BACKGROUND (REGULATORY HISTORY)

I. Ejection Fraction Assessments (from the Original NDA review)

Despite the improved visualization of the endocardial border, AF0150 contrast did not appear to improve the ability for the echocardiologists to accurately assess the ejection fractions (EF). According to the action letter sent by the Division in August 2000, the data provided by the sponsor for the structural indication of improved EBD and EFwas inadequate due to

(1) absence of end-systolic and end-diastolic assessments;

(2) inability to correlate EBD with EF due to the absence of calculations used to objectively measure cardiac volumes – particular end-systolic and end-diastolic volumes; and

(3) AF0150-enhanced EBD did not translate into improved accuracy for EF measurements when compared with non-enhanced (baseline) EBD.

II. Segmental Wall Motion Assessments (from the Original NDA Review)

A secondary endpoint, assessment of segmental wall motion (SWM), appeared to show a statistically significant improvement in inter-reader agreement when using AF0150, as compared to baseline non-contrast poorly visualized echocardiograms. However, only 26 patients were evaluated with MRI. This number was considered to be too small upon which any conclusion could be drawn. Based upon the review, the action letter entailed the following recommendations:

- **Segmental Wall Motion (No action items listed)**
  - Lack of sufficient data for validation
  - Insufficient number of patients (N = 26) for support of improved assessment

III. Safety (from the Original NDA Review)

Safety issues were identified that included the following recommendations:

1. Particle size: Upper range
   a) A micro-circulation study
   b) A chronic pulmonary hypertension animal model study
   c) CMC specifications: Upper particle size limits

2. QTc interval prolongation – 77 patients (17%)
   a) QTc monitored in ongoing Phase 2 multi-center study, detecting coronary artery disease in myocardial contrast study

3. Package insert: Dosage and administration section, entailing the labeling of the dose necessary for specific weights
   a) Provide efficacy and safety information to support the weight range from 40 kilograms to 168 kilograms

4. Integrated Summary of Safety Update

IV. Responses by Alliance

An industry meeting held Nov. 3, 2000 was a preliminary attempt by the sponsor to address the issues raised in the action letter. Four additional teleconferences were focused upon discussions on the relationships between EBD (a structural endpoint) and — ' SWM (functional endpoints). Again, the sponsor did not want to pursue —but SWM data was requested by the Division with any literature support to seek for a "trend" between EBD and SWM. However, the division stated that either the action letter be followed regarding the primary endpoint o: r a new option: Segmental wall motion may be re-assessed with radionuclide ventriculography (RVG) as the standard of truth since there were only 26 patients with MRI, in demonstrating the accuracy of the SWM assessments with 2D-echo. The sponsor did not send any data demonstrating any relationship between 2D-echo SWM readings compared with RVG.

The division suggested that, if re-reading th — data is still considered not feasible by the sponsor, then the SWM 2D-echo results could be compared with the RVG results. The sponsor stated that re-reading for — SWM would be too problematic, but that SWM could be reassessed on the 26 patients who had MRI performed.

The teleconference from Dec, 18, 2000 led to the current submission (initially sent as a correspondence on Feb. 5, 2001), consisting of previously reviewed data and newly submitted articles. Essentially, the teleconference focused upon guidelines which stated that data derived from peer-reviewed literature which can be used serve to support the approval of a product.[2] All articles are listed and discussed in the Literature Review subsection of the Efficacy Review section.

In addition, responses to clinical issues identified previously in the action letter pertaining to the following disciplines were submitted by the sponsor:

1. Chemistry, manufacturing, and controls (CMC)
2. Pharmacology (pre-clinical) and toxicology
3. Clinical pharmacology (pharmacokinetics)

[2] From "Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products", US DHHS, FDA, May 1998, clinical 6.

14

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REASON:

_____ b(2) 'low'

✓ b(4) CCI

_____ b(4) TS

_____ b(5) Deliberative Process; Attorney Client and Attorney Work Product Privilege

_____ b(6) Personal Privacy

_____ b(7) Law Enforcement Records

5-18

SAFETY SUMMARY AND RECOMMENDATIONS

No new data has been provided since the previous review. In addition, there were no new data provided from the ongoing study, and no annual report was provided. The sponsor did provide responses to clinical safety issues pertaining to QTc-interval evaluation, chemistry, pharm/tox, and clinical pharmacology.

1. **QTc interval evaluation**: The sponsor stated that QTc intervals will be monitored in the ongoing study entitled [redacted] " (IMUS- [redacted] submitted to IND [redacted] Serial No. 074, March 22, 2000). A 12-lead ECG will be recorded at baseline and at 5 and 15 min, 1 and 24 hr post-Imavist™ for all subjects and every min during the 6-min adenosine infusion. Additionally, Stage 2 subjects in the study will be monitored at 5, 10, 15, and 30 min post-adenosine infusion.

2. **Chemistry issues**: The issues regarding the particle size upper range have been addressed, reviewed and deemed acceptable by the chemistry reviewer.

3. **Pharm/Tox issues**: The issues regarding microcirculation studies have been addressed, reviewed and deemed acceptable by the pharm/tox reviewer. In addition, there appears to be an acceptable response regarding plans to develop a chronic pulmonary hypertension animal model, with a post-approval study.

4. **Pharmacokinetic issues**: The issue regarding the efficacy and safety information to support the listed weight range from 40 to 168 kg has been addressed, reviewed and deemed acceptable by the biopharmacology reviewer.

Other recently approved agents of the same class have studied high-risk patient populations such as patients with chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF) and pulmonary hypertension (PHTN) with adequate pre-clinical buttressing. The Imavist™ program has not comprehensively evaluated such conditions either in the clinical (refer to Table 23, page 44 of previous review) or pre-clinical trials. Digression from such safety approach is not recommended; therefore, a special clinical safety study involving patients with COPD and PHTN may be required prior to approval.

Recommendations:

1. Provide data (QTc intervals) from the ongoing study.
2. Need a new study for evaluating patients with high-risk pulmonary diseases.

3. Additional information regarding QTc interval monitoring is required in all studies and cardiovascular electrophysiological studies may be required depending on the data from ongoing studies.

OVERALL RECOMMENDATION

The sponsor now proposes a structural claim for the product. The data is not supportive of a functional claim. The data does not support a trend towards a functional claim upon which such a structural claim may be approved. The current recommendations stand.

LABELING SECTION

I. Proposed Insert Indication

Original proposal:

Recent proposal: "...for use in patients with suboptimal echocardiograms to opacify the left ventricle, which enhances the delineation of the LV endocardial borders."

II. Issues

In the context of an "approvable" recommendation, it is premature to attempt to address labeling issues, as the data is not supportive towards the sought structural claim.

APPENDIX:

I. *References Submitted By The Sponsor:*

The submitted literature is listed in the appendix, where the scientific issues and relevant cardiac imaging information with references were presented and discussed. Four research articles, 1 abstract, 2 book chapters, and 2 sets of guidelines were referenced by the sponsor. These are listed below.

1. **Research Articles:**

   a) Urena, PE, Lamas, GA, Mitchell G, et al. "Ejection Fraction by Radionuclide Ventriculography and Contrast Left Ventriculogram: A Tale of Two Techniques." ***JACC*** 1999; 33 (1): 180-5.

   b) Yang PC, Kerr AB, Liu AC, et al. "New real-time interactive cardiac magnetic resonance imaging system complements echocardiography." ***JACC*** 1998; 32: 2049 – 2056.

   c) Nagel E and Fleck E. "Functional MRI in ischemic heart disease based on detection of contraction abnormalities." ***J Magnetic Resonsance Imaging*** 1999; 10: 411-7.

   d) Hundley WG, Kizilbash AM, Afridi I, et al. "Effect of contrast enhancement on transthoracic echocardiographic assessment of left ventricular regional wall motion." ***Am J Cardiology*** 1999; 84: 1365 – 1368.

2. **Abstracts:**

   a) Thomson HL, Avierinos JF, Breen JF, and Sarano ME. "Improvement in the Accuracy of Echocardiographic Assessment of Left Ventricular Remodeling with Contrast: A Prospective Blinded Study Comparing Echocardiography with and without Contrast and Electron Beam CT." An abstract in the ACC Program Planner; JACC (no date).

3. **Guidelines:**

   a) "ACC/AHA Guidelines for the Clinical Application of Echocardiography." ***Circulation*** 1997; 95: 1686 – 1744.

   b) FDA Draft Guidance for Industry. Developing Medical Imaging Drugs and Biological Products. October 1998, revised June 2000.

