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APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-191

Statistical Review(s)

STATISTICAL REVIEW AND EVALUATION

NDA#:	21-191
Sponsor:	Alliance Pharmaceutical Corp.
Drug:	Imavist (AFO150; Perflexane-Phospholipid Microbubbles for Injection)
Drug Class:	1S
Indication:	Ultrasound contrast agent to delineate the left ventricular endocardial borders.
Submission Date:	April 5, 2002
Documents Reviewed:	AZ Vols. 1, 2, 3 (April5, 2002) and N-000-BS (April 18, 2001)
Medical Reviewer:	Bernard Parker, M.D., HFD-160
Statistical Reviewer:	Sonia Castillo, Ph.D., HFD-715
Keywords:	Clinical studies, NDA review

CONTENTS

1 Background and Indication

The sponsor submitted this amendment to NDA 21-191 for Imavist, an echocardiographic contrast agent, in response to the Agency's second approvable letter of February 6, 2002. In the letter, the Agency stated that the application lacks sufficient information to establish the clinical utility of endocardial border delineation (EBD). A subset analysis in 26 patients compared to MRI provides preliminary evidence of improvement in visualization of sub-optimal EBD segments converted to visualized and evaluable for wall motion. However, the exclusion of minimally visualized segments from analyses is unacceptable because Imavist is proposed for use in suboptimal ultrasound examinations.

To address this deficiency, the Agency stated that the sponsor must perform a re-read of the images for the 26 patients with MRI data. The analysis must include the patient level and segment level results when at least 2 adjacent segments were scored as suboptimal EBD at baseline. To establish clinical utility, the change in interpretability of these segments must be linked to improvement in wall motion evaluation.

In this amendment, the sponsor provides responses to the segmental wall motion issues. The sponsor claims that the evidence presented in this amendment with the information in the NDA demonstrate the proposed indication for left ventricular EBD, a structural endpoint supported by functional SWM information. The sponsor's proposed indication is:

[redacted]

2 Segmental Wall Motion Analyses

2.1 Statistical Reviewer's Comments

The sponsor decided not to conduct a reread of the echocardiography images for the subset of 26 patients with MRI data. Instead, they chose to reanalyze the existing data.

From a statistical view, the study results submitted by the sponsor are descriptive only and meant to provide a trend of clinical utility. Given the nature of the information, this reviewer performed the following: verified the tables

presented by the sponsor, gave a quick synopsis of the table, and calculated additional tables requested by the Medical Reviewer. For further interpretation of the tables, refer to the Medical Reviewer's document.

2.2 Sponsor's Analyses

Following is a list of procedures used to calculate the tables for segmental wall motion (SWM) analyses:

- For each reader, echocardiography views (apical 2-chamber, apical 4-chamber, and apical long axis) are classified as "Normal" or "Abnormal" based on the wall motion scores from the MRI data. Abnormal views are those having any segment with a SWM score of 2 to 5 from the MRI.
- Views are defined as non-evaluable if two or more consecutive segments have an EBD score of 0 or 1; and as evaluable if no consecutive segments have an EBD score of 0 or 1.
- Patients are classified as evaluable if they have 2 or 3 evaluable views for that read.

Tables of unanimous agreement are not discussed since percent outcomes with unanimous agreement (pre vs post) is not an endpoint that the division would accept as a surrogate for diagnostic performance. In tables using the entire population, values for normal and abnormal views/patients are not reported because the diagnosis is based on the post-contrast blinded echo evaluation, not a standard of truth. All other tables are summarized below.

- Table 1.1a – Study IMUS-008 (N=26): Fraction of Cardiac Views for Which EBD Converted From Non-Evaluable (Pre-Contrast) to Evaluable (Post-Contrast)

  The conversion rate for the three blinded readers varies from 52% to 84% for the 4-chamber view; from 26% to 61% for the 2-chamber view; and from 44% to 77% for the long axis view.

- Table 1.3 – Studies IMUS-007 and IMUS-008 (N=409): Fraction of Cardiac Views for Which EBD Converted From Non-Evaluable (Pre-Contrast) to Evaluable (Post-Contrast)

  For study IMUS-007, the conversion rate for the three blinded readers varies from 57% to 66% for the 4-chamber view; from 38% to 52% for the 2-chamber view; and from 29% to 81% for the long axis view. For study IMUS-008, the conversion rate for the three blinded readers varies from 52% to 88% for the 4-chamber view; from 60% to 80% for the 2-chamber view; and from 51% to 88% for the long axis view.

- Table 1.2 – Study IMUS-008 (N=26): Fraction of Patients Whose EBD Converted From Non-Evaluable (Pre-Contrast) to Evaluable (Post-Contrast)

  The conversion rate for the three blinded readers varies from 43% to 79%.

- Table 1.4 – Studies IMUS-007 and IMUS-008 (N=409): Fraction of Patients Whose EBD Converted From Non-Evaluable (Pre-Contrast) to Evaluable (Post-Contrast)

  For study IMUS-007, the conversion rate for the three blinded readers varies from 55% to 60%. For study IMUS-008, the conversion rate for the three blinded readers varies from 40% to 85%.

- Table 1.5a – Study IMUS-008 (N=26): Echo SWM Agreement with MRI using Cardiac Views That Were Non-Evaluable Pre-Contrast and Evaluable Post-Contrast

  At baseline, the percentage of evaluable segments for the three blinded readers varies from 56% to 70% for the 4-chamber view; from 44% to 73% for the 2-chamber view; and from 54% to 88% for the long axis view. At post contrast, the percentage of evaluable segments for the three blinded readers varies from 66% to 76% for the 4-chamber view; from 64% to 79% for the 2-chamber view; and from 76% to 90% for the long axis view.

- Table 1.7a – Study IMUS-008 (N=26): Echo SWM Agreement with MRI using All Cardiac Views

  At baseline, the percentage of evaluable segments for the three blinded readers varies from 43% to 67% for the 4-chamber view; from 57% to 66% for the 2-chamber view; and from 48% to 63% for the long axis view. At post contrast, the percentage of evaluable segments for the three blinded readers varies from 65% to 76% for the 4-chamber view; from 67% to 75% for the 2-chamber view; and from 75% to 77% for the long axis view.

2.3 Analyses Requested by Medical Reviewer

Following is a list of procedures used to calculate the additional tables for segmental wall motion (SWM) analyses:

- An echo or MRI view is classified as normal only if all segments in the view are scored as "1".

- An MRI view is classified as abnormal if at least one of the segments is scored as 2 to 5.
- Using Medical Reviewer input, an echo view is classified as abnormal if:

  At least one segment is scored as abnormal (score of 2 to 5) and the segment is not necessarily the same segment as the MRI diagnosis or the same score as the MRI score. The presence of adjacent, non-evaluable segments is acceptable because from a clinical perspective, the patient would require further follow-up.
- An echo view is classified as not diagnosable if none of the segments are scored as abnormal and there are non-evaluable segments present.
- A patient is classified as normal only if all 16 segments in the three views (echo or MRI) are scored as "1".
- A patient is classified as abnormal if at least one view (echo or MRI) is classified as abnormal.
- A patient is classified as not diagnosable if none of the echo views are classified as abnormal and at least one of the echo views is not diagnosable.

Table 2.1 presents the number of normal and abnormal views as determined by MRI.

Table 2.1
Study IMUS-008
Number of Normal and Abnormal Views as Determined by MRI Diagnosis (N=26)

	Number of Normal Views	Number of Abnormal Views
4-Chamber	16	10
2-Chamber	17	9
Long Axis	24	2

Source: Statistical Reviewer's listing.

Table 2.2 presents the number of patients with 2 or more adjacent segments that are not evaluable post contrast by view for each blinded reader. There are more patients with 2 or more adjacent segments that are not evaluable post contrast in the 2-chamber view compared to the other two views.

Table 2.2
Study IMUS-008
Number of Patients with 2 or More Adjacent Segments That Are Not Evaluable* Post Contrast (N=26)

	Number of Patients with Non-Evaluable Adjacent Segments					
4-Chamber View						
Number of Adjacent Segments	2	3	4	5	6	Total
Reader 1	4	1	3	0	3	11
Reader 2	4	1	2	0	4	12
Reader 3	2	0	1	0	1	4
2-Chamber View						
Number of Adjacent Segments	2	3	4	5	6	Total
Reader 1	5	3	3	0	5	16
Reader 2	4	4	3	1	7	19
Reader 3	6	1	2	0	0	9
Long Axis View						
Number of Adjacent Segments	2	3	4	Total		
Reader 1	4	0	9	13		
Reader 2	5	1	8	14		
Reader 3	4	1	0	5		

Source: Statistical Reviewer's listing.
* Either all non-evaluable segments at baseline did not become evaluable post contrast, some segments got worse post contrast, or a combination of not becoming evaluable and getting worse.

Table 2.3 presents shift tables for the number of diagnosable and non-diagnosable views at baseline and post-contrast by view for each reader. A view is diagnosable if the view is correctly diagnosed as either normal or abnormal when using the MRI SWM result for that view. Otherwise, the view is non-diagnosable. Of those views that are not diagnosable at baseline, all views for Readers 1 and 3 have a greater than 30% conversion to diagnosable while Reader 2 has between 5% and 25% conversion.

Table 2.3
Study IMUS-008
Shift Tables for the Number of Views that Are Diagnosable (Yes / No) Using MRI SWM Results as Standard of Truth for Baseline versus Post Contrast Echo Images (N=26)

			4-Chamber		2-Chamber		Long Axis	
			Post Contrast					
			N	Y	N	Y	N	Y
Reader 1	Baseline	N	14	9	13	6	17	8
		Y	1	2	1	6	0	1
			N	Y	N	Y	N	Y
Reader 2	Baseline	N	17	4	20	1	18	6
		Y	2	3	1	4	0	1
			N	Y	N	Y	N	Y
Reader 3	Baseline	N	13	6	9	11	12	12
		Y	1	6	0	6	0	2

Source: Statistical Reviewer's listing.

Table 2.4 presents shift tables for the number of diagnosable and non-diagnosable patients at baseline and post-contrast for each reader. A patient is diagnosable if they are correctly diagnosed as either normal or abnormal when using the MRI SWM result for that patient. Otherwise, the patient is non-diagnosable. Of those patients who are not diagnosable at baseline, Readers 1 and 3 have a greater than 30% conversion to diagnosable while Reader 2 has 6% conversion.

Table 2.4
Study IMUS-008
Shift Tables for the Number of Patients Who Are Diagnosable (Yes / No) Using MRI SWM Results as Standard of Truth for Baseline versus Post Contrast Echo Images (N=26)

		Reader 1		Reader 2		Reader 3	
		Post Contrast					
		N	Y	N	Y	N	Y
Baseline	N	10	6	17	1	11	5
	Y	2	7	3	5	1	8

Source: Statistical Reviewer's listing.

