops. The ultrasound-exposed albumin control sample had low particle counts in the 3 to 10 µm range. The effect of exposure to ultrasound power on AF0150 microbubble counts was not discernible in the 0.0 and 0.2 MI samples; however, the effects were slightly notable at 0.5 MI and were most notable at the 1.0 and 1.7 MI exposures. Due to the low number of particles counted in the > 10 µm bubble measurement over the 6 minute ultrasound exposure time, only a general downward trend in bubble counts was observed, and no effect from power was discernible. The volume median diameter of AF0150 microbubbles remained consistent over the 0 to 6 minute interval for the various MI settings tested. While the system attempted to mimic *in vivo* physiological conditions, it did not simulate pulsatile blood pressure, lung filtration, or lung gas exchange. The results from this study indicate that circulation of AF0150 microbubbles at 37°C in a 6% albumin solution and in the sustained presence of clinically relevant ultrasound power levels results in slow dissolution of microbubbles at lower MI settings and accelerated dissolution at the highest MI settings. No generation of microbubbles in any size range was observed (> 10 µm, 3 to 10 µm, or total bubble counts).

REVIEWER'S COMMENT ON REFERENCE #3: ***As stated by the Applicant, it is noted that this in vitro study does not adequately mimic the in vivo situation (pulsatile blood pressure, lung filtration, or lung gas exchange, etc.). Additionally it is noted from the study report that the ultrasound-exposed albumin control sample had a large median diameter of 14.1 to 19.6 µm. The Applicant stated that this***

represents "the non-bubble background state." This information is somewhat perplexing since the measured microbubble median diameter is in the 3 – 10 μm range. However, it is possible that this may be due to the nature of the bubble counting machine ([redacted]), which the particles are measured (or counted) as they traverse through an orifice of the machine. The detected differences in the electrical current applied at the orifice are converted to counts or into measurement (e.g., volume diameter). In all, the study report appears acceptable.

Reference #4 used in the text Volume 8, page 6:

Research and Development Technical Report

DEPARTMENT: **STUDY DATES:** November – December, 1998
REPORT NUMBER: EB-98-22 **REPORT DATE:** June 16, 1999

TITLE: Effect of Hypertension on AF0150-Enhanced Doppler Signal in Rabbits and Video Intensity Levels in Swine

Introduction: Ultrasound imaging is a technique currently used to assess cardiac function (e.g., echocardiography). AF0150 is an intravenous (IV) contrast agent being developed to enhance ultrasound images. The stability of AF0150 microbubbles *in vivo* is important for facilitating contrast-enhanced ultrasound imaging. Applied external pressure affects microbubble stability. High pressures generated within the myocardium and/or the systemic vasculature in patients with systemic hypertension may affect the stability of the microbubble and, therefore, contrast enhancement with AF0150.

Purpose: The purpose of the present study was to assess the effect of changes in systemic arterial blood pressures following IV administration of AF0150 on both Doppler signal responses in rabbits and left ventricular cavity video intensity levels in swine.

Methods: In Part I, seven (7) rabbits (3.5 ± 0.6 kg) were anesthetized, intubated and instrumented for hemodynamic monitoring and AF0150 administration. A [redacted] Doppler flow cuff transducer [redacted]) was used for the measurement of carotid artery blood flow. During control (normotension) and hypertension (IV phenylephrine-induced increase in systemic vascular resistance), Doppler signal responses were measured following bolus IV injections of AF0150 at either 0.2 mg/kg (n=5) or 1.0 mg/kg (n=4). Two of the rabbits were used in both AF0150 dosing protocols. In Part II, four (4) swine (21.9 ± 1.4 kg) were anesthetized, intubated, mechanically ventilated, and instrumented for hemodynamic monitoring and AF0150 administration. A 3.2 MHz ultrasound probe was positioned over the heart (closed chest) to obtain echocardiographic images using the [redacted] ultrasound imaging system [redacted] During control and hypertension, images of the left ventricular cavity were collected during fundamental ultrasound imaging at three different mechanical indices (MI: 0.2, 0.6, 1.0) at baseline and following bolus IV injections of AF0150 (0.25 mg/kg).

Results: Phenylephrine infusion maintained a hypertensive state with, on average, a 138% and 42% increase in systolic arterial pressure for rabbit and swine, respectively, with no significant effect on heart rate. Further, phenylephrine increased left ventricular pressure by approximately 44% in swine. The clinical level of hypertension had no significant effect on carotid artery Doppler flow signal in the rabbit following bolus IV injections of AF0150 at either 0.2 mg/kg or 1.0 mg/kg. Hypertension also had no significant effect on video intensity levels in the left ventricular cavity of swine following 0.25 mg/kg bolus IV injections of AF0150.

Conclusions: Results from this study indicate that AF0150-induced contrast enhancement is not affected by high left ventricular and arterial pressures associated with systemic hypertension. These data suggest that AF0150, administered at doses ranging from 0.2 to 1.0 mg/kg, remains stable under these conditions and can be used effectively for imaging patients with hypertension.

REVIEWER'S COMMENT ON REFERENCE #4: Overall, the study report appears to be adequate. It should be noted that, for the 0.2 mg/kg dose rabbit group, there was a noticeable difference in the Doppler signal response measured for the 20-40 sec group between the control and the hypertensive groups. The impact of the difference in this group can not be discussed due to lack of information, however, this difference may not be critical since other Doppler groups should no differences between the control and hypertensive groups.

Reference #5 used in the text Volume 8, page 6:

Research and Development Technical Report

DEPARTMENT: **STUDY DATE:** May 14, 1998
REPORT NUMBER: EB-98-16 **REPORT DATE:** February 3, 1999

TITLE: Effect of External Pressure Applied to Constituted AF0150 on Doppler Response in Rabbits

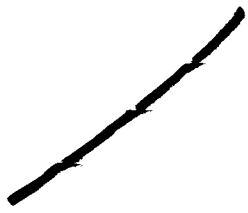
Background: Ultrasound imaging is a technique currently used to assess cardiac function and perfusion (echocardiography), vascular patency, organ perfusion and tissue characterization. Much research has focused on the development of a safe and effective intravenous contrast agent to enhance sonographic images. AF0150, an ultrasound contrast agent being developed by Alliance Pharmaceutical Corp., is a sterile, nonpyrogenic powder comprising hollow microspheres that contain perfluorohexane (PFH) vapor and nitrogen gas. When constituted with sterile water, AF0150 forms a dispersion of highly echogenic microbubbles in solution. The stability of the microbubbles is highly dependent on the balance of forces surrounding the gas-filled bubbles. External pressures applied to the bubbles (e.g., during administration) may alter the efficacy of AF0150.

Purpose: The purpose of the present study was to determine the extent to which varying external pressures, applied to constituted AF0150, affect efficacy of this agent as measured by Doppler flow signal in the rabbit.

Methods: One (1) rabbit was anesthetized, intubated via tracheotomy and allowed to free-breathe. The jugular vein was cannulated for the administration of AF0150. Heart rate was monitored using a pulse oximeter placed on the ear. A [redacted] Doppler flow cuff transducer was placed around the carotid artery for the measurement of carotid artery blood flow. AF0150 constituted with sterile water for injection (WFI) was subjected to 5 different pressures (760, 842, 925, 1008 and 1090 mm Hg). Following baseline measurements, Doppler signal data was evaluated in response to 1.0 mg/kg bolus injections of AF0150. Mean Doppler data ± SEM was calculated for injections administered at each pressure level (total of 15 injections).

Results: Compared to ambient pressure levels (total pressure = 760 mm Hg), total pressures of 842, 925 and 1008 mm Hg applied to constituted AF0150 had no effect on the Doppler signal response to a 1 mg/kg bolus injection of AF0150 for 2 minutes. However, at the highest level of pressure applied to constituted AF0150 (total pressure = 1090 mm Hg), peak Doppler response was reduced by approximately 21%.

Conclusions: The results of this study suggest that high levels of external pressure applied to constituted AF0150 may affect contrast enhancement as measured by Doppler signal analysis.



REVIEWER'S COMMENTS ON REFERENCE #5: Overall the study report appears to be adequate. It is noted that one rabbit was used in the experiment; this rabbit received a total of 15 injections. It is also noted that at the highest level of pressure applied to constituted Imavist (total pressure was 1090 mm Hg), peak Doppler response was reduced by approximately 21 %.

APPENDIX #3

DMPC METABOLISM

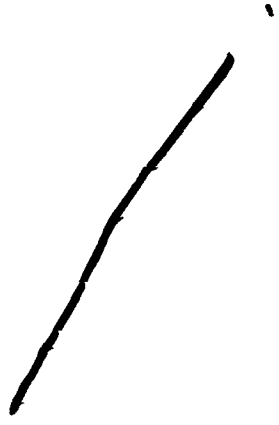
Reference #6 used in the text Volume 8, page 7:

Research and Development Technical Report

DIVISION: **DEPARTMENT:**
REPORT NUMBER: PRD-01-15 **REPORT DATE:** May 1, 2001

TITLE: Effect of Phospholipase D on Natural and Semi-synthetic Phosphatidylcholine

ABSTRACT: AF0150 contains dimyristoylphosphatidylcholine (DMPC), which is a semi-synthetic phosphatidylcholine. To demonstrate *in vitro* that DMPC metabolism follows the same pathway as that of naturally occurring phospholipids, DMPC and egg yolk L-alpha-phosphatidylcholine (EYP) were each exposed to enzymatic degradation by phospholipase D. Degradation by phospholipase D was selected because it attacks the characteristic phosphatidylcholine backbone, rather than the less specific hydrolysis of ester linkages to release the fatty acid side chains. The results of this study demonstrate that the *in-vitro* susceptibility of DMPC to degradation by phospholipase D is not different from that of EYP, a naturally occurring phosphatidylcholine.