4. **Book Chapters:**

   a) Vuille C and Weyman AE. "Chapter 20: Left Ventricle I: General Considerations, Assessment of Chamber Size and Function." In **Principle and Practice of Echocardiography, 2nd Ed** (1994); ed. Weyman AE; pp 575 - 624.

   b) Nidorf SM and Weyman AE. "Chapter 21: Left Ventricle II: Quantification of Segmental Dysfunction." In **Principle and Practice of Echocardiography, 2nd Ed** (1994); ed. Weyman AE; pp 625 - 655.

II. *References (by the Clinical Reviewer):*

1. **ACC/AHA Guidelines for the Clinical Application of Echocardiography.** ***Circulation***. 1997; 95 (6):1686-1744.

2. **ACC/AHA Guidelines for the Clinical Application of Echocardiography: Executive Summary.** ***JACC***. 1997; 29 (4): 862-79.

3. **ACC/AHA Guidelines for the Evaluation and Management of Heart Failure.** ***JACC***. 1995; 26 (5): 1376-98.

4. Beller GA, "Chapter 13: Relative Merits of Cardiovascular Diagnostic Techniques", pp 422 - 438. In **Heart Disease: A Textbook of Cardiovascular Medicine, 6th Edition** (2001), ed. Braunwald E, Zipes DP, and Libby P.

5. Berman DS, Shaw LJ, and Germano G, "Nuclear Cardiology", pp 525 – 566. In **Hurst's The Heart, 10th Edition** (2001), ed. Fuster V, Alexander RW, and O'Rourke RA.

6. Greenburg SB and Sandhu SK. "Ventricular Function." In **The Radiologic Clinics of North America: Cardiac Radiology** 37 (2): 341 – 59 (March 1999).

7. "Heart Failure", page 40, of the **Medical Knowledge Self-Assessment Program® Cardiovascular Medicine, 12th Edition** (2001); ed. Hatem CH and Kettyle WM.

8. Katz AS et al; "Chapter 27: Echocardiographic Assessment of Ventricular Systolic Function", pp 297 – 324. In **Marcus Cardiac Imaging, 2nd Edition** (1996), ed. Skorton, DJ.

9. Lejemtel TH, Sonnenblick EH, Frishman WH: "Diagnosis and Management of Heart Failure"; p 691. In **Hurst's The Heart, 10th Edition** (2001), ed. Fuster V, Alexander RW, and O'Rourke RA.

10. Raphael MJ, Donaldson RM. "The Normal Heart: Methods of Examination", pp 513 - 4. In **A Textbook of Radiology and Imaging, 5th Edition** (1993), ed. Sutton D.

11. Skorton DJ et al. "Chapter 1: Goals of Cardiac Imaging", p 3. In **Marcus Cardiac Imaging, 2nd Edition** (1996), ed. Skorton DJ (consulting editor: Braunwald E).

12. Urena, PE, Lamas, GA, Mitchell G, et al. "Ejection Fraction by Radionuclide Ventriculography and Contrast Left Ventriculogram: A Tale of Two Techniques." **JACC** 1999; 33 (1): 180-5.

TABLE A : PHASE 3 STUDIES –
% of Segments Not Visualized in Screening (Non-Contrast) Echocardiograms

Echo View	Segment #	Cardiac Wall		IMUS-007 Results		IMUS-008 Results	
		Wall	Segment	Qualify.	Confirm.	Qualify.	Confirm.
Apical 4-chamber	Segment 1	Septal	Basal	12 (6%)	14 (7%)	25 (12%)	26 (14%)
	Segment 2		Middle	11 (5%)	11 (6%)	6 (3%)	11 (6%)
	Segment 3		Apical	76 (37%)	67 (34%)	48 (24%)	59 (32%)
	Segment 4	Lateral	Apical	130 (63%)	122 (63%)	127 (63%)	117 (63%)
	Segment 5		Middle	138 (67%)	129 (67%)	177 (87%)	147 (79%)
	Segment 6		Basal	139 (68%)	133 (69%)	163 (80%)	134 (72%)
Apical 2-chamber	Segment 7	Inferior	Basal	17 (8%)	23 (12%)	35 (17%)	35 (19%)
	Segment 8		Middle	12 (6%)	10 (5%)	14 (7%)	16 (9%)
	Segment 9		Apical	68 (33%)	6 (34%)	66 (32%)	61 (33%)
	Segment 10	Anterior	Apical	142 (69%)	132 (68%)	129 (64%)	111 (59%)
	Segment 11		Middle	143 (69%)	135 (70%)	153 (75%)	138 (74%)
	Segment 12		Basal	142 (69%)	136 (70%)	167 (82%)	137 (73%)
Apical long axis (not evaluated in these "screening" Studies)	Segment 13	Posterior	Basal	Not Applicable	Not Applicable	Not Applicable	Not Applicable
	Segment 14		Middle				
	Segment 15	Anterior	Middle				
	Segment 16	septal	Basal				

Source: Protocol IMUS-007 – Vol. 90 pp 188–189; Protocol IMUS-008 – Vol. 107 pp176 – 177.

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ON ORIGINAL

Table B: No. of Patients w/ 2D-Echo Segmental EBD Scores* of 2 or 3 per Reader (N = 26)

Cardiac Wall	Cardiac Segment	Coronary Territory	Seg No.	Reader 1		Reader 2		Reader 3	
				Baseline	Contrast	Baseline	Contrast	Baseline	Contrast
			Apical 4 – chamber segments						
Septal	Basal	RCA	1	6	19	5	15	4	21
	Middle	LAD	2	12	22	13	21	13	25
	Apical	LAD	3	5	16	12	17	7	23
Lateral	Apical	LAD	4	2	15	4	11	2	24
	Middle	LCX	5	3	19	3	18	2	22
	Basal	LCX	6	0	15	2	16	4	21
			Apical 2 – chamber segments						
Inferior	Basal	RCA	7	14	19	1	15	16	24
	Middle	RCA	8	20	21	15	16	21	26
	Apical	LAD	9	7	12	10	10	8	19
Anterior	Apical	LAD	10	2	9	3	7	2	20
	Middle	LAD	11	6	14	4	9	5	20
	Basal	LAD	12	2	14	2	11	3	19
			Apical Long axis segments						
Posterior	Basal	RCA	13	5	12	1	12	7	23
	Middle	LCX	14	6	15	1	12	7	24
Antero-Septal	Middle	LAD	15	9	15	9	14	12	22
	Basal	LAD	16	3	13	6	15	12	23
Total			**All**	**102**	**250**	**91**	**219**	**125**	**356**

Source : Sponsor's data.
* Scores: "2" = good EB delineation; "3" = excellent EB delineation
Note: No suboptimal segments were assessed.

TABLE C: Centers/Sites for Protocol No. IMUS-008-USA

Site	Center	Location	Investigator	No. of Subjects AF0150 (n = 232)
20	University of Massachucetts Medical Center	Worcestor, MA	Gerard Aurigemma, MD	22
21	Vanderbilt University Medical Center	Nashville, TN	Benjamin Byrd, MD	3
22	University of Texas Southwestern Medical Center	Dallas, TX	Paul Grayburn, MD	24
23	Bowman Gray School of Medicine	Winston-Salem, NC	Dalane Kitzman, MD	45
24	University of Alabama at Birmingham	Birmingham, AL	Navin Nanda, MD	33
25	Washington University	St. Louis, MO	Julio Perez, MD	14
26	Johns Hopkins Bayview Medical Center	Baltimore, MD	Edward Shapiro, MD	7
27	San Diego Cardiovascular Associates	Encinitas, CA	David Hill, MD	37
28	Scripps Clinic and Research Foundation	La Jolla, CA	David Rubenson, MD	9
29	Hartford Hospital	Hartford, CT	Linda Gillam, MD	8
30	Southpoint Cardiology Associates	Jacksonville, FL	Stephen A. Stowers, MD	30

* Shaded areas are those centers which were evaluated by the Agency's inspection team.