A comparison of *Imavist* to DEFINITY™, another echocardiographic contrast agent, with respect to the wall motion agreement with the MRI assessment of normal versus abnormal wall motion for patients whose endocardial border delineation converted from non-evaluable at baseline to evaluable post-contrast of interest. Using the subjects in the sponsor's Table 1.2, the wall motion agreement with the MRI assessment (i.e., normal versus

abnormal) improved in 46%, 10%, and 26% of the patients for Reader 1, Reader2, and Reader 3, respectively. The values for DEFINITY™ are from 42% and 71%, depending on the reader. Also, for Reader 2, 20% of the patients had an obscured post-contrast image, thus making the image non-evaluable. The values for DEFINITY™ are from 13% and 37%, depending on the reader.

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Concur with review

STATISTICAL REVIEW AND EVALUATION

NDA#:	21-191
Sponsor:	Alliance Pharmaceutical Corp.
Drug:	Imavist (AFO150; Perflexane-Phospholipid Microbubbles for Injection)
Drug Class:	1S
Indication:	Ultrasound contrast agent to delineate the left ventricular endocardial borders.
Submission Date:	August 16, 2001
Medical Reviewer:	Bernard Parker, M.D., HFD-160
Statistical Reviewer:	Sonia Castillo, Ph.D., HFD-715
Keywords:	Clinical studies, NDA review

CONTENTS

1 Background and Indication

The sponsor submitted this amendment to NDA 21-191 for Imavist, an echocardiographic contrast agent, in response to the Agency's approvable letter of August 14, 2000. In the letter, the Agency requested that the sponsor conduct for each trial a repeat blinded read of left ventricular (LV) endocardial border delineation (EBD) at end-systole and end-diastole and blinded reader calculations of the LV ejection fraction measurements. The Agency also stated that there was a lack of sufficient data for validation, that is, there were insufficient number of patients (N=26) for support of improved assessment of segmental wall motion (SWM).

In this amendment, the sponsor provides responses to the left ventricular ejection fraction and segmental wall motion issues. The sponsor claims that the evidence presented in this amendment with the information in the NDA demonstrate the proposed indication for left ventricular EBD, a structural endpoint supported by functional SWM information. The sponsor's new proposed indication is:

2 Sponsor Responses

2.1 Ejection Fraction

The sponsor decided that nothing further would be gained by rereading the echocardiography images in achieving the ejection fraction endpoint

2.2 Segmental Wall Motion

The sponsor restated the result that the number of segments visualized and available to score for SWM was significantly increased with contrast compared to baseline, i.e., improved EBD with contrast.

The sponsor presented inter-observer agreement analyses of the echo segmental wall motion (SWM) data between the three blinded readers in each study [IMUS-007 (N=206) and IMUS-008 (N=203)] using all patients to assess the clinical utility of Imavist. The first analysis used the mean number of segments for which there was agreement without contrast and compared it with the mean number of segments for which there was agreement with contrast for readers 1 vs. 2, 1 vs. 3, and 2 vs. 3. The second analysis used the percentage of segments visualized per patient ([number of segments that agree]/[number of segments visualized by at least one reader] x 100) that agree before and after contrast. The results for both analyses were significant and the sponsor concludes that:

> ... the addition of Imavist ... significantly improves the level of reproducibility and agreement among blinded readers for the assessment of the regional functional endpoint, SWM. ... This finding substantiates the clinical utility for the use of a contrast agent and is consistent with the improvement in EBD visualization scores previously discussed. As such it serves as a measure of clinical usefulness in support of a structural indication for Imavist.

The sponsor also presented a subset analysis to validate the accuracy of the improvement seen in SWM with contrast. The SWM echo data from a subset of 26 patients at one center in study IMUS-008 were compared to their MRI SWM data. These 26 patients were the only ones that had both echo and MRI SWM data. The sponsor stated that this sample of 26 patients was representative of the overall study population because they were representative in terms of demographics. The sponsor assumes that the results for this subset of 26 patients should be valid for the study population as a whole.

The total EBD score for each patient (sum of 16 segments) was plotted against the percentage of segments that agree in the SWM scores assigned utilizing both MRI and echocardiography. A Pearson's correlation coefficient (R) was calculated. This analysis was done separately for the non-contrast and contrast data for each blinded reader. The sponsor claims there is a strong correlation between EBD score and the percentage of segments that agree between MRI and echocardiography (R ranging between 0.61 and 0.76). The sponsor concludes that:

> EBD must be visualized [improvement in EBD scores] to evaluate SWM and that contrast improves the ability to accurately read SWM [using MRI as a gold standard].

The sponsor also presented an ANOVA model analysis to look at the percentage of segments that agreed between echo and MRI both without and with contrast. For all three blinded readers there was greater than a doubling of segments that agreed after the administration of contrast vs. the non-contrast images. This result was statistically significant for each blinded reader when looking at all segments combined or when evaluating regional function.

2.3 Sponsor Conclusions

The sponsor concludes that the results of two Phase 3 trials demonstrate that Imavist improves the delineation of the endocardial border, the primary endpoint. The findings of several analyses demonstrate that the secondary functional endpoint of SWM was met in the study population with further substantiation of the validity of these results through a sub-study utilizing MRI as a comparator. This result reflects the clinical usefulness of EBD and is supportive of a structural (EBD) indication.

3 Statistical Reviewer Evaluation

3.1 Comments on Sponsor's Analyses

The following are comments about the sponsor's analyses:

- All of the sponsor's SWM analyses were not done on baseline and post-contrast segment data that were matched within a patient on a segment by segment basis. These analyses do not adequately assess the added benefit of Imavist when compared to the baseline images.
- The inter-observer agreement analyses of SWM between the three blinded readers were performed without respect to a standard of truth.
- A large number of baseline segments were not evaluated for SWM from the echo images (see below), which results in incomplete echo SWM data for all patients in both studies. This incomplete data is used in the sponsor's SWM analyses and thus make the results of these analyses not accurate.
- It is not known how the 26 patients who had both EBD and MRI data were selected (all were from one center in study IMUS-008). Not knowing how these patients were selected may invalidate the assumption that the SWM results for the 26 patients should be valid for the whole study population.

3.2 Incomplete Segmental Wall Motion Data

The sponsor states that:

> SWM scores were recorded for all segments for which the blinded reader recorded an EBD visualization score of 1 or greater.

This is not the case. As reported in the original statistical review, for the blinded reader's assessment of segmental wall motion (SWM), the study report stated that:

> For segments evaluated for EBD as 0 or 1 (no delineation or unable to assess function), the electronic CRF was programmed to accept only a score of 0, "not visualized" when scoring motion. For segments evaluated for EBD as 2 or 3 (able to assess function), the electronic CRF was programmed to accept only a score greater than zero.

In addition, an overview of the data shows that all segments that were rated as 0 or 1 for EBD had a SWM score of 0 for all patients in both studies.

Although a segment may have an echo SWM score of 0, the score is not a true reader score because the blinded reader did not evaluate the segment. Since the blinded readers evaluate EBD to determine the effect of contrast on the echo images and since what they observe for EBD affects the visualization of wall motion, the electronic CRF should not have been pre-programmed as described above. The blinded reader should have assessed each segment for SWM. Thus, these echo SWM scores of 0 can be viewed as potential data that the blinded reader did not get an opportunity to generate.

Table 3.1 presents the distribution of segments with baseline EBD scores of 0 or 1 and thus the number of incomplete data for baseline SWM evaluation in the 26 patients with SWM MRI data. Since the analyses presented below use paired data (pre- and post-contrast within a patient) for each segment, the number of incomplete baseline SWM data reflects the number of incomplete data for each segment. Almost all segments for all readers have 50% or greater incomplete data.

Table 3.1
Study IMUS-008: Number of Segments with EBD Scores of 0 or 1 at Baseline for Each Blinded Reader from 26 Patients with SWM MRI Data

	Number of Baseline Segments with an EBD Score of 0 or 1		
	Reader 1	Reader 2	Reader 3
4 – chamber segments			
1	20	21	22
2	14	13	13
3	21	14	19
4	24	22	24
5	23	23	24
6	26	24	22
2 – chamber segments			
7	12	25	10
8	6	11	5
9	19	16	18
10	24	23	24
11	20	22	21
12	24	24	23
Long axis segments			
13	21	25	19
14	20	25	19
15	17	17	14
16	23	18	14

Source: Statistical Reviewer's listing.

3.3 Wall Motion Analysis by Segment

Although there is incomplete SWM echo data for segments, paired analyses on a segment by segment basis using the data at hand can provide useful information about any trend that may be present. Recall that segmental wall motion was evaluated by three independent blinded readers using a 5-point scale (0 = segment not visualized, 1 = Normal, 2 = Hypokinesis, 3 = Akinesis, 4 = Aneurysmal).

The outcome measure in this analysis is the percentage of patients with matching (with MRI) wall motion (abnormal/normal) post-contrast compared to baseline by segment. McNemar's test was used to test for the differences in agreement between baseline and post-contrast wall motion evaluation. All non-evaluable echo segments (score of 0) are included in the analysis as mismatches with MRI. Table 3.2 presents the significance levels for the difference between baseline and post-contrast by segment, that is, the improvement in wall motion agreement with MRI at post-contrast compared to baseline. Only Reader 3 showed improvement for almost all segments while Reader 1 and Reader 2 showed improvement for 6 of 16 segments (segments 1, 3, 5, 12, 13, and 14 for Reader 1; and segments 1, 4, 5, 6, 12, and 13 and 14 for Reader 2).

Table 3.2
Study IMUS-008: Segmental Wall Motion Agreement With MRI by Reader When Segments are Classified as Either Normal or Abnormal (N=26)

	Significance (NS/S[‡])		
Segment	**Reader 1**	**Reader 2**	**Reader 3**
4-chamber			
1	S*	S*	S**
2	NS	NS	NS
3	S*	NS	S**
4	-[#]	S*	S**
5	S**	S**	S**
6	-	S**	S**
2-chamber			
7	NS	-	NS
8	NS	NS	NS
9	NS	NS	S*
10	NS	NS	S**
11	NS	NS	S**
12	S**	S*	S**
Long axis			
13	S**	S**	S**
14	S**	-	S**
15	NS	NS	S*
16	S*	NS	S**

Source: Statistical Reviewer's listing.
* Significance level between 0.01 and 0.05
** Significance level less than 0.01
‡ NS = Not statistically significant; S = Significantly improved post-Imavist; based on McNemar's Test
# - = Not enough cell observations to conduct McNemar's Test.

3.4 Wall Motion Analysis by Normal/Abnormal Patient Status

Wall motion at baseline is compared to wall motion post-Imavist in normal and abnormal patients separately. Of the 26 patients with MRI segmental wall motion data, 12 are classified as normal and 14 are classified as abnormal. A patient was considered normal if all 16 segments were normal. A patient was considered abnormal if any segment was abnormal. All patients had complete MRI SWM data. All 14 abnormal patients had abnormal segments scored as 2 = hypokinesis.

For the echo SWM data, the same definition for normal and abnormal described above was used for the unpaired blinded echo results. Otherwise, if non-evaluable segments were present and none were abnormal, the patient was considered non-evaluable.

