TABLE 27 : 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.

The next table illustrates the specific segments reviewed by the blinded readers, to define those views deemed poorly visualized in the baseline echocardiograms when comparing with the screening (qualifying and confirmatory) echocardiograms. The FDA statistical reviewer revealed notable differences between the readers of IMUS-007 and IMUS-008 with regards to the readings of the screening and baseline echocardiograms; the readers in IMUS-008 had

- A higher number of segments considered "poorly visualized" at baseline, and ...
- A higher number of "poorly visualized" readings of the screening echocardiograms in those segments that were considered "well-visualized" readings by the core laboratory.

The blinded readers also felt that significantly more segments were considered poorly visualized among those segments where the screening echos were seldom viewed as poorly visualized. This may be attributed to the fact that the screening echos used different technicians and different machine settings from the baseline echos. On the other hand, this may also be interpreted as a potential for bias by having more segments that will be improved after AF0150 contrast is applied.

TABLE 28 : PHASE 3 STUDIES –
Summary of Segments Not Visualized on the Screeing Echocardiogram, Compared with Baseline (non-contrast) Echocardiogram

Apical Views	Study IMUS-007 (N = 206)				Study IMUS-008 (N = 203)			
	Screen*	Reader #			Screen*	Reader #		
(Segs.)	Echo	1	2	3	Echo	1	2	3
4-chamber								
1	12 (6%)	27 (13%)	18 (9%)	45 (22%)	25 (12%)	135 (67%)	134 (66%)	158 (78%)
2	11 (5%)	28 (14%)	16 (8%)	41 (20%)	6 (3%)	98 (48%)	89 (44%)	104 (51%)
3	76 (37%)	151 (73%)	46 (22%)	108 (52%)	48 (24%)	151 (74%)	111 (55%)	125 (62%)
4	130 (63%)	157 (76%)	107 (52%)	154 (75%)	127 (63%)	170 (84%)	153 (75%)	152 (75%)
5	138 (675)	146 (71%)	109 (53%)	145 (70%)	177 (87%)	178 (88%)	175 (86%)	178 (88%)
6	139 (68%)	149 (72%)	132 (64%)	153 (74%)	163 (80%)	193 (95%)	184 (91%)	178 (88%)
2-chamber								
7	17 (8%)	30 (15%)	17 (8%)	49 (24%)	35 (17%)	114 (56%)	177 (87%)	86 (42%)
8	12 (6%)	25 (12%)	20 (10%)	29 (14%)	14 (7%)	81 (40%)	91 (45%)	59 (29%)
9	68 (33%)	172 (84%)	80 (39%)	122 (59%)	66 (33%)	150 (74%)	117 (58%)	139 (69%)
10	142 (69%)	165 (80%)	129 (63%)	158 (77%)	129 (64%)	172 (85%)	161 (79%)	158 (78%)
11	143 (69%)	150 (73%)	116 (56%)	149 (72%)	153 (75%)	162 (80%)	166 (82%)	154 (76%)
12	142 (69%)	160 (78%)	130 (63%)	161 (78%)	167 (82%)	182 (90%)	180 (89%)	172 (85%)

Source: Modification of the FDA statistical reviewer (Table 3.8), page 14, and in Volume 44 p 91 (Table VII.5)
* The screening echos for IMUS-007 and IMUS-008 are qualifying results.

Table 28 illustrates individual ***segments*** not visualized from continuous mode ECHO, compared with non-visualized segments based upon each blinded reader.

Endocardial Border Delineation

All data illustrated in the ensuing efficacy reports are focusing upon the fundamental continuous mode 2-D echocardiography results, primarily because this mode provides results which address the primary efficacy endpoints. In addition, the fundamental gated mode was used in this study to answer secondary efficacy endpoints (see the Appendix A for gated data). As pointed out by the sponsor, gated mode is not commonly used at bedside in the community. Finally, those data recorded as mean data were listed instead of the median data, but median data collected and submitted are so closely similar to the mean data that there is virtually no difference between the mean and median results.

The next table illustrates the results of the reading of the baseline, non-contrast control echocardiograms (n-ECHO), where 3 apical views were investigated prior to use of AF0150.

