CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-191

Pharmacology Review(s)

BACKGROUND

This is the 3rd submission for the NDA 21-191, *Imavist* (AF0150), in response to comments indicated in the Approvable Letter dated February 6, 2002. The sponsor proposed to change the drug name to *Imagent*. Therefore, in this review, *Imavist*, *Imagent* and AF0150 will be used interchangeably.

The sponsor was asked to address the following pharm/tox issues raised from the 2nd submission review in the AE letter:

II. CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS, AND PHARMACOLOGY/TOXICOLOGY

A. The submission lacks data on the Pharmacokinetics (PK) of Imavist in pulmonary impaired patients.

In the action letter of August 14, 2000, these data were requested. In lieu of human data, the resubmission included a commitment to provide similar data in dog studies to be completed postmarketing. This request amounts to a waiver of human data based on animal data. Since the animal data are not available for review, it is not possible to determine their acceptability or to determine the appropriateness of a waiver of human data. Therefore, before approval either the results in humans with the potential to have decreased expiration of oxygen or in an appropriate animal model must be submitted. Positive controls (e.g., carbon dioxide diffusion capacity) must be used. If animal data are to be submitted, the protocol must be submitted for our review before implementation.

.....

IV. POST MARKETING COMMITMENTS – The following study agreements are requested:

A. We acknowledge the December 12, 2001, submission of your postmarketing commitment to complete a subacute/chronic pulmonary hypertension study in dogs. We advise you that comments on the preclinical dog study protocol will be issued in a separate letter. Additionally, in order for the commitment to be adequate, revise the commitment to add the following time frames:

The study will be implemented within 4 months of protocol agreement. The results will be submitted within 4 months of study completion.

B. Recent proprietary data suggests that several microsphere products may reside in the body for prolonged periods after imaging is lost and after the fluorocarbon is eliminated. It appears that the microspheres exchange oxygen and carbon dioxide. In order to address this commit to the following:

The completion of a non-clinical study to determine the fate of the activated microspheres, characterizing the length of microsphere persistence and the potential for microsphere gas exchange. Submit draft protocols within 6 months of approval

with initiation of the studies within 6 months of agreement on protocol design. Submit final study reports within one year of study initiation.

C. Recent literature data suggest that ultrasound-triggered microsphere cavitation induced vascular endothelium damage (endothelial hemorrhage) in vivo and ex vivo. The long-term effects of such damage have not been established. In order to determine whether Imavist causes similar effects, commit to the following:

   To study the cavitation effects of Imavist on vasculature with an animal study. If endothelial damage is seen, a subsequent study to evaluate the long-term effects will be conducted.

PHAR/TOX REVIEW

Sponsor's response to Clinical Pharmacology and Pharm/Tox issue in the Section II of the AE letter:

A study report titled "*Effects of Minute Ventilation on Perfluorohexane Elimination after Intravenous Administration of AF0150 in Healthy, Anesthetized and Ventilated Beagle Dogs.*" was provided in the volume 4 of the current submission. This study report is identical to the one submitted to the Agency on March 15, 2002, and the review for this study was completed on March 26, 2002 (see pharm/tox review of March 26, 2002 for detail). It was concluded that this study provided very limited information to address the issue raised during the 1st and 2nd review cycles about effects of pulmonary impairments on PK of PFH, due to multiple deficiencies (inappropriate animal model, insufficient observation period, missing pre-injected PFH measurement for recovery calculation and missing blood PFH kinetics).

A teleconference was held with the sponsor on April 1, 2002 to discuss this study together with clinical COPD results. The following recommendation from the Agency was made to the sponsor: PK study of *Imavist* should be conducted using a pulmonary embolism animal model as a phase IV commitment, and the results from the PK study will determine if further clinical study is necessary to address effects of Imavist on compromised pulmonary subjects. The sponsor accepted our recommendation during the teleconference and has made clear commitment in the current submission (as seen below).

Sponsor's response to Post-marketing commitments in the Section IV of the AE letter

A. We acknowledge the December 12, 2001, submission of your postmarketing commitment to complete a subacute/chronic pulmonary hypertension study in dogs. We advise you that comments on the preclinical dog study protocol will be issued in a separate letter. Additionally, in order for the commitment to be adequate, revise the commitment to add the following time frames:

   The study will be implemented within 4 months of protocol agreement. The results will be submitted within 4 months of study completion.

The sponsor committed to conduct a study in dogs with subacute pulmonary hypertension. The study protocol was submitted on December 21, 2001 as a supplemental submission (as per the Agency's request) during the 2nd cycle review. The comments on the protocol were sent to the sponsor on March 18, 2002. The sponsor agreed to revise the protocol to incorporate the Agency's comments, particularly including PK study of PFH in blood and expired air. The sponsor also committed that a small clinical PK study in COPD subjects will be conducted, depending on the results from the dog study. This was the agreement that the sponsor made during the teleconference of April 1, 2002. The sponsor will follow the timeline that the AE letter requested to complete this commitment.

Reviewer's Comments: the sponsor's response is acceptable.

B. Recent proprietary data suggests that several microsphere products may reside in the body for prolonged periods after imaging is lost and after the fluorocarbon is eliminated. It appears that the microsphere exchange oxygen and carbon dioxide. In order to address this commit to the following:

   The completion of a non-clinical study to determine the fate of the activated microspheres, characterizing the length of microsphere persistence and the potential for microsphere gas exchange. Submit draft protocols within 6 months of approval with initiation of the studies within 6 months of agreement on protocol design. Submit final study reports within one year of study initiation.

The sponsor has not yet identified an analytical method to address the fate of the microsphere, but will continue to investigate potential methods and submit protocols post approval according to timeline the AE letter requested.

Reviewer's Comments: the sponsor's response is acceptable. The following suggestions will be forwarded to the sponsor: the existing 2 study systems that the sponsor submitted in the original NDA could be considered for study design.

i. *In vitro* simulated circulation model (as described in the report #RE-99-46) in the presence of different partial pressures of O2 and CO2 in circulated solution (preferably plasma instead of albumin-saline).

ii. *In vivo* Doppler signal monitoring under different partial pressures of O_2 and CO_2 in arterial blood (as described in report #EB-98-15).

D. Recent literature data suggest that ultrasound-triggered microsphere cavitation induced vascular endothelium damage (endothelial hemorrhage) in vivo and ex vivo. The long-term effects of such damage have not been established. In order to determine whether Imavist causes similar effects, commit to the following:

To study the cavitation effects of Imavist on vasculature with an animal study. If endothelial damage is seen, a subsequent study to evaluate the long-term effects will be conducted.

Reviewer's Comments: The sponsor agreed to conduct a study to address this issue post approval, but did not provide any information about methods and timeline. The sponsor will be advised to provide study plans during phase IV period.

OVERALL SUMMARY

The sponsor provided an appropriate response to issues in two section of the Approvable Action Letter of February 6, 2002: the clinical pharmacology and pharm/tox issue in the section II and postmarket commitments in the section IV. There are no outstanding pharm/tox issues for this NDA at the present.

RECOMMENDATION

From pharm/tox perspective, this NDA is Approval. The following comments will be forwarded to the sponsor for study design of phase IV commitment (sent with a separated letter from Action Letter):

1. To address the commitment B (fate of microbubbles), the following 2 study systems submitted in the original NDA could be considered:

   i. *In vitro* simulated circulation model (as described in the report #RE-99-46) in the presence of different partial pressures of O2 and CO2 in circulated solution (preferably plasma instead of albumin-saline).

   ii. *In vivo* Doppler signal monitoring under different partial pressures of O_2 and CO_2 in arterial blood (as described in report #EB-98-15).

2. To address the commitment D (cavitation and endothelium damage), a detailed study plan (methodology and timeline) should be provided.

SIGNATURES

Reviewer:

Jin Chen, *MD, PhD, MPH* — Date
Reviewing Pharmacologist

Team Leader:

Adebayo Laniyonu, *PhD* — Date
Acting Supervisory Pharmacologist

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

/s/

Jin Chen
5/7/02 01:33:25 PM
PHARMACOLOGIST

Adebayo Laniyonu
5/9/02 12:14:14 PM
PHARMACOLOGIST
I concur with Dr. Chen's recommendation.

Supervisory Pharmacologist Memorandum

NDA:	21-191
Drug:	Imavist (AFO 150)
Sponsor:	Alliance Pharmaceutical Corp
Submission Type:	Resubmission of NDA (October 30, 2001)
Method of administration:	Intravenous

AFO 150 (Imavist) is a perfluorohexane-phospholipid microbubble contrast agent indicated for improved delineation of left ventricular endocardial border [redacted]

An approvable letter was sent out to Alliance on August 14, 2000, in which 4 pharm/tox-related issues were raised. Two of the issues were requested to be addressed prior to approval and the other two could be addressed after approval. The resubmission contains the sponsor's response to these requests.

The issues that needed to be addressed by the sponsor prior to approval included:

1. A microcirculation study with an intraarterial mode of administration
2. Submission of available data on blood gas analysis in non-anesthetized animals.

The issues that could be addressed after approval included:

1. Studies in animal models with chronic pulmonary hypertension
2. A description of the pathogenesis and consequences of macrophage vacuolization and cecal lesions noted in toxicology studies.

The reviewing pharmacologist, Dr. Jin Chen, has written a detailed review of the sponsor's resubmission, including the above-mentioned issues. This memo is aimed at highlighting the outcome of the resubmission review.

1. The microcirculation study submitted was adequately designed and conducted. The study was conducted using the cremaster muscle of normal and hyperlipidemic rats dosed intraarterially with 40 mg/kg Imavist (30 X clinical dose based on body surface area) or with polystyrene particles as positive control. The results indicated that between 0.4 and 2% of the capillaries in the microcirculation bed were plugged by lodged microbubbles (maximal size 12 microns). The effect was transient, due to the deformability of the bubbles, and by 25 minutes after dosing, normal flow was established in the capillaries. This effect was more pronounced in hyperlidemic rats. A total of 0.1% of the total microbubbles injected was determined to be lodged in

the capillaries. This study was deemed adequate and the effect seen is not considered to be clinically significant.