TABLE D: Tabulated Review of the Published Reports Submitted by the Sponsor

Authors	Title	Design	Truth Standard	No. of Pts	Results Analyses	Conclusion	Medical Officer Remarks
Urena, PE, Lamas, GA, Mitchell G, et al. ***JACC*** 1999; 33 (1): 180-5.	"Ejection Fraction by Radionuclide Ventriculography and Contrast Left Ventriculogram: A Tale of Two Techniques."	Multi-center (50 sites); randomized; determine accuracy of measuring the ejection fraction using radionuclide ventriculography	Contrast left ventriculogram	688 patients who had recent myocardial infarctions.	Multivariate analysis: Assessing linear correlation coefficient to volumetric EF. Correlation between RVG and cath: R = 0.42	Although correlation between RVG and ventriculography was statistically significant, it was less than desirable from a clinical standpoint.	1. Multiple sites with diverse group of operators in the acquisition of both RVG and ventriculography data. 2. Echocardiography, with or without contrast, was not evaluated in the study.
Yang PC, Kerr AB, Liu AC, et al. ***JACC*** 1998; 32: 2049 – 2056.	"New real-time (NRT) interactive cardiac magnetic resonance imaging (CMRI) system complements echocardiography."	Single-center, to determine the clinical utility of NRT cardiac MRI system with echocardiography	2-D echocardiography	85 patients, randomly selected and divided into 3 groups: (1) those with optimal echo's; (2) suboptimal echo's; (3) patients with either severe pulmonary disease or congenital heart disease	2% agreement btw. 2 readers: • Echo vs Echo: 94% • Echo vs CMRI: 92% • CMR vs CMR; 93% Visualization of wall segments in the 3rd group: Best visulaizaiton with CMRI use over 2D-echo	The clinical utility for NRT-CMRI is for evaluation of those patients with poorly visualized echocardiograms.	1. Use of 2D-echo as a "gold standard" is based upon an earlier publication (1993), and based upon cost and convenience, not necessarily upon accuracy. However, when presented with 2D-echo limitations, such as poor visualization, 2D-echo can be replaced by NRT-CMRI. 2. The authors state that 10 – 15% patients undergoing 2D-echo have poorly visualized results.

TABLE D: Tabulated Review of the Published Reports Submitted by the Sponsor (continued)

Authors	Title	Design	Truth Standard	No. of Pts	Results Analyses	Conclusion	Medical Officer Remarks
Nagel E and Fleck E. ***J Magnetic Resonsance Imaging*** 1999; 10: 411-7.	"Functional MRI in ischemic heart disease based on detection of contraction abnormalities."	No designed mentioned, but data was derived from a prospective study. This involved comparison of dobutamine-stress echocardiography (DSE), vs dobutamine-stress MRI (DSMR), which were then compared with coronary angiography	Coronary angiogram	172 patients who had moderate coronary artery disease (>50% as seen by coronary angiography)	DSMR had statistically ($p < 0.05$) significant improvement than DSE in sensitivity (86% vs 74%), specificity (86% vs 70%) and diagnostic accuracy (86% vs 73%).	DSMR may be used to determine stress-induced wall motion abnormalities in patients with poorly visualized echocardiograms.	1. Exclusion of patients due either to poor 2D-echo image (10% of the DSE pts) or claustrophobia or severe obesity (10% of the DSMR patients). 2. Article mentions of use of either harmonics or contrast for suboptimal echos, and about tagging for MRI.
Hundley WG, Kizilbash AM, Afridi I, et al. ***Am J Cardiology*** 1999; 84: 1365 – 1368.	"Effect of contrast enhancement on transthoracic echocardiographic assessment of left ventricular regional wall motion."	Randomized, blinded, at 2 study sites. To determine the clinical utility of contrast 2D-echocardiography.	Magnetic resonance imaging	40 patients referred for routine 2D-echo for assessment of LV systolic function. Segmental wall motion (SWM) was assessed.	Post-contrast Improvement in (1) endocardial border delineation (pre- = 78%; post- = 98%); (2) distiguishing normal from abnormal (from 65% to 88%); (3) improved MRI-concordance post-contrast (78%, vs 65% pre-contrast); and (4) improved inter-reader agreement post-contrast.	With contrast-enhanced 2D-echo, there is an Improvement in inter-reader agreement and in visualizing and differentiating segmental wall motion.	1. Dodeca-fluoro-pentane emulsion (Echogen®) was the contrast agent used. 2. One author was a PI for an Imavist™ phase 3 study (IMUS-008).
Thomson HL, Avierinos JF, Breen JF, and Sarano ME. An abstract in the ACC Program Planner; JACC (no date).	"Improvement in the Accuracy of Echocardiographic Assessment of Left Ventricular Remodeling with Contrast: A Prospective Blinded Study Comparing Echocardiography with and without Contrast and Electron Beam CT."	Single site; non-randomized; blinded; determine accurate assessment of left ventricular indices using haromonic echo with and without contrast.	Electron beam CT (computed tomography)	25 patients	Compared harmonics with and without contrast, with electron beam CT (gold standard).	Harmonic imaging used with contrast allows accurate assessment of LV volumes with echo.	1. Harmonics not a part of this NDA. 2. Small number of patients. 3. Not published yet.

TABLE E: Updated Integrated Safety Summary
Enrolled Subjects Receiving Various Doses/Modes of Imavist™ (AF0150)

AF0150 Dose	Administration	Dose	Protocol	No. of Subjects
Single Dose	IV Bolus	0.125 mg/kg	IMUS – 007	213
			IMUS – 008	232
			IMUS – 001	12
		0.5, 1.0, 2.0, or 4.0 mg/kg	IMUS – 001	28
		0.5 and 1.0 mg/kg	IMUS – 003	4
	***Updated no.→**	1.0, 2.0, or 4.0 mg/kg	IMUS – 013	28
			IMUS – 014	12
		4.0 mg/kg	IMUS – 012	10
	IV Infusion	4.0 mg/kg	IMUS – 001	4
		80 mg	IMUS – 003	2
	***Updated no.→**	Up to 4.0 mg/kg	IMUS – [redacted]	14
			IMUS – 014	14
Multiple Dose	IV Bolus	0.125 + 0.25 + 0.5 mg/kg	IMUS – 018	18
		4.0 + 4.0 mg/kg	IMUS – 012	3
	IV Bolus + IV Infusion	0.25 mg/kg bolus + up to 80 mg infusion	IMUS – 002	41
		1.0 mg/kg bolus + up to 160 mg infusion	IMUS – 003	41
Saline (Control)	IV Bolus	0.2 mL/kg	IMUS – 001	20
			IMUS – 007	81

Source: Volume 1, p 061 (Table C.2.), with data referenced to Section 1.IIIC, Table C.2.

ADDENDUM (POST-INTERNAL REVIEW / DISCUSSION):

On January 10, 2002, an internal discussion regarding the reviews took place. Concerns were raised regarding the response to the submission and whether the response will be consistent with responses to other echocardiographic microbubble contrast agents reviewed in the Division. A recently reviewed microbubble contrast agent was approved based upon evaluation of 2 contiguous segments considered suboptimal by 2D-echo. In addition, for that product, any segment considered to be visualized was evaluable, unlike the Imavist® studies, where a computer-generated kick-out occurred for segments considered either non-visualized (EBD score of 0) or fairly / mildly visualized (EBD score of 1).

Therefore, the discussion ended with the recommendation that the SWM data from the 26 patients be re-evaluated for post-Imavist® 2D-echo improvement in correctly assessing for SWM, based upon full re-reads for fairly visualized (EBD score of 1), contiguous (≥ 2) segments. If the results are not supportive, then a new study for the demonstration of improved accurate assessment for SWM post-Imavist® would need to be discussed and performed.

In addition, from a safety standpoint, it was agreed that the following must be submitted:

- A safety update
- A review of arterial 02 saturation in COPD patients
- Phase IV commitments for pharmacokinetic analyses of pulmonary patients, then, pending the results, a possible new study evaluating that patient population
- Post-marketing safety study for risk management.

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/s/

Bernard Parker
1/29/02 04:52:52 PM
MEDICAL OFFICER
Please note the addendum (based upon the internal meeting on Jan. 10, 2002) at the end of the review.
Note: An addendum (based upon the internal meeting on Jan. 10, 2002) has been added at the end of the review.

Ramesh Raman
2/4/02 03:22:23 PM
MEDICAL OFFICER
Concur in essence. The robustness of the results on the 26 patients with the suggested re-analyses methods may support the sought anatomic EBD claim. However, adequately well controlled new study/s would be required to validate a functional claim.

Patricia Love
2/4/02 03:50:16 PM
MEDICAL OFFICER
Agree with essence and with Dr. Raman's comments. Please see the Division Directors Memo to the File for final decision. In DFS 02/04/00.

NDA 21-191

Medical Review Document

IMAVIST™ FOR INJECTION

(AF0150; PERLEXAN-PHOSPHOLIPID MICROBUBBLES)

Medical Reviewer: Bernard Parker, M.D. /S/
Statistical Review: Sonia Castillo, Ph.D.
Team Leader: Sally Loewke, M.D. /S/ MD
Division Director: Patricia Love, M.D., M.B.A.

* I agree with Dr Parker's recommendation of approvable. However, if the new re-read analysis continues to show no improvement over baseline of the enhanced LVEF values in spite of improved enhanced LVEB delineation, additional studies will be needed.