TABLE 29: PHASE 3 STUDIES –
 # Subjects With Varying Nos. of Segments Poorly Visualized (EBD score of 0 or 1) at Baseline*

# Poorly Visualized Segments	Study IMUS-007 (N = 206) Reader #, n (%)			Study IMUS-008 (N = 203) Reader #, n (%)		
	1	2	3	1	2	3
0 through 1	8 (4%)	42 (20%)	17 (8%)	3 (2%)	6 (3%)	4 (2%)
2 through 9	176 (85%)	146 (71%)	157 (76%)	98 (48%)	106 (52%)	117 (58%)
10 through 12	22 (11%)	18 (9%)	32 (16%)	102 (50%)	91 (45%)	82 (40%)

Source: FDA statistical reviewer – Table 3.7 on page 13.

* The baseline assessments are not meant to be confused with the screening assessments; baseline studies involved 3 apical views, while screening assessments involved 2 apical views. _

Note that, in the above table, the blinded readers in IMUS-007 felt that 71% (Reader 2) up to 85% (Reader 1) of the 206 subjects had 2 – 9 poorly visualized segments in the Baseline echocardiograms. Therefore, most of the enrolled subjects fit the entry criteria to the blinded readers. However, the blinded readers in IMUS-008 felt that 48% to 58% of the 203 subjects had 2 – 9 poorly visualized segments. These same blinded readers felt that 40% - 50% of the 203 subjects had 10 – 12 segments poorly visualized in the baseline echocardiograms. Therefore, the readers felt that almost half of the subjects entered into IMUS-008 had more poorly visualized segments noted. This baseline assessment is not meant to be confused with the screening assessments; baseline studies involved 3 apical views, while screening assessments involved 2 apical views. Nevertheless, this data may introduce bias into the study by setting the stage for pending visual improvement after use of the AF0150 contrast agent.

The next 2 tables demonstrate the change in the **mean score** of **individual** segments after AF0150 administration when compared to "Baseline" scores. All the EBD data for the next set of tables were analyzed for both studies using the "no-change" scenario only because the populations from the "no change scenario" and "worst case scenario" are virtually the same. Within both studies, there was noted a statistically significant difference in visualizing the EBD post-AF0150 (c-ECHO) in comparison with non-contrast Baseline ECHO (n-ECHO). All segments that were previously considered the most frequently poorly visualized were noted to have statistically significant improvements in visualization of the respective walls. Only in IMUS-007 were there a few segments in which no statistically difference was noted; those segments were not among the most frequently poorly visualized segments as listed earlier. In addition, the Agency clinical reviewer felt that a visual score difference of ≥ 0.5 could be used as the point of *clinically* (as opposed to *statistically*) significant improvement in visualization of the various segments.

As was mentioned before, the readers were to read the n-ECHO and c-ECHO images and score each of the 16 segments for the baseline (n-ECHO) and AF0150 treated (c -) ECHO as follows:

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

TABLE 30 – PROTOCOL *IMUS-007*: Endocardial Border Delineation – Individual Segment Mean Change from Baseline Score ‡ (continuous mode in 3 apical view, *N = 206*)

View	Reader 1			Reader 2			Reader 3		
(Seg.)	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
View†	21.4	31.9	10.5	26.3	31.2	4.9	19.7	29.0	9.3
Apical 4-chamber segments									
View†	8.4	12.6	*4.3*	10.3	12.3	*2.0*	7.7	11.7	*4.0*
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***
Apical 2-chamber segments									
View†	7.5	11.4	*3.9*	9.7	11.5	*1.8*	7.2	10.0	*2.9*
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***
Apical long axis segments									
View†	5.5	7.9	*2.4*	6.3	7.4	*1.1*	4.8	7.3	*2.5*
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*

Derived from Volume 90, pp 224 – 264; bolded numbers represent segments demonstrating clinically significant improvement with AF0150 administration (Difference ≥ 0.5).

* p-value ***not*** significant at the 0.05 level.

† The "View" data actually is the sum of the scores across all segments per view (2nd, 3rd, 4th) and across all views (1st); all results are significant at a p value of 0.001.

‡ Calculated base on a scale of 0 = no delineation; 1 = mild/ fair delineation; 2 = moderate / good delineation; 3 = excellent delineation.

Included within the tables above (IMUS-007) and below (IMUS-008) is the total EBD score and change score for the individual ***views*** (in contrast to ***segments***, as discussed above). The "view"

data was found in the submission as follows: IMUS-007-USA in volume 90, pp 259-264; and IMUS-008-USA in volume 107, pp 247-252.