2. The sponsor did not provide additional data regarding blood gas analysis in non-anesthetized animals. However, since this parameter could be assessed in clinical studies, there are no outstanding issues regarding this question, at the present time.
3. Regarding studies in an animal model with chronic pulmonary hypertension, the sponsor committed to perform a study in normal dogs injected with microspheres to simulate acute pulmonary hypertension. This type of study had been conducted by other sponsors of microbubble agents, therefore the study will be acceptable, pending review of the protocol.
4. Regarding the vacuolation of the macrophages, the sponsor attributed this finding to the HES (Hydroxyethyl Starch) in the formulation. Although this is a possibility, one cannot rule out the fact that microbubbles are injectable particulates, and therefore likely taken up by RES cells. This uptake could also potentially lead to macrophage vacuolation. Regarding the cecal lesions, the sponsor did not think that the findings were of toxicological consequence, since the lesions were only seen in mice, and were not dose-related. Although the finding may not be of concern to humans, due to species differences, cecal lesions have been reported with other microbubble contrast agents, and are therefore considered to be directly related to the drug administration. This question (#3) will be considered to be adequately addressed by the sponsor at the present time.

In conclusion, the sponsor has adequately responded to the pharm/tox issues raised in the approvable letter of August 14, 2000. No new changes to the pharm/tox sections of the package insert, resulting from the resubmission, are recommended. There are no outstanding issues from the pharm/tox persepective, that need to be resolved prior to approval. From the pharm/tox perspective, and we therefore recommend Approval of Imavist, pending resolution of issues from clinical, chemistry, biopharm, statistics and microbiology.

11/2/01.

Nakissa Sadrieh, Ph.D.
Supervisory Pharmacologist

PHARMACOLOGY AND TOXICOLOGY SAFETY REVIEW

Division of Medical Imaging and Radiopharmaceutical Drug Products • HDF-160 • CDER/FDA

NDA NUMBER **NDA 21-191**

DRUG NAME ***Imavist* (AF0150)**

SUBMISSION TYPE

Series No.	Letter Date	Stamp Date	Contents	Action
N-000-BP	October 26, 2001	October 30, 2001	Responses to requests	Review
N-000-A2 (vol. 1 and 7)	August 16, 2001	August 20, 2001	Response to Approvable letter dated August 14, 2000	Review

Relevant IND **IND** [redacted]

SPONSOR

Alliance Pharmaceutical Corp
3040 Science Park Rd
San Diego, CA 92121

Draft Date: **October 16, 2001**

Complete Date: **October 22, 2001**

Is submission approvable? **Yes**
Comments to Review Team **Yes**
Comments to Sponsor **No**

PHARM/TOX REVIEWER **Jin Chen, *MD, PhD***

REVIEW TEAM

Medical Officer: Bernard Parker, MD
Chemistry: Milagros Salazar, PhD
Statistician:
Project Manager: Tia Harper-Velazquez, PharmD

Table of Content

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BACKGROUND

The initial NDA for Imavist (AF0150) was submitted on October 11, 1999 and the review of pharm/tox studies was completed on July 25, 2000. The overall summary and conclusion on pharm/tox section were briefed to ODE-IV office on August 4, 2000, as follows:

Most studies, particularly those pivotal studies, in this NDA are adequate. There are no major deficiencies in pharm/tox section. No critical toxic effects were found; the safety margin was high, which mostly determined by transient effects. The pharmacology and toxicology section in this NDA is **approvable**. The following issues still need to be addressed.

1. A microcirculation study with AF0150 should be submitted **before approval**. As requested on March 30, 2000's T-Con, AF0150 microbubble behavior and effects on blood flow and capillary endothelial cells need to be evaluated in this study. Pathological conditions (such as atherosclerosis, hypertension, hyperlipidemia) and pharmacological cardiovascular stress should also be considered.

2. Pulmonary artery pressure and blood gas analysis were studied with *Imavist* in a thromboxane-induced pulmonary hypertension animal model and normal animals. Studies in chronic or subacute pulmonary embolism animal model are suggested to further assess potential pulmonary impact of *Imavist*. Also, it will be more valuable blood gas analysis is performed in non-anesthetized animals. These studies may be submitted **during post approval.**

3. Macrophage vacuolation and cecal lesions were found in some animal species. However, underlying mechanisms are unclear. The sponsor needs to comment on the potential impact of AF0150 on the physiology of monocytes/macrophages and cecum/appendix in humans. Related clinical observations should be discussed and correlated. Further studies, using *ex vivo* or *in vitro* systems, are suggested to understand mechanisms of cecal lesion and to test monocytes/macrophage function, particularly the bubble growth in the lesion tissue. These studies should be submitted **during post approval**.

4. Microbubble profile-related issues need to be clarified **before approval**:

   i. There was high variation in PFH levels of reconstituted AF0150 from vial to vial but the mg/ml was constant. The dosage in most pharm/tox studies was verified by osmolality measurement. How can PFH levels in each vial be correlated to osmolality measurements and microbubble profile? How does this affect the conversion of mg/kg AF0150 to bubble count/kg?

   ii. The reconstituted AF0150 concentrations of 40 or 20 mg/ml (400 or 200 mg fill per vial) were used in pharmacology & toxicology studies. This was different from the clinical concentration, 10 mg/ml (200mg fill vial). Possible differences in microbubble behavior (*in vivo* and *in vitro*) in these different preparations (fill sizes and concentration) need to be addressed.

5. Significant renal toxicity of AF0150 was noted in a rat study with saline volume challenge test (Study #IMUS-044-TOX). The NOAEL was less than 4 mg/kg, which is converted to human equivalent dose of <0.65 mg/kg and human dose multiple of <5 folds. The sponsor needs, **before approval**, to address this issue by correlating this finding with renal observations in clinical trials, particularly in those patients with decreased renal function.

6. AF0150 was studied in animals given pharmacological stress agents, but not administrated a physical stress test (such as treadmill). Neither stress test with AF0150 were evaluated in

humans (as indicated in the labeling). Since it is conceivable that AF0150 will be used with either pharmacological or physical stress tests in a clinical setting, the sponsor needs to comment on this. Physical stress test with *Imavist* in animals should be conducted **before approval if the indication is for stress tests**.

7. Potential drug-drug interaction and drug-food interaction of AF0150 with other medications was not evaluated. However, the target patients may be treated with certain medications for cardiovascular or pulmonary diseases. The sponsor needs to address if and how common medications used in target patients may interact with AF0150, pharmacologically and chemically. As described in the labeling, patients included in the phase 3 trials had the following medical conditions: hypertension (60%), CAD (40%), COPD (20%) and LVEF <50% (20%). It could be useful for the sponsor to demonstrate if there was any correlation between the observed clinical adverse effects and the medications in these subjects before, during and after AF0150 administration. This could be narrowed down to specific classes of drugs for drug-drug interaction.

An approvable letter for this NDA was issued to the sponsor on August 14, 2000. In the letter the sponsor was requested to address the following pharm/tox issues:

II. NON-CLINICAL PHARMACOLOGY/TOXICOLOGY

The application lacks sufficient detail to complete the non-clinical assessment.

A. ISSUES TO BE ADDRESSED BEFORE APPROVAL

1. **Perform a microcirculation study to assess the potential for coalescence, clumping, and aggregation as well as to visualize microspheres as they transverse vessels. We note your commitment to complete such a microcirculation study. As requested in our March 30, 2000, teleconference, this study should include a direct arterial injection of IMAVIST. Also, based upon data in the NDA, in addition to evaluating the microcirculation of normal animals, the use of animals with compromised vasculature (e.g., atherosclerosis) is recommended.**

2. **Provide available data on blood gas analysis in non-anesthetized animals receiving IMAVIST.**

B. ISSUES THAT MAY BE ADDRESSED EITHER BEFORE OR AFTER APPROVAL.

1. **Studies in chronic or subacute pulmonary embolism animal models are needed to further assess the potential pulmonary impact of IMAVIST in chronically compromised patients.**

2. **Macrophage vacuolation and cecal lesions were found in some animal species. However, underlying mechanisms are not identified. The pathogenesis and clinical consequences of these abnormalities should be addressed.**

If these issues are not addressed before approval, please commit to addressing them after approval.

In this submission package, the sponsor provided responses to the above pharm/tox issues under non-clinical section.

PHARM/TOX DATA REVIEW

FDA Issue A1: Perform a microcirculation study to assess the potential for coalescence, clumping, and aggregation as well as to visualize microspheres as they transverse vessels. We note your commitment to complete such a microcirculation study. As requested in our March 30, 2000, teleconference, this study should include a direct arterial injection of IMAVIST. Also, based upon data in the NDA, in addition to evaluating the microcirculation of normal animals, the use of animals with compromised vasculature (e.g., atherosclerosis) is recommended.

Sponsor Response: a microcirculation study was conducted using cremaster muscle microcirculation system in both normal and tansgenic hyperlipidemic rats. The full study reported was provided in this submission, as reviewed below:

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

Study no:	IM-4000 (Report # ER-00-01)
Volume # and page #:	7 of 16, pp007-053
Conducting laboratory and location:	
Date of study initiation:	June 200 – February 2001
GLP compliance:	No
QA report:	No
Drug lot #:	Lot # UA10003
Formulation/vehicle:	AF0150

Methods

Ex vivo microcirculation study in cremaster muscle of normal rats and genetically hyperlipidemic rats using intravital [redacted] microscopy.

Animals: Total 48 male rats were used, including normal Wistar rats (248-342 g body weight, 8-11 week-old) and genetically altered hyperlipidemic rats (564-807 g body weight, 16-37 week-old). The hyperlipidemic rats were developed from crossbreeding female Zucker Diabetic Fatty (ZDF) and male Spontaneously Hypertensive Heart Failure (SHHF), maintained on a feed supplemented with fat. Blood lipids and glucose profiles from the normal and hyperlipidemic rats were measured and summarized in the following Table 1. In addition, 13 Wistar rats were used as blood donor for preparing [redacted] RBC.