The results for normal/abnormal patient analysis are shown in Table 3.3. In both groups, wall motion at baseline was compared to wall motion at post-contrast. Results of mismatches and non-evaluable patients are not presented. This analysis shows that wall motion agreement with MRI on a patient level at baseline and post-contrast trended in the same direction across reader. It appears that post-Imavist images identified more normal patients correctly than abnormal patients compared to baseline. But in abnormal patients the trend was reversed. Baseline ultrasound was more effective in detecting wall motion abnormality than contrast enhanced ultrasound. Thus the benefit of using Imavist could

be to identify those patients with normal wall motion. However, the two trials submitted in this NDA were not designed to test this hypothesis with true normal patients. In addition, mismatches, that is, false positives and false negatives, also limit the efficacy of Imavist in wall motion evaluation.

Table 3.3
Study IMUS-008: Echo Agreement with MRI for Wall Motion on a Patient Level by Normal/Abnormal Classification from MRI Result

MRI Result*	Unpaired Blinded Echo Results at:	Reader 1	Reader 2	Reader 3
Normal (n=12)	Baseline	0 (0%)	0 (0%)	0 (0%)
	Post-Imavist	4 (33%)	1 (8%)	4 (33%)
Abnormal (n=14)	Baseline	10 (71%)	8 (57%)	9 (64%)
	Post-Imavist	9 (64%)	6 (43%)	9 (64%)

Source: Statistical Reviewer's listing.
* MRI assessed by an independent core laboratory.

3.5 Comments on Wall Motion Analysis

The following are the statistical reviewer's comments:

1. Paired analyses of segmental wall motion with respect to a MRI standard give results that are not consistent across blinded readers. Wall motion agreement with MRI at post-Imavist compared to baseline was improved for almost all segments (13 of 16 segments) for one blinded reader while the other two blinded readers' results were marginal (improvement shown in 6 of 16 segments).
2. The subgroup analysis in normal/abnormal patients showed that post-Imavist agreed more often with MRI over baseline ultrasound in normal patients only. In abnormal patients, post-Imavist was inferior to baseline ultrasound in detecting abnormal wall motion for all three blinded readers.

4 Recommendation

There is no further evidence in this submission from segmental or patient level analysis in support of the EBD structural indication. The sponsor's data does not show improved agreement in echo evaluated wall motion with MRI evaluated wall motion. In addition, no wall motion improvement with Imavist in abnormal patients makes the clinical utility of Imavist and its use for endocardial border delineation questionable.

Data from a new study in a patient population similar to that used in the NDA would be required to support the EBD structural indication.

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Sonia Castillo
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Concur with review.

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STATISTICAL REVIEW AND EVALUATION

MAY 5 2000

NDA#: 21-191

SPONSOR: Alliance Pharmaceutical Corp.

DRUG: Imavist (AFO150; Perflexane-Phospholipid Microbubbles for Injection)

DRUG CLASS: 1S

INDICATION:

DOCUMENTS REVIEWED: Volumes 1.1 and 1.131 to 1.199; and documents NC (stamp date 12-23-99), N-000-BS (stamp date 1-14-00), and N-000-BS (stamp date 2-7-00).

DATES: **Date received by Medical Division, HFD-160:** October 14, 1999
Date received by Division of Biometrics, HFD-715: October 20, 1999
User Fee Date: August 4, 2000

MEDICAL REVIEWER: Bernard Parker, M.D., HFD-160

STATISTICAL REVIEWER: Sonia Castillo, Ph.D., HFD-715

Major Review Issues:

- Preselection of images precludes a fully blinded evaluation of ejection fraction.

Key words: clinical studies, NDA review, image evaluation, post-hoc analyses, pooling, per protocol

Study IMUS-007: A Multicenter, Saline-controlled Study of AFO150 in the Echocardiographic Assessment of Left Ventricular Function in Patients with Suboptimal Noncontrast Images
AND
Study IMUS-008: A Multicenter, Open-label Study of AFO150 in the Echocardiographic Assessment of Left Ventricular Function in Patients with Suboptimal Noncontrast Images.

1 INTRODUCTION

This statistical review of the NDA application for Imavist, a perflexane-phospholipid microbubbles for injection product, evaluates the data to provide evidence of efficacy to support approval of the application. The review describes the study conduct, efficacy data collected, the sponsor's efficacy analyses and results, the statistical reviewer's evaluation of the studies and the sponsor's analyses, and the statistical reviewer's exploratory efficacy analyses and conclusions.

The following terms are used interchangeably throughout the review:

- clinical trial, trial, pivotal study, and study
- Imavist, AFO150
- echocardiogram and echo

1.1 Indication

The sponsor has proposed the following indication for their product, AFO150 (Imavist), a suspension of echogenic microbubbles of perfluorohexane vapor and nitrogen gas:

2 DESCRIPTION OF STUDIES

The sponsor submitted two pivotal studies, IMUS-007 and IMUS-008. The primary objectives of studies IMUS-007 and IMUS-008 are to assess the safety and efficacy of AF0150 in improving the evaluation of cardiac function, as measured by endocardial border delineation (EBD) and ejection fraction (EF) based on resting two-dimensional gray-scale continuous echocardiograms. The secondary objective is to evaluate the efficacy of AFO150 in improving the evaluation of segmental wall motion (SWM).

2.1 Study Design

Studies IMUS-007 and IMUS-008 are Phase-3, multi-center safety and efficacy studies of subjects with sub-optimal non-contrast echocardiograms. A sub-optimal echocardiogram is defined as the inability to visualize at least 2, and no more than 9, of the segments in the apical 4- and 2-chamber views of a non-contrast echo. Study IMUS-007 is a saline-controlled study where subjects randomly received either saline or AFO150. A saline control is included for safety purposes only. Study IMUS-008 is an open-label study where all subjects received AFO150. Table 2.1 presents an overview of the design of the two clinical studies.

Table 2.1
Overview of Studies IMUS-007 and IMUS-008

Study No.	No. of Centers	Design*	Treatment Groups	Sample Size Enrolled: ITT# Population	ITT Subgroups: a) Male/Female b) White/Black/Oth
IMUS-007	18	MC,R,DB, PC	Saline AFO150	*81:0 213:206 Total – 294:206*	*a) 129 / 77 b) 174 / 22 / 10*
IMUS-008	11	MC,DB	AFO150	*Total – 232:203*	*a) 138 / 65 b) 166 / 32 / 5*

** MC: Multicenter; R: Randomized; DB: Double-blind; PC: Placebo control(for safety evaluation only)*
 # ITT: intent-to-treat

Subjects must have had a referring clinical diagnosis for which an echocardiographic exam is indicated. Approximately 290 male or female subjects in Study IMUS-007, and approximately 230 male or female subjects in Study IMUS-008 at least 18 years of age with a sub-optimal echocardiogram are enrolled. Female subjects are non-pregnant and non-lactating.

A qualifying echocardiogram was done on the day of screening to determine if the subject had a sub-optimal echo. A confirmatory echocardiogram was done on the day of dosing prior to initiation of study to verify that the subject did have a sub-optimal non-contrast echo. For the qualifying and confirmatory echoes, the investigators determined if a segment was either "visualized" or "not visualized." The determination of these criteria was left to the investigator's judgement. The sponsor advised the investigator that if a "portion" of a segment is not seen, it is to be scored as "not visualized" (Relayed during a telephone conference with the sponsor on March 9, 2000).

All subjects needed to have a gated radionuclide ventriculography (RVG) study done within 48 hours prior to or after the study. In study IMUS-008, gated MRI, was done at 2 selected clinical sites on all subjects enrolled at those sites. The RVG and MRI were used as truth standards for left ventricular ejection fraction and/or wall motion.

In Study IMUS-007, the first 160 enrolled subjects were randomized in a 1:1 ratio to receive a single bolus intravenous (IV) injection of either AF0150 (0.125 mg/kg) or saline. The next 130 subjects received AF0150. In Study IMUS-008, all subjects received a single bolus IV injection of AF0150.

2.1.1 Echocardiographic Imaging

A resting, baseline two-dimensional echocardiogram using continuous and gated imaging was done within 1 hour of dosing. All images were recorded on S-VHS videotape. Three views were collected in the following order: (1) apical 4-chamber, (2) apical 2-chamber and (3) apical long axis. Baseline images were acquired using protocol recommended machine settings, which are held constant for the contrast image acquisition.

After the baseline images were acquired, the subject received either an injection of AFO150 or saline. The contrast images were then immediately collected in the same way as the baseline images. After collecting the required views, imaging continued in continuous mode in the apical 4-chamber view to evaluate the duration of contrast enhancement. The investigator noted on the case report form the time that each view was captured and the duration of useful imaging, defined as the time during which contrast images were obtained.

2.1.2 Tape Preparation

An imaging core laboratory made digital copies of the echocardiogram tapes, which were masked of site and subject information. For the study images, all subjects had 4 separate tapes made for use in the blinded read: baseline continuous, baseline gated, contrast continuous, and contrast gated. Each tape had the three cardiac views imaged (4-chamber, 2-chamber, and long axis views). Each tape was assigned a unique random number to determine the order of viewing.

In addition, an independent blinded echocardiologist reviewed all baseline and contrast apical 4- and 2-chamber views in both continuous and gated modes. For each view, a single cardiac cycle that was free from artifact and arrhythmia, and that allowed the endocardium to be most fully delineated was

chosen and the end-diastolic and end-systolic frames identified. Per protocol, the blinded readers were to use these frames for their evaluation of EF. Instead, the study report stated that a core laboratory image specialist drew the outline of the endocardium on the selected frames. The core laboratory then digitized these selected frames for each of the views. These digitized images, with the endocardial border traced, were presented to each reader for the calculation of EF.

2.1.3 Blinded Reader Training

The blinded readers received training to standardize the wall segments that were assessed and the definition of the scores applied to grade endocardial border delineation (EBD). Each training session used the same series of examples of contrast and non-contrast images. Examples were provided with an explanation of the appropriate scoring of each segment using the scoring definitions described in Section 2.2.1. The readers scored examples of echocardiographic images. The training sessions lasted from 2 to 3 hours and were conducted by [redacted]

2.1.4 Change in Echocardiographic Imaging

There was a change in the echocardiographic image acquisition. During the early conduct of the Phase 3 studies, the sponsor was notified by several investigators enrolling subjects that in some subjects there was marked attenuation followed by rapid disappearance of contrast following administration of AF0150. An investigation by the sponsor concluded that this was due to ultrasound destruction of the AF0150 microbubbles from higher power ultrasound scanning.

Several of the investigational sites were using the new [redacted] ultrasound machine [redacted] whose default settings provided a power increase of 33% - 50% over previous [redacted] machines. The original Phase 3 protocols state that machine power settings are optimized prior to recording baseline echocardiograms and are not changed for contrast echocardiograms. Due to the situation encountered with the [redacted] and to ensure standardization of baseline optimization, the protocols were amended to include guidelines for the machine settings.