REVIEWER'S COMMENT: The study report appears to be adequate.

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/s/

David Lee
11/26/01 05:27:54 PM
BIOPHARMACEUTICS

John P. Hunt
11/27/01 08:18:19 AM
BIOPHARMACEUTICS

Clinical Pharmacology and Biopharmaceutics Review

NDA:	**21-191**
Type of Submission:	1S
Generic Name:	**AFO150**
Other Name(s):	Perflexane-phospholipid microbubbles
Formulation; **Prescription Status;** **Strength** **Route of Administration**	A kit containing a vial of 200 mg of powder, 20 ml of sterile water for injection RX 200 mg intravenous
Brand Name:	**Imavist™**
Sponsor:	Alliance Pharmaceutical Corp., 3040 Science Park Rd, San Diego, CA
Submission Date(s):	Date of Submission: 10/11/99 Stamp Date: 10/14/99
Review Date:	8/11/00
Reviewers:	David J. Lee, Ph.D.

A. INTRODUCTION

Alliance Pharmaceutical Corp. has submitted a New Drug Application (21-191) on 10/11/99, and is seeking approval of Imavist™ (Imavist) as an ultrasound drug.

Imavist *(AFO150)* is indicated for use in patients with suboptimal echocardiograms to opacify the left ventricle (LV). Imavist, microbubbles, is an echogenic intravenous ultrasound contrast agent that improves the ability to assess cardiac function through improved delineation of the LV endocardial borders and improved ability to evaluate segmental wall motion (SWM). The recommended dose is 0.125 mg/kg (0.00625 ml/kg or 6.25×10^6 microbubbles/kg) administered as an intravenous bolus over a period of not less than 10 seconds.

Clinical trials with Imavist were developed and performed under IND [redacted] The to-be-marketed formulation was used in all clinical studies. To support the Human Pharmacokinetics and Bioavailability section of NDA 21-115, the Applicant has submitted 3 studies (See Pharmacokinetics Section of this review):

Study 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 AFO150 in Healthy Adult Volunteers);
Study IMUS-001-USA (A Single-Blind, Dose-Ranging, Placebo-Controlled, Safety, and Contrast-Enhancement Study in Normal Volunteers Receiving AFO150 Administered by Intravenous Injection);
Study IMUS-018-USA (An Open-Label Dose- Titration Study of 3 Doses of AFO150 in the Echocardiographic Assessment of Patients with Left Ventricular Dysfunction).

B. QUESTIONS

What is Imavist?

Imavist for injection, is a sterile nonpyrogenic powder (dimyristoylphosphatidylcholine (DMPC) phospholipid, starch, poloxamer 188) with a perflexane, an n-perfluorohexane, headspace that, upon constitution, forms microbubbles used for contrast enhancement during ultrasound imaging procedures. Microbubbles are filled with fluorohexane gas that is presumed to provide a highly compressible surface which reflects ultrasound waves.

How does Imavist work?

Imavist is presumed to increase the ultrasound reflectivity of blood which enhance the ultrasound signals within a vessel, tissue or cavity. As with other microbubbles, Imavist relies on the compressibility of gases (perflexane vapor and air) enclosed within microbubbles to enhance the reflectivity of ultrasound waves. In the past some microbubbles used air only as enclosed gas within microbubbles.

The diluted vapor of perflexane is used to provide longevity to the microbubbles, as compared to that of air-filled microbubbles; additionally, the diluted perflexane vapors are presumed to increase the available time for contrasted imaging while limiting the potential side effects (bubble growth). For further information please see *BACKGROUND, Theoretical: Microbubble Thermodynamics - Microbubble Diffusion Equilibrium* section.

Is there a PK/PD relationship for Imavist?

There is no PK/PD data for Imavist. A phase I pharmacokinetic study, *Study IMUS-012-USA*, examined Imavist at a dose of 4 mg/kg, which is 32-fold higher than the recommended dose. Additionally there is no dose ranging study to address linearity or accumulation issues. However, there is pharmacodynamic dose-response information from *Study IMUS-018-USA (See below, Optimal dose for Imavist question).*

What is the optimal dose for Imavist?

The recommended dose is 0.125 mg/kg (0.00625 ml/kg or 6.25×10^6 microbubbles/kg) administered as an intravenous bolus over a period of not less than 10 seconds.

From submitted studies in Section 6, a phase I/II study, *Study IMUS-018-USA*, examined AFO150 at doses of 0.125, 0.25 and 0.5 mg/kg. The Applicant stated that 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., ejection fraction 20% to 40%). Little improvement in opacification was derived from using higher AFO150 doses (i.e., 0.25 and 0.5 mg/kg). Nevertheless, a dose-dependent increase in both duration of attenuation and duration of useful contrast enhancement was observed with increasing AFO150 dose (from 0.125 to 0.5 mg/kg).

Eventually, in phase III studies, the recommended dose, 0.125 mg/kg, was studied. With information presented in Section 6 (PK study was conducted with 4 mg/kg), and since there is no PK/PD data, it is difficult to comment if the 0.125 mg/kg is the optimal dose for Imavist.

Is metabolism information available for phospholipid or surfactant, i.e., DMPC used in the microbubbles?

This surfactant is a semi-synthetic compound representing a member of compounds (phospholipids) that occur naturally in the environment. It is reported that the amount of DMPC that would be administered in a single dose is approx. 400- to 1000- fold less than the amount of phospholipids naturally present in the serum.

The literature data* showed that phospholipids are nontoxic. Phospholipids, which are the main constituents of natural cellular membranes, have been used in a wide variety of pharmaceutical, nutritional, and cosmetic products.

The metabolism of phospholipids involve the phospholipases A1, A2, C, and D. Phospholipases A1 and A2 are responsible for cleaving the fatty acid ester bonds, while phospholipases C and D cleave at the head group phosphoester bonds. The hydrolysis products formed are available for further degradation or

for reincorporation into the same or other lipids. Thus, phospholipases A1 and A2 release fatty acids that can be degraded by beta-oxidation or reincorporated into phospholipids, triglycerides, sphingomyelin, etc. Phospholipase C forms diacylglycerol, which can act as a mediator of cell signal transduction and/or may be further metabolized by diacylglycerol lipase to generate free fatty acids. Phosphatidic acid, which is initially generated by the action of phospholipase D, is also active in cell signal transduction and can be further metabolized to diacylglycerol.

For comparison, amount of DMPC contained in a 10-mL vial of AF0150, is approximately 11 mg; the level of phospholipids present in the serum is 150 to 380 mg/dL. Presuming an adult human has approximately 3 L of serum, the total amount of DMPC contained in a vial of AFO150 would contribute approximately 11 mg *DMPC/3* L of serum or 0.37 mg/dL, which is approximately 400- to 1000-fold less than that normally present in the serum. However, it should be noted DMPC is a 'semi-synthetic' phospholipid, and that it is not the magnitude of the amount that warrants safety, but, the 'sensitivity' derived from the immune response. Unfavorable immune response may develop from the "nominal" amount of semi-synthetic phospholipids. The reader should seek for Pharm/Tox review on this matter.

Is metabolism information available for perflexane (PFH) gas?

Several literature articles* have reported that no metabolites of PFH were detected in various tissues or urine post administration. Since PFH has a relatively high vapor pressure and a low boiling point, it is expected that PFH would be eliminated rapidly as an unchanged drug in expired air.

*Literature references:

1. Dodd DE, Brashear WT, Vinegar A. Metabolism and pharmacokinetics of selected halon replacement candidates. *Toxicol Lett.* 1993; 68: 37-47.
2. Parnham MJ, Ghyczy M, Wendel A. Environmental effects of phospholipids used in liposomes: relevance for safety. In: Barenholz, Y, Lasic, DD, eds. *Handbook of Nonmedical Applications of Liposomes.* New York, NY: CRC Press; 1996; 81-94.
3. Lopez-Berestein G, Kasi L, Rosenblum MG, et al. Clinical Pharmacology of 99mTc-labelled liposomes in patients with cancer. *Cancer Res.* 1984; 44: 375-378.
4. Allen, TM. Toxicity and systemic effects of phospholipids. In: Cevc G, ed. *Phospholipids Handbook.* New York, NY: Marcel Dekker; 1993; 801-816.
5. Scherphof, GL. Phospholipid metabolism in animal cells. In: Cevc G, ed. *Phospholipids Handbook.* New York, NY: Marcel Dekker; 1993; 777-800.
6. Lehmann HP, Henry, JB. SI units. In: Henry JB, ed. *Clinical Diagnosis and Management by Laboratory Methods.* 18th ed. Philadelphia, PA: W.B. Saunders Company; 1991; 1370-1382.
7. Alliance Pharmaceutical Corp., Internal Report IMUS-041-ADME. Elimination of perfluorohexane from AFO150 expired air in rats with pharmacokinetic evaluation of perfluorohexane in blood. August 1999. *(Included in Section 5, Nonclinical Pharmacology and Toxicology)*
8. Wei K, Jayaweera AR, Firoozan S, et al. Basis for detection of stenosis using venous administration of microbubbles during myocardial contrast echocardiography: bolus or continuous infusion? *J Am Coll Cardiol.* 1998; 32:252-60.