Table 1. Blood Metabolic Parameters in Normal and Hyperlipidemic Rats

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Normal *n=6*	75 ± 6	95 ± 12	153 ± 5
Hyperlipidemic *n=21*	431 ± 61*	3484 ± 381*	538 ± 15*

†From the literature normal blood parameter values for adult male rats:[13]
Cholesterol = 40-130 mg/dL
Triglycerides = 26-145 mg/dL
Glucose = 50-135 mg/dL
*p<0.05, vs. the Normal group.

Animal Preparation: Non-fasted rats were anaesthetized by IM with Na Pentobarbital, allowed to free breath room-air and maintained at 37°C on circulating water heating pad throughout the study. The left femoral artery, femoral vein and carotid artery were isolated and cannulated for monitoring of BP and blood sampling (the femoral artery), administration of fluorescence-labeled RBC and anesthetic (the femoral vein) and injection of AF0150 and polystyrene microparticles (the carotid artery catheter advanced to the arch of aorta with approximately 3-5 mm "free-end"). All catheters were maintained patent with heparinized saline.

Cremaster Muscle Preparation: the cremaster muscle of the right testicle was prepared according to the method described in the literature (Baez S: An Open Cremaster Muscle Preparation for the Study of Blood Vessels by in vitro Microscopy. *Microvasc Res* 5: 384-394, 1973). The capillary diameter was 5-8 um (5.4±0.2 um), which is comparable to human capillary (4-9 um in diameter). There are no arteriovenus anastomoses in the microvasculature of the cremaster muscle. During surgical preparation and throughout the study, the cremaster muscle was continuously superfused with Krebs-Henseleit buffer at 37°C equilibrated with 95% N2 and 5% O2.

Intravital Microscopy: a customized intravital microscope , [redacted] and the stage holding the rat was mounted in the microscope. The images were stored in video tape and CD-ROM for analysis.

Administrations: AF0150, 40 mg/kg (2 ml/kg), or equivalent saline was injected through carotid artery catheter into aorta, with total injection time of 25 seconds (the rate, the size of needle and tube were considered to minimize microbubble damage during delivery).

Validation of Microcirculation System: The cremaster muscle circulation system was validated with stiff , [redacted] polystyrene microparticles by IA injection in both normal and hyperlipidemic rats. The diameter of the microparticles was 10.2 ± 0.9 um and number of the particles was calculated to be equivalent to the minimum number of AF0150 microbubble with diameters >10um that would be expected in the 40 mg/kg AF0150 dose (3.8 x 106 microbubbles > 10um for each ml).

Observations and times:

Observation parameters included systematic hemodynamics (BP and HR), microdynamics (blood flow velocity in arterioles and capillaries), adhesion, aggregation, and coalescence of microbubbles in the microvasculature. The experimental protocols and observation duration were summarized in the following ***Tables 2 and 3***.

Table 2. Animal Groups and Observations

Group (Number of Animals)	Primary Objective of Experiments
High-Speed Camera Experimental Series	
Normal (n=6) Hyperlipidemic (n=5)	Visualize AF0150 microbubbles during "first pass" transit through the muscle capillary network after IA injection using a high-speed camera system.
Early Scan Experimental Series	
Normal (n=2) Hyperlipidemic (n=2)	Visualize and count number of lodged AF0150 microbubbles in capillaries by scanning muscle immediately after and a few minutes after IA injection.
Arteriolar/Venular Experimental Series	
Normal (n=6)	Measure arteriolar and venular diameters and blood flow velocities after IA injection of saline and AF0150.
Capillary Experimental Series	
Normal (n=7) Normal + PAF (n=6) Hyperlipidemic (n=8) Hyperlipidemic + PAF (n=6)	Measure number of perfused capillaries and capillary RBC velocity after IA injection of saline and AF0150. Scan muscle microcirculation after AF0150 injection for lodged, adhered, aggregated, and coalesced AF0150 microbubbles. In some groups, PAF was applied topically to the muscle to induce acute inflammation.

IA = intra-arterial PAF = platelet-activating factor

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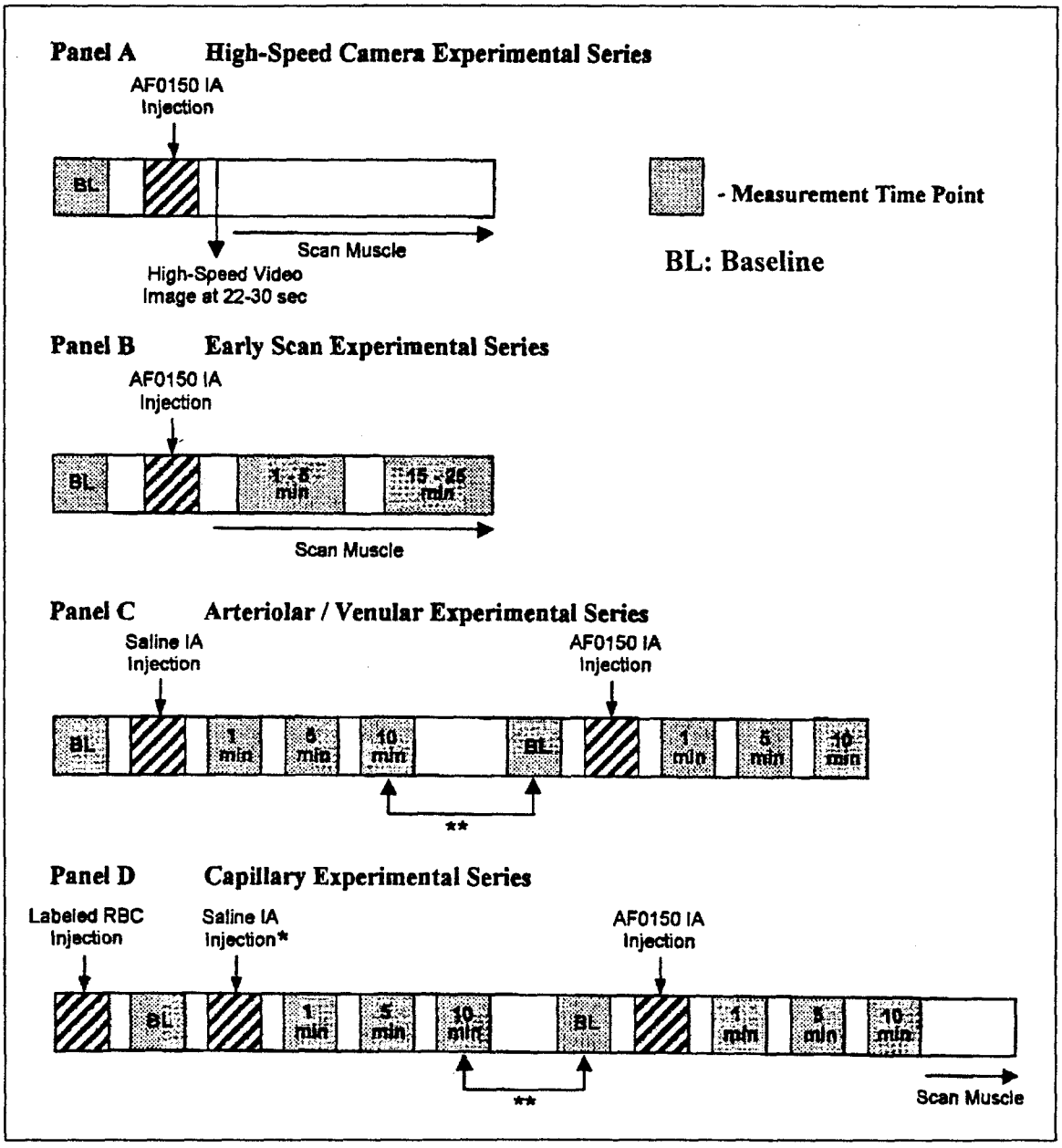
Table 3. Experimental Protocols and Observation Times



Results:

Systemic Hemodynamics: there were no significant changes in BP and HR in normal and hyperlipidemic rats between pre-dose (baseline) and post dose (up to 10 min) of AF150 and between saline and AF0150 IA administration.

Microhemodynamics: Findings included first-pass transit, microbubbles lodging, arteriolar/venular diameters and blood flow velocity, capillary perfusion and capillary RBC velocity.

Validation of Cremaster Muscle Microcirculation System: stiff polystyrene microparticles (10 um) at the same delivery condition as AF0150 induced permanent plugging of capillaries. The approximate percentage of plugged microvessels was estimated as 0.5-1%. There was no significant difference in the mean number of plugged microparticles between normal and hyperlipidemic rats, as seen in the ***Table 4.***

Table 4. Validation of Cremaster Muscle Microcirculation System with Polystyrene Microparticles

Group	Number of Plugged Microparticles§§	
	Mean ± SEM	Range
Normal *n=7*	31 ± 5	[redacted]
Hyperlipidemic *n=7*	24 ± 7	[redacted]

§The number of microparticles injected IA was taken as the expected minimum number of AF0150 microbubbles with diameter >10 μm.
§§Determined by scanning 33 to 50% of the entire cremaster muscle in each animal.
No intergroup statistically significant differences were found.

First-pass transit observation: using high-speed camera, digital images were taken every <0.1 seconds starting at 25 seconds after IA injection of AF0150 in normal and hyperlipidemic rats. Various sizes of AF0150 microbubbles were first seen during 8 seconds of digital recording and moved freely and independently through the microvessels. Transient lodging/plugging of microbubbles were observed, which lasted seconds to a few minutes. However, nature (quantity and quality) of lodged/plugged microbubbles were not specified in this experiment. There was no growth in microbubble size, and no aggregation or coalescence. The size of larger microbubbles tended to decrease prior to resuming movement through capillaries.

Microbubble Lodging: number of lodged microbubbles in capillaries were counted during muscle scanning at 1-5 minutes (5-10% cremaster muscles) and 15-25 minutes (33-50% cremaster muscles) in both normal and hyperlipidemic rats (***Tables 5 and 6***).

Higher cumulative number of lodged microbubbles was noted in hyperlipidemic rats at 1-5 minutes post AF0150, as presented in ***Table 5***. The majority of lodged microbubbles was a 1:1 correlation with capillaries, i.e., the lodged microbubbles represented the number of microbubble-lodged capillaries. The total number of capillaries scanned from 5-10% the cremaster muscle was estimated at minimum of 1000. Therefore, approximately 0.4-2.1% of capillaries were impacted by microbubble lodging.