Sponsor:
Alliance Pharmaceutical Corporation; San Diego, CA 92121

Manufacturers:

2. Alliance Pharmaceutical Corporation; San Diego, CA 92121

Related Drugs:
1. Definity NDA # 21-064
2.
3. Optison NDA # 20-899
4.

Indication:

Date Submitted: October 11, 1999
User Fee Date: August 14, 2000

NDA 21-191

IMAVIST™ (AF0150; PERFLEXAN-PHOSPHOLIPID MICROBUBBLES) FOR INJECTION

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

NDA 21-191: IMAVIST™
(AF0150; PERFLEXAN-PHOSPHOLIPID MICROBUBBLES) FOR INJECTION

Manufacturers: [redacted] Alliance Pharmaceutical Corporation
San Diego, CA 92121

ABSTRACT

IMAVIST™ [AF0150 (perflexane-phospholipid microbubbles) for injection] is an intravenous ultrasound contrast agent developed by Alliance Pharmaceutical Corporation (San Diego, CA). AF0150 is a sterile, non-pyrogenic powder whose critical compenents include **perflexane**, a stabilizing gas diluted into N_2, and **dimyristoyl phosphatidylcholine (DMPC)**, a semi-synthetic phospholipid surfactant. Upon re-constitution with sterile water (final concentration of 10 mg/mL), the AF0150 is re-suspended, yielding DMPC-shelled microbubbles filled with perflexane/N_2 and air in an iso-osmotic solution, buffered to physiological pH.

In two pivotal phase 3 studies, patients with stable cardiac function but poorly visualized echocardiograms received a single dose of 0.125 mg/kg of AF0150. A significant improvement in delineating the endocardial border (EBD) was demonstrated when using AF0150 as a contrast agent in fundamental continuous (and also gated) 2-D echocardiography, especially in those segments with the greatest frequency of poor visualization. This was proven in both studies by evaluating the improvement in visualizing (1) individual segments, (2) individual views, and (3) comparing the proportion of improved images visualized before and after AF0150 contrast was administered. Statistical significance was demonstrated at a p-value = 0.01 and better. Subset analyses based upon age, race, and gender strengthened those conclusions, although not one particular subset demonstrated greater improvement using AF0150. However, in spite of the statistically significant improvement in visualizing the endocardial border, AF0150 contrast does not appear to improve the ability for the echocardiologists to accurately assess the ejection fractions (EF). Subset analyses did not identify any population subset that could benefit in terms of EF assessment from use of this product. Thus, the only primary endpoint that has proven efficacy is an improved EBD, with no subset demonstrating greater efficacy. Assessment of segmental wall motion (a secondary endpoint) appeared to show a statistically significant improvement when using AF0150, as compared to baseline non-contrast poorly visualized echocardiograms. There was also a statistically significant higher percentage of segments in the AF0150-contrasted echocardiograms in agreement with MRI studies as compared with non-contrasted baseline echocardiograms' agreement with MRI studies. However, 26 patients were evaluated with MRI; this number is too small to draw reliable conclusions upon. Therefore, with respect to the secondary endpoint of segmental wall motion, the data provided is testimonial

(subjective) and not with a ***universally accepted*** standard of truth (objective) and therefore unreliable.

Finally, the anatomic endpoint of EBD has been assumed to be a surrogate for the 2 functional endpoints of EF and SWM. EBD was proven but EF did not improve; therefore, we question the surrogate. In order to correlate EBD's role in assessing EF, 2 values -- the end-systolic (ES) and end-diastolic (ED) ventricular volumes [derived from both fundamental modes (continuous and gated) of 2-dimensional echocardiography] – need to be analyzed. Although the sponsor provided EF data *per se*, a database is needed for ES and ED to help correlate EF with EBD. Improved estimations of ES and ED volumes should lead to better estimations for EF. Therefore, at this point, EBD does not appear to be useful as a surrogate for EF prediction. Because the estimation of EF did not improve after use of the contrast agent, the question arises as to the value of EBD as a surrogate in determining the function of the heart when the contrast agent is used in 2-D echocardiography. Given the poor EF data, the Agency needs, at a minimum, a re-read of the EBD data at end-systole and at end-diastole, as well as a blinded-reader EF calculation.

At the proposed clinical dose of 0.125 mg/kg, AF0150 produces few adverse events, with few patients manifesting symptoms related to gas embolism; most are mild and sporadic. Patients with recent cardiac disease, especially related to coronary artery disease, have a greater propensity toward developing arrhythmias, possibly related to the contrast agent or to disease. In addition, a trend toward an increase in the QTc interval was noted in subjects receiving AF0150. However, for the pivotal Phase 3 study IMUS-007-USA, the incidence of QTc interval prolongation in subjects randomized to both AF0150-treated and saline-treated arms was virtually the same; for both Phase 3 studies, the QTc interval increases were not statistically or clinically significant and therefore were not recorded as clinical adverse events. Follow-up with Phase 4 studies (post-approval) may be used to address both concerns in subjects with stable and unstable cardiac disease. The dosage of 0.125 mg/kg appears to be safe in the majority of patients with stable cardiac disease; no subpopulation of patients (i.e., gender, race, age) can be distinguished as having an increased chance of AE's, due to low numbers enrolled in the studies.

The overall recommendation is that this product might be approvable, in spite of the lack of evidence of improving efficacy in determining the cardiac function (EF), with and without EBD data, in patients with stable cardiac disease. There is demonstrated efficacy for EBD as a primary endpoint; however, with the lack of improvement in EF calculations, the value of EBD as a surrogate for functional assessment is in question. A re-read of the EBD data at end-systole and

at end-diastole, as well as a blinded-reader EF calculation, will be requested. The product is relatively safe, with a propensity toward increasing the QTc interval in cardiac patients, though not clinically significant and not noted to be significantly greater than QTc prolongation noted in subjects receiving saline.

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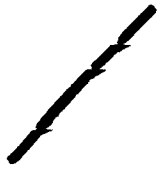
I. PROPOSED PACKAGE INSERT INDICATION



II. REGULATORY HISTORY

In the initial clinical development, there were 2 planned applications for the use of AF0150 as a contrast agent:

(1) for echocardiographic studies to improve assessment of functional and perfusion abnormalities in acute myocardial infarction patients; and

(2) for radiographic studies to determine the ability of AF0150 to improve assessment of vascular flow abnormalities and characterization of space-occupying lesions.

Both planned applications were to use gray-scale and color Doppler techniques. Below is a tabulation of the different studies planned to answer for the different variables in each plan, as discussed in an End-Of-Phase 2 meeting (November 13, 1997).

TABLE 1: DEVELOPMENTAL HISTORY (BY YEAR AND PHASE/STUDY) OF IMAVIST™

Phase	1996	1997	1998	1999	2000
Safety Study					
1	IMUS-001				
Cardiac Function Studies					
2		**IMUS-002** LV function and myocardial perfusion			
3		**IMUS-007 & IMUS-008** Cardiac function		**NDA** Cardiac function	
Myocardial Perfusion Studies					
2		**IMUS-** Cardiac Perfusion			
3			**IMUS-009 & IMUS-010** Myocardial Perfusion		
Diagnostic Radiology Studies					
2		**IMUS-003** Liver and Kidney Tumors	**IMUS-013 & IMUS-014** Breast & Prostate Tumors		
3					

Source: Volume 44, p 024 (Table II.2.)
* Studies have not been conducted.

This New Drug Application (NDA) was submitted to demonstrate use of AF0150 as an ultrasound contrast agent for improving assessment of endocardial border delineation and cardiac function in patients with suboptimal echocardiograms.

III. OVERVIEW OF ECHOCARDIOGRAPHY

Ultrasound constrast agents have been developed to allow for a more accurate assessment of anatomic definition, flow quantitation, and tissue perfusion of an organ or system when utilizing ultrasound as a diagnostic imaging modality. Thus, ultrasound contrast agents may increase the ultrasonic reflectivity of both the vascularity of an organ and of the organ itself (helping to differentiate normal and diseased tissue).

According to the sponsor, the 1st ultrasound contrast agent was discovered serendipitously when saline was injected during an M (motion)-mode echocardiographic procedure. This resulted in visualization of a cloud of microbubbles of gas originally dissolved in the saline. Other injectable solutions that subsequently demonstrated this similar quality for ultrasound include indocyanine green, dextrose, sorbitol, and radiographic contrast media. Limitations of the earlier forms of microbubble contrast included (1) inconsistent bubble size, (2) very short vascular residence times, and (3) inability to pass through smaller vessels. The recently developed agents were produced to overcome these obstacles; they consist of microbubbles stabilized as various emulsions (i.e., emulsions of serum albumin, liposomes collagen spheres, and combinations). The 1st approved ultrasound contrast agent was *Albunex*®, which consists of albumin-based air-filled microbubbles, introduced in 1994 by Molecular Biosystems Inc., San Diego, CA. In 1997, Molecular Biosystems introduced its 2nd ultrasound contrast agent called *Optison*®, an agent composed of perfluoropropane-filled albumin microspheres.