What is the conclusion from Study 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 AFO150 in Healthy Adult Volunteers)?

The Applicant stated that AF0150 was well tolerated in this study. The disappearances of PFH from both blood and expired air appears to follow first-order kinetics. Approximately 75% of the PFH was eliminated through expired air within 3 hours post injection, and 87% was eliminated within 24 hours. PFH clearance values obtained from expired air and blood were comparable, however, more variation of blood clearance was noted. There appears to be statistically significant differences in the Kel of PFH from expired air between the genders, however, the clinical significance is unknown. There were no statistically differences between the genders in the rate and extent of PFH exposure in blood.

Mean ± SD and Coefficient of Variance (%) of PFH in Blood and Air PK parameters

Parameter	Blood	Expired Air
AUC 0-24 (ng*hr/ml)	3.7 ± 1.4 (38.9)	3.3 ± 0.7 (21.2)
AUC 0-inf (ng*hr/ml)	4.2 ± 2.2 (52.4)	3.4 ± 1.3 (20.9)
Vd (L)	2960.0 ± 2595.2	NA
Tmax (min)a	2.0	1.5
Cmax (ng/ml)	28.0 ± 28.6	27.8 ± 8.3
T1/2 (hrs)	5.3 ± 6.1	9.0 ± 5.0
MRTlast (hrs)	2.7 ± 3.6	1.6 ± 0.5
CL (L/hr)	716.3 ± 735.3	603.7 ± 93.9
%PFH (0-3 hr)	NA	74.6 ± 17.6
%PFH (0-inf)	NA	87.2 ± 19.3

a: median Tmax
NA: not applicable

What additional information is needed for Imavist?

Pharmacokinetic information is needed for the patients with pulmonary deficiency, due to the fact that PFH is eliminated primarily via the lungs. Additional information is requested under Comments to the Applicant section.

C. TABLE OF CONTENTS

D. BACKGROUND

1. CLINICAL PROGRAM DEVELOPMENT RATIONALE

In general, ultrasound contrast agent development has focused on increasing the ultrasound reflectivity of blood, thereby enhancing the signals in proportion to the amount and velocity of moving blood within a vessel, tissue, or cavity, which allows for a more accurate assessment of anatomic definition. In this regard, the microbubble must be of a size to allow it to pass unimpeded through the pulmonary circulation, be nontoxic, and remain in concentrations that permit vascular and tissue augmentation of signals for a period of time sufficient to obtain the diagnostic information.

To date, the ability to quantitate cardiac function has been limited by the inability to define precisely the endocardial border around the entire perimeter of the ventricle on images of the heart. Therefore, the Phase III cardiology program was designed to demonstrate the ability of AFO150 in contrast echocardiography:

1) To improve the ability to assess cardiac function by enhancing endocardial border delineation, improving the determination of ejection fraction, and improving the ability to assess and evaluate segmental wall motion, and

2) To obtain an acceptable safety profile and minimal risk to the patient.

2. Product Design Rationale

As with other microbubbles, AFO150 relies on the compressibility of gases (perflexane vapor and air) enclosed within microbubbles to enhance the reflectivity of ultrasound waves. In the past some microbubbles used air only as enclosed gas within microbubbles.

The Applicant stated that AFO150, the diluted vapor of perflexane is used to provide longevity to the microbubble, compared to that of an air-filled microbubble. The Applicant further speculated that diluted perflexane vapors increase the available time for contrasted imaging while limiting the potential side effects (bubble growth), as seen in other agents in this class.

The Applicant stated that the microbubble is the active moiety of AFO150; two critical components (perflexane vapor and DMPC) help define, maintain, and/or stabilize the microbubble.

Rationale for Perflexane and DMPC usage

Perflexane inside the microbubble must be present in the gaseous state to exert the stabilizing pressure that resists collapse pressures exerted on the microbubble. Perflexane has a vapor pressure of 223 torr at 25°C, which is more than nine times greater than that of room temperature water.

Vials are filled with the hollow, porous micro spheres ([redacted]) and then gassed with a perflexane vapor/N2 mixture (N2 is a diluent for perflexane). During the gassing step, the voids of the microspheres are filled with the perflexane vapor/N2 mixture. The perflexane vapor/N2 mixture inside the microspheres is trapped when the microbubbles are formed upon constitution with Sterile Water for Injection (SWFI), USP. After the microbubbles have been formed, the N2 in the microbubbles quickly reaches equilibrium (through the gas permeable DMPC membrane) with the air in the water. The water-insoluble perflexane resists dissolving out through the membrane, thereby providing an extended lifetime to the microbubble.

The concentration of perflexane was chosen to ensure that the perflexane exists as a vapor and will not condense at the recommended temperatures of storage, constitution, and administration of AF0150. Vials are filled with a mixture of perflexane vapor in N2, which contains nominally 2.4 mg perflexane/mL (approximately 33 mg perflexane per vial).

The second critical component of the AFO150 microbubble is that which controls the membrane surface tension. The surfactant, DMPC, was added to lower surface tension and has been shown experimentally to control the surface tension of the microbubble. Therefore, perflexane vapor and DMPC are the critical components of the AFO150 microbubble.

3. Theoretical : Microbubble Thermodynamics - Microbubble Diffusion Equilibrium

The Applicant provided the following information regarding microbubbles dynamics:

The internal gas pressure within the microbubbles after constitution is higher than in the vial headspace due to the membrane surface tension. The membrane surface acts like the skin of a balloon, pressuring the gases inside (Laplace pressure). The DMPC film on the bubble minimizes surface tension, but the surface tension pressure is still appreciable (calculated at 104 torr for a typical AF0150 microbubble). After injection into the bloodstream, the microbubble has an additional blood pressure force driving it toward dissolution. The blood pressure (approximately 100 torr) is additive to the surface tension pressure, raising the internal total gas pressure of the microbubble. The cumulative pressure above atmospheric inside the microbubble is approximately 204 torr. Thus, the total gas pressure inside the microbubble is equal to 964 torr (204 torr plus 760 torr of ambient atmospheric pressure).
Blood is saturated with air in the lungs at nearly one atmosphere (760 torr). If microbubbles are filled only with air (i.e., no perflexane vapor is present in the microbubble), the air inside the bubble rapidly diffuses out, due to the 204 torr excess pressure, causing the microbubble to shrink and collapse.

When an AF0150 microbubble is injected, the total gas pressure within the microbubble also becomes 964 torr, consisting of 760 torr partial pressure for air and 204 torr of perflexane vapor partial pressure (Figure I, Panel B). In this case, the low water solubility of perflexane vapor prevents the dissolution of air, because the partial pressure of air inside the microbubble is maintained at 760 torr. Thus, perflexane vapor prevents rapid microbubble shrinking and collapse. Equilibrium is established whereby the water-soluble gasses from SWFI or blood diffuse in and out of the bubble; simultaneously, the perflexane vapor provides opposing pressure to the membrane surface tension and blood pressure forces that push the bubble towards collapse.

Low, but finite solubility of perflexane allows it to leave the microbubble over time, exiting the body through the lung. As perflexane vapor slowly dissolves from the microbubble, its internal support pressure reduces, causing the water-soluble gasses to dissolve and re-equilibrate with blood. This re-equilibration decreases the size of the microbubble, until the perflexane vapor is concentrated to the point where it again matches the dissolution forces. With respect to the bubble size, the membrane surface tension pressure is inversely proportional to the bubble diameter, which means the membrane surface tension pressure increases as the microbubble continues to slowly shrink. Increases in the total internal pressure continue to be counterbalanced by an increase in perflexane vapor partial pressure inside the microbubble. In all, perflexane provides microbubble stability during bubble dissolution.

After more than 90% of the perflexane vapor has left the microbubble, the perflexane vapor concentration inside the microbubble eventually reaches the saturated vapor pressure of perflexane at body temperature, causing it to condense into a submicron emulsion particle. As perflexane is a liquid at body temperature (boiling point of 57°C), these submicron liquid droplets cannot re-vaporize into new microbubbles. Regardless of the form (vapor or liquid), all perflexane leaves the body via the lung.

The diluted perflexane vapor vial headspace concentration is targeted at 2.4 mg perflexane/mL (17% v/v) which yields a microbubble partial pressure of 204 torr once injected. Controlling the amount of perflexane in the product restricts the potential for bubble growth. By contrast, if microbubbles were filled with a pure perfluorocarbon (PFC) gas or vapor (100% v/v), once injected they would absorb gases from the blood to a partial pressure of 760 torr. Since the solubility of most PFCs in water is low, this would result in a substantial increase in microbubble size, in order to maintain total pressures equal to the sum of surface tension, arterial and atmospheric pressures. Additional PFC from a volatile emulsion would cause much greater growth.

In conclusion, the Applicant stated that the perflexane vapor concentration in AF0150 was selected to be the amount that stabilizes the microbubbles after injection, without the potential for microbubble growth. Once constituted, AF0150 contains approximately 0.01% w/v perflexane (100 µg/mL), the minimal, controlled amount required to stabilize the approximate 9.8 x 10^8 microbubbles/mL that it contains.

E. GENERAL REVIEW COMMENTS

1. PFP GAS PROTEIN BINDING INFORMATION

There is no PFH gas binding and distribution information in the submitted application. The Applicant should submit any supporting data, e.g., in vitro, on PFH binding to proteins. In the past, perfluorocarbon gas binding information has been asked from the applicants. It should be noted, however, that literature information suggests that perfluorocarbon gases appear not to bind to plasma proteins.