The largest bubbles lodged in capillaries were 10-12 um. The size distribution of lodged bubbles was summarized in ***Table 6***.

The lodged microbubbles tended to deform (shrink and change in shape) prior to passing the capillaries. There were no lodged, adhered, aggregated or coalesced microbubbles at 15-25 minutes. The report did not indicate if the number of lodged microbubbles included results from the above first-pass transit observation.

The estimated fraction of lodged microbubbles over injection microbubbles (40 mg/kg by intra-aorta) was 0.1%.

Arteriolar/Venular Diameters and Blood Flow Velocity: there was significant increase in the diameters of arterioles and venules after IA injection AF0150 during the 10-miniute observation in normal rats (hyperlipidemic rats were not observed) as compared to baseline (for arterioles and venules) and to saline injection (venules). Blood flow velocity in the corresponding vessels increased after AF0150 injection in both arterioles and venules as compared to baseline and saline injection groups. No explanation was provided other than a large variation in velocity of one venule.

Table 5. Lodging of AF0150 Microbubbles in Rat Cremaster Muscle Microvessels

Group	**Number of Lodged AF0150 Microbubbles** *Time After IA Injection for Muscle Visualization and Microbubble Count (min)*	
	1-5‡	*15-25*‡‡
Normal		
Rat #1	4	0
Rat #2	8	0
Hyperlipidemic		
Rat #1	10	0
Rat #2	21	0

‡Estimate that 5 to 10% of the cremaster muscle was scanned during the 4 minutes.
‡‡The cremaster muscle was scanned until 33 to 50% of the entire muscle had been visualized.

Table 6. Size Distribution of Lodged Microbubbles in Capillaries

Number of Stationary ("Lodged") Microbubbles According to Size*			
Animal**	Size		
	<7 μm	≥7 μm and <10 μm	≥10 μm and <12 μm***
Normal Rat #1	5	1	0
Normal Rat #2	3	3	3
Hyperlipidemic Rat #1	5	3	2
Hyperlipidemic Rat #2	6	7	4

* In situations when the microbubble was deformed into an elliptical shape, the long axis was measured.
** Animals are those in Table III of report ER-00-01.
*** No microbubbles were observed of size >12 μm.

Capillary perfusion and RBC velocity: there were slight and transient decreases (5-11%) in number of perfused capillaries in both normal and hyperlipidemic rats with or without PAF challenge as compared to baseline at 1 minute post AF0150 and saline administration. AF0150 injection tended to decrease capillary perfusion more (1-2%) as compared to saline control group, as seen in ***Table 7***. In some rats, lodged microbubbles were visible in the non-perfused capillaries.

Table 7. Changes in Capillary Perfusion after AF0150 and Saline Administration

Group	Time After IA Injection (min)	Number of Perfused Capillaries		Perfused Capillaries (% of Baseline)	
		Saline	AF0150	Saline	AF0150
Normal *Saline n=41 AF0150 n=55*	**Baseline**	10.3 ± 0.9	9.2 ± 0.6	100 ± 0	100 ± 0
	1	10.3 ± 0.9	8.7 ± 0.8	100 ± 0	94 ± 3
	5	10.0 ± 0.7	8.8 ± 0.8	98 ± 2	96 ± 3
	10	9.8 ± 0.6	9.0 ± 0.6	96 ± 3	98 ± 2
Normal + PAF *Saline n=38 AF0150 n=54*	**Baseline**	9.5 ± 0.6	9.0 ± 0.4	100 ± 0	100 ± 0
	1	9.3 ± 0.6	8.3 ± 0.8	98 ± 3	92 ± 6
	5	9.3 ± 0.6	8.5 ± 0.7	98 ± 3	94 ± 4
	10	8.8 ± 0.9	8.3 ± 0.8	92 ± 3	92 ± 6
Hyperlipidemic *Saline n=32 AF0150 n=52*	**Baseline**	8.0 ± 0.4	8.0 ± 0.6	100 ± 0	100 ± 0
	1	8.0 ± 0.4	8.0 ± 0.5	100 ± 0	95 ± 4
	5	8.0 ± 0.4	8.8 ± 0.6	100 ± 0	100 ± 0
	10	7.3 ± 0.9	8.8 ± 0.6	90 ± 7	100 ± 0*
Hyperlipidemic + PAF *Saline n=32 AF0150 n=47*	**Baseline**	8.0 ± 0.7	7.8 ± 0.4	100 ± 0	100 ± 0
	1	7.8 ± 0.7	6.8 ± 0.7	96 ± 4	89 ± 6
	5	7.8 ± 0.7	7.8 ± 0.4	96 ± 4	100 ± 0
	10	7.8 ± 0.9	7.8 ± 0.4	96 ± 4	100 ± 0

Data are means ± SEM.
*p <0.05, vs. the Saline group.
The "n" for each group is the total number of capillaries analyzed from 4 rats receiving saline and 6 rats receiving AF0150.

The RBC velocity slightly decreased in both saline and AF0150-treated normal rats following PAF challenge. In hyperlipidemic rats, the velocity values were very variable; AF0150 treatment tended to increase RBC velocity in the rats without PAF application. However, PAF treatment decreased the velocity in both saline and AF0150 group, consistent with observations in normal rats. This is likely due to increased WBC-endothelium interaction in postcapillary venules following topical application of PAF to the cremaster muscle. There were no significant changes in RBC velocity within capillaries between saline and AF0150 treatment, in normal rats during the 10-minute observation.

Summary

Effects of AF0150 microbubbles on microhemodynamics were tested with a cremaster muscle microcirculation system in normal and hyperlipidemic rats. AF0150, at 40 mg/kg, saline, or [redacted] polystyrene microparticles (as a positive control) were injected intra-arterially (to aorta) with 10-minutes observation post dosing. Transillumination and [redacted] intravital

microscopy coupling with video system and computer-based imaging analysis was used to image blood perfusion and diameters of arterioles, venules and capillaries; velocity of [redacted] RBC in capillaries; and lodging, adhering, aggregating and coalescing of microbubbles in the microvessels. Acute inflammatory challenge to cremaster muscle with topical application of PAF was included in monitoring of capillary flow dynamics. The following results were observed:

1. Majority (99.9%) of AF0150 microbubbles freely and independently passed through microvessels with RBC after intra-aorta injection of 40 mg/kg AF0150 in both normal and hyperlipidemic rats.

2. Few microbubbles (approximately 0.1%, with size up to 12 um) transiently lodged in capillaries of both normal and hyperlipidemic rats in the first 5 minute observation, with higher incidence in hyperlipidemic rats. The lodged microbubbles tended to deform (shrink and change in shape) prior to passing the capillaries. The microbubble lodging tended to decrease capillary perfusion at 1 minute post AF0150 injection with a maximal decrease by 1-2% as compared to saline control.

3. No significant aggregated and coalesced microbubbles were found in the microvessels from both normal and hyperlipidemic rats during 10-minute observation.

4. The cremaster muscle microcirculation model was validated with [redacted] polystyrene microparticles, which demonstrated permanent plugging of 0.5-1% capillaries.

Conclusion

1. AF0150 microbubbles can transiently lodge in capillaries following intra-arterial injection, which tends to decrease capillary perfusion in normal and hyperlipidemic rats. Hyperlipidemic conditions seemed more favorable to lodging of microbubbles.

2. There was no evidence that AF0150 microbubbles aggregated or coalesced in microvessels based on the cremaster muscle microcirculation system.

Reviewer's Comments

1. The study appropriately assessed the effects of AF0150 microbubbles on microhemodynamics and the behavior of the microbubbles in microvessels. However, concurrent application of ultrasound was not included in the study to evaluate potential endothelium damage induced by microbubble cavitation. A long-term follow-up in clinic for potential effects of microbubbles cavitation on vasculature (such as vasculitis), particularly coronary vessels, is recommended.

2. The sponsor provided appropriate responses to those comments and requests raised following a preliminary review (review draft) of the submission. The comments raised by the reviewer were faxed to the sponsor on Oct 17, 20001, and a copy of the faxed is attached to this review, as Appendix 1.

FDA Issue A2: Provide available data on blood gas analysis in non-anesthetized animals receiving IMAVIST

Sponsor Response: There are no blood gas data available from nonanesthetized animals given *Imavist.* Animals in the nonclinical studies that evaluated efficacy were anesthetized at the time of drug administration for the purpose of the imaging procedure. Animals in the GLP toxicology studies were nonanesthetized at the time of *Imavist* administration; however, blood gas determinations were not part of the safety assessment

Reviewer's Comments: further studies may not be necessary if potential impacts of AF0150 on blood gases were addressed in clinical trials.

FDA Issue B1: Studies in chronic or subacute pulmonary embolism animal models are needed to further assess the potential pulmonary impact of IMAVIST in chronically compromised patients.

Sponsor Response 1 [*in submission N-000-A2, August 16, 2001****]***: To date, no validated chronic pulmonary embolism animal model has been identified. Efforts continue to evaluate subacute pulmonary embolism models found in the literature. When, and if, an appropriate model is identified, a protocol will be submitted to the FDA for review and comment prior to conducting the study.

Sponsor Response 2 [*in the submission N-000-BP, October 26, 2001****]***: an acute pulmonary embolism study in normal dogs will be conducted. Microspheres of appropriate size will be directly injected into pulmonary circulation to produce pulmonary embolism (with pulmonary artery pressure of 25-30 mmHg). The animals will then receive escalating doses of AF0150 followed by cardiopulmonary monitoring. The study design and protocol will be submitted to the Agency for review.

Reviewer's Comments: there are several animal models in the literature, including acute, subacute and chronic models. The sponsor was advised to re-search literature and propose a brief study plan using those animal models (forwarded by fax on October 17, 2001, as seen in the Appendix 1). In the response to our comments, the sponsor planned to conduct a study in acute pulmonary embolism dogs and will submit the study protocol. In the original NDA, the sponsor submitted a study conducted in thromboxane-induced pulmonary hypertension rabbit model. There were no remarkable findings in mean blood pressure, pulmonary artery pressure, heart rate and blood gases within 10-minute observation. Taken together, the current response is acceptable.