Echocardiography uses ultrasound to image the heart and great vessels, and contrast echocardiography permits the identification of such structures as the interatrial septum, left main coronary artery, and coronary sinus. For background information regarding echocardiography, the table below defines 3 types of echocardiographic studies.

TABLE 2: ECHOCARDIOGRAPHS – STANDARD MODES

TYPE / MODE	TYPE OF RESOLUTION	TYPE OF DISEASE EVALUATED
M (motion)- mode	Provides an "ice-pick" view of the heart, with temporal resolution.	Widely used to measure left ventricular size, wall thickness, and function only when ventricular shape is normal and systolic movement is relatively symmetrical; also used, in conjunction with angiography and hemodynamic assessments, in evaluating abnormalities of prosthetic valves.
Two-dimensional (2-D)	Provides two distance dimensions, with spatial resolution of structural movement in real time.	Recognizes etiologies of mitral regurgitation; structural abnormalities with the aortic regurgitation; evaluates the volume and function of the left ventricle (particularly in patients with asymmetric conraction due to ischemic heart disease); improves imaging of the right-sided heart vessels (tricuspid and pulmonary valve disease).
Doppler	Permits detection of blood flow velocity and turbulence.	Permits evaluation of regurgitation by mapping the extent of turbulence within a cavity and/or vessel. It is the most useful initial diagnostic procedure in chronic heart failure because it can assess systolic and diastolic function, chamber dimensions, ventricular wall thickness, and significant valvular disease.

(Information derived from Harrison's Textbook of Medicine)

According to the sponsor, the ability to evaluate the left ventricular function is dependent on the ability to precisely define the endocardial border around the entire perimeter of the ventricle on 2-D images of the heart. This detection may be suboptimal in 50% of subjects; therefore, subsequent assessment of cardiac function, segmental wall motion abnormalities, and estimation of the ejection fraction would be difficult.

IV. PRODUCT CHARACTERIZATION

A. CHEMISTRY

AF0150 (perflexane-phospholipid microbubbles) for injection is an intravenous ultrasound contrast agent developed by Alliance Pharmaceutical Corporation (San Diego, CA); its intended trade name is *Imavist*™. AF0150 is a sterile, non-pyrogenic powder whose formulation (Vol. 1, p. 69) utilizes the following **(critical components bolded)**:

(1) **perflexane**, a perfluorinated alkane stabilizing gas which is diluted into N_2;
(2) **dimyristoyl phosphatidylcholine (DMPC)**, a semi-synthetic phospholipid surfactant;
(3) m-hydroxy-ethyl starch (HES),
(4) sodium chloride (an inactive component, ';
(5) phosphate buffers (inactive component, '; and
(6) poloxamer 188 (an inactive component).

Upon constitution with sterile water, the AF0150 is re-suspended, yielding DMPC-enrobed microbubbles filled with perflexane/N_2 and air in an iso-osmotic solution, buffered to physiological pH.

For the clinical studies reported by the sponsor, a single formulation of AF0150 was used. In the studies, AF0150 was supplied in vials containing either 100 mg or 200 mg of the dried powder. Vials containing 100 mg of dry powder were constituted with 10 mL sterile water to a final concentration of 10 mg/mL. The mean number of microbubbles/mL is @ 9.8×10^8.

TABLE 3: IMAVIST™ (AFO150) MICROBUBBLE PARAMETERS

MICROBUBBLE PARAMETERS	
Size	
• Volume median diameter (μm)	
• Range: Lowest to maximum	Not reported by sponsor.
Number	
• Total counts/mL	(mean – 9.8×10^8)
• Counts/mL (3 to 10 μm)	
• Counts > 10 μm	

The FDA chemistry reviewer pointed out 2 size distribution curves incorporated into the application, based upon a study (n = 36 vials). Two figures were illustrated, the first being a size distribution curve of constituted AF0150 which was averaged by measuring channels (bins) > 2 μm-sized bubbles. The second figure was a size distribution curve of constituted AF0150 which was averaged by measuring channels (bins) > 10 μm-sized bubbles. Bubble sizes > 20 μm accumulated in the last channel (the 20 μm channel), thus potentially larger sizes than those listed may be present. Data from the 36 vials were averaged; no error bars were included in these plotted curves, thus an even greater number of bubbles larger than capillary diameter (7 – 10 μm) may have been present. Such bubbles increase the risk for clinical adverse events if administered into human subjects. The sponsor mentioned that the counts of > 20 μm-sized bubbles might actually be background noise (e.g. due to electronic noise signals and the water diluent particle background) rather than those larger bubbles.

Finally, the sponsor provided a table within the submission (Volume 1, p 081 – Table 22: AF0150 Lot Disposition) which illustrates the different lot numbers for the AF0150 (with differing sub-lots for the perflexane and DMPC, per AF0150 lot) used in stability studies and clinical studies. There is concern about the relationship of the pilot and commercial lots to the investigational lots; if the testing methodologies are not sufficiently established for confirmation of this, then additional bridging studies may be needed.

B. NON-CLINICAL PHARMACOLOGY

The FDA biopharmacology reviewer pointed out that, within the general pharmacology studies, the intravenous administration of AF0150 microbubbles appeared effective to enhance ultrasound

signals in animal models. However, the reviewer stated that the numbers of animals used in the major studies were not high enough for statistical analyses, despite similar trends noted in the studies. Additionally, Doppler measurements of carotid artery blood flow (instead of left ventricular cavity imaging) was used without a proper explanation for equivalence. Finally, SEM (standard error of the mean) data was used instead of the more appropriate standard deviation (SD) data, thereby making the data appear better.

At various intravenous doses, AF0150 demonstrates ***no significant*** adverse effects, as tabulated below:

TABLE 4: Organ-System Safety Evaluation of AF0150

ORGAN-SYSTEM	VARIABLES INCLUDED	DOSE (mg/kg)		ANIMAL STUDIES
Cardio-vascular	Blood pressure, pulse, cardiac output, and pulmonary artery pressure	20 mg/kg	52-fold proposed clinical dose (PCD)	Anesthetized rabbits
		1.6 mg/kg 40 mg/kg	6.9-fold PCD 104-fold PCD	Anesthetized dogs Non-anesthetized monkeys
Blood gases	PaO_2, $PaCO_2$, and pH		up to 52-fold 6.9-fold	Anesthetized rabbits Anesthetized dogs during 60-minutes post-dosing observation
ECG	Including QTc	20 mg/kg 40 mg/kg	86-fold PCD 104-fold PCD	Anesthetized dogs Non-anesthetized monkeys with the 60-minute post-dosing observation
Renal *†		4 – 100 mg/kg	5 – 130-fold PCD	Rats
Neurological or Behavioral †		Up to 16 mg/kg	20-fold PCD	Rats
Gastro-intestinal charcoal transit		Up to 100 mg/kg	130-fold PCD	Rats during the 30-minute post-dosing observation
Hematology	Decrease in WBC and platelets	20 mg/kg 20 mg/kg	52-fold PCD 86-fold PCD	Rabbits Dogs

PCD = Proposed Clinical Dose

* NOAEL (no observed adverse effect level) was < 4 mg/kg (5-fold PCD) after intravenous administration.

† The AF0150 microbubbles tended to increase kidney and brain ischemia following intra-arterial injection at a dose up to 16 mg/kg (20-fold PCD)

Regarding the pharmacokinetics study (Protocol IMUS-012-USA), the major elimination route of perfluorohexane (PFH) was through the lungs. Approximately 90% of the PFH was exhaled within 3 hours post-dosing of 20 mg/kg (26-fold) IV AF0150 in the rat. PFH in blood decreased by 78% at the 1st 2 minutes post-dosing as compared to measurement at time 0, and became non-detectable by 24 hours. The terminal serum half-life of PFH was 88 minutes. (The kinetic profiles of PFH in both exhaled air and blood may be seen in the later review in this document.)

Regarding the toxicology of AF0150, the intravenous administration of AF0150 induced transient toxic effects tabulated below. The NOAEL (no observed adverse effect levels) are listed along with the toxicities noted in the respective animal studies.