2. PHARMACOKINETICS OF DMPC, THE MICROBUBBLE SHELL

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. The Applicant stated that surfactant is a semi-synthetic compound representing a member of compounds (phospholipids) that occur naturally in the environment. Additionally, the Applicant reported that the amount of DMPC that would be administered in a single dose is approx. 400- to 1000- fold less than the amount of phospholipids naturally present in the serum. This Reviewer agrees with the Applicant's comment. However, the Applicant should submit any supporting data, e.g., in vitro metabolism, on DMPC phospholipid. Again, in the past, phospholipid metabolism information has been requested and submitted by the applicants.

3. PHARMACOKINETICS OF THE *INTACT* MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of Imavist microbubbles, i.e., the "intact PFH- filled" microbubbles. However, the Applicant did assess the pharmacokinetics of PFH gas, some of which may or may not have been encapsulated.

Currently this reviewer is not aware of any analytical assay method, which could be utilized to detect this product's microbubbles in vivo. Therefore, it may not be possible at this time to characterize "intact" microbubbles in vivo due to the lack of assay methodology. However, the Applicant is encouraged to explore in vitro methods to provide information on the microbubbles in terms of microbubble "fragility and stability." One such in vitro method that can be explored is the microbubble "fragility" test: Addition of the microbubbles in blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. In addition, the Applicant may explore relevant animal models (e.g., microscopic examination of nail-bed capillary or cannulated cat mesenteric artery), if any. The Applicant is encouraged to correspond with the Pharm/Tox review team to explore the feasibility of using animal models to obtain microsphere fragility information.

<u>Toxicity due to the intact microbubbles</u>
There are pharmacology and toxicology safety concerns due to the intact microbubbles. At this time the reader is referred to the Pharm/Tox Review and encouraged to follow the Pharm./Tox concerns outlined within the text.

4. Blood Assay

It appears that QC was tested with only one QC concentration value, ——— ng/ ——ml of blood. Additionally, it appears that samples were analyzed at three different starting days, 6/29, 7/6, and 7/12.

The Applicant needs to submit the standard curves and QC runs from days 7/6 and 7/12. Additionally, profiles from blood assay should be submitted (as in the expired air samples).

However, the assay methodology appears acceptable from the submitted data (standard curve generation, peak separation between PFH and [redacted] and repeated measurement of [redacted] ng/[redacted] ml of blood, etc.)

5. Expired air Assay

As stated above in the blood samples, it appears that QC was tested with only one QC concentration value, [redacted] ng/[redacted] ml of blood. The Applicant needs to submit a table of calculated PFH air concentration data, as in the vol. 35, p. 206. The submitted table is incomplete.

However, the assay methodology appears acceptable from the submitted data (standard curve generation, peak separation between PFH and [redacted], and repeated measurement during the sample analysis.

6. Pharmacokinetic information in patients with pulmonary deficiency

Pharmacokinetic information is needed for the patients with pulmonary deficiency, due to the fact that PFH is eliminated primarily via the lungs. Additional information is requested under Comments to the Applicant section.

F. COMMENTS TO THE APPLICANT

Since there are issues that need to be sufficiently addressed by the Applicant, the following comments should be forwarded to the Applicant, as appropriate.

1. PFP GAS PROTEIN BINDING INFORMATION

There is no PFH gas binding and distribution information in the submitted application. The Applicant should submit any supporting data, e.g., in vitro, on PFH binding to proteins.

2. PHARMACOKINETICS OF INTACT MICROBUBBLES

The NDA submission does not contain any data to describe the "fate" of Imavist bubbles, i.e., the "intact PFH- filled" microbubbles. Ideally "intact" microbubble pharmacokinetic information is needed with respect to the dosage proposed in the package insert. The Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method in order to definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. In addition, the Applicant is encouraged to explore in vitro methods to provide information on the microbubbles in terms of microbubble "fragility and stability." One such in vitro method that can be explored is the microbubble "fragility" test: Addition of the microbubbles in blood or plasma followed by microscopic examination to gather information in terms of microbubble population, the rate and time of disappearance, duration of microbubble detection, % aggregation or coalescence rate, etc. Furthermore, the Applicant may explore relevant animal models (e.g., microscopic examination of nail-bed capillary or cannulated cat mesenteric artery), if any. Once such information is obtained the data/information should be submitted to the agency for review.

3. PHARMACOKINETICS OF DMPC, THE MICROSPHERE SHELL

It should be noted that lipid components of the microbubble shell have not been characterized in the current submission. It is requested that supportive information, e.g., in vitro, regarding DMPC metabolism should be submitted.

4. PFH GAS ASSAY INFORMATION

For blood samples, it appears that samples were analyzed at three different starting days, 6/29, 7/6, and 7/12. The Applicant should submit the following assay information:

a. Standard curves and QC runs from days 7/6 and 7/12
b. Profiles from blood assay (as in the expired air samples)

For expired air samples, the Applicant needs to submit a table of calculated PFH expired air concentration data, as in the vol. 35, p. 206. It should be noted that the submitted table is incomplete.

5. Pharmacokinetic information in patients with pulmonary deficiency

Pharmacokinetic information is needed for the patients with pulmonary deficiency, due to the fact that PFH is eliminated primarily via the lungs.

G. RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation II (OCPB/DPE-2) has reviewed NDA # 21-191 for Imavist which was submitted by the Applicant on 10/11/99.

The current NDA submission, The Human Pharmacokinetics and Bioavailability Section, contains perfluorohexane pharmacokinetic information and negligible PK/PD data. Ideally "intact" microbubble pharmacokinetic information should have been provided with respect to the dosage proposed in the package insert.

In order to obtain this information in the future, the Applicant should agree to continue to develop an analytical method(s) or to modify the existing analytical method so it could definitively characterize the pharmacokinetics of "intact" microbubbles in vivo. Once such information is obtained the data/information should be submitted to the agency for review.

Regarding the approval status of the application, this submission is considered acceptable. The items covered under 'Comments to the Applicant' section and the Labeling Comments (to be covered under a separate review) should be conveyed to the Applicant as appropriate.

/S/ 8/11/00

David J. Lee, Ph.D. Date
Team Leader
Division of Pharmaceutical Evaluation II
Office of Clinical Pharmacology and Biopharmaceutics

/S/ 8/15/00

FD – John Hunt, Deputy Director Date

OCPB Briefing Meeting: 8/8/00; Attendees: John Hunt, David Lee

CC: NDA 21-191 (orig., 1 copy)
HFD-160 (Division file, THarper)
HFD-850 (Lesko, Huang, LEEP)
HFD-870 (Huang, LeeD, HUNT)
Central Document Room (Barbara Murphy)

H. INDICATIONS AND USAGE (According to the Package Insert)

I. DOSAGE AND ADMINISTRATION (According to the Package Insert)

The recommended dose is 0.125 mg/kg (0.00625 ml/kg or 6.25×10^6 microbubbles/kg) administered as an intravenous bolus over a period of not less than 10 seconds. Imavist must be used within 30 minutes of constitution. Discard any unused portion. Additionally, the PI stated Imavist is for single use only (a single vial must not be used for more than one patient).

J. CHEMISTRY

1. PERFLEXANE (PFH) GAS CHEMISTRY

Description and Characterization

PFH Molecular Formula:	C_6F_{14}; CF_3-CF_2-CF_2-CF_2-CF_2-CF_3
Chemical Name:	tetradecafluorohexane
IUPAC Name:	perfluorohexane
CAS registry number:	355-42-0
USAN name:	perflexane

Physical Characteristics

Color and appearance:	Clear, colorless liquid
Molecular Weight:	338.04 amu
Boiling Point:	57°C @ 1 atm
Freezing Point:	-90°C @ 1 atm
Specific gravity:	1.684 @ 25°C
Ostwald coefficient:	2.3×10^{-5} @ 25°C
	1.03×10^{-4} @ 37°C
Solubility in water:	2.7×10^{-7} mol/L @ 25°C
Vapor pressure:	129 torr @ 13°C
	223 torr @ 25°C
	367 torr @ 37°C
Stability:	Inert. Degradation dose not occurs. Pharmaceutical stability is considered infinite.

IUPAC: International Union of Pure and Applied Chemistry
CAS: Chemical Abstracts Service
USAN: United States Adopted Name

2. Dimyristoylphosphatidylcholine (DMPC)

Description and Characterization

Chemical Name:	1,2-dimyristoyl-sn-glycero-3-phosphocholine
IUPAC Name:	1,2-di(tetradecanoyl)-sn-glycero-3-phosphcholine
CAS registry number:	18194-24-6
Common name:	Dimyristoylphosphatidylcholine
Abbreviation:	DMPC

Physical Characteristics

Color and appearance:	White to off-white free flowing powder
Melting point range:	240 - 242
Formula weight, amu	677.96
Specific optical rotation	
Critical micelle concentration:	9.4×10^{-9} mol/L

3. FORMULATION

For 200 mg fill size:

Component	mg/vial (dry weight)
Perflexane	
DMPC	
NaCl (tonicity agent)	
Na2HPO4 (buffering agent)	
NaH2PO4 (buffering agent)	
Poloxamer 188 (wetting agent)	
HES (bulking agent)	

Injectate Characteristics (10 ml reconstitution)	Concentration
Perflexane	
DMPC	
Mean Number of Microbubbles*	

*The majority of bubbles are < 6 μm in diameter.