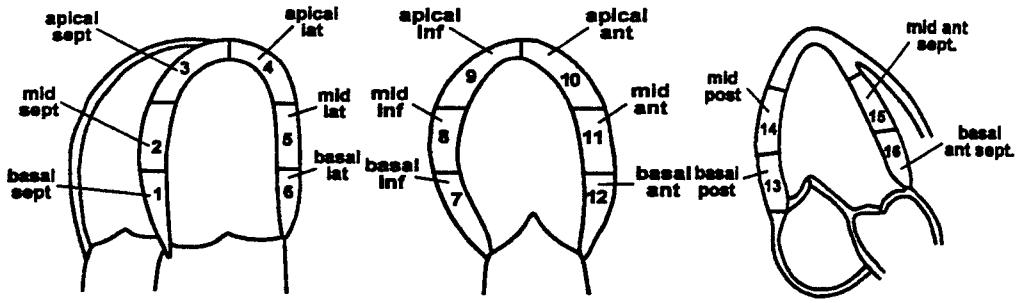
2.2 Efficacy Outcomes

Three independent echocardiologists, blinded to subject and diagnostic information, read the images for all subjects. Baseline and contrast images were randomly displayed. All views for either baseline or contrast were displayed simultaneously. Continuous images were evaluated first followed by the gated images. Endocardial border delineation (EBD) and segmental wall motion (SWM) were evaluated and scored together from the presentation of any given continuous image. SWM was not evaluated for the gated images. Separate image evaluations were done for the evaluation of ejection fraction (EF) during separate blinded read sessions.

2.2.1 Endocardial Border Delineation and Segmental Wall Motion

Each blinded reader independently assessed all images for EBD and SWM for each of 16 segments. These segments are presented in Figure 2.1. The blinded readers used laminated cards printed with the scoring definitions to evaluate and score the images. The scores were given verbally to a core laboratory specialist who entered the scores into the electronic case report form (CRF).

Figure 2.1
Segments in the Three Apical Views (sept = septal, lat = lateral, ant = anterior, post = posterior)



Apical 4-chamber **Apical 2-chamber** **Apical Long Axis**

First, for each segment, EBD was scored for both continuous and gated images as:

0 = no delineation
1 = mild or fair delineation (inadequate to assess function)
2 = moderate or good delineation (adequate to assess function)
3 = excellent delineation (excellent demonstration of borders throughout the cardiac cycle)
N = no view available for segment

Then, for each segment, SWM was scored for the continuous images only as:

0 = Segment not visualized
1 = Normal
2 = Hypokinesis
3 = Akinesis
4 = Dyskinesis
5 = Aneurysmal
N = No view available for segment

In addition, for segments evaluated for EBD as 0 or 1 (no delineation or unable to assess function) for EBD, the electronic CRF was programmed to accept only a score of 0, "not visualized" when scoring motion. For segments evaluated for EBD as 2 or 3 (able to assess function), the electronic CRF was programmed to accept only a score greater than zero. This programming for the electronic CFR was not per protocol.

2.2.2 Ejection Fraction

Each blinded reader randomly evaluated the single baseline and contrast still frames (end-diastolic and end-systolic). Continuous loops of baseline and contrast images of the 4- and 2-chamber views were displayed simultaneously on the screen with the selected still frames. Per protocol, each of the blinded readers were to draw their own region of interest (ROI) on the still frames for use in the calculation of ejection fraction.

Instead, the study report stated that the blinded reader evaluated the accuracy of the drawn ROI on the single still frames. If the blinded reader disagreed with the frames selected, new frames were selected. If the blinded reader selected new frames, or disagreed with the ROI, the ROI was redrawn. The final ROI was used in the calculation of EF for that reader. The core laboratory used computer software to calculate EF based on a 3-D image and using the biplanar method.

Ejection fraction from the RVG images was done at the core laboratory. A blinded technologist drew ROIs from selected end-diastolic and end-systolic frames. An independent, blinded nuclear cardiologist reviewed the selected frames and determined if the drawn ROI was accurate. If the

cardiologist selected new frames or disagreed with the ROI, the ROI was redrawn. Once selection of the ROI was finalized, EF was calculated by software.

2.2.3 MRI Images

Per protocol (Vol. 188, page 166), ejection fraction was to be calculated by the core laboratory from the MRI images. Instead, the study report stated that an independent blinded cardiologist, with expertise in MRI, evaluated "tagged" and "untagged" MRI images for SWM.

2.3 Efficacy Analysis

All statistical tests are two-sided and performed at the 0.05 significance level, except for tests of interaction, which are performed at the 0.10 significance level. Results are calculated for each blinded reader separately. The intent-to-treat population for efficacy evaluation includes all subjects receiving AF0150 who did not have their images acquired with the [redacted] ultrasound machine. There are three other populations of interest but this review focuses only on the intent-to-treat population. The three other populations are: 1)the intent-to-treat population excluding patients that had a change in their cardiac medications that would affect cardiac function; 2) those patients that had a change in their cardiac medications that would affect cardiac function; and 3) all patients who received AF0150 (including all patients enrolled prior to Amendment 2).

2.3.1 Sample Size

The sample size for EBD was calculated using Phase 2 study estimates, the paired t-test, type I error of 0.025, power of 0.80, EBD change score (paired difference) of 0.15, and standard deviation of paired difference of 0.447. The sample size was 85 patients that had evaluable data from baseline and post-contrast echoes and RVG images.

The sample size for EF was calculated using the sample size estimate for the McNemar test, type I error of 0.025, power of 0.80, baseline echo agreement with RVG of 61%, post-contrast echo agreement with RVG of 74%, and joint agreement between baseline and post-contrast echo and RVG of 50%. The sample size was 160 patients that had evaluable data from baseline and post-contrast echoes and RVG images. The sample size for EF was based upon a type I error of 0.05 since it was assumed that the results from EF would produce the larger observed p-value when compared to the p-value from EBD.

For evaluation of efficacy, the sample size of 160 patients was used since it is the larger of the two sample size estimates for EBD and EF. To account for patient drop-out and images that were not evaluable, approximately 40 additional patients were enrolled for a target enrollment of approximately 200.

In addition, 80 patients were enrolled into the study that received saline injection only for purposes of safety. Also, approximately 10 patients were enrolled into the study prior to Amendment 2, received AF0150 and had their images acquired with the [redacted] ultrasound machine, for a total study enrollment of 290.

2.3.2 Primary Endpoints

Primary efficacy analyses are based on the continuous echo images, so this review will focus on continuous images only. The study objective is to demonstrate efficacy of AF0150 with at least one

of the primary endpoints, either EBD or EF or both. Statistically significant study results must be found for at least 2 of the 3 blinded readers to declare efficacy.

2.3.2.1 Endocardial Border Delineation

Each of the 16 segments from the blinded read of the baseline and contrast echocardiograms was assigned one of the following scores:

0 = no delineation	2 = moderate or good delineation	N = no view for segment
1 = mild or fair delineation	3 = excellent delineation	

For each subject's baseline and contrast echo images, the total EBD score was calculated by adding the score from each of the 16 segments. A within subject EBD change score, contrast minus baseline total EBD, was calculated. Missing values were accounted for using 2 approaches, a no-change and a worst-case scenario. In the no-change scenario, if either the baseline or contrast value, but not both, was missing, then the missing value was replaced by the non-missing value. If both were missing, then both were set to 0 (no delineation). In the worst-case scenario, if the value for an individual segment was missing, the missing baseline value was set to 3 (excellent delineation) and the missing contrast value was set to 0 (no delineation).

Post Hoc Analyses

The EBD score was analyzed using analysis of variance (ANOVA) methods with the following general linear model that was not specified in the protocol:

EBD score = investigational site + subject within site + visit + visit-by-site interaction + experimental error, where visit distinguished baseline and contrast results.

The null hypothesis is that the overall mean change score denoted by visit is equal to 0. If the null hypothesis is rejected, efficacy is shown if the overall mean change is positive. A difference among investigational sites is tested with the interaction effect, pooling centers with small sample sizes.

Other Endocardial Border Delineation Analyses

Several exploratory analyses (by cardiac view, segment, gender, race, and age) were performed for the efficacy evaluation of EBD. The analysis of the by-view EBD change score was the same as the primary EBD efficacy analysis. A *post hoc* analysis of the EBD change score presented by individual segments, a WLS method for categorical data was used as follows:

Individual segment EBD change score = overall mean change + investigational site + experimental error.

2.3.2.2 Ejection Fraction

The percent EF values from the blinded read of the echocardiographic images and from gated radionuclide ventriculography (RVG, a standard of truth for measurement of EF) were categorized into one of the following EF classes:

1. >65 %	3. 45-54%	5. 25-34%
2. 55-65%	4. 35-44%	6. <25%

Two 6 x 6 contingency tables were constructed by cross classifying the 6-categories of the EF classes: 1) baseline echo vs. RVG, and 2) contrast echo vs. RVG. From these tables, a third contingency table was constructed comparing the agreement between each echo method and RVG. Each subject was assigned to one of the following 4 categories of this third table:

1. Baseline ECHO & RVG agree, & contrast ECHO & RVG agree.
2. Baseline ECHO & RVG disagree, & contrast ECHO & RVG agree.
3. Baseline ECHO & RVG agree, & contrast ECHO & RVG disagree.

4. Baseline ECHO & RVG disagree, & contrast ECHO & RVG disagree.

There was agreement if echo and RVG EF results were assigned the same EF classes. There was disagreement if echo and RVG EF results were assigned different EF classes.

For the analysis of EF, subjects with a technically inadequate RVG or with missing RVG data were excluded from the analysis, since no standard of truth was available for comparison. Missing values in the echo data were accounted for using 2 approaches, a no-change and a worst-case scenario. In the no-change scenario, if data were missing for either the baseline or contrast echocardiogram, the missing value was set to be the same as the non-missing value. If data were missing from both the baseline and contrast echocardiograms, then both were set to the category of "echocardiogram and RVG agree." In a worst-case scenario, if baseline data was missing, then the data was set to agree with RVG. If contrast data was missing, the data was set to disagree with RVG.

An analysis of the discordant pairs in categories 2 and 3 was tested using McNemar's test. The null hypothesis was that the percent agreement for contrast echo when compared to RVG was not different than the percent agreement for the baseline echo when compared to RVG. The alternative hypothesis was that the percent agreement for contrast echo when compared to RVG was different than the percent agreement for the baseline echo when compared to RVG. If the null hypothesis was rejected, efficacy was shown if the contrast echo showed greater percent agreement with RVG than the baseline echo percent agreement with RVG.

2.3.3 Secondary Outcomes

2.3.3.1 Segmental Wall Motion

The 16 segments were categorized for both baseline and contrast continuous images as follows:

0 = Segment not visualized	3 = Akinesis	N = No view for segment
1 = Normal	4 = Dyskinesis	
2 = Hypokinesis	5 = Aneurysmal	

For each subject, the number of segments not visualized (category 0) was calculated. The analysis was similar to the one done for the total EBD score (ANOVA model), with a null hypothesis of no difference between baseline and contrast with respect to number of segments not visualized.

For the subgroup of subjects who had a MRI, the following analysis was performed using the untagged MRI data. For each subject, the number of segments assigned to the same category on both baseline echo and MRI was calculated. Also, the number of segments assigned to the same category on both contrast echo and MRI was calculated. If a segment from an untagged MRI was assessed as "no view available," then the assessment of the tagged MRI for the same segment was used in the analysis. The per protocol analysis was the same as for EF, with the null hypothesis being no difference between baseline and contrast with respect to number of segments that agreed with MRI (Volume 188, page 174). Instead, the study report stated that the analysis was the same as for total EBD score (ANOVA model). The null hypothesis was that there was no difference between baseline and contrast with respect to number of segments that agreed with MRI.