FDA Issue B2: Macrophage vacuolation and cecal lesions were found in some animal species. However, underlying mechanisms are not identified. The pathogenesis and clinical consequences of these abnormalities should be addressed.

Sponsor Response 1 *[in submission N-000-A2, August 16, 2001]*: the presence of vacuolated macrophages in the spleen, lymph nodes, liver, and various other organs of rats treated with *Imavist* is believed to be a consequence of the normal clearance process for this product and/or its components. Due to the particulate nature of the microbubbles, *Imavist* is most likely cleared from the circulation by the mononuclear phagocytic cells in the primary organs of the reticuloendothelial system (RES). Studies in mice, rats, rabbits, and dogs have shown that following IV administration, hydroxyethyl starch (HES), a component commonly used in synthetic plasma volume expanders, is accumulated by macrophages and parenchymal cells in the organs of the RES, frequently resulting in vacuolation of both cell types. HES, which is used in *Imavist* to impart structural strength to the dry, preconstituted microspheres, is not considered a component of the microbubble once constituted, however, it is injected along with microbubbles. The persistence of HES in the RES appears to be species dependent. Rats have been shown to have a relatively slow elimination process for HES, its approximate half-life in spleen and liver is 64 and 132 days, respectively, whereas dogs exhibit a more rapid elimination from the body. Based on this information, it is speculated that the vacuolated macrophages observed in both the single-dose and repeated-dose rat studies are the result of accumulated HES, and that the persistence of the vacuoles is due to the slow elimination process for HES in this species. Furthermore, there was no indication in any of the studies that the presence of the vacuolated macrophages impaired organ function or adversely affected the health of the animals.

The cecal lesion, consisting of diffusely thickened cecum walls containing clear fluid-filled cysts, were observed only in male mice. The relationship of these changes to administration of *Imavist* is unclear since they were found in two and four males in groups given 400 and 1600 mg/kg, respectively, and not in any of the males given 800 mg/kg or in any females at the dose levels evaluated. Although the etiology of these findings is unknown at this time, the changes were not considered to be toxicologically significant since they were not dose-related, there was no apparent adverse effect on the health of the animals that had the changes, and findings similar to those observed in the mice have not been noted in other animal species or other studies conducted with this product.

The clinical consequences, if any, of these findings are unclear at the present time. Both the presence of vacuolated macrophages and the cecal changes appear to be species specific and had little or no effect on the health of the animals. Investigation into the possible underlying mechanisms of these findings will continue with any significant results being reported to the FDA.

Sponsor Response 2 *[in the submission N-000-BP, October 26, 2001]*: HES is a synthetic plasma volume expander, with trade names as Hespan and Hextedn. The recommended clinical dose is 400-800 mg/kg, which is 800-1600 fold HES (0.5 mg/kg) of 0.125 mg/kg AF0150. Vacuolated RES in various organs, including liver, spleen, lymph nodes and bone marrow have been reported in humans after HES administration. Staining with Periodic Acid Schiff (PAS) and immunochemistry suggest the presence of HES within the vacuoles.

Reviewer's Comments: the sponsor provided a brief literature review in the "Response 2" to demonstrate that HES, a commonly used synthetic plasma volume expander, induced similar vacuolated cells in various organs in human, and the recommended dose of HES in human is 800-1600 fold higher than HES formulated in the *Imavist*. It appears that there is correlation between HES and the vacuolation of RES. However, vacuolation of RES has also been found in other microbubble products which are not formulated with HES, suggesting that other components in

addition to HES are involve in the vacuolation. It is likely that this was due to direct phagocytosis and thus clearance of intact microbubbles by the RES. It is recommended that the functional impact and fate of the vacuolation in RES still need to be appropriately followed up during clinical application.

Species difference in cecal lesion was indicated in the original NDA, and also noted in other microbubble products. Potential impact on human can not be ruled out. Appropriate follow-up in humans is recommended.

OVERALL SUMMARY

The sponsor provided responses to four pharm/tox issues which were included in the approvable letter of August 2000. The four issues were 1) *microcirculation study, 2) blood gas analysis in non-anesthetized animals, 3) study using chronic or subacute PE animal models and 4) pathogenesis of macrophage vacuolation and cecal lesion.* The first two issues were required to be addressed before approval and the last two before or after approval (but with commitment). All issues except #2 (the blood gas) were appropriately addressed in the two submissions (N-000 BP, 10/26/01 and N-000 A2, 8/16/01).

1. Microcirculation study (required before approval): A full study report was submitted to assess microbubble behavior in microvessels and potential impact on microhemodynamics using cremaster muscle microcirculation model in normal and hyperlipidemic rats. The model was validated with [redacted] polystyrene microparticles. The major findings were that AF0150 microbubbles transiently lodged in approximately 0.6% of the microvessels in normal rats and 1.6 % of the microvessels in hyperlipidemic rats. The size of lodged microbubbles was <7um to 12 um. The lodged microbubbles tended to transiently (1 minute) decrease capillary perfusion (by 1-2% as compared to saline control). No adherence and aggregation or coalescence of AF0150 microbubbles were observed in the microvessels of both normal and hyperlipidemic rats during 10-minute observation.

Concurrent application of ultrasound was not included in the microcirculation study to evaluate potential endothelium damage induced by microbubble cavitation. A long-term follow-up in clinic for potential effects of microbubble cavitation on vasculature (such as vasculitis), particularly in coronary vessels, is recommended.

2. Blood gas analysis in non-anesthetized animals (required before approval): this issue was not addressed; no data were submitted; no new studies were conducted or planned. Although this issue was not appropriately addressed from the pharm/tox perspective, appropriate justification based on human experience from clinical trials may be adequate.

3. Study using chronic or subacute pulmonary embolism (PE) animal models (required before or after approval): the sponsor committed to conduct study with an acute pulmonary embolism dog model after approval. A brief study plan was provided. The response is acceptable.

4a. Macrophage vacuolation (required before or after approval): The sponsor provided a brief literature review to indicate that HES, as a synthetic plasma expander, induces vacuolated cells in various organs in human. The clinical dose of HES for plasma expansion is 800-1600 fold higher than HES formulated in *Imavist*. However, the vacuolation in RES were also found in other microbubbles formulated without HES. It is recommended that the functional impact and fate of the vacuolation in RES still need to be appropriately followed up during clinical application.

4b. Cecal lesion (required before or after approval): the sponsor's response is acceptable. However, the species difference in cecal lesion, which was also noted in other microbubble products, does not rule out that there may be potential impact on humans. Appropriate follow-up in humans should be planned and a commitment should be made to address potential long-term impact on cecal pathophysiology, particularly in patients with previous cecal and appendix inflammation.

RECOMMENDATION

From the pharm/tox perspective, we recommended Approval. However, the following potential risks in human may need to be assessed during clinical application of *Imavist*:

1. Potential long-term effects of microbubble lodging and cavitation-triggered endothelium damage of coronary vessels in humans, particularly in those patients with hyperlipidemia.

2. Potential long-term effects of the *Imavist* on cecal pathophysiology in humans, particularly in those patients with history of cecal and appendix inflammation.

3. Potential long-term functional impact of the vacuolation on macrophages/monocytes.

SIGNATURES

Reviewer:

Jin Chen, MD, PhD
Reviewing Pharmacologist

NOV. 5, 2001
Date

Team Leader:

Nakissa Sadrieh, PhD
Supervisory Pharmacologist

11/20/01
Date

CC: list
Original NDA; HFD-160/Division Files/Review Team

Review and Evaluation of Pharmacology and Toxicology Data

Division of Medical Imaging and Radiopharmaceutical Drug Products • HFD-160

1. **Reviewer:** **Jin Chen, MD, PhD, MPH**
   Pharmacologist

2. **Electronic File Number:**

3. **NDA Number:** **21-191**

4. **Serial Number:** **N000**
   Date: **October 11, 1999**
   Type of submission: **Original NDA**

5. **Information to Sponsor:** **Yes (X) No ( )**

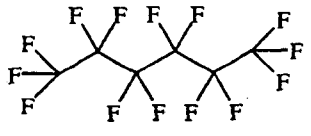
6. **Completion Date:** **July 25, 2000**

7. **Sponsor:** **Alliance Pharmaceutical Corp**
   3040 Science Park Rd
   San Diego, CA 92121

8. **Manufacturer for drug substance:**

9. **Drug:**
   Trade Name: ***Imavist™***
   Code Name: **AF0150**
   Chemical name: **Perflexane-phospholipid microbubbles**
   CAS number: **355-42-0 (Perflexane);18194-24-6 (DMPC)**
   Molecular Weight: **338.04 (Perflexane); 677.96 (DMPC)**
   Structures:

Perflexane



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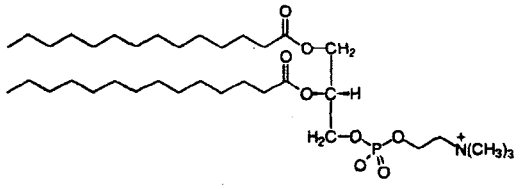

14. **Relevant IND/NDA/DMF:** **IND** ~~~

TABLE OF CONTENT

15. Drug Class: **Ultrasound Contrast Agent**

16. Indication:

/

17. Clinical Formulation (and components, 200mg Fill Size):

Each kit of *Imavist™* contains one vial of AF0150, one vial of 20-ml SWFI, one vented dispensing pine and one 10-ml disposable syringe. Planned Clinical Dose (PCD) was 0.125 mg (whole power) per kg body weight.