Microbubble Size Volume median diameter (μm)	
Total counts /ml	5.9 to 13.7 x 10^8
Counts /ml - 3 to 10 μm	
Counts > 10 μm (%)	

Determination of microbubbles: A particle-counting instrument was used. Additionally, this same methodology was employed to determine the total number of large bubbles (>10 μm). The specification for bubble counts/ml greater than 10 μm in size is less than [redacted] % of the total injected dose. The Applicant stated that, in the pulmonary vasculature, there is approx. 280×10^9 capillaries, with diameters between 8 – 16 μm. Since AFO150 mean bubble size is approx. 4 – 5 μm and since there are very few bubbles greater than 10 μm ([redacted] %), it is likely that majority of pulmonary capillaries will not be occluded. In order to count the bubbles, the constituted product is diluted [redacted] fold, to avoid coincidence counting of microbubbles. Additionally, the Applicant stated that size and distribution does not appreciably change (growth and coalescence) over the 30 minute postconstitution period. Imavist is to be used within 30 minutes of constitution.

K. ANALYTICAL METHODS

1. Assay Methodology

Redacted 2

pages of trade

secret and/or

confidential

commercial

information

Reviewer's Comment: **As stated above in the blood samples, it appears that QC was tested with only one QC concentration value, — ng/ — ml of blood.**

a. The Applicant needs to submit a table of calculated PFH air Concentration data, as in the vol. 35, p. 206. The submitted table is incomplete.

However, the assay methodology appears acceptable from the submitted data (standard curve generation, peak separation between PFH and — ', and repeated measurement during the sample analysis.

L. OVERALL PHARMACOKINETIC/PHARMACODYNAMIC INFORMATION

1. PHARMACODYNAMIC

a. Study IMUS-001-USA

A Single-Blind, Dose-Ranging, Placebo-Controlled, Safety, and Contrast-Enhancement Study in Normal Volunteers Receiving AFO150 Administered by Intravenous Injection

Overall conclusion: The Applicant stated that AF0150 was well tolerated at all the doses used in this study. The adverse events 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, including results of standard tests of coagulation, were observed during the study. Furthermore, AF0150 was not associated with clinically significant changes in vital signs, respiratory function, SaO2, and ECG findings.

Reviewer's Comment: This conclusion should be evaluated by the Reviewing Medical Officer. Additionally, videodensity measurements are not reported. Therefore, this study should be considered only for the safety purpose.

b. Study IMUS-018-USA

An Open-Label Dose- Titration Study of 3 Doses of AFO150 in the Echocardiographic Assessment of Patients with Left Ventricular Dysfunction

Overall Conclusion: The Applicant stated that intravenous administration of AFO150, at 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 AFO150 doses (i.e., 0.25 and 0.5 mg/kg). However, a dose-dependent increase in both duration of attenuation and duration of useful contrast enhancement was observed with increasing AFO150 dose.

Reviewer's Comment: This conclusion should be evaluated by the Reviewing Medical Officer.

2. PHARMACOKINETICS

Study 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 AFO150 in Healthy Adult Volunteers

PFH.

Many literature data reported that no metabolites of PFH were detected in various tissues or urine post administration. Since PFH has relatively high vapor pressure and low boiling point, it is expected that PFH would be eliminated rapidly as an unchanged drug in expired air.

Metabolism and Pharmacokinetics of DMPC

This surfactant is a semi-synthetic compound representing a member of compounds (phospholipids) that occur naturally in the environment. It is reported that the amount of DMPC that would be administered in a single dose is approx. 400- to 1000- fold less than the amount of phospholipids naturally present in the serum.

The literature data showed that phospholipids are remarkably nontoxic. Phospholipids, which are the main constituents of natural cellular membranes, have been used in a wide variety of pharmaceutical, nutritional, and cosmetic products.

Most naturally occurring phospholipids have a very low solubility in aqueous solutions, and, due to the amphiphilic nature of these molecules, they aggregate into large ordered structures. Because of their nontoxic nature and physicochemical properties, phospholipids have been used as major constituents of most liposomes. The pharmacokinetics, organ distribution, and 24-hour urinary excretion of 99mTc-labelled multilamellar liposomes, composed of DMPC and dimyristoylphosphatidylglycerol (7:3 molar ratio), was reported from a clinical study in seven patients with cancer - The reported data indicated that administration of the phospholipid liposomes, at doses of 150 to 450 mg/m2 of body surface area, was not associated with short- or long-term side effects, as assessed by clinical observation and chest X-rays, as well as by hematological, renal, and liver function tests. For comparison, at these doses, patients were injected with approximately 270 to 800 mg of phospholipid microsomes, which is 25 to 70x the maximum amount (approximately 11 mg) of DMPC contained in a vial of AFO150. The plasma disappearance curve was biphasic (half-life (α = 5.53 min, half-life β = 289 min) and appeared to closely fit a two-compartment mathematical model. The short plasma half-life α matched the kinetics of uptake by the liver and spleen, which reached a peak and then plateau within the first 5 minutes following injection. Twenty-four hours after injection, liposomes were localized in organs rich in reticuloendothelial cells, i.e., liver (44.5 ± 9.1%), spleen (25.5 ± 7.7%), lung (14.5 ± 4.9%), and bone marrow. The reason for the lack of noticeable toxicity associated with single dose of phospholipids has been suggested to be the capacity of the reticuloendothelial system (RES), i.e., the capacity of RES to regenerate binding sites and metabolize ingested material, and to recruit new RES cells from bone marrow, to proliferate Kupffer cells *in situ*, and, possibly derivate Kupffer cells from blood monocytes.

The metabolism of phospholipids involve the phospholipases A1, A2, C, and D. Phospholipases A1 and A2 are responsible for cleaving the fatty acid ester bonds, while phospholipases C and D cleave at the head group phosphoester bonds. The hydrolysis products formed are available for further degradation or for reincorporation into the same or other lipids. Thus, phospholipases A1 and A2 release fatty acids that can be degraded by beta-oxidation or reincorporated into phospholipids, triglycerides, sphingomyelin, etc. Phospholipase C forms diacylglycerol, which can act as a mediator of cell signal transduction and/or may be further metabolized by diacylglycerol lipase to generate free fatty acids. Phosphatidic acid, which is initially generated by the action of phospholipase D, is also active in cell signal transduction and can be further metabolized to diacylglycerol. (Reference #5)

For comparison, amount of DMPC contained in a 10-mL vial of AF0150, is approximately 11 mg; the level of phospholipids present in the serum is 150 to 380 mg/dL. Assuming an adult human has approximately 3 L of serum, the total amount of DMPC contained in a vial of AFO150 would contribute only approximately 11 mg *DMPC/3* L of serum or 0.37 mg/dL, which is approximately 400- to 1000-fold less than that normally present in the serum.

M. INDIVIDUAL STUDY REPORTS

1. Study 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 AFO150 in Healthy Adult Volunteers

Overall conclusion: The Applicant stated that AF0150 was well tolerated in this study. The disappearances of PFH from both blood and expired air appears to follow first-order kinetics. Approximately 75% of the PFH was eliminated through expired air within 3 hours post injection, and 87% was eliminated within 24 hours. PFH clearance values obtained from expired air and blood were comparable, however, more variation of blood clearance was noted. There appears to be statistically significant differences in the Kel of PFH from expired air between the genders, however, the clinical significance is unknown. There were no statistically differences between the genders in the rate and extent of PFH exposure in blood.

Reviewer's Comment: The study report submitted by the Applicant appears to be adequate.

Objectives: To obtain PK parameters, blood and pulmonary clearance, of PFH after bolus i.v. injection of 4 mg/kg (0.2 mL/kg) AFO150 in healthy adult volunteers.

Study Design: Open-Label, Phase I study.

Subjects: Healthy subjects; Age 23-55 years old (mean age 35 years); mean weight of 75.1 ± 15.8 kg (range 49-96 kg); mean height 172 ± 8.6 cm (range 160-188 cm);

The study was conducted in two phases: pilot phase included 2 subjects and pivotal phase included 10 subjects.
Safety was assessed through 24 hours post injection based on AEs, clinical laboratory values, vital signs, oxyhemoglobin saturation (SaO2), 12-lead ECGs, neurologic evaluations including cranial nerve examinations and mental status testing, and continuous cardiac monitoring.

Drug product: AFO150 was supplied in a 200 mg vial and reconstituted with 10 ml SWFI to a final concentration of 20 mg/ml. Lot No#18027.

Dosing: All subjects received bolus i.v. injection at a rate not to exceed 25 ml/min. A 30 ml saline flush was administered immediately following AFO150 injection. It is noted that doses from 0.125 to 4.0 mg powder/kg doses were used in clinical studies. For this study 4 mg/kg dose was selected in order to include the upper dose range of the clinical studies and to increase the signal for the analytical methods for expired air and blood.