3 RESULTS OF STUDIES IMUS-007 AND IMUS-008

This section presents information about studies IMUS-007 and IMUS-008, and the sponsor's results and conclusions.

3.1 Study Site and Subject Information

3.1.1 Principal Investigators, Blinded Readers, and Image Selectors

Eighteen principal investigators (P.I.) participated in study IMUS-007 and 11 P.I.s participated in study IMUS-008. Each site had one P.I. and the majority of sites had multiple subinvestigators.

Table 3.1 presents the blinded readers that participated in studies IMUS-007 and IMUS-008. Each study had three blinded readers and all are from Philadelphia, Pennsylvania.

Table 3.1
Blinded Readers for Studies IMUS-007 and IMUS-008

Study	Blinded Reader	Institutional Affiliation	Location
IMUS-007	[redacted] M.D.	Temple School of Medicine	Philadelphia, PA 19140
	[redacted] M.D.	Allegheny General Hospital	Philadelphia, PA 19129
	[redacted] M.D.	Albert Einstein Medical Center	Philadelphia, PA 19141
IMUS-008	[redacted], M.D.	University of Pennsylvania	Philadelphia, PA 19104
	[redacted], M.D.	Albert Einstein Medical Center	Philadelphia, PA 19141
	[redacted] M.D.	Hahnemann University	Philadelphia, PA 19102

Table 3.2 presents the blinded selectors for the echocardiogram images and the blinded RVG and MRI readers for each study. Dr. [redacted] one of the blinded selectors, also trained all the blinded echocardiogram readers and trained the other blinded selector, Dr. [redacted]

Table 3.2
Blinded Selectors, RVG Readers, and MRI Reader for Studies IMUS-007 and IMUS-008

	Blinded Selector	Blinded RVG Reader	Blinded MRI Reader
IMUS-007	[redacted] M.D. Allegheny General Hospital Philadelphia, PA 19104	[redacted] M.D. Albert Einstein Medical Center Philadelphia, PA 19141	
IMUS-008	[redacted] M.D. Thomas Jefferson Univ. Hospital Philadelphia, PA 19107	[redacted] M.D. Temple University Medical Center Philadelphia, PA 19140	[redacted] M.D. University of Pennsylvania Philadelphia, PA 19104

3.1.2 Subject Enrollment and Disposition

Table 3.4 presents a summary of the subject enrollment and disposition for each study. A total of 294 subjects are enrolled at 18 study sites in study IMUS-007 and a total of 232 subjects are enrolled at 11 study sites in study IMUS-008. There are 213 subjects (80 with saline) in study IMUS-007 and 232 subjects in study IMUS-008 who are treated.

Table 3.4
Studies IMUS-007 and IMUS-008: Summary of Subject Enrollment and Disposition

Population	IMUS-007	IMUS-008	Total
Number of Subjects Enrolled	294	232	526
Number of Subjects Treated	213*	232	445
Number (%) of Subjects in ITT Efficacy Population	206 (96.7)	203 (87.5)	409 (91.9)
Number (%) of Subjects Who Completed Study	211 (99.1)	226 (97.4)	437 (98.2)
Number (%) of Subjects Who were Imaged Prior to Amendment 2	7 (3.3)	29 (12.5)	36 (8.1)
Number (%) of Subjects Who Did Not Complete Study	2 (1.0)	6 (2.6)	8 (1.8)
Reasons for Not Completing:			
Religious beliefs	1 (0.5)	0	1 (0.2)
Refusal to undergo gated RVG	1 (0.5)	0	1 (0.2)
Traffic accident / Work schedule	0	1 (0.4)	1 (0.2)
Lost to Follow-up	0	1 (0.4)	1 (0.2)
IV failure, no contrast seen	0	1 (0.4)	1 (0.2)
RVG cancelled or not done	0	3 (1.3)	3 (0.7)

Source: Figures 10A, Figures 10B, and report text; Vol. 1.168, pages 050 - 051 and Vol. 1.185, pages 047 - 048.
Note: All percentages are relative to the number of subjects treated.
* In Study IMUS-007, 81 subjects were randomized to saline for safety purposes only.

3.1.3 Subject Enrollment in the Intent to Treat Population

Table 3.3 presents the number of subjects enrolled in the intent to treat (ITT) population at each study site in studies IMUS-007 and IMUS-008.

Table 3.3
Summary of the Number of Subjects at Each Study Site in the Intent to Treat Population

Study IMUS-007 N=206		Study IMUS-008 N=203	
Site No.	Number of Subjects	Site No.	Number of Subjects
2	9 (4.4)	20	19 (9.4)
3	46 (22.3)	22	16 (7.9)
4	17 (8.2)	23	40 (19.7)
5	24 (11.6)	24	24 (11.8)
6	10 (4.8)	25	14 (6.9)
7	4 (1.9)	26	7 (3.4)
8	7 (3.4)	27	37 (18.2)
9	7 (3.4)	28	8 (3.9)
10	29 (14.1)	29	8 (3.9)
11	3 (1.5)	30	30 (14.8)
12	7 (3.4)		
13	14 (6.8)		
14	6 (2.9)		
16	23 (11.2)		

Source: Statistical Reviewer's listing

The ITT population in each study is 206 subjects in study IMUS-007 and 203 subjects in study IMUS-008. The number of subjects by site ranged from 3 to 46 in study IMUS-007 and from 7 to 40 in study IMUS-008.

3.1.4 Subject Demographics

Table 3.5 presents a summary of the subject demographics for the ITT population. In study IMUS-007, there are more males (62.6%) than females (37.4%), the majority of subjects are white (84.5%), and 68% of the subjects are less than 65 years old. In study IMUS-008, there are more males (68%) than females (32%), the majority of subjects are white (81.8%), and 55% of the subjects are less than 65 years old.

Table 3.5
Studies IMUS-007 and IMUS-008: Subject Demographics for ITT Population

	IMUS-007 (N=206)	IMUS-008 (N=203)	Total (N=409)
Gender – N (%)			
Male	129 (62.6)	138 (68.0)	267 (65.3)
Female	77 (37.4)	65 (32.0)	142 (34.7)
Race – N (%)			
White	174 (84.5)	166 (81.8)	340 (83.1)
Black	22 (10.7)	32 (15.8)	54 (13.2)
Other	10 (4.8)	5 (2.5)	15 (3.7)
Age (years) – N (%)			
< 65	140 (68.0)	112 (55.2)	252 (61.6)
≥ 65	66 (32.0)	91 (44.8)	157 (30.4)

Source: Tables 11A, Vol. 1.168, page 053 and Vol. 1.185, page 050.

3.2 Sponsor's Efficacy Results

All efficacy results are for the ITT population. For all efficacy analyses, the following sites are pooled into the following groups because they had the smallest number of subjects enrolled:

Study IMUS-007 – Group 1: Sites 11, 12, and 14; Group 2: Sites 7, 8, and 9
Study IMUS-008 – Group 1: Sites 26, 28, and 29

Since the "Worst Case Scenario" results were similar to the "No Change Scenario" results, only the "No Change Scenario" results are presented.

3.2.1 Endocardial Border Delineation (EBD)

3.2.1.1 Primary Efficacy Analysis of the Total Endocardial Border Delineation Change Score

The final model for analysis of the change from baseline of the total EBD score is an ANOVA with investigational site, subject within site, visit, and visit-by-site interaction factors. The results of this primary efficacy analysis are presented in Table 3.6. For all blinded readers in both studies, the contrast echo total EBD score was significantly larger than the baseline echo total EBD score.

Table 3.6
Studies IMUS-007 and IMUS-008: Summary of Analysis of Change in Total EBD Score

		IMUS-007 (N=206)			IMUS-008 (N=203)		
Parameter	Statistic	Reader 1	Reader 2	Reader 3	Reader 1	Reader 2	Reader 3
Baseline Echo Total EBD Score	Mean	21.4	26.3	19.7	15.4	14.3	21.2
	SD	6.61	6.21	8.40	8.38	8.00	4.72
	Median	22.0	26.0	20.0	14.0	14.0	21.0
	(Min, Max)			[redacted]			
Contrast Echo Total EBD Score	Mean	31.9	31.2	29.0	26.2	23.2	29.8
	SD	9.29	7.90	7.94	10.81	8.77	4.88
	Median	32.5	31.0	30.0	27.0	25.0	32.0
	(Min, Max)			[redacted]			
Total EBD Change Score	Mean	10.5	4.9	9.3	10.8	8.9	8.6
	SD	8.02	6.03	7.47	8.74	6.87	4.89
	Median	11.0	4.0	10.0	11.0	9.0	9.0
	(Min, Max)			[redacted]			
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Source: Tables IV.6 and IV.7, Vol. 131, pages 060 – 061.
Total EBD Score for each subject is the sum of the EBD scores for the 16 segments.
Total EBD Change Score is the Contrast Echo Total EBD Score minus the Baseline Echo Total EBD Score.
p-value for within subject Total EBD Change Score from ANOVA model with effect for site.

3.2.1.2 Secondary Endocardial Border Delineation Results: Baseline and Qualifying Echocardiograms

Per protocol, all subjects have between 2 to 9 segments not visualized on their qualifying echocardiogram . Table 3.7 presents the number of segments that are poorly visualized on the baseline echocardiogram in the 2-chamber and 4-chamber views, the two views in the qualifying echo. In study IMUS-007, between 8% to 16% of baseline echocardiograms have 10 to 12 segments poorly visualized, and between 4% to 20% of echocardiograms have 0 to 1 segments poorly visualized. In study IMUS-008, between 40% and 50% of baseline echocardiograms have 10 to 12 segments poorly visualized, and between 1% to 3% of echocardiograms have 0 to 1 segments poorly visualized. One possible explanation for the baseline echo having less than 2 or more than 9 segments poorly visualized is that different technicians, machine settings, and image evaluators are used for the qualifying and baseline echoes.

Table 3.7
Studies IMUS-007 and IMUS-008: Number of Subjects Who Had Varying Numbers of Segments That Were Poorly Visualized (EBD score of 0 or 1) at Baseline

	Study IMUS-007 (N=206)			Study IMUS-008 (N=203)		
	n (%)			n (%)		
Number of Segments Poorly Visualized at Baseline	Reader1	Reader 2	Reader 3	Reader 1	Reader 2	Reader 3
0 - 1	8 (3.9)	42 (20.4)	17 (8.2)	3 (1.5)	6 (3.0)	4 (2.0)
2 - 9	176 (85.4)	146 (70.9)	157 (76.2)	98 (48.3)	106 (52.2)	117 (57.6)
10 - 12	22 (10.7)	18 (8.7)	32 (15.5)	102 (50.2)	91 (44.8)	82 (40.4)

Source: Statistical reviewer's listing.

Table 3.8 presents a by-segment summary of the number of segments not visualized on the qualifying non-contrast and baseline echocardiograms. Across all blinded readers in both studies, the qualifying and baseline echo segments that consistently have more numbers not visualized are 4, 5, and 6 in the 4-chamber view; and 10, 11, and 12 in the 2-chamber view. The blinded readers in study IMUS-008 tended to rate more baseline segments not visualized than were not visualized on the qualifying echo for segments 1, 2, and 3 in the 4-chamber view; and segments 7, 8, and 9 in the 2-chamber view. In general, the blinded readers in study IMUS-008 have more numbers of segments not visualized in the baseline echo than the blinded readers in study IMUS-007. Also, for each study, pairs of readers did not consistently agree in the number of segments not visualized, in general, for most segments.