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TABLE 5: Toxicologic Evaluations Using AF0150

ANIMAL	SYMPTOM/SIGN	DOSE (mg/kg)		PROTOCOL
Single Dose (Acute) Toxicity Studies				
Mice	Hypoactivity; dyspnea	NOAEL = 800 mg/kg	518-fold PCD	IMUS-037-TOX
	Cecal inflammation (at necropsy)	NOAEL = 200 mg/kg	130-fold PCD	IMUS-037-TOX
Rats	Blushing of lips, nose, ears, paw and tail	NOAEL = 400 mg/kg	518-fold PCD	IMUS-010-TOX
	Macrophage vacuolation in the spleen and mesentaric LNs	All dose levels (50 – 400 mg/kg)		IMUS-011-TOX
Dogs	Slight decrease in platelets; slight increase in serum triglyceride and alanine aminotrasferase (ALT)	NOAEL = 200 mg/kg	865-fold	IMUS-039-TOX
	Vomiting, excessive salivation, hypoactivity, and injected sclera	No NOAEL		IMUS-039-TOX
Multiple Dose Toxicity Studies				
Dogs	Vomiting, pale mucous membrane hypoactive or uncoordinated behavior	NOAEL = 25 mg/kg/day	108-fold PCD	IMUS-014-TOX, IMUS-027-TOX
Rats	Vacuolated macrophages in multiple tissues; esp. spleen and lymph nodes (not the bone marrow)	All AF0150 doses levels		IMUS-013-TOX
	Eosinophilic infiltration in mesenteric lymph nodes and perivascular area in the lungs	> 200 mg/kg/day (NOAEL = 50 mg/kg/day)	HED: 310 mg/kg/day HDM: 65-fold	IMUS-013-TOX
	Increased extramedullary hematopoiesis in the spleen	> 200 mg/kg/day (NOAEL = 50 mg/kg/day)	HED: 310 mg/kg/day HDM: 65-fold	IMUS-013-TOX
	↓ Serum chemistries: 1. Renal – creatinine 2. Nutrition – total protein and globulin 3. LFTs – AST, ALT, alkaline phosphatase	NOAEL = 50 mg/kg/day	65-PCD	IMUS-013-TOX

Microbubble profile data were submitted by FAX on April 6, 2000, as requested by the pharm/tox reviewer; below is a tabulation of the size distribution and counts/mL of AF0150. Less than 0.2% of all microbubbles were > 10 μm in all time-points after reconstitution. In study RE-99-47 (a pharmacology study), there were 2 peaks in populations of microbubbles: the 1st at bubbles sizes between 1 – 1.5 μm and the 2nd between 4 – 5 μm in diameter. Total counts/mL at 60 minutes post-reconstitution statistically decreased; no significant difference was noted at 30 minutes.

TABLE 6: Size Distribution & Counts/mL of AF0150 Microbubbles Post-Reconstitution

Time Post-Reconstitution	Microbubble Size (μm) and Counts/mL (x 10^6)		
	Whole Range	3 – 10 μm	> 10 μm
0 minutes	979 ± 1.17	181 ± 0.158 (18.5%)*	1.51 ± 0.607 (0.15%)
30 minutes	912 ± 1.09	173 ± 0.25 (19.0%)	1.36 ± 0.806 (0.15%)
60 minutes	858 ± 1.02	173 ± 0.25 (20.2%)	1.50 ± 0.739 (0.17%)

Source: FDA pharm/tox reviewer summary, page 13 (Table 5).
* Data in parenthesis are % of bubbles at that size over total bubbles (whole range).

Effects of the ultrasonic waves upon the AF0150 microbubbles were tested in study RE-99-46 (another pharmacologic study); in this *in vitro* study, a bovine albumin solution was the "circulatory system". In the study, there was a slight shift in bubble sizes toward the smaller sizes when using sustained ultrasound; the exception was when the highest power (mechanical index of 1.7) was used. At the mechanical index (MI) of 1.7, bubble sizes increased from 4.1 to 6.1 μm in range. Microbubble counts for small- and large-sized bubbles decreased with increasing ultrasound exposure time and power levels.

AF0150 had no significant adverse effects on fertility, teratology, embryonic (early and later) and fetal development in the rat when administered doses of 65 – 259 fold the PCD (based on body surface area). In rabbits, the dose of 200 mg/kg/day (518-fold PCD) led to a slightly increased incidence of external, visceral and skeletal malformations. At the same dose in rats (259-fold PCD), a lower live-birth (and higher still-birth) rate was noted. There was minimal maternal toxicity noted in all 4 reproductive toxicity studies with doses up to 200 mg/kg/day. No significant genotoxicity was recorded after the standard battery of genotoxicity studies.

Thus, issues that need to be addressed are the following (obtained from the Agency pharm/tox review):

1. Blood gas analyses need to be performed in non-anesthetized animals (dogs or monkeys) following IV injection of AF0150 at the highest dose of at least 100-fold PCD with more than 24-hour observation.
2. Renal functions need to be evaluated with lower AF0150 dose ranges in order to achieve the NOAEL. Animal species besides rat may be considered, such as the dog or monkey.
3. The fate of DMPC and Poloxamer-188 following IV administration need to be addressed.
4. There was great variation in PFH levels of reconstituted AF0150 from vials to vials but mg/mL concentrations were constant. The dosage in most pharm/tox studies was verified by osmolality measurement. PFH levels must be correlated to osmolality measurements and microbubble profiles; the impact upon conversion from mg/kg to bubble counts/kg must be identified.
5. AF0150 microbubble behavior and effects on blood flow and capillary endothelial cells need to be evaluated with microcirculation tests, particularly under pathological conditions (such as atherosclerosis, hypertension, and hyperlipidemia) and pharmacological cardiovascular stress.
6. The sponsor is suggested to indicate the potential impact of AF0150 on physiology of the monocyte/macrophage and cecum/appendix in the labeling since "species-specific" macrophage vacuolation and cecal inflammation are mechanistically unclear.
7. Weak teratology and post-natal toxicity are suggested to be included in the labeling, and lack of maternal toxicity at the highest dose in all studies may need to be indicated in the labeling.

8. Local skin pathological reactions by peri-venous injections of AF0150 need to be indicated in the labeling.

C. HUMAN PHARMACOKINETICS AND BIOAVAILABILITY

Please refer to the Clinical Pharmacology Protocols IMUS-001 and IMUS-012 in this review.

D. PEDIATRIC WAIVER

E. FINANCIAL DISCLOSURE

A copy of the Financial Disclosure certification was submitted within the 1st volume of the NDA. In the certification, the Chief Scientific Officer (Artemios B. Vassos, MD) states that no financial information was required to be submitted by any clinical investigator. This was because the Phase 3 studies IMUS-007-USA and IMUS-008-USA were ***completed*** as of February 2, 1999 (the revised rule on Financial Disclosure by Clinical Investigators (63FR72171, December 31, 1998) 21 CFR 54 requires disclosure for ***ongoing*** studies as of February 2, 1999). In addition, Alliance certifies the following:

- There were no financial arrangements between the sponsor and any of the clinical investigators that would bias the clinical studies, and
- There was no proprietary interest on the part of any clinical investigator.

V. CLINICAL STUDIES

For synopses in tabular form, refer to

- Appendix C, which summarizes the plans for all protocols
- Appendix D, which summarizes the numbers of subjects recorded as having adverse events (total, moderate, and severe)
- Appendix E, which records all moderate and severe adverse events for all Phase 1, 2, and 3 studies lists the individual subjects with the respective adverse events (not including the 120-day safety update, which illustrates 3 additional studies not included in this submission)

A. TITLE -- PROTOCOL # IMUS-001-USA

A Single-Blind, Dose-Ranging, Placebo-Controlled, Safety, and Contrast Enhancement Study in Normal Volunteers Receiving AF0150 Administered by Intravenous Injection

STUDY DESIGN

A single-center [redacted], single-blind, randomized, placebo-controlled, dose-ranging, 3-staged Phase 1 study investigating safety.

40 pts → Bolus AF0150 (0.125, 0.5, 2.0, or 4.0 mg/kg in 10 sec x 1 dose)
20 pts → Placebo (0.2 mL 0.9% NaCl)
4 pts → Infusion (4.0 mg/kg over 10 min)

Stage 1: 24 subjects randomized to either AF0150 or placebo, followed by U/S evaluation for visual clearance of agent.
Stage 2: 20 subjects randomized similarly, followed by contrast imaging of the heart.
Stage 3: 20 subjects randomized similarly, followed by contrast imaging of the abdominal region.