Component	Composition* (*mg/vial*)
Active Components	
Perflexane#	/
DMPC	
Inactive Components	
NaCl	!
Na2HPO4	
NaH2PO4	
Poloxamer 188	
HES	

DMPC: 1,2-Dimyristoyl-sn-glycero-3-phosphocholine
HES: m-Hydroxyethyl starch
* Dry weight basis;
# Added as a mixture of perflexane/N2 during filling

18. Route of Administration: **Intravenous injection**

19. Proposed clinical protocol: **N/A**

20. Studies reviewed within this submission:

GENERAL PHARMACOLOGY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg)	GLP	Review Page#
EB-95-08 Vol.009 P077-097	08/95	Dose-response of Doppler to AF0150	Rabbit 6-Bolus 6-Inf	2.5; 5; 10 Bolus Infusion	No	12
EB-97-17 Vol.009 P098-107	01/97; 08/98	Dose-Response of Doppler to AF0150 (3 studies)	Rabbit 6-A;1-B 1-C	0.059-5.0 Bolus	No	15
EB-95-28 Vol.009 P108-122	10/95-04/96	Effects of AF0150 injection dose and mode on Doppler and echocardiography	Swine 6-Dopp 2-2nd H	0.13; 0.66; 1.3; Bolus Infusion	No	16
EB-95-25 Vol.009 P123-135	10/95	Dose-response of hemodynamic and echocardiography to AF0150	Dog 6	0.03; 0.09; 0.3; 0.6 Bolus	No	19
RMI-97-01 Vol.009 P136-139	01/97	Computer program for Doppler signal analysis	ND	ND	No	22
RMI-97-02 Vol.009 P140-144	05/97	Computer program for Doppler signal analysis	ND	ND	No	23
PSM-97-04 Vol.009 P145-146	07/97	Effects of AF050 fill size and reconstituted concentration on echocardiography	Swine 2	160-240 mg/kg/Hr Infusion	No	24
EB-97-16 Vol.009 P147-158	07/97	Effects of AF050 fill size and reconstituted concentration on Doppler signals and ultrasound power resistance	Rabbit 4	1.0 Bolus	No	25
EB-98-14 Vol.009 P159-171	04-09/98	Effects of AF0150 reconstituted with pretreated water (tension and temperature) on Doppler signal	Rabbit 3	0.2, 1.0 Bolus	No	27
EB-98-17 Vol.009 P172-183	02-03/98	Effects of AF0150 constitution conditions (time and vial inversion) on Doppler signal	Rabbit 4	1.0	No	29
RE-99-47 Vol.009 P184-205	ND	Effects of different AF0150 microbubble size on Doppler signal	Rabbit 2/3	Not specified	No	30
RE-99-46 Vol.009 P213-222	03/99	Effects of ultrasound power on AF0150 microbubble size *in vitro*	N/A	0.056 mg/ml (*In vitro*)	No	32
EB-97-04 Vol.009 P223-236	ND	Effects of ultrasound power on AF0150-induced Doppler signal	Rabbit 7/6	1.0 Bolus	No	34

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg)	GLP	Review Page#
EB-97-20-Vol.009 P237-247	ND	Effects of ultrasound power and frequency on AF0150 Doppler signal (amended study)	Rabbit 3	1.0 Bolus	No	36
EB-98-18 Vol.009 P248-259	10/98	LV cavity imaging using [redacted]	Swine 2	0.125; 0.25 Bolus	No	37
EB-98-19 Vol.009 P260-269	07/98	LV cavity imaging using [redacted]	Swine 2	0.125 Bolus	No	39
EB-98-20 Vol.009 P270-282	06/98	LV cavity imaging using [redacted] with different imaging frame rate	Swine 3	0.125; 0.25 Bolus	No	40
EB-98-16 Vol.009 P283-292	05/98	Effects of pressure-pretreated AF0150 solution on Doppler signal	Rabbit 1	1.0 Bolus	No	42
EB-98-22 Vol.009 P293-307	11-12/98	Effect hypertension on AF0150-induced Doppler signal and cardiac imaging	Rabbit 7 Swine 4	0.2; 1.0(rabbits); 0.25(Swine) Bolus	No	43
EB-98-15 Vol.009 P308-322	01-03/98	Effects of high O2 inhalation on AF0150-induced Doppler and cardiac imaging	Swine 4	0.125; 0.25 Bolus	No	47

SAFETY PHARMACOLOGY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
Cardiovascular Safety						
EB-95-19 Vol009 P324-334	10/94 07/95	HR, MAP and blood cell. (anesthetized; up to 1-hr post dosing observation)	Rabbit 3 Control 24 AF0150	20 (52) IV bolus	No	61
EB-97-13 Vol. 009 p335-348	ND	Hemolysis, Hematology and Hemodynamics with ultrasound application, (anesthetized, up to 4-hr post dosing observation)	Dogs 4 males	20 (86) IV infusion (1mg/kg/min, 20 min)	No	63
EB-95-25 Vol.009 P123-135	10/95	Dose-response of hemodynamic and echocardiography. (anesthetized, 1-min post dosing observation)	Dog 6	0.03, 0.09, 0.3, 0.6 (-2.6) IV bolus	No	19
EB-95-27 Vol.009 P349-362	11/95 - 03/96	Hemodynamics (HR, BP, CO, PAP), PO2 and Echocardiography. (anesthetized, up to 10-min post dosing observation)	Dog 4	0.4, 0.8, 1.2, 1.6 (-6.9) IV bolus	No	66

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
EB-98-05 Vol009 P363-374	ND	Hemodynamics under adenosine-induced CV stress. (anesthetized, up to 1-hr post dosing observation)	Rabbit 4-5/group	2, 20 (5.2, 52) IV bolus	No	69
EB-98-06 Vol.009 P375-389	ND	Hemodynamics under Dipyridamole-induced CV stress (anesthetized, up to 1-hr post dosing observation)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	No	71
EB-98-07 Vol.009 P390-398	12/97 - 01/98	Hemodynamics under Arbutamine-induced CV stress (Anesthetized)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	No	74
EB-98-08 Vol.009 P399-408	08/98	Hemodynamics under Dobutamine-induced CV stress (Anesthetized)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	No	76
EB-98-13 Vol.009 P409-421	03-04/98	Pulmonary Artery Pressure in the U46619-induced Pulmonary Hypertension (anesthetized, up to 10-min post dosing observation)	Rabbit 3 control 4 mild 4 moderate	1, 4, 10 (2.6, 10.4, 26) IV bolus to all groups	No	78
BS-97-09 Vol.010 P016-028	05/97	Effect on the 99mTC-Sestamibi (MIBI) cardiac imaging (anesthetized, 30-min post dosing observation)	Rabbit 32 4 control 6 ischemia	0.5, 2.0 (1.3, 5.2)	No	81
IMUS-016-TOX Vol.022 P182-286	10/95	Hemodynamics in conscious monkeys (non-anesthetized, up to 1-hr post dosing observation)	Cynomolgus monkeys 4/group	0, 10, 20, 40 (0-104) IV bolus, all doses/animal	Yes	83
IMUS-035-TOX Vol.023 P001-197	01/98	Toxicity study with concurrent application of high power ultrasound. (anesthetized, up to 24-hr post dosing observation)	Dogs Male 3/group	0, 20 (0, 86) IV infusion	Yes	88
Neurological and Behavioral Toxicity						
PSM-98-01 Vol 009 P323-323	12/ 98	Neurotoxicity following intracarotid injection (abstract only)	Rat (1 control, 5 AF0150)	0.125; 2.0; 4.0; 8.0; 16.0 (0.16-20)	No	92
IMUS-042-TOX Vol.024 p001-358	02/99	Acute toxicity following intra-arterial injection (Functional Observational Battery; Spontaneous Locomotor Activity). (8-day post dosing observation	Rats 5/sex/group	0, 4, 16 (0-20) carotid artery catheterization	Yes	93
IMUS-043-TOX Vol.025 p001-038	02/99	Gross behavioral and physiological evaluation using "Primary Observation Test" (Irwin Test).(2.5-hr post dosing observation)	Rats Male 6/group	0, 4, 40, 100 (0-130) IV bolus	Yes	99

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
Renal Toxicity						
IMUS-044-TOX Vol.025 p038-080	02/99	Effects on Renal Function in Saline-Loaded Rats; with 24-hour post dose observation	Rats Male 8/group	0, 4, 40, 100 (0-130)	Yes	102
Gastrointestinal Toxicity						
IMUS-045-TOX Vol.025 p081-116	02/99	Effects on the Gastrointestinal Transit of a Charcoal Meal. (30-minute observation)	Rats Male 8/group	0, 4, 40, 100 (0-130)	Yes	105
Others						
IMUS-046-TOX Vol.025 p117-156	03/99	Respiration Rate and Body Temperature. (15-min post dosing observation)	Rats Male 8/group	0, 4, 40, 100 (0-130)	Yes	108

TOXICOLOGY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
Single Dose Toxicity Studies						
IMUS-037-TOX Vol.010 P179-225	05-06/98	Acute Toxicity Single Dose, IV (Termination on day 15; 14-day observation)	Mice 5/sex/group	0, 200, 400, 800, 1600 (0-1037)	Yes	117
IMUS-010-TOX Vol.010 P226-343	07-08/95	Acute Toxicity Single dose, IV (Termination on day 15; 14-day observation)	Rats 5/sex/group	0, 200, 400, 800, 1600 (0-2073)	Yes	119
IMUS-011-TOX Vol.011 P001-276; Vol.012 P001-326	08/95	Expanded Acute Toxicity Single dose, IV (14-day observation, Termination on days 2, 8, and 15)	Rats 20/sex/group	0, 50, 200, 400 (0-518)	Yes	120
IMUS-012-TOX Vol.013 P001-399	08/96	Expanded Acute Toxicity Single dose, IV (14-day observation, Termination on days 2, 8, and 15)	Dogs 6/sex/group	0, 50, 100, 200 (0-864)	Yes	123
IMUS-039-TOX Vol.014 P001-333	06-07/98	Expanded Acute Toxicity Single dose, IV 14-day observation	Dogs 5/sex/group	0, 200, 400 (0-1731)	Yes	125