Table 3.8
Studies IMUS-007 and IMUS-008: A By-Segment Summary of the Number of Segments Not Visualized on the Qualifying Non-Contrast Echocardiogram and the Baseline* Echocardiogram

	Study IMUS-007 N=206				Study IMUS-008 N=203			
	Qualifying Echo	Reader 1 n (%)	Reader 2 n (%)	Reader 3 n (%)	Qualifying Echo	Reader 1 n (%)	Reader 2 n (%)	Reader 3 n (%)
4-chamber segments								
1	12 (5.8)	27 (13.1)	18 (8.7)	45 (21.8)	25 (12.3)	135 (66.5)	134 (66.0)	158 (77.8)
2	11 (5.3)	28 (13.6)	16 (7.8)	41 (19.9)	6 (3.0)	98 (48.3)	89 (43.8)	104 (51.2)
3	76 (36.9)	151 (73.3)	46 (22.3)	108 (52.4)	48 (23.6)	151 (74.4)	111 (54.7)	125 (61.6)
4	130 (63.1)	157 (76.2)	107 (51.9)	154 (74.8)	127 (62.6)	170 (83.7)	153 (75.4)	152 (74.9)
5	138 (67.0)	146 (70.9)	109 (52.9)	145 (70.4)	177 (87.2)	178 (87.7)	175 (86.2)	178 (87.7)
6	139 (67.5)	149 (72.3)	132 (64.1)	153 (74.3)	163 (80.3)	193 (95.1)	184 (90.6)	178 (87.7)
2-chamber segments								
7	17 (8.3)	30 (14.6)	17 (8.2)	49 (23.8)	35 (17.2)	114 (56.2)	177 (87.2)	86 (42.4)
8	12 (5.8)	25 (12.1)	20 (9.7)	29 (14.1)	14 (6.9)	81 (39.9)	91 (44.8)	59 (29.1)
9	68 (33.0)	172 (83.5)	80 (38.8)	122 (59.2)	66 (32.5)	150 (73.9)	117 (57.6)	139 (68.5)
10	142 (68.9)	165 (80.1)	129 (62.6)	158 (76.7)	129 (63.5)	172 (84.7)	161 (79.3)	158 (77.8)
11	143 (69.4)	150 (72.8)	116 (56.3)	149 (72.3)	153 (75.4)	162 (79.8)	166 (81.8)	154 (75.9)
12	142 (68.9)	160 (77.7)	130 (63.1)	161 (78.2)	167 (82.3)	182 (89.7)	180 (88.7)	172 (84.7)

Source: Table IV.5, Vol. 131, page 059 and statistical reviewer's listing.
* Baseline echo segment was considered not visualized if the EBD score is 0 or 1.

3.2.1.3 Secondary Endocardial Border Delineation Results: Segmental Analysis

The mean scores of endocardial border delineation (EBD) at baseline, after administration of AFO150, and of the change from baseline are presented by blinded reader and by segment in Tables 3.9 and 3.10 for both studies.

For each segment, the mean EBD scores following AFO150 were greater compared to baseline for all blinded readers in both studies ($p<0.001$). AFO150 provided improvement from baseline in EBD scores across all 12 segments, and thus by cardiac view. In addition, for both cardiac phases and both readers in both studies, segments 4, 5, and 6 in the 2-chamber view; segments 10, 11, and 12 in the 4-chamber view; and segments 13 and 14 in the long-axis view had mean changes that were larger than the other segments in that view.

Table 3.9
Study IMUS-007: Endocardial Border Delineation -Individual Segment
Mean Change from Baseline Score# (N=206)

	Reader 1			Reader 2			Reader 3		
	Baseline	AF0150	Difference	Baseline	AF0150	Difference	Baseline	AF0150	Difference
4-chamber segments									
1	1.99	2.50	*0.52*	2.10	2.16	*0.06**	1.84	2.30	*0.46*
2	1.99	2.52	*0.53*	2.14	2.29	*0.15*	1.94	2.44	*0.50*
3	1.06	1.69	*0.63*	1.81	1.89	*0.09**	1.39	1.61	*0.22*
4	0.97	1.68	*0.71*	1.45	1.83	*0.38*	0.86	1.31	*0.45*
5	1.19	2.13	*0.94*	1.44	2.14	*0.70*	0.92	2.03	*1.11*
6	1.19	2.12	*0.93*	1.34	2.00	*0.66*	0.77	2.01	*1.24*
2-chamber segments									
7	1.89	2.30	*0.40*	2.02	2.10	*0.08**	1.72	2.07	*0.35*
8	1.96	2.36	*0.41*	2.03	2.17	*0.14*	1.92	2.21	*0.29*
9	0.87	1.47	*0.60*	1.61	1.71	*0.10**	1.24	1.26	*0.01**
10	0.79	1.44	*0.65*	1.31	1.65	*0.33*	0.74	1.00	*0.26*
11	1.00	1.92	*0.91*	1.39	1.96	*0.57*	0.84	1.76	*0.92*
12	1.00	1.88	*0.88*	1.31	1.90	*0.59*	0.70	1.72	*1.02*
Long axis segments									
13	1.23	1.93	*0.69*	1.42	1.84	*0.42*	1.06	1.88	*0.82*
14	1.19	1.95	*0.76*	1.45	1.87	*0.42*	1.01	1.85	*0.83*
15	1.50	1.97	*0.47*	1.68	1.83	*0.15*	1.28	1.77	*0.49*
16	1.57	2.01	*0.44*	1.76	1.83	*0.07**	1.43	1.80	*0.37*

Source: Derived from sponsor Table 2.4a, pages 024 – 039, Vol. 132; and modifications by statistical reviewer (The statistical reviewer used 2 decimal places in the presentation of the table instead of the 1 decimal place used in the sponsor table table and adjusted p-values for multiple comparisons using the Hochberg Step-up procedure. See Section XX.XX.).

* p-value not significant at the 0.05 level.

Calculated based on a scale of: 0 = no delineation; 1 = mild or fair delineation; 2 = moderate or good delineation; 3 = excellent delineation; N = no view available.

Table 3.10
Study IMUS-008: Endocardial Border Delineation -Individual Segment
Mean Change from Baseline Score# (N=203)

	Reader 1			Reader 2			Reader 3		
	Baseline	AF0150	Difference	Baseline	AF0150	Difference	Baseline	AF0150	Difference
4-chamber segments									
1	1.20	1.94	*0.74*	1.14	1.67	*0.52*	1.21	1.88	*0.67*
2	1.50	2.13	*0.64*	1.44	1.84	*0.40*	1.49	1.93	*0.44*
3	1.03	1.55	*0.52*	1.30	1.62	*0.32*	1.37	1.84	*0.46*
4	0.77	1.45	*0.67*	0.89	1.45	*0.56*	1.23	1.82	*0.60*
5	0.74	1.79	*1.05*	0.63	1.51	*0.89*	1.10	1.88	*0.78*
6	0.36	1.65	*1.28*	0.45	1.45	*1.00*	1.10	1.88	*0.78*
2-chamber segments									
7	1.37	1.86	*0.48*	0.83	1.54	*0.71*	1.56	1.92	*0.36*
8	1.69	2.02	*0.33*	1.38	1.67	*0.29*	1.70	1.95	*0.24*
9	0.96	1.37	*0.41*	1.14	1.33	*0.19*	1.30	1.80	*0.50*
10	0.71	1.28	*0.56*	0.76	1.20	*0.44*	1.20	1.78	*0.58*
11	0.83	1.61	*0.77*	0.65	1.26	*0.61*	1.23	1.84	*0.62*
12	0.51	1.47	*0.96*	0.47	1.23	*0.76*	1.13	1.83	*0.69*
Long axis segments									
13	0.69	1.54	*0.85*	0.51	1.32	*0.80*	1.33	1.89	*0.56*
14	0.89	1.67	*0.78*	0.67	1.39	*0.72*	1.35	1.89	*0.53*
15	1.17	1.54	*0.37*	0.98	1.33	*0.35*	1.43	1.83	*0.39*
16	0.97	1.35	*0.38*	1.10	1.41	*0.32*	1.47	1.83	*0.35*

Source: Derived from sponsor Table 2.4b, pages 040 – 055, Vol. 132; and modifications by statistical reviewer (The statistical reviewer used 2 decimal places in the presentation of the table instead of the 1 decimal place used in the sponsor table and adjusted p-values for multiple comparisons using the Hochberg Step-up procedure. See Section XX.XX.).

* p-value not significant at the 0.05 level.

Calculated based on a scale of: 0 = no delineation; 1 = mild or fair delineation; 2 = moderate or good delineation; 3 = excellent delineation; N = no view available.

3.2.1.4 Secondary Endocardial Border Delineation Results: Sub-optimal to Optimal Images

The results of the analysis for a baseline sub-optimal image becoming optimal with AFO150 for endocardial border delineation for all three readers for all segments are presented in Tables 3.11 and 3.12. The difference between the proportion of all images rated as optimal by AFO150 and the proportion of all images rated as optimal by both baseline and AFO150 is the proportion of all images that were rated as sub-optimal at baseline that then became optimal after AFO150. That is, the difference is the benefit provided by AFO150 because AFO150 changed a sub-optimal baseline image to an optimal image. This can be said because AFO150 imaging always followed baseline imaging.

Table 3.11
Study IMUS-007: The Proportion of All Images (N=206) where AFO150 Provided Added Benefit Compared to Baseline

	Reader 1			Reader 2			Reader 3		
	Proportion of Images Rated as Optimal by:			Proportion of Images Rated as Optimal by:			Proportion of Images Rated as Optimal by:		
	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)
4-chamber segments									
1	0.96	0.87	*0.09*	0.90	0.86	*0.04**	0.94	0.77	*0.17*
2	0.97	0.86	*0.11*	0.95	0.91	*0.04**	0.97	0.80	*0.17*
3	0.69	0.23	*0.46*	0.83	0.72	*0.11**	0.61	0.38	*0.23*
4	0.69	0.22	*0.47*	0.76	0.42	*0.34*	0.42	0.18	*0.24*
5	0.81	0.28	*0.53*	0.85	0.46	*0.39*	0.90	0.29	*0.61*
6	0.79	0.27	*0.52*	0.77	0.35	*0.42*	0.86	0.25	*0.61*
2-chamber segments									
7	0.91	0.81	*0.10**	0.89	0.86	*0.03**	0.94	0.75	*0.19*
8	0.94	0.86	*0.08*	0.93	0.88	*0.05**	0.95	0.84	*0.11*
9	0.55	0.14	*0.41*	0.70	0.53	*0.17**	0.39	0.23	*0.16**
10	0.53	0.15	*0.38*	0.63	0.33	*0.30*	0.29	0.15	*0.14**
11	0.73	0.25	*0.48*	0.78	0.43	*0.35*	0.80	0.26	*0.54*
12	0.71	0.21	*0.50*	0.74	0.35	*0.39*	0.76	0.21	*0.55*
Long axis segments									
13	0.73	0.31	*0.42*	0.70	0.39	*0.31*	0.83	0.37	*0.46*
14	0.73	0.29	*0.44*	0.73	0.42	*0.31*	0.81	0.32	*0.49*
15	0.77	0.55	*0.22*	0.76	0.63	*0.13**	0.79	0.47	*0.32*
16	0.77	0.60	*0.17*	0.76	0.69	*0.07**	0.80	0.59	*0.21*

Source: Derived from sponsor Table 2.5a, pages 056 – 071, Vol. 132.
* p-value not significant at the 0.05 level.