Volumes of AFO150 injected to each subject:

Subject number	Volume of AFO150 Administered	Dose
101	19.246 ml	2556.25
102	17.246 ml	2290.61
103	15.246 ml	2024.97
104	17.246 ml	2290.61
105	18.246 ml	2423.43
106	18.246 ml	2423.43
107	14.086 ml	1870.90
108	11.086 ml	1472.44
109R	14.246 ml	1892.15
110R	10.086 ml	1339.62
112R	10.086 ml	1339.62
113	18.246 ml	2423.43

Dose Calculations: average µg/ml for dose : 132.82 ± 13.952 µg/ml (CV% : 10.5)

Date	Subject No.:	Average µg/ml
5/12/99	1 2	110.83
5/20/99	3 4 5	133.10
5/24/99	6 7 8 9	148.41
6/21/99	9R 10R 12R 13	132.07

R: re-dose; No PK parameters were collected from Subjects 110, 111 and 112 initial dosing. These subjects were dosed all in the same day. The Applicant suspected of improper dosing and thus, they were re-dosed. Subject 109 was dosed again, serving as a positive control.

Blood samples

Predose (time 0), 2, 5, 10, 15, 20, 25, 30, 45 minutes, 1, 4, 8, and 24 hours post-dose. At each sampling time, 2 mL blood samples were collected at each time point in syringes and 0.5 ml of the collected sample was immediately injected into each of 3 headspace vials. Samples were vortexed and stored refrigerated until shipment to [redacted] for [redacted] analysis of PFH.

Expired Air samples

Collected at start of injection and collected continuously for 3 hours in 1 min. intervals for the first 5 min., at 2.5 min. intervals for second 5 min., at 1.25 min. intervals for the next 50 min. and at 20 min. intervals for the next 120 min. Additional samples at 4, 5, 6, 8, 24 hours post dosing. Samples were collected in Tedlar bags of 12 to 200 L volumes, which were filled to approximately 50 to 75% of capacity. Subjects remained sitting for the first 3 hours post dosing and for 5 min. prior to any expired air sample collection thereafter. Samples were sent to [redacted], for [redacted] analysis of PFH.

Methods to measure

C_{PFH} was an average of multiple analyses by [redacted]

Study Population Healthy adult volunteers.

Pharmacokinetic Data Analysis

PFH model-independent PK variables : AUC 0-24, AUC 0-inf, Tmax, Cmax, T1/2, MRTlast (from time 0 to last quantifiable time), Vz (apparent volume of distribution), CLsys(total), CLlung, % PFH(0-3hr), and %PFH(0-inf). WinNonlin 2.1 [redacted]).

% PFH(0-3hr) calculation : PFHbag = (Vout x Cpfh) / 1000 unit: microgram = (ml x ng/ml) / 1000
%PFH(0-inf)

Assay

PFH concentration in blood samples was determined using a validated [redacted] method using high resolution selected ion monitoring (SIM) mode.

Determination of PFH concentrations in expired air was accomplished using a validated [redacted] method.

Analysis
Pharmacokinetic parameters were analyzed by dose and sex. Two tests were conducted: 1) F-test for comparison of variances between men and women, and 2) T-test for comparison of means between men and women.

Results.

1. Safety Results: There were no deaths or serious AEs associated with this study.

2. Mean PK overall summary table

Mean ± SD and Coefficient of Variance (%) of PFH in Blood and Air PK parameters

Parameter	Blood	Expired Air
AUC 0-24 (ng*hr/ml)	3.7 ± 1.4 (38.9)	3.3 ± 0.7 (21.2)
AUC 0-inf (ng*hr/ml)	4.2 ± 2.2 (52.4)	3.4 ± 1.3 (20.9)
Vd (L)	2960.0 ± 2595.2	NA
Tmax (min)a	2.0	1.5
Cmax (ng/ml)	28.0 ± 28.6	27.8 ± 8.3
T1/2 (hrs)	5.3 ± 6.1	9.0 ± 5.0
MRTlast (hrs)	2.7 ± 3.6	1.6 ± 0.5
CL (L/hr)	716.3 ± 735.3	603.7 ± 93.9
%PFH (0-3 hr)	NA	74.6 ± 17.6
%PFH (0-inf)	NA	87.2 ± 19.3

a: median Tmax
NA: not applicable

3. PFH Blood pharmacokinetic parameters

PFH Blood - all subjects

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	Vd (L)	CL (L/hr)
N	12	12	12	12	12	12	12	12	12
Mean	3.7	4.2	2.0	28.0	1.9	5.3	2.7	2960.0	716.3
SD	1.4	2.2	-	28.6	4.4	6.1	3.6	2595.2	735.3
Min									
Max									
CV %	38.9	52.4	-	102.2	236.1	114.7	133.5	87.7	102.6

PFH Blood – men

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	Vd (L)	CL (L/hr)
N	7	7	7	7	7	7	7	7	7
Mean	**3.6**	**3.8**	**2.0**	**33.1**	**3.1**	**2.8**	**1.3**	**2163.1**	**936.4**
SD	1.6	1.9	-	**34.7**	**5.6**	**4.2**	**2.1**	**2683.4**	**916.1**
Min									
Max									
CV %	**44.9**	51.7	-	**104.9**	**181.1**	**146.7**	**160**	**124.1**	**97.8**

PFH Blood – women

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	Vd (L)	CL (L/hr)
N	5	5	5	5	5	5	5	5	5
Mean	**3.7**	**4.8**	**1.6**	**20.8**	**0.15**	**8.7**	**4.7**	**4075.7**	**408.2**
SD	1.2	2.6	0.9	**18.2**	**0.13**	**7.1**	**4.6**	**2247.8**	**156.3**
Min									
Max									
CV %	33.8	54.5	55.9	**87.4**	**83.5**	**81.1**	**98.4**	55.2	38.3

4. PFH expired air pharmacokinetic parameters

PFH Expired air – all subjects

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	CLlung (L/hr)	%PFH 0-3hr	%PFH 0-24hr	%PFH 0-inf
N	12	12	12	12	12	12	12	12	12	12	12
Mean	**3.3**	**3.4**	**1.5**	**27.8**	**0.18**	**9.0**	**1.6**	**603.7**	**74.6**	**86.4**	**87.2**
SD	0.7	0.7	-	**8.3**	**0.32**	**5.0**	**0.5**	**93.9**	**17.6**	**20.2**	**19.3**
Min											
Max											
CV %	21.2	20.9	-	29.7	**181.5**	55.1	32.3	15.6	23.6	23.4	22.1

PFH expired air – men

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	CLlung (L/hr)	%PFH 0-3hr	%PFH 0-24hr	%PFH 0-inf
N	7	7	7	7	7	7	7	7	7	7	7
Mean	**3.7**	**3.7**	**1.5**	**29.0**	**0.26**	**6.0**	**1.4**	**619.7**	**81.0**	**94.1**	**94.1**
SD	0.5	0.5	-	**4.1**	**0.41**	**3.1**	**0.4**	**105.0**	**6.1**	**6.7**	**6.7**
Min											
Max											
CV %	13.0	13.8	-	14.2	**156.7**	51.9	27.4	16.9	7.5	7.1	7.1

PFH expired air – women

	AUC 0-24 (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	Tmax (min)a	Cmax (ng/ml)	Kel (1/hr)	T1/2 (hrs)	MRTlast (hrs)	CLlung (L/hr)	%PFH 0-3hr	%PFH 0-24hr	%PFH 0-inf
N	5	5	5	5	5	5	5	5	5	5	5
Mean	**2.8**	**2.9**	**1.5**	**26.1**	**0.058**	**13.1**	**2.0**	**581.3**	**65.6**	**75.6**	**77.5**
SD	0.7	0.7	-	**12.5**	**0.023**	**4.0**	**0.6**	**81.6**	**25.0**	**28.3**	**27.5**
Min											
Max											
CV %	25.7	25.0	-	47.8	39.1	30.8	29.3	14.0	38.0	37.5	35.5

5. Comparison of PK parameters

AUC 0-24 (ng*hr/ml)

	Blood **all subjects**	Blood men	Blood women	Expired air **all subjects**	Expired air men	Expired air women
	AUC 0-24 (ng*hr/ml)	AUC 0-24 (ng*hr/ml)	AUC 0-24 (ng*hr/ml)	AUC 0-24 (ng*hr/ml)	AUC 0-24 (ng*hr/ml)	AUC 0-24 (ng*hr/ml)
N	12	7	5	12	7	5
Mean	**3.7**	**3.6**	**3.7**	**3.3**	**3.7**	**2.8**
SD	1.4	1.6	1.2	0.7	0.5	0.7
Min						
Max						
CV %	38.9	44.9	33.8	21.2	13.0	25.7

AUC 0-inf (ng*hr/ml)

	Blood all subjects	Blood men	Blood women	Expired air all subjects	Expired air men	Expired air women
	AUC 0-inf (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	AUC 0-inf (ng*hr/ml)	AUC 0-inf (ng*hr/ml)
N	12	7	5	12	7	5
Mean	**4.2**	**3.8**	**4.8**	**3.4**	**3.7**	**2.9**
SD	2.2	1.9	2.6	0.7	0.5	0.7
Min						
Max						
CV %	52.4	51.7	54.5	20.9	13.8	25.0

Tmax (min)

	Blood all subjects	Blood men	Blood women	Expired air all subjects	Expired air men	Expired air women
	Tmax (min)a	7	Tmax (min)a	Tmax (min)a	Tmax (min)a	Tmax (min)a
N	12	**2.0**	5	12	7	5
Mean	**2.0**	-	**1.6**	**1.5**	**1.5**	**1.5**
SD	-	0.0	0.9	-	-	-
Min						
Max						
CV %	-		55.9	-	-	-

Cmax (ng/ml)

	Blood all subjects	Blood Men	Blood women	Expired air all subjects	Expired air men	Expired air women
	Cmax (ng/ml)	Cmax (ng/ml)	Cmax (ng/ml)	Cmax (ng/ml)	Cmax (ng/ml)	Cmax (ng/ml)
N	12	7	5	12	7	5
Mean	**28.0**	**33.1**	**20.8**	**27.8**	**29.0**	**26.1**
SD	**28.6**	**34.7**	**18.2**	**8.3**	**4.1**	**12.5**
Min						
Max						
CV %	102.2	104.9	87.4	29.7	14.2	47.8

Kel (1/hr)

	Blood all subjects	Blood men	Blood women	Expired air all subjects	Expired air men	Expired air women
	Kel (1/hr)	Kel (1/hr)	Kel (1/hr)	Kel (1/hr)	Kel (1/hr)	Kel (1/hr)
N	12	7	5	12	7	5
Mean	**1.9**	**3.1**	**0.15**	**0.18**	**0.26**	**0.058**
SD	**4.4**	**5.6**	**0.13**	**0.32**	**0.41**	**0.023**
Min						
Max						
CV %	**236.1**	**181.1**	**83.5**	**181.5**	**156.7**	39.1

Note: It appears that Kel and T1/2 (below) values are different for men and women. Additionally, SD is larger in men than women. The difference of Kel, and, thus, T1/2 may not be clinically significant.