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
Multiple Dose Toxicity Studies						
IMUS-013-TOX Vol.015 P001-305 Vol.016 P001-361	08-10/95	Repeated Dose Toxicity Daily dosing for 17 and 29 days; (Termination on days 17, 30 and 44 with 15-day recovery)	Rats 20/sex/group	0, 50, 200, 400 (0-518)	Yes	127
IMUS-027-TOX Vol.017 p001-055	06/95	Repeated Dose Toxicity Daily dosing for 7days; No recovery period	Dogs 3/sex/group	10, No control (43)	No	130
IMUS-014-TOX Vol.017 P056-055 Vol.018 P001-269	09-10/95	Repeated Dose Toxicity Daily dosing for 14 and 28 days; (Termination on days 14, 28 and 42 with 14-day recovery after 28-day dosing)	Dogs 8/sex/group	0, 25, 50, 100 (0-433)	Yes	132
Pharmacokinetics/Toxicokinetics						
IMUS-041-TOX Vol.032 p080-179	12-02/99	PFH Elimination from Expired Air and Blood	Rats 7/sex (Expired Air) 5/sex (Blood)	20 (26)	Yes	53

SPECIAL TOXICOLOGY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
Local Tolerance						
IMUS-028-TOX Vol.018 p270-336	02/97	local irritation with a single intravenous, perivenous, intra-arterial injection (1, 4, 15 days post dosing)	Rabbits 3/sex/group	0, 2, 20 (0-52)	Yes	140
IMUS-038-TOX Vol.018 p337-392	05-06/98	local tolerance with a single intramuscular injection (1, 4, 8, 15 days post dosing)	Rabbits 3/timepoint	0, 1 (0-2.6)	Yes	142
Immunotoxicology						
IMUS-021-TOX Vol.019 P001-051	11/96	Antigenicity Study: Active Systemic Anaphylaxis (ASA); Passive Cutaneous Anaphylaxis (PCA)	Guinea Pigs 5 male/group	20 (for IV) 2 (for SC) ±FCA (35)	Yes	144

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg) (HDM)	GLP	Review Page#
IMUS-029-TOX Vol.019 P052-129	01-02/97	Dermal Sensitization	Guinea pig 20-AF0150 10-Control 4-Screen	20 mg/ml to saturate filter papers ±FCA	Yes	147
CMB-96-14 Vol.101 P010-015	03-06/96	TNF-α production by blood cells *in vitro*	Human Rat blood	0.5 mg/ml	No	150
BC-95-17-Amended Vol.010 P001-009	ND	*In vitro* Activation of Complement (C3)	Human plasma	0.5 mg/ml	No	149
Single Dose Acute Toxicity with CV Stress Pretreatment						
IMUS-018-TOX Vol.019 P130-384	10/96	Acute toxicity study with adenosine pretreatment. 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	Yes	151
IMUS-019-TOX Vol.020 p001-257	10/96	Acute toxicity study with dipyridamole pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	Yes	153
IMUS-036-TOX Vol.022 P001-181	03/98	Acute toxicity study with arbutamine pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	Yes	155
IMUS-020-TOX Vol.021 p001-257	10-11/96	Acute toxicity study with dobutamine pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	Yes	157
Microbubble Size Profiles						
AC-00-08	04/00	Bubble Size and Distribution at 0, 30 and 60 minutes Post-reconstitution	N/A	N/A	No	159
Dosage Conversion	04/00	Conversion of AF0150 dose from mg/kg to bubbles/kg	N/A	N/A	N/A	164

REPRODUCTIVE TOXICITY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	Dose (mg/kg/day) (HDM)	GLP	Review Page#
IMUS-022-TOX Vol.025, P170-279; Vol.026 P001-328	01/99	Fertility and early embryonic development (Segment I)	Rats 25/Sex	0, 50, 100, 200 (0, 65, 123, 260) Dosing 2 wks for female and 4 wks for males pre-mating till gestation day 7.	Yes	165
IMUS-023-TOX Vol.027 p001-350	09/98	Teratology (Segment II)	Rats 25 Female	0, 50, 100, 200 (0, 65, 123, 260) Dosing on gestation day 6-17; Sacrifice on gestation day 20	Yes	170
IMUS-024-TOX Vol.031 P001-337	09/98	Teratology (Segment II)	Rabbits 22 Female	0, 50, 100, 200 (0, 130, 260, 518) Dosing on gestation day 7-20; Sacrifice on gestation day 29	Yes	174
IMUS-025-TOX Vol.028 P001-389; Vol.029 P001-449; Vol.030 P001-434	09/98	Pre- & Postnatal Development (Segment III)	Rats 25 Females (F0) 25/Sex Offspring (F1) for assessing development	0, 50, 100, 200 (0, 65, 123, 260) dosing on gestation day 6 to lactation 20; Sacrifice on lactation day 21. No treatment on offspring.	Yes	178

GENETIC TOXICOLOGY STUDIES

Report # Vol # Page #	Study Date (M/Y)	Study Type	Species (no.)	AF0150 Dose	GLP	Review Page#
IMUS-015-TOX Vol.031 P338-378	09-11/95	Bacterial Reverse Mutation Test (Ame's)	TA98, TA100, TA1535 TA1537 WP2uvrA	0, 1-20 mg/plate (with/without S9)	Yes	188
IMUS-031-TOX Vol.032 P001-028	08-09/97	Chromosomal Aberration Assay *in vitro*	Human whole Blood culture (lymphocyte)	0, 2-5 mg/ml (with/without S9)	Yes	189
IMUS-030-TOX Vol.032 P029-051	08/97	Micronucleus Assay *in vivo*	Mouse 6/sex/group	200-800 mg/kg (-518-fold PCD) Single dose, IV	Yes	191
IMUS-032-TOX Vol.032 P052-079	08-09/97	Forward Mutation Assay *in vitro*	L5178Y TK+/- Mouse Lymphoma cell line	0, 1-5 mg/ml (with/without S9)	Yes	193

21. Disclaimer-use of sponsor's material:

Some of the information contained in this review is taken from the sponsor's NDA submission.

22. Introduction/Drug History:

AF0150 (*Imavist™*) is a new molecular entity. It is a perflexane-phospholipid microbubble agent for intravenous injection [redacted]

AF0150 consists of two critical (active) components, perflexane and dimyristoylphosphatidyl-choline (DMPC), and some inactive components. The perflexane presents in the vapor (gaseous) mixed with N2 in the AF0150 vial. All other components are in the porous microspheres generated during [redacted] of AF0150 manufacturing. Upon reconstitution with Sterile Water For Injection (SWFI), the microbubbles are formed and the perflexane/N2 mixture inside the microbubbles is trapped. The N2 in the microbubbles quickly reaches equilibrium (through the gas permeable membrane) with the air in the water. The water-insoluble perflexane resists dissolving out through the membrane, thereby providing an extend lifetime to the microbubble. DMPC (a choline phospholipid), a surfactant, controls (lowers) the microbubble membrane surface tension and thus maintain microbubble persistence.

23. Previous clinical experience:

There is no previous clinical experience with this drug.

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25. GENERAL PHARMACOLOGY

Report Number: EB-95-08
Dose Response Effects of Intravenous AFO150 Administrated as a Bolus or a Constant Infusion on Doppler Signal Enhancement in Rabbits.

Report Location:	Vol.009, p077-097
Report date:	December 4, 1995
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	Not specified (August 1995?)
GLP Compliance:	No
AF0150 Lot number:	ZZ15036

Specific Aim

To determine the effect of dose and administration mode (IV bolus or infusion) of AF0150 on Doppler flow signal enhancement in rabbits.

Methods

Animal Preparation: 12 Rabbits (New Zealand White, weighting 3.2-3.7 kg [*Reviewer comment: sex was not specified*]) were anesthetized with Ketamine/Xylazine/Acepromazine. The right carotid artery and the right jugular vein were exposed using a routine procedure. The right jugular vein was catheterized for AF0150 administration. A Doppler flow cuff transducer was placed around the exposed carotid artery for monitoring carotid artery flow. The rabbits were randomly divided into two groups with 6 per group.

AF0150 Administration: AF0150 was reconstituted in SWFI to the final concentration of 20 mg/ml. Rabbits received duplicate injection of 2.5, 5, or 10 mg/kg as either a bolus (n=6 per dose group) or a 10-min infusion at rates of 0.25, 0.5 and 1.0 mg/kg/min (n=6 per infusion rate group) through catheterized right jugular vein. Doppler signals were collected every second for approximately 120 seconds prior to injection of AF0150 and through 600 seconds (bolus injection group) or 800 seconds (infusion group) from the onset of each AF0150 administration. Each animal received a total six injection (duplicate injection of each of 3 doses).

Doppler Signal Analysis and Data Processing: Doppler signals of carotid artery blood flow was monitored using Doppler before and after AF0150 administration and the signal output intensity (mV) was measured and expressed as Root Mean Square (RMS). RMS values were sampled at a rate of one per second. To correct baseline change (residual bubbles from previous injection), all postinjection RMS values were subtracted from a mean baseline value generated by averaging all preinjection RMS values (approximately 120 seconds). The resulting net RMS was used to calculate RMA ratio by dividing the net RMS at each time point with the initial baseline RMS value:

$$\text{RMS ratio} = (RMS_t - RMS_{bl})/RMS_{bli}$$

Where RMS_t: RMS value at time t (t=0-600 seconds postinjection)
RMS_{bl}: preinjection baseline for each specific injection
RMS_{bli}: initial baseline prior to any injections.

The data (mean of duplicate injections) from each animal were plotted as RMS ratio over time (seconds). The area under the curve (AUC) and signal enhancement half-life or mean maximal signal enhancement were calculated.

Results

Bolus injection dose-response: AUC (area under the curve) and half-life of Doppler signal enhancement in carotid artery blood flow increased with increasing AF0150 dose after a bolus injection to the jugular vein on the same side, as seen in *Figure 1.*

Infusion injection dose-response: the AUC and the maximal signal enhancement increased with increasing AF0150 dose or infusion rate after IV injection, as seen in *Figure 2.*

Discussion and Comments

1. Intravenous injection of AF0150 via both bolus and infusion appeared to produce a dose-dependent increase in Doppler signal of carotid artery blood flow in rabbits.

2. Doppler signal was recorded from the carotid artery on the same side where the jugular vein was cannulated and injected with AF0150. It was not clarified if the microbubbles in the jugular vein interfered with Doppler signals from the carotid artery.