For all three readers, the proportion of images that were optimal at baseline and then became sub-optimal after AFO150 was less than 0.10 for 4-chamber view, less than 0.09 for the 2-chamber view (except for Reader 3, segment 9 which was 0.18), and less than 0.08 for the long axis view in Study IMUS-007. In Study IMUS-008, the proportion was less than 0.07 for 4-chamber view, less than 0.10 for the 2-chamber view (except for Reader 2, segment 9 which was 0.12), and less than 0.08 for the long axis view.

AFO150 provided more optimal images than at baseline on a segment-by-segment basis in both studies ($p \leq 0.001$ for most segments). The extent of the added benefit of AFO150 varied. In each study, for each blinded reader, the segments that benefited the most with AFO150 compared to baseline were segments 4, 5, 6 in the 2-chamber view, segments 10, 11, 12 in the 4-chamber view, and segments 13, 14 in the long axis view.

Table 3.12
Study IMUS-008: The Proportion of All Images (N=203) where AFO150 Provided Added Benefit Compared to Baseline

	Reader 1			Reader 2			Reader 3		
	Proportion of Images Rated as Optimal by:			Proportion of Images Rated as Optimal by:			Proportion of Images Rated as Optimal by:		
	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)	AF0150 Only	Baseline and AF0150	Difference (AF0150 Benefit)
4-chamber segments									
1	0.70	0.25	*0.41*	0.67	0.31	*0.36*	0.88	0.22	*0.66*
2	0.77	0.46	*0.31*	0.80	0.53	*0.27*	0.93	0.48	*0.45*
3	0.54	0.21	*0.33*	0.65	0.39	*0.26*	0.84	0.37	*0.47*
4	0.51	0.13	*0.38*	0.53	0.22	*0.31*	0.83	0.24	*0.59*
5	0.65	0.11	*0.54*	0.59	0.13	*0.46*	0.87	0.12	*0.75*
6	0.58	0.05	*0.53*	0.57	0.09	*0.48*	0.86	0.12	*0.74*
2-chamber segments									
7	0.70	0.34	*0.36*	0.60	0.11	*0.49*	0.92	0.57	*0.35*
8	0.76	0.51	*0.25*	0.70	0.48	*0.22*	0.94	0.70	*0.24*
9	0.45	0.19	*0.26*	0.44	0.30	*0.14**	0.81	0.30	*0.51*
10	0.38	0.10	*0.28*	0.39	0.16	*0.23*	0.79	0.22	*0.57*
11	0.57	0.16	*0.41*	0.47	0.14	*0.33*	0.85	0.24	*0.61*
12	0.51	0.10	*0.41*	0.46	0.07	*0.39*	0.83	0.15	*0.68*
Long axis segments									
13	0.52	0.10	*0.42*	0.47	0.07	*0.40*	0.89	0.34	*0.55*
14	0.61	0.18	*0.43*	0.54	0.12	*0.42*	0.89	0.36	*0.53*
15	0.53	0.28	*0.25*	0.52	0.26	*0.26*	0.83	0.43	*0.40*
16	0.40	0.16	*0.24*	0.59	0.30	*0.29*	0.83	0.47	*0.36*

Source: Derived from sponsor Table 2.5b, pages 072 – 087, Vol. 132.
* p-value not significant at the 0.05 level.

3.2.2 Ejection Fraction

Tables 3.13 and 3.14 present the results of the analysis comparing the echocardiogram ejection fraction with the ejection fraction from the standard of truth, RVG. For both studies, the results are not significant for concluding that the contrast echo EF is better than the baseline echo EF when both are compared to the RVG EF.

Recall that the EF categories for both the echo and RVG EF values are as follow:

1. >65 % 3. 45-54% 5. 25-34%
2. 55-65% 4. 35-44% 6. <25%

An echo EF agrees with the RVG EF if they both have the same EF category, otherwise they disagree. In both studies, both the baseline and contrast echo EFs disagree with the RVG EF about 42% to 47% of the time. Also, about 10% to 15% of the time, both baseline and contrast echo EFs agree with the RVG EF. For all blinded readers in both studies, the baseline echo EF agrees with the RVG EF while the contrast echo EF disagrees with the RVG EF about 20% to 25% of the time.

Table 3.13
Study IMUS-007: Ejection Fraction Agreement by Ejection Fraction Categories

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

Source: Table IV.17, Vol. 131, page 072.
p-value from McNemar's test.

The sponsor also presents an analysis of EF which is similar to the per protocol analysis except that instead of using agreement based on the EF categories, agreement was based on if the echo EF estimate was within +/- 5% of the RVG EF estimate or not. That is, an echo EF value that was within +/- 5% of the RVG EF was considered to agree. As with the per protocol analysis, no significant difference is noted.

Table 3.14
Study IMUS-008: Ejection Fraction Agreement by Ejection Fraction Categories

		Reader 1		Reader 2		Reader 3	
		Contrast Echo and RVG		Contrast Echo and RVG		Contrast Echo and RVG	
		Agree n (%)	Disagree n (%)	Agree n (%)	Disagree n (%)	Agree n (%)	Disagree n (%)
Baseline Echo and RVG	Agree	21 (11.2)	42 (22.3)	22 (11.7)	40 (21.3)	22 (11.7)	38 (20.2)
	Disagree	45 (23.9)	80 (42.6)	48 (25.5)	78 (41.5)	46 (24.5)	82 (43.6)
p-value		0.748		0.394		0.075	

Source: Table IV.18, Vol. 131, page 073.
p-value from McNemar's test.

Table 3.15 presents the simple correlation (r) between the echo EF and RVG EF for the baseline and contrast echoes. The correlation is poor, about 0.50 for all blinded readers in both studies. This result is consistent with the above agreement rates in Tables 3.13 and 3.14.

Table 3.15
Studies IMUS-007 and IMUS-008: Correlation (r) between EF by Echocardiogram and RVG

Study	Reader	Baseline r	Contrast r
IMUS-007	Reader 1	0.53	0.50
	Reader 2	0.47	0.49
	Reader 3	0.56	0.51
IMUS-008	Reader 1	0.55	0.53
	Reader 2	0.50	0.49
	Reader 3	0.51	0.53

Source: Table IV.19, Vol. 131, page 074

3.2.3 Secondary Efficacy Results: Segmental Wall Motion

Table 3.16 presents the results of the analysis of segmental wall motion (SWM) agreement of echocardiogram with MRI. Only study IMUS-008 collected MRI data. For all blinded readers, the overall mean number of segments that agree with MRI for wall motion is greater ($p<0.001$) for the contrast echo than for the baseline echo.

Table 3.16
Study IMUS-008: Summary of Analysis of Segmental Wall Motion Agreement of Echocardiogram with MRI

		IMUS-008 (N=26)		
	Statistic	Reader 1	Reader 2	Reader 3
Baseline Echo	Mean	12.5	14.2	17.8
	SD	11.32	15.57	15.78
Contrast Echo	Mean	41.1	38.9	61.8
	SD	33.83	30.43	30.89
Change from Baseline Echo to Contrast Echo	Mean	28.6	24.8	44.0
	SD	31.28	28.42	28.75
	p-value	<0.001	<0.001	<0.001

Source: Tables IV.27, Vol. 131, page 083.
The Change from Baseline Echo to Contrast Echo is defined as the Contrast Echo number of segments that agree with MRI minus the Baseline Echo number of segments that agree with MRI.
p-value for within subject number of segments that agree with MRI from ANOVA model with effect for site.

3.2.4 Information about Duration of Useful Enhancement

Table 3.17 presents the duration of useful contrast enhancement, defined as the time during which contrast enhanced images are obtained, as assessed by the principal investigators. In both studies, the mean duration of useful contrast enhancement is about 2.5 minutes.

Table 3.17
Studies IMUS-007 and IMUS-008: Duration of Useful Contrast Enhancement as Assessed by the Principal Investigator

Duration of useful contrast enhancement in minutes (seconds)	Study IMUS-007 (N=197)	Study IMUS-008 (N=200)
Mean	2.68 (160.8)	2.58 (154.8)
St. Dev.	1.69 (101.4)	1.45 (87.0)
Minimum	[redacted]	
Median	2.28 (136.8)	2.31 (138.6)
Maximum	[redacted]	

Source: Tables 10.1a and 10.1b, Volume 132, pages 301 and 302.

4 REVIEWER'S COMENTS ON STUDY DESIGN AND SPONSOR ANALYSES

1. The assumption of independent trials may not hold for studies IMUS-007 and IMUS-008. For both studies, the blinded selectors and all the blinded readers are from the Philadelphia area and some belong to the same institution. Table 4.1 presents all the blinded selectors and blinded readers grouped by institution. In addition to having multiple readers and/or selectors at the same

institution, one of the Blinded Selectors (Dr. [redacted] conducted the training sessions for all the blinded echo readers and trained the blinded selector for study IMUS-007.

Table 4.1
Studies IMUS-007 and IMUS-008: Listing of Blinded Reader and Blinded Selector Institution Affiliation

Institution City, State, Zip Code	Study	Reader/Selector Name	Study Role
Albert Einstein Medical Center Philadelphia, PA 19141	IMUS-007	M.D.	Blinded Echo Reader
	IMUS-008	M.D.	Blinded Echo Reader
	IMUS-007	M.D.	Blinded RVG Reader
Allegheny General Hospital Philadelphia, PA 19104 / 19129	IMUS-007	M.D.	Blinded Echo Reader
	IMUS-007	, M.D.	Blinded Echo Selector
Hahnemann University Philadelphia, PA 19102	IMUS-008	, M.D.	Blinded Echo Reader
Temple School of Medicine Philadelphia, PA 19140	IMUS-007	M.D.	Blinded Echo Reader
	IMUS-008	, M.D.	Blinded RVG Reader
Thomas Jefferson Univ. Hospital Philadelphia, PA 19107	IMUS-008	, M.D.	Blinded Selector, Trainer for All Blinded Echo Readers, Trainer for Blinded Selector in IMUS-007
University of Pennsylvania Philadelphia, PA 19104	IMUS-007	M.D.	Blinded Echo Reader
	IMUS-008	M.D.	Blinded MRI Reader

2. The pooling of sites with small numbers of subjects is not specified in the protocol and is done in a *post hoc* manner for all efficacy analyses. Data from the sites with the smallest number of enrolled subjects are pooled into one group. There are two pooled groups in IMUS-007 (Sites 1,11,12,14 and Sites 7, 8, 9) and one pooled group in IMUS-008 (Sites 21, 26, 28, and 29). No rationale for pooling the sites into the two groups that were formed in study IMUS-007 is presented in the study report. The study report does not give an explanation why these sites did not enroll a sufficient number of subjects. Differences across these sites are not evaluated or presented to justify the pooling of the sites into one site. The protocol should specify a minimum number of subjects to be enrolled per site and describe a procedure to assure adequate enrollment at each site.