T1/2 (hrs)

	Blood all subjects	Blood men	Blood women	Expired air all subjects	Expired air men	Expired air Women
	T1/2 (hrs)	T1/2 (hrs)	T1/2 (hrs)	T1/2 (hrs)	T1/2 (hrs)	T1/2 (hrs)
N	12	7	5	12	7	5
Mean	**5.3**	***2.8***	**8.7**	**9.0**	**6.0**	***13.1***
SD	**6.1**	**4.2**	**7.1**	**5.0**	**3.1**	**4.0**
Min						
Max						
CV %	**114.7**	**146.7**	**81.1**	55.1	51.9	30.8

MRTlast (hrs)

	Blood **all subjects**	Blood men	Blood women	Expired air **all subjects**	Expired air men	Expired air women
	MRTlast (hrs)	MRTlast (hrs)	MRTlast (hrs)	MRTlast (hrs)	MRTlast (hrs)	MRTlast (hrs)
N	12	7	5	12	7	5
Mean	**2.7**	**1.3**	**4.7**	**1.6**	**1.4**	**2.0**
SD	**3.6**	**2.1**	**4.6**	**0.5**	**0.4**	**0.6**
Min						
Max						
CV %	**133.5**	**160**	**98.4**	32.3	27.4	29.3

CL (L/hr) vs. **CLlung (L/hr)**

	Blood **all subjects**	Blood men	Blood women	Expired air **all subjects**	Expired air men	Expired air women
	CL (L/hr)	CL (L/hr)	CL (L/hr)	**CLlung (L/hr)**	**CLlung (L/hr)**	**CLlung (L/hr)**
N	12	7	5	12	7	5
Mean	**716.3**	**936.4**	**408.2**	**603.7**	**619.7**	**581.3**
SD	**735.3**	**916.1**	**156.3**	**93.9**	**105.0**	**81.6**
Min						
Max						
CV %	**102.6**	**97.8**	38.3	15.6	16.9	14.0

Note: Overall, CLlung values approach the total CL plasma value. However, for women, blood CL is larger than expired air CLlung for women. The Applicant did not provide any explanation on this matter. It is, however, noted that minimal value for blood CL is lower than the Lung CL, thus, pushing the trend of the mean toward lower side.

Discussion

It should be noted that standard deviation values for parameters are relatively large; this may be due to assay sensitivity or due to improper sample collection, e.g., not enough samples, miscalculation of volumes, etc. However, from assay methodology section, the methodology for both blood and expired air appears to be adequate. Additionally, it appears that adequate samples were collected for both blood and expired air.

However, looking at individual PK parameters, it appears that there are inter-subject variability for both blood and expired air.

The following observations are made from the study results:

1. The decline of PFH in both blood and expired air appears to follow first-order kinetics. Approximately 75% and 87% of PFH were eliminated through expired air within 3 hours and within 24 hours, respectively. Therefore, it appears that PFH was readily eliminated from the lungs following the AFO150 i.v. administration;

2. Volume of Distribution for AFO150 is approximately 3000 L. This indicates that, immediately following the injection, PFH appears to be eliminated by the lungs, thus, giving a large Volume of distribution;

3. PFH blood data indicated that the standard deviation (SD) and coefficient of variation (CV%) values for Cmax, kel, T1/2, MRT, Vd and CL parameters are extremely large. Overall values obtained from men showed greater variation than women. It is apparent that the inter-subject variability is large. The large variation in the SD and CV% is expected, especially for the blood samples due to the difficulties in measuring PFH gas, which may be present in the negligible amount in the blood, and this is a typical characteristic for the microbubble drug category. However, the minimum and maximum values

observed are unusually too large. Perhaps, in order to decrease the SD and CV%, a larger sample size may be required for this drug product.

F- and T-tests indicated that there were no statistical differences between men and women in the rate and extent of PFH exposure in blood system. Statistical tests may not show any differences perhaps due to the large spread in the values. However, Kel, T1/2, MRT, Vd and CL point estimates, i.e., the mean values, are drastically different for men and women.

4. Overall PFH expired air data appear to be less variable than blood data. The standard deviation (SD) and coefficient of variation (CV%) values for Kel parameter are large; the value obtained from men showed greater variation than women.

   F- and T-tests indicated that no statistically significant difference was detected for CLlung. However, there were statistically significant differences in the extent of exposure (AUC) and terminal half-life of PFH from expired air between men and women. The mean value for Kel is different as well. However, the impact of these differences is not known and may not be clinically significant.

2. Study IMUS-001-USA

A Single-Blind, Dose-Ranging, Placebo-Controlled, Safety, and Contrast-Enhancement Study in Normal Volunteers Receiving AFO150 Administered by Intravenous Injection

> **Overall conclusion:** The Applicant stated that AF0150 was well tolerated at all the doses used in this study. The adverse events 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, including results of standard tests of coagulation, were observed during the study. Furthermore, AF0150 was not associated with clinically significant changes in vital signs, respiratory function, SaO2, and ECG findings.
>
> ***Reviewer's Comment***: This conclusion should be evaluated by the Reviewing Medical Officer. Additionally, videodensity measurements were not measured. Therefore, this study should be considered only for the safety purpose.

Objectives: To investigate the safety of intravenously administered AFO150 at 4 dose levels in normal, healthy volunteers; To assess indirectly visual clearance (from the heart) of AFO150 on 2-D gray-scale ultrasound; To determine the potential efficacy of AFO150 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: Open-Label, Phase I study receiving AF0150 either as a bolus or as an infusion or placebo (0.9% sodium chloride [NaCl]) injection.

Investigator: [redacted] **Study Center:** [redacted] **Studied Period** (date of first enrollment to date of last completed): 3/2796 to 6/5/96

Methodology: Safety was to be evaluated by assessment of adverse events, vital signs, clinical laboratory tests (hematology, blood chemistry, and coagulation parameters), arterial oxyhemoglobin saturation (SaO2) by pulse oximetry, respiratory status (respiratory rate and expired carbon dioxide [C02J levels), and ECG changes using both 12-lead and ambulatory monitoring (Holter monitor). In addition, complement (C3, C3a, C4, and CH50) and lymphokine (tumor necrosis factor-a [TNF-a)) levels were to be evaluated. Assessments were to be performed at screening (2 to 21 days prior to administration of study drug), on admission to the clinical research unit (day before administration of study drug), at baseline (within 2 hours prior to injection), and at specified times following injection and up to 7 days after administration of the study drug.

Enrollment study was to take place in 3 separate stages. Stage 1: to undergo ultrasound evaluations of visual clearance of the contrast agent. Stage 2 : to undergo contrast imaging of cardiac regions. Stage 3: to undergo imaging of abdominal regions.

Stage I: The first 24 subjects enrolled in the study were to be randomized to receive either AF0150 or placebo. Based on an escalating-dose design, subjects randomized to AF0150 were to receive 1 of 4 doses (0.125, 0.5, 2.0, or 4.0 mg powder/kg body weight: 4 subjects per dose level), starting with the lowest dose. Dosing was to progress to each subsequent level following review of available safety data through Day 3 of the study. Within each AF0150 dose level, subjects were to be randomly assigned to receive either AF0150 or placebo (0.2 ml/kg body weight) to be administered as a bolus over a period of approximately 10 seconds.

Stages 2 and 3: The next 18 subjects (per stage) were to be randomized to receive either AFOI50 at 1 of 3 doses (0.125.0.5, or 4.0 mg powder/kg; 4 subjects per dose group) or placebo (0.2 ml/kg; 6 subjects). Doses were to be administered as a single bolus injection over a period of 10 seconds. After completion of dosing of the 18 subjects (per stage), 2 additional subjects (per stage) were to be enrolled to receive a 4.0-mg powder/kg dose of AF0150 as a slow infusion over a period of approximately 10 minutes. For Stages 2 and 3, assignment to all treatment groups was to be randomized, except the 4.0-mg powder/kg infusion group, which was to be studied after all other dose groups were studied.

Subjects and Inclusion Criteria: Subjects were to be 18 to 45 years old, male or female, 45 to 100 kg in body weight, and in good health, based on medical history, laboratory assessments, and physical examination.