ADDENDA

Amendment 1 was produced for the following changes:

1. To include in the treatment plans
   a) a 3rd dose level (0.5 mg/kg) for Stage 1 subjects
   b) add 2 placebo subjects to each escalation group in Stage 1
   c) require that dosing at each subsequent Stage 1 dose level not begin until safety data through Day 1 were reviewed by Alliance and submitted to FDA
2. To include in the study monitoring plans
   a) Collect additional blood samples at 24 and 72 hours and Day 7
   b) Add continuous monitoring of respiratory rate and use of a capnometer device [redacted] along with monitoring of ventilatory status for 15 to 30 minutes following drug administration
   c) Include tumor necrosis factor (TNF-α) measurements at 2, 8, and 24 hours, and 3 and 7 days post-dosing
   d) Include Holter (cardiac) monitoring for 2 hours before injection and x 8 hours post-injection
   e) Require safety data be reviewed by Alliance for safety issues that would preclude advancement to the next phase of the study

RESULTS:

SUBJECTS ENROLLED

Normal adult volunteers, 64 patients in total, were enrolled, as illustrated in the table below (% male gender in parenthesis).

TABLE 7: ENROLLMENT PER STAGE IN PROTOCOL IMUS-001*

Drug and Dose Administered	Stage 1 Clearance Imaging	Stage 2 Cardiac Imaging	Stage 3 Abdominal Imaging	Total
AF0150 (bolus)				
• 0.125 mg/kg	4 (50%)	4 (50%)	4 (100%)	12 (66.7%)
• 0.5 mg/kg	4 (75%)	4 (25%)	4 (50%)	12 (50%)
• 2.0 mg/kg	4 (0%)	0	0	4 (0%)
• 4.0 mg/kg	4 (50%)	4 (75%)	4 (0%)	12 (41.7%)
AF0150 (infusion)				
• 4.0 mg/kg	0	2 (50%)	2 (0%)	4 (25%)
Placebo (bolus)				
• 0.2 mL/kg	8 (37.5%)	6 (50%)	6 (33.3%)	20 (40%)
Total	24 (41.7%)	20 (50%)	20 (40%)	64 (43.8%)

Source : Volume 84 p 031 – Table IV.
* % male gender in parenthesis

Efficacy Results

As tabulated above, with the exception of the 2.0 mg/kg bolus AF0150 group, 12 subjects were enrolled into each of the 4 bolus-treatment groups, such that 4 subjects were enrolled for each stage of the trial at each dose. Thus, 40 patients received bolus AF0150 treatment, administered over a 10-second period, followed by a saline flush manually. Four patients received infusional AF0150 treatment, administered over a 10-minute period, and 20 subjects received placebo. From an efficacy standpoint, substantial attenuation of the signal occurred after dosing at higher levels, such that, almost complete black-out of the image occurred. It was found that minutes were required for the attenuation to diminish allowing contrast in the ventricular cavity and myocardium to be visualized. From this qualitative review of all of the cardiac videotapes, it was determined that sufficient contrast with minimal attenuation time could be obtained with the lowest dose of AF0150 tested (0.125 mg/kg), leading to the decision to proceed with the 0.125 mg/kg dose in the pivotal echocardiographic studies.

For abdominal imaging, it appears that not all organs and structures were viewed for all subjects and qualitative evaluations of both gray-scale and Doppler images were anecdotal.

For the cardiac (videodensitometry) images, it can be seen in the data that a significant period of no increase in Gray-Scale Units (GLU) is observed after time zero. It would appear that this represents the time of attenuation until some enhancement can be observed in the ventricle. Because attenuation was greater and longer for the higher doses (as can clearly be seen on review of the original videotapes), the interpretation of the data with respect to dose-response effects on contrast are confounded. From these data, one cannot distinguish whether lower GLU values represent less contrast or more attenuation.

Adverse Events (AE's)

I. Clinical AE's

All subjects were included into the safety summary. There were no deaths or serious adverse events encountered in this study; all other adverse events were assessed as mild to moderate in intensity, with none considered serious.

Adverse events (AE's) were reported by 10 (23%) of the 44 subjects treated with AF0150; the most commonly reported AE was headache (4 patients = 9 %), while 1 (5%) patient in the placebo group reported headache. The headaches were treated with acetaminophen and were mild and transient; in addition, there were 2 patients [ID# 026 (→ 0.5 mg/kg bolus) and 063 (→ 4.0 mg/kg infusion)] who were listed as having pre-AF0150-treated headaches who had headaches but were not listed in the AE profiles. Taste perversion was noted in 1 (2%) of the 44 AF0150-treated subjects, and in 2 (10%) of the 20 placebo-treated subjects. The perversion of taste was transient, lasting from 0.5 seconds to 1 minute in the AF0150-treated subject, and 45 seconds and 1 minute in the 2 placebo-treated subjects.

TABLE 8: IMUS-001 -- Adverse Events Reported (All Subjects)

ID #	Tx-Group	AE	Severity	Time of Onset §	Duration	AF0150 - Related
AF0150 BOLUS						
001	0.125 mg/kg	Taste perversion	Mild	10 sec.	0.5 – 1 sec.	Possible
009	0.5 mg/kg	Headache	Mild	2 days	4 hours	Not Likely
010		Hiccup	Mild	23 hours	18 hours	NL
		Hiccup	Mild	2 days	5 hours	NL
026		Fever (38.6°C→ NSAID)	Mild	8 hours	14.6 hours	NL
052		Conjunctivitis	Moderate	4 days	5 days	NL
017	2.0 mg/kg	Headache	Moderate	3 days	1.2 days	NL
048	4.0 mg/kg	Headache	Mild	1 hour	3 hours	NL
AF0150 INFUSION						
043	4.0 mg/kg	Vasodilation	Moderate	2 days	1 minute	NL
		Vasodilation	Moderate	2 days	1 minute	NL
		Vasodilation	Moderate	2 days	1 minute	NL
		Vasodilation	Moderate	3 days	1 minute	NL
063		Headache (required tx)	Mild	10.7 hour	2.5 hours	NL
		Pain	Mild	7 days	24 minutes	NL
064		Dizziness	Mild	0.3 hours	40 minutes	Possible
		Nausea	Mild	1.2 hours	8 hours	Possible
PLACEBO BOLUS						
006	0.2 mL/kg	Taste perversion	Mild	10 sec.	1 minute	NL
012		Postural Hypotension	Mild	6.7 hours	11.8 hours	NL
014		Parosmia	Mild	10 sec.	10 seconds	NL
015		Headache	Mild	4 hours	30 minutes	NL
		Taste perversion	Mild	10 sec.	45 seconds	NL
040		Dry skin	Mild	3.8 hours	2.6 hours	NL
042		Diarrhea	Mild	6.8 hours	2.2 days	NL

Source: Volume 84 p 043 (Modification of Table XIII).
§ Time of onset relative to injection.

II. Laboratory AE's

Hematology: Decreases in selected panels (hemoglobin, hematocrit, and RBC count) from normal baseline levels to levels < 18% of normal were noted in both AF0150-treated and placebo-treated subjects. All events exhibited no distinct pattern with regards to timing or dose and were not clinically significant. There were no significant post-treatment decreases in reticulocyte counts, white blood cell counts (including differentials), and platelet counts noted. One subject (Subject 004 → 0.125 mg/kg bolus) had a decrease from baseline (157 K/μL) to a nadir at 5 minutes post-dosing (146 K/μL) which returned to normal 4 hours later.

Complement: There were no clinically or laboratory significant changes noted with changes in complement relative to baseline. During evaluation of C3, C4, and CH50 levels, only CH50 levels demonstrated fluctuation within the 1st 2 hours post-treatment in the subjects in the 4.0 mg/kg infusion group.

Mean C3a levels increased from baseline levels post-bolus AF0150 groups (except the 4.0 mg/kg group) which gradually decreased but remaining above the mean baseline levels. Increased C3a levels were also noted in the 4.0 mg/kg infusion group, but the mean C3a levels decreased below baseline at 2 hours. This is also noted with regards to the 4.0 mg/kg bolus group. This is illustrated below in tabular form. No clear evidence of systemic change was noted in the vital signs and hematologic parameters in relation to any complement changes in this study.

TABLE 9: IMUS-001-USA -- Mean C3a Values (ng/mL) at Specified Time Points

TIME	AF0150 (MG/KG) BOLUS								INFUSION		PLACEBO (ML/KG)	
	0.125		0.5		2.0		4.0		4.0		0.2	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Baseline	12	239	12	255	4	237	12	368	4	310	20	303
5 min.	12	300	10	258	4	602	10	369	4	273	20	290
15 min.	11	291	12	316	4	432	12	380	4	365	20	288
30 min.	12	272	12	302	4	393	12	394	4	324	20	339
60 min.	12	261	12	277	4	378	12	442	4	317	20	277
120 min.	12	252	12	285	4	284	12	323	4	256	19	251

Lymphokine: No significant changes were noted with tumor necrosis factor (TNF-α); no AE's occurred related to any changes of lymphokine levels.