3. The ANOVA model used for the primary efficacy analysis of EBD is not the model specified in the protocol. No documentation describing the change in model is provided in the application. The *post hoc* model uses the total EBD score instead of the protocol specified change in EBD score and includes a nested model with an interaction term. The per protocol (in the statistical analysis plan submitted in the pre-NDA Meeting Briefing Document on June 28, 1999 under IND [redacted] Serial No. 070) ANOVA model for the EBD change score analysis is:

EBD change score = (overall mean change) + investigational site + experimental error

The model used for analysis and presented in the study report, based on the total EBD score, is:

EBD score = investigational site + subject within site + visit + visit-by-site interaction + experimental error
where visit distinguished baseline and contrast results

The use of an ANOVA model is not appropriate for analysis of the segmental EBD score data. This comment was sent to the sponsor in the statistical comments for Serial Number 031, 12-18-97 submission. It was also recommended that the analysis be done on a by-segment basis and on a by-

view basis for each subject using the EBD segment scores. The by-segment analysis is preferred because there needs to be a 1-to-1 correspondence of the segments to assess the change per segment, not the sum of 16 segments for all three cardiac views as used in the analysis of the total EBD score.

4. The blinded readers should have selected their own frames for the evaluation of EF and should have outlined the endocardial border. Because the blinded readers evaluate EBD with and without contrast, the EBD evaluation has a direct effect on which frames the blinded reader thinks are best for outlining the endocardial border. Having the blinded readers select their own frames provides further information about the effect of contrast on the evaluation of EF.

One result of providing pre-selected and pre-inscribed frames is that a majority of the time, a single subject's EF value was the same for all three blinded readers. Also, having two intermediaries, the blinded selector who chose the best images for evaluation of EF and the blinded technician who outlined the endocardial border, made the evaluation by the blinded readers unnecessary. This process of determining EF does not provide information on how the blinded readers' assessment of EBD with and without contrast affects their ability to determine the ROIs used to calculate EF.

The sponsor stated during a T-con on March 9, 2000 that the blinded readers did not draw the ROIs for all images because the software used is complicated. This comment does not make sense because the blinded readers did redraw the ROIs for those technician drawn ROIs they disagreed with during the blinded read. Also, per protocol (Volume 171, pages 162), the blinded readers were to outline the endocardial border but this was changed without justification by the sponsor.

5. The MRI data for segmental wall motion (SWM) are not adequate for use in the analyses done by the sponsor because of the following 6 reasons:

1. MRI images were of two types, "tagged" and "untagged". According to the sponsor, tagged MRI images are used to evaluate wall thickness, an anatomical attribute, and untagged MRI images are used to evaluate segmental wall motion. The study report stated that the untagged MRI data are used for the analysis of SWM and if the untagged image data were not available, the tagged image data are used (Volume 185, pages 031 and 040).
2. The MRI data set consists of the untagged and tagged MRI data for 26 subjects from one study site (site no. 23). For the tagged data set, 3 subjects have no data for all MRI evaluations. For the untagged data set, 15 subjects have no data for all MRI evaluations and 2 subjects have no data for one cardiac view. Since untagged data are missing for 15 of the 26 subjects (57.7%) and untagged data is to be used for the analyses, the untagged data in this study is not adequate for analysis. The small number of untagged MRI data from only one site is not sufficient to show any treatment effect.
3. During a t-con with the sponsor on March 9, 2000. the sponsor stated that the investigative site was asked to collect untagged MRI images. Tagged MRI images could be collected but not until the untagged ones were collected. The sponsor could not give a reason for the missing untagged MRI image data.
4. MRI data was not collected from 2 trials to provide replication of the results.
5. Not all subjects enrolled at the two study sites that collected MRI data had an MRI performed, as per protocol. No reason is presented in the study report for the lack of data.
6. The study report states that 2 subjects had missing data for untagged segments and the data for tagged segments was used (Volume 185, page 060). This does not match what is seen in the MRI data set.

Given the above, the data is not adequate and the results of segmental wall motion analysis are questionable.

6. For the blinded reader's assessment of segmental wall motion (SWM), the study report stated in Vol. 131 on pages 079 to 080 that:

> *For segments evaluated for EBD as 0 or 1 (no delineation or unable to assess function) for EBD, the electronic CRF was programmed to accept only a score of 0, "not visualized" when scoring motion. For segments evaluated for EBD as 2 or 3 (able to assess function), the electronic CRF was programmed to accept only a score greater than zero.*

This procedure was not described in the protocol. Since the blinded readers evaluate EBD to determine the effect of contrast on the echo images and since what they observe for EBD affects the visualization of wall motion, the electronic CRF should not have been pre-programmed as described above. The blinded reader should assess each segment for SWM without pre-defining what the response should be. If an analysis is wanted where EBD scores of 0 or 1 are defined to have a SWM score of 0, then this should be done at the data analysis stage.

7. Per protocol, a claim for segmental wall motion is not sought. The sponsor stated in a document submitted April 27, 1999 under IND [redacted] serial no. 064, on page 01-013 that they do not intend to include SWM in the labeling. In addition, in the briefing document (serial no. 070, June 28, 1999, under IND [redacted] page 01-005) submitted for the pre-NDA meeting, the sponsor stated that the proposed indication for AFO150 is as follows:

[redacted]

In the application, the proposed indication has changed to the following:

[redacted]

5 REVIEWER'S ANALYSES AND CONCLUSIONS

5.1 Endocardial Border Delineation

The statistical reviewer analyzed the individual segmental EBD score data. The analysis consisted of taking the difference between contrast and baseline EBD score for each of the 16 segments individually for each subject. The overall mean change from baseline was calculated for each segment and tested using a two-sided t-test. Since there were 16 segments, p-values were adjusted for multiple comparison using the Hochberg Step-up procedure. The results are the same as presented in Tables 3.9 and 3.10.

The statistical reviewer concurs with the sponsor's results for the individual segment EBD score analysis based on the suboptimal and optimal segment images. These results and the results for the individual segmental EBD score analysis based on the overall mean change from baseline provide supporting evidence that AFO150 improves the delineation of the endocardial border compared to using no contrast agent. Per the Division, end-systole and end-diastole results for individual segment EBD score data are needed to demonstrate efficacy for AFO150.

5.2 Ejection Fraction

The statistical reviewer conducted an exploratory analysis to investigate how well the baseline and contrast echocardiograms EFs compare to the RVG EF. The baseline and contrast echo EFs are classified as being within ± 5% of the RVG EF or not. Table 5.1 presents a listing of the number of echo EF estimates that are within ± 5% of the RVG EF or not for the baseline and contrast echoes from the no change scenario.

Table 5.1
Studies IMUS-007 and IMUS-008: Ejection Fraction from Baseline and Contrast Echocardiograms Compared to RVG Ejection Fraction (No Change Scenario)

	Baseline			Contrast		
	Reader 1 n (%)	Reader 2 n (%)	Reader 3 n (%)	Reader 1 n (%)	Reader 2 n (%)	Reader 3 n (%)
IMUS-007 (N=191)						
Difference < -5%	56 (29.3)	58 (30.4)	56 (29.3)	63 (33.0)	54 (28.3)	63 (33.0)
Difference between -5% and 5%	*71 (37.2)*	*74 (38.7)*	*70 (36.7)*	*64 (33.5)*	*59 (30.9)*	*65 (34.0)*
Difference > 5%	64 (33.5)	59 (30.9)	65 (34.0)	64 (33.5)	78 (40.8)	63 (33.0)
IMUS-008 (N=188)						
Difference < -5%	86 (45.8)	77 (41.0)	79 (42.0)	51 (27.1)	45 (23.9)	49 (26.1)
Difference between -5% and 5%	*67 (35.6)*	*70 (37.2)*	*67 (35.6)*	*63 (33.5)*	*64 (34.1)*	*61 (32.4)*
Difference > 5%	35 (18.6)	41 (21.8)	42 (22.4)	74 (39.4)	79 (42.0)	78 (41.5)

Source: Statistical reviewer's exploratory analysis.

In both studies for both the baseline and contrast echo EF estimates, all readers the echo EF is within ± 5% of the RVG EF about one-third of the time and is not within 5% of the RVG EF about two-thirds of the time. In study IMUS-007, all readers tend to underestimate and overestimate EF about the same when using both baseline and contrast echoes. In study IMUS-008, all readers tend to underestimate EF more often than overestimate EF when using the baseline echo; and tend to overestimate EF more often than underestimate EF when using the contrast echo.

Either overestimating or underestimating the ejection fraction within 5% of the RVG EF 66% of the time is not evidence that the echocardiogram estimate of EF is consistent with the RVG EF. Also, the improvement in visualizing the endocardial border (from the significant EBD analysis) does not contribute to agreement between echo EF and RVG EF. There is no clear reason for the discrepancy. This may be due to the procedure for evaluating EF from the echocardiogram. The blinded readers did not select the frames to use for inscribing the endocardial border for the calculation of ejection fraction and did not inscribe the endocardial border.

Given the sponsor and statistical reviewer's results for the analyses of ejection fraction, using AFO150 to improve measurement of ejection fraction has not been demonstrated.

5.3 Segmental Wall Motion

The inadequacies in the MRI data and echocardiogram data for segmental wall motion (SWM) are described in Section 4; the results of the SWM analyses do not have a clear interpretation. Thus, using AFO150 to assess segmental wall motion has not been demonstrated.

6 RECOMMENDATION

From a statistical standpoint, the sponsor has provided studies that are well controlled but not adequate to show evidence for efficacy in support of their claims for endocardial border delineation, ejection fraction, and segmental wall motion. The following is a list of the inadequacies:

1. End-systolic and end-diastolic endocardial border delineation data were not collected to fully assess the effect of AFO150 in improving the evaluation of endocardial border delineation compared to baseline.
2. Pre-contrast and post-contrast values of ejection fraction are not statistically different; thus AFO150 does not appear to improve the measurement of ejection fraction.
3. The data provided to determine if AFO150 can assess segmental wall motion is not adequate.

The following are recommended to address the inadequacies for the claims endocardial border delineation, ejection fraction, and segmental wall motion claims sought:

1. A reread of the echocardiograms is needed to collect end-systolic and end-diastolic data. Analyses by segment are needed for the EBD score and the sub-optimal/optimal data.
2. A reread of the echocardiograms is recommended with the blinded readers selecting their own frames to assess ejection fraction and drawing the region of interest for use in the calculation of ejection fraction.
3. MRI data should be collected for all patients from two independent trials to fully assess the segmental wall motion claim. In addition, the blinded readers should evaluate segmental wall motion for all segments using a scoring procedure acceptable to the agency.

4-18-00

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Concur:

4-18-00

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cc:
Archival NDA 21-191
HFD-160/P. Love/S. Loewke/B. Parker/T. Harper-Velazquez
HFD-715/File Copy/[redacted]/M.Welch/M. Sobhan/S. Castillo

S. Castillo/Microsoft Word/ 4/17/00
This review contains 26 pages of text and tables.