Demography and Other Baseline Characteristics: There were 36 (56%) female and 28 (44%) male subjects. Sixty-three subjects (98%) were identified as Caucasian and one (2%) was Black (Age: From 18 to 45 years with a mean of 26 ± 7 years (mean ± SD); Weight: From 49 to 96 kg with a mean of 69 ± II kg; Height: From 152 to 190 cm with a mean of 170 ± 9 cm). All subjects were normal, healthy volunteers and presented with no clinically significant underlying disease or medical conditions. None of the concomitant medications received by the subjects was considered relevant to the study.

Test Product, Dose and Mode of Administration, Batch Number: AF0150, lot number UAI6010, was used in this study. AFO150 was to be prepared by constituting a 200-mg vial with 10.0 mL of sterile water for injection (WFI) to a final concentration of 20 mg/mL.

Dose and Mode of Administration: 0.125, 0.5, 2.0, or 4.0 mg powder/kg body weight, 4 subjects per dose level. AFOI50 was given as a single intravenous administration either as a bolus (over approximately 10 seconds) or infusion (over approximately 10 minutes). Placebo was administered as a bolus injection. The number of subjects who received AFOI50 at different doses or placebo were as follows:

Treatment	Number of Subjects
AFOI50 (mg powder/kg body weight)	
Bolus: 0.125	12
0.5	12
1.0	4
4.0	12
Infusion: 4.0	4
Placebo (0.9% NaCl in mL/kg body weight)	
Bolus: 0.2	20

Criteria for Evaluation: Safety: Vital signs and clinical laboratory tests, including hematology, coagulation, blood chemistry, and urinalysis, were to be measured preinjection and at specified times postinjection. Complement (C3, C3a, C4, and CH50) and lymphokine TNF-a levels were to be obtained. SaO2 by pulse oximetry, respiratory status (respiratory rate and expired CO2), and electrocardiogram

(ECG) changes were to be evaluated. Subjects were to be monitored for adverse events.

Efficacy: Fundamental 2-D gray--scale ultrasound imaging was to be performed prior to, during, and following administration of the study drug. ***Videotape images were to be digitized to quantify changes in gray-scale from anatomic regions of interest (ROIs).*** The effectiveness of the compound as an ultrasound contrast agent was to be evaluated by assessing the extent of contrast enhancement, as measured by the level and duration of the videodensity signal at various times postinjection from the left heart chamber, myocardium, liver, right kidney, spleen, and other vascular structures within the abdomen. ***Videodensitometric analysis was to be conducted using the .***

Statistical Methods: Statistical hypothesis testing was not performed.

Results: The following information was stated by the Applicant

1. Safety Result Ten (23%) of 44 AF0150-treated subjects reported at least 1 adverse event. These adverse events were assessed as mild to moderate in intensity and none was considered serious by the investigator. Three events (21%), taste perversion (Subject 001, 0.125-mg/kg bolus), dizziness, and nausea (Subject 064, 4.0-mg/kg infusion) were assessed as possibly related to AFO150. The rest of the adverse events reported were assessed as unlikely related to study drug. Only two subjects required treatment for their adverse events; fever and headache were treated with acetaminophen or ibuprofen.

Six (30%) of 20 placebo-treated subjects reported at least 1 adverse event. All events were considered mild in intensity and nonserious.

The most commonly reported adverse event was headache, which was noted in 4 (9%) of 44 AF0150-treated subjects and 1 (5%) of 20 placebo-treated subjects. The only other adverse event 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 adverse event, taste perversion, was transient, lasting from 0.5 sec to 1 minute in the AF0150-treated subject and 45 seconds and 1 minute in the placebo-treated subjects. All other adverse events were reported for 1 subject each: for AFO150-treated subjects, hiccup, fever, conjunctivitis, vasodilatation, pain, dizziness, and nausea were reported; for placebo-treated subjects, parosmia, postural hypotension, diarrhea, and dry skin were reported.

Mean values for all hematology parameters changed little during the study. There were subjects who manifested decreases in hemoglobin, hematocrit, and RBC count from normal baseline levels at one or more post-dosing intervals in both the AFOI50-treated and placebo-treated subjects. However, there was no distinct pattern with regard to timing of the decrease in these parameters and the findings do not suggest a clinically significant effect of AFO150. No clinically significant changes in values were observed in standard tests for coagulation, blood chemistry, complement (C3, C3a, C4, and CH5o) levels, and urinalysis. There was also no evidence of systemic release of lymphokine as measured by TNF-a. Furthermore, AFOI50 was not associated with clinically significant challenges in vital signas, respiratory function, SaO2, or ECG findings.

2. Efficacy Results: This single blind, placebo-controlled, Phase I study was conducted to assess primarily safety and preliminary efficacy of AF0150. ***Contrary to protocol plan, videodensity measurements are 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, e.g., patients with suboptimal echocardiograms or patients with suspected abdominal organ lesions.

3. Study IMUS-018-USA

An Open-Label Dose- Titration Study of 3 Doses of AFO150 in the Echocardiographic Assessment of Patients with Left Ventricular Dysfunction

> **Overall Conclusion:** The Applicant stated that intravenous administration of AFO150, at 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 AFO150 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 AFO150 dose.
>
> ***Reviewer's Comment***: This conclusion should be evaluated by the Reviewing Medical Officer.

Objectives: To assess the ability of AF0150 to opacify the left ventricular (LV) cavity in fundamental continuous (real-time) and fundamental gated (end-diastolic and end-systolic) imaging modes with three doses of AF0150 (0.125, 0.25, and 0.5 mg/kg); To assess the duration of attenuation and the duration of useful contrast enhancement of the left ventricle using fundamental continuous imaging, and assessment of safety.

Study Design: An open-label, Phase 2, dose-ranging study to assess the efficacy and safety of AFOIS0 in subjects with LV dysfunction. No placebo control was used in this study.

Investigators: **Study Center(s):**

Studied Period (date of first enrollment to date of last completed) : 11 August 1998 to 16 October 1998.

Methodology: Each subject received three incremental doses of AF0150 with a 100 minute interval between each dose and following the last dose during which resting contrast echocardiography and safety assessments were performed. Safety was assessed up to 24 hours after administration of the initial dose. Efficacy was assessed based on videodensitometry measurements and blinded review of the echocardiographic images. Videodensitometry was performed for LV opacification and duration of useful contrast enhancement. The reviewer was blinded to subject and dose.

Subjects and Inclusion Criteria: Number or Subjects (planned and analyzed): Eighteen subjects were planned and enrolled. All subjects were evaluable for efficacy (at each dose level) and safety. The study population consisted of 13 (72%) male and 5 (28%) female subjects and the mean age was 67.3 ± 10.6 years (range 44 to 87 years). Mean height was 172.8 ± 9.2 cm (range 162 to 187 cm) and mean weight was 80.8 ± 11.4 kg (range 61.7 to 100.7 kg). Male or female subjects ranging in age from 18 to 80 years with LV dysfunction as evidenced by an estimated ejection fraction (EF) of 20% to 40%.

Test Product, Dose and Mode or Administration, Batch No.: *Lot number UA18010* was used in this study. AF0150 was prepared by constitution of 200 mg dry powder with 10 mL of sterile water for injection to a final concentration of 20 mg/mL. Each subject was to receive three intravenous bolus doses of AFO150 in the following sequence: 0.125,0.25, and 0.5 mg/kg. All but 1 subject was dosed according to the protocol (I subject received approximately half the AF01 50 dose at each dose level and was included in the analysis as intent-to-treat).

Duration or Treatment: Each bolus dose was injected over approximately 10 seconds with a 10-minutc interval between each dose.

Criteria for Evaluation: Efficacy: Efficacy was assessed by the ability of each dose of AFOI50 to opacify the left ventricle. In addition, the duration of attenuation and the duration of useful contrast enhancement were assessed for each dose.

Safety: Safety was based on adverse events (AEs) and hematology, coagulation, blood chemistry, urinalysis, vital signs, and electrocardiogram (ECG) measurement.

Statistical Methods: Data were summarized with descriptive statistics. For the primary efficacy endpoint, LV opacification, differences among doses and views were tested using mixed effects analysis of variance (ANOVA); comparisons included fixed effects for dose and view and random effects for subject, and a comparison without a fixed effect for view. For the secondary endpoints, duration of attenuation and duration of useful contrast enhancement, differences among doses were tested using mixed effects ANOVA; the comparison included fixed effects for dose and random effects for subject. The analysis was to be performed separately for quantitative (videodensitometry) and qualitative (blinded review) measures. A significance level of 0.01 was used.

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. 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, LV opacification 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.

Analysis of the effect of view on qualitative LV opacification across all doses showed that the apical 4-chamber view was significantly better (P=0.0004) than the apical long axis view in continuous mode. When the effect of dose was analyzed for the qualitative data for each view, the only statistically significant difference observed was in the gated mode; qualitative LV opacification increased significantly (P=0.0013) from the 0.125- to 0.5-mg/kg dose groups for the apical 2-chamber view in gated mode.

Duration or attenuation: A statistically significant *(P=0.0001)* dose-dependent increase in duration of attenuation was observed with each increasing dose level.

Duration or useful contrast enhancement: Mean duration of useful contrast enhancement increased in a dose dependent manner at each dose level for both the quantitative and qualitative assessments. 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: AFO150 was well tolerated in this study with no serious or severe adverse events reported. AEs were reported in 2 (11%) of 18 subjects and consisted of one event each of moderate hypoalkemia 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 no significant electrocardiographic changes from baseline were reported for ECGs performed during the study.

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