3. The gender of rabbits was not specified.

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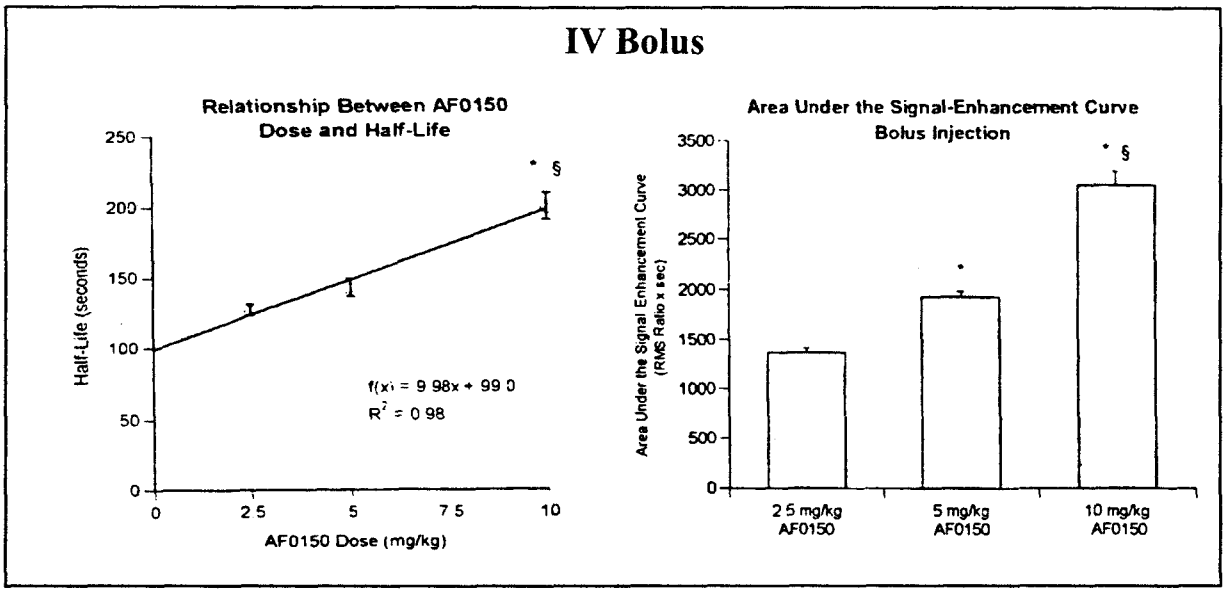

Figure 1. Dose-response of Doppler signal enhancement in rabbits after **IV bolus injection** of AF0150 at 2.5, 5 or 10 mg/kg. ***Left Panel,*** dose-dependent increase in the half-life of Doppler signal enhancement; ***Right Panel,*** dose-dependent increase in AUC (calculated from Doppler signal intensity *vs.* time curve).

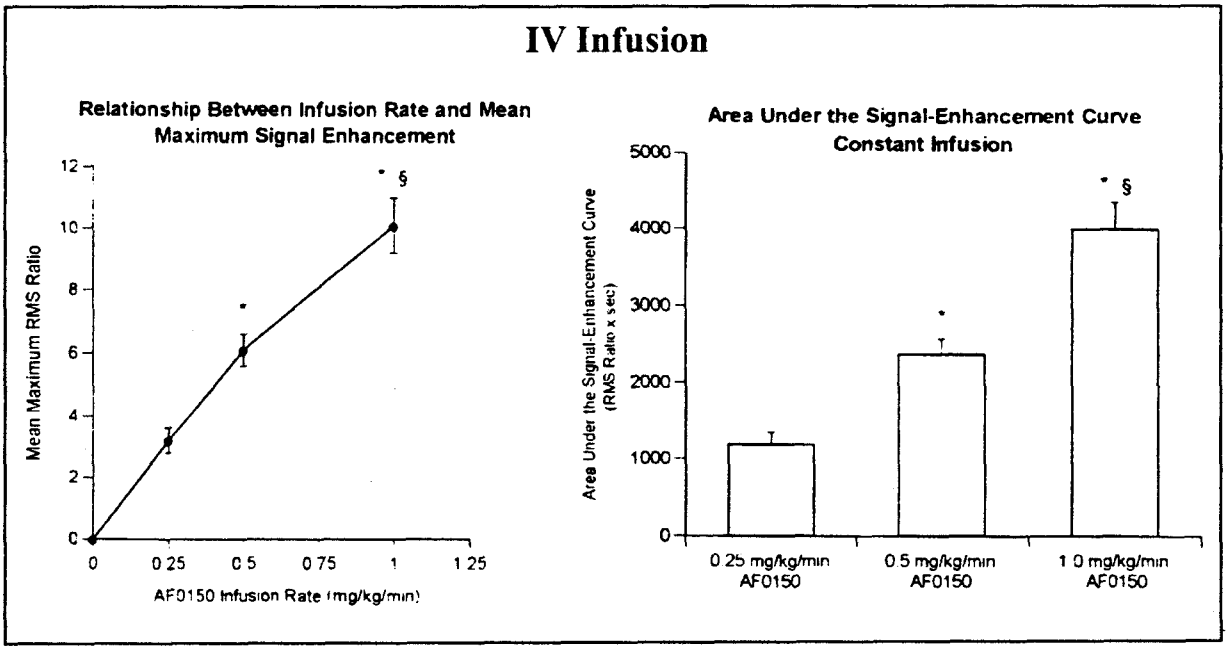

Figure 2. Dose-response of Doppler signal enhancement in rabbits after **IV infusion** of AF0150 at 2.5, 5 or 10 mg/kg (0.25, 0.5, or 1.0 mg/kg/min). ***Left Panel,*** dose-dependent increase in maximal Doppler signal enhancement; ***Right Panel,*** dose-dependent increase in AUC (calculated from the Doppler signal intensity *vs.* time curve).

Report Number: EB-97-17

Dose Response Effects of Intravenous AFO150 on Doppler Signal Enhancement in Rabbits.

Report Location: Vol.009, p098-107
Report date: May 24, 1999
Study Facility: Alliance Pharmaceutical Corp
In-life phase: January 30-May 23, 1997 and Aug 26-28, 1998
GLP Compliance: No
AF0150 Lot number: UA16064

Specific Aim

To present a retrospective evaluation of the dose-response relationship of AF0150 on Doppler signal response in rabbits (pooled data from 3 different studies conducted at different times)

Methods

Animal Preparation: New Zealand White rabbits (3.3-3.9kg) of either sex were anesthetized and intubated (but allowed to freely breathe) in all three different studies conducted at different times (Table 1). The jugular vein [*the side was not specified*] was cannulated for AF0150 administration. A Doppler flow cuff transducer was placed around the exposed carotid artery for monitoring Doppler signal of carotid artery blood flow.

Table 1. Study Design

Study ID	Study Date	AF0150 Dose (mg/kg)	AF0150 Lot No	Number of Rabbits	Dosing Repeat No
A	Jan 97	0.2, 0.5, 1, 2, 5	ZY16042	6	1
B	Aug 98	0.125, 0.25, 0.5, 1	UA16063	1	2
C	Aug 98	0.059, 0.118, 0.178, 0.296	ZY18020	1	3

AF0150 Administration: Three different lots of AF0150 were used in these studies, as indicated in Table 1. UA16063 (100 mg fill), ZY16042A (100 mg fill) and ZY18020 (200 mg fill) were reconstituted in 10 ml SWFI to a final concentration of 20 mg/ml (for 200 mg fill) or 10 mg/ml (for 100 mg fill). Each rabbit was given a bolus IV injection of AF0150 at different subsequent doses (after Doppler signal returned to baseline in-between).

Doppler Signal Analysis and Data Processing: Doppler signal of carotid artery blood flow was recorded using [redacted] Doppler [redacted] before (for baseline) and after AF0150 administration. The signal output intensity (mV) was measured and expressed as Root Mean Square (RMS). RMS values were sampled at a rate of one per second for 150 seconds post-injection. "Peak Doppler Signal" and "Doppler Signal Persistence" were evaluated at 20-40 seconds and 100-120 seconds post dosing, respectively. Data from 3 different studies were pooled and summarized in tabular and graphical form.

Results

IV bolus injection of AF0150 dose-dependently increased both Doppler peak signals (20-40 seconds post-dosing) and persistent signals (100-120 second post-dosing) of carotid artery blood flow, with good reproducibility in 3 different studies.

Discussion and Comments

This study basically reproduced the dose-response results presented in Study EB-95-08. Enhancement of Doppler signal in remote sites other than carotid artery such as cardiac (in clinical indications) needs to be evaluated. Also, the sponsor needs to clarify why a total of 8 rabbits were used to complete the study but collected data from only 6 rabbits.

Report Number: EB-95-28
Effect of dose and mode of administration of AF0150 on Doppler signal enhancement and echocardiography of the left ventricle cavity and myocardium with comparison of imaging modality on swine.

Report Location:	Vol.009, p108-122
Report date:	May 31, 1999
Study Facility:	Alliance Pharmaceutical Corp
In-life phase:	October 1995 – April 1996
GLP Compliance:	No
AF0150 Lot number:	ZZ16005, ZZ16007, ZY15104C and F ZY15109A and B, ZY15074, ZY15099A

Specific Aim

To evaluate the effect of AF0150 dose and administration mode on Doppler signal and ultrasound image quality during continuous fundamental contrast echocardiography of the left ventricular cavity and myocardium. And to compare harmonic imaging during both continuous and intermittent ultrasound transmission.

Methods

Animal preparation: 9 swine (14-20 kg body weight, either sex) were anesthetized. Left jugular vein was cannulated for AF0150 infusion and a 10 MHz pulsed Doppler flow cuff transducer was placed around left carotid artery for Doppler flow signal measurement. A 2.5 MHz ultrasound probe was positioned over the heart at a depth of approximately 6-9 cm for closed chest ultrasound imaging. Of the nine animals, 6 were used for Doppler and fundamental imaging analysis and 3 were used for second harmonic imaging analysis.

AF0150 administration: each animal was given 3 IV injections of AF0150 at doses of 0.13, 0.66 or 1.3 mg/kg at both a 5-second bolus and 1-minute infusion followed by 1 ml saline flush with