Coagulation: There was little to no variation for mean PT, aPTT, and fibrinogen levels; no AE's occurred related to any changes in these parameters.

Chemistries: Mean values for blood chemistry parameters (liver functions tests, renal function tests, serum proteins, and electrolytes) were within normal limits at all time points. No AE's occurred related to any of the changes.

Urinalysis: No clinically significant changes were observed.

III. Post-dosing ECG Abnormalities

TABLE 10: POST-AF0150 DOSING ECG ABNORMALITIES

Subject	Group	Hrs. Post-Tx	Abnormality	Comment
003	Placebo	8	Sinus bradycardia; increased QRS voltage, non-specific ST-wave changes	Not clinically significant; abnormalities present upon entry
015	Placebo	1	Sinus bradycardia with sinus arrhythmia and 1° AV block	AV block evident on admission
028	Placebo	8	Non-specific T-wave abnormality	Not clinically significant
044	AF0150 4.0 mg/kg infusion	8	Possible ectopic atrial bradycardia	Not clinically significant
045	AF0150 4.0 mg/kg bolus	8	Sinus arrhythmia with normal sinus rhythm	Within normal limits

Of note, there is an increased incidence of ectopic beats noticed with infusion when compared with placebo and with bolus. This is possibly attributed to one patient enrolled and randomized to the 4.0 mg/kg infusion group – subject # 063 (Volume 87 p 274) – who had, at baseline , 41 ectopic beats over a 2 hour period. This patient, unlike other patients who had < 5 ectopic beats, had a clinically significant rise in the number of ectopic beats – 81 between 0 to 2 hours; and 60 over 2 to 4 hours post-AF0150 – before decreasing to baseline levels at 4 to 6 hours post-AF0150. Thus, it appears that this patient is the outlying influence for the rise in ectopic beats for this group. In addition, a patient enrolled and randomized to the 0.125 mg/kg dose group – Subject # 046 (Volume 87 p 269) – had a baseline ectopic beat number of 17 (hours –2 to 0). That subject had a rise of ectopy to 24 at 0 to 2 hours and later 46 at 2 to 4 hours, reaching a peak of 81 at 6 to 8 hours. Therefore, subjects with a propensity for ectopy should be closely monitored when being given AF0150 due to the potential for arrhythmias.

IV. Vital Signs

There were no clinically significant changes in any vital signs post-therapy, nor any differences between vital signs after placebo and AF0150 administration. One subject, Subject # 026, enrolled into the 0.5 mg/kg group), was listed as having an AE due to a rise in temperature at 38.6°C (= 101.5°F; 1.2° C above baseline) noted 8 hours after AF0150 dosing. The subject received 400 mg ibuprofen and the fever resolved 24 hours post-dosing.

V. Respiratory Function

Excluding the 14 subjects in Stage 3 of the study who were instructed to hold their breaths (one of which had a decrease of expired CO_2 by 28% relative to baseline), there were 5 subjects recorded to have > 20% decrease in respiratory rate from baseline. There is no trend noted for association of the study drug with the events.

TABLE 11: IMUS-001-USA – Subjects with Decreased Resp. Rates Post-AF0150

Dose Group	Subject	Time Point	Mean Resp. Rate	Mean CO_2
0.5 mg/kg	009	10 – 15 min post	14 (from 19 at baseline)	49 (from 52 at baseline)
	037	0 – 5 min post	15 (from 19 at baseline)	51 (from 53 at baseline)
2.0 mg/kg	017	15 – 20 min post	13 (from 17 at baseline)	39 (from 52 at baseline)
Control	012	15 – 20 min post	18 (from 25 at baseline)	57 (from 51 at baseline)
	042	10 – 15 min post	12 (from 17 at baseline)	47 (from 49 at baseline)

VI. Arterial Oxyhemoglobin Saturation

Excluding those subjects in the 2.0 mg/kg group, SO_2 varied little across time points. This "exclusion" is due to one subject – #013 (in the 2.0 mg/kg group) – whose 1-hr value of 86% brought down the group as a whole at 1 hour. Otherwise, the mean values ranged from 97% to 99.5%. There were 4 subjects whose SO_2 values were below 95%, all of whom had values at 94% reported at baseline and/or at 1 or 2 post-dosing time points: #013 (mentioned above); #034 (who received 0.125 mg/kg); #018 (2.0 mg/kg); and #019 (4.0 mg/kg).

CONCLUSION

AF0150 was well-tolerated at all doses (up to 4.0 mg powder/kg body weight as bolus or infusion), with adverse events predominantly mild in intensity and considered unrelated to administration of AF0150. The sponsor did not provide quantitative efficacy data, but stated from a qualitative perspective that the lowest bolus dose tested for AF0150, 0.125 mg/kg, provides "good" imaging for cardiac imaging with a "reasonable" duration of imaging time following a "minimal" period of attenuation. In addition, data was not formally analyzed and videodensitometry measurements were not reported, all contrary to the protocol plan. The sponsor felt that, because the enrolled subjects for this protocol were all normal volunteers, the results would not be representative of results needed to approve the product for a different target population. Subjects with a propensity for ectopy should be closely monitored when given AF0150 due to the potential for arrhythmias.

B. TITLE – PROTOCOL # IMUS-002-USA

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

STUDY DESIGN

A multicenter (all 7 centers were in the U.S.), open-label, 2-staged Phase 2 study investigating safety in such patients (see title); there is no placebo-control group.

Stage 1: The "pilot stage", enrolling 10 patients with suspected myocardial perfusion defects encompassing ≥ 20% of the left ventricle.* These patients are referred for nuclear perfusion study prior to discharge from hospital; evaluating left-ventricular function, comparing bolus versus infusional AF0150 contrast.

* Function inferred from results of cardiac isozyme levels, and echocardiogram and ECG results.

Stage 2: The "open stage", enrolling 30 patients but without regards to presence or extent of any existing myocardial perfusion abnormality; comparing AF0150-2D-echo results with ^{99m}Tc sestamibi-SPECT imaging results.

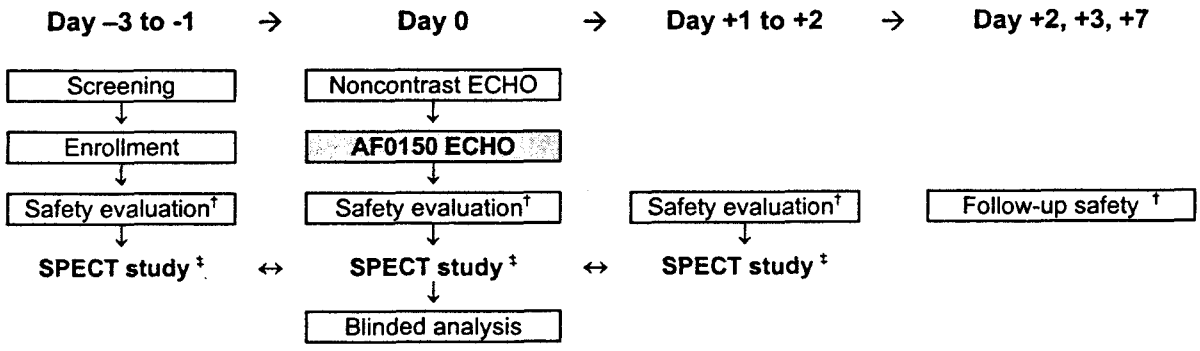
All subjects were to receive 2 injections of AF0150. The 1st treatment -- IV bolus of 0.25 mg/kg over 30 seconds. The 2nd treatment – an IV infusion [redacted] of up to 80 mg AF0150 over 10 minutes. Both doses were to be administered on the same day.

OBJECTIVES

Primary: Safety of dose strategy for patients undergoing contrast echocardiography after myocardial infarction.

Secondary: Ability to improve assessment of left ventricular function (when compared with non-contrast echocardiography); demonstrate the ability to assess myocardial perfusion consistent with nuclear perfusion scintigraphy.

STUDY FLOW CHART



† Safety evaluations were prior to AF0150 dosing, at 5 and 15 minutes after AF0150 dosing, and on Days 1, 2, 3, and 7.

‡ To be conducted within 48 hours prior to or following, but more than 2 hours immediately prior or following, Day 0 ECHO.

Images were read by 3 blinded readers (blinded to subject and imaging period, i.e. noncontrast or contrast images). For ejection fraction determination, readers were not completely blinded to subject and imaging period. Inter- and intra-observer variability was determined for SWM. Myocardial perfusion abnormalities in both non-contrast and contrast ECHO was evaluated in the