TABLE 31 – PROTOCOL *IMUS-008*: Endocardial Border Delineation – Individual Segment Mean Change from Baseline Score ‡ (continuous mode in 3 apical view, *N = 203*)

View	Reader 1			Reader 2			Reader 3		
(Seg.)	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
View†	15.4	26.2	10.8	14.3	23.2	8.9	21.2	29.8	8.6
Apical 4-chamber segments									
View†	5.6	10.5	*4.9*	5.8	9.5	3.7	7.5	11.2	3.7
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***
Apical 2-chamber segments									
View†	6.1	9.6	*3.5*	5.2	8.2	*3.0*	8.1	11.1	*3.0*
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***
Apical long axis segments									
View†	3.7	6.1	*2.4*	3.3	5.5	*2.2*	5.6	7.4	*1.8*
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*

Derived from Volume 107, pp 214 – 252; bolded numbers represent segments demonstrating clinically significant improvement with AF0150 administration (Difference ≥ 0.5).

* p-value ***not*** significant at the 0.05 level.

† The "View" data actually is the sum of the scores across all segments per view (2nd, 3rd, 4th) and across all views (1st); all results are significant at a p value of 0.001.

‡ Calculated base on a scale of 0 = no delineation; 1 = mild/ fair delineation; 2 = moderate / good delineation; 3 = excellent delineation.

The data illustrated in the above tables demonstrate that the blinded readers agree that AF0150 administration led to a statistically significant improvement in virtually all the segments for IMUS-007 and all segments for IMUS-008. Clinically significant improvement, defined as a difference ≥ 0.5, was also noted in both studies. In IMUS-007, segments 5, 6, 11 and 12 had clinically improved across all 3 readers; in IMUS-008, segments 4, 5, 6, 11, 12, 13, and 14 had clinically improved across all 3 readers. Thus, virtually all of the segments that were noted to be poorly

visualized (with the notable exception being segment 10 for both studies) had both clinical and statistically significant improvement in visualization after AF0150 administration.

Tables illustrating the data for the fundamental gated mode readings in a similar manner to the above tables for fundamental continuous mode are included within Appendix A. There was greater evidence, however, of better visualization with baseline n-ECHO readings than visualization with the c-ECHO. Thus, some of the differences are denoted with negative signs; because the analyses were 2-tailed analyses, some of the "negative" differences are noted to be statistically significant from a negative standpoint. These results may be worse based upon the fact that the gated mode was used minutes later after the fundamental continuous mode was used, after much of the microbubbles had disintegrated. Thus, the gated data is flawed; this is also illustrated in the next set of data, illustrated below.

The next table depicts the proportion of all images in each study where AF0150 provided added benefit to viewing the previously poorly viewed echocardiograms (shifts in EBD by segment from suboptimal to optimal in fundamental continuous mode). Individual reader evaluations by segment are presented for the number and percent of subjects whose segment scores shifted from ***suboptimal*** visualization (score of 0 – 1) to ***optimal*** visualization (score 2 – 3) after AF0150 administration. For each reader is a "Difference" column, which depicts that portion of images which were suboptimal at baseline that became optimal after AF0150.

		Post-AF0150 Visualization	
		Suboptimal	**Optimal**
Baseline Visualization	**Suboptimal**		***Difference***
	Optimal		**BL + AF †**
			AF0150 ‡

† Proportion of images rated as optimal by both baseline and AF0150
‡ Proportion of images rated as optimal by AF0150 ***only***

From the fundamental continuous mode, almost all segments had a statistically significant shift of improvement in IMUS-007 (Reader 1 = 16 segments; Reader 2 = 9 segments; Reader 3 = 14 segments) and IMUS-008 (Reader 1 = 16 segments; Reader 2 = 15 segments; Reader 3 = 16 segments).

TABLE 32: Endocardial Border Delineation – Image Improvement Proportion of Images (continuous mode in 3 apical view)

View (Seg.)	Reader 1 BL + AF†	Reader 1 AF0150‡	Reader 1 Differ.	Reader 2 BL + AF†	Reader 2 AF0150‡	Reader 2 Differ.	Reader 3 BL + AF†	Reader 3 AF0150‡	Reader 3 Differ.
PROTOCOL *IMUS-007* (N = 206)									
Apical 4-chamber segments									
1	0.87	0.96	*0.09*	0.86	0.90	***0.04****	0.77	0.94	*0.17*
2	0.86	0.97	*0.11*	0.91	0.95	***0.04****	0.80	0.97	*0.17*
3	0.23	0.69	*0.46*	0.72	0.83	***0.11****	0.38	0.61	*0.23*
4	0.22	0.69	*0.47*	0.42	0.76	*0.34*	0.18	0.42	*0.24*
5	0.28	0.81	*0.53*	0.46	0.85	*0.39*	0.29	0.90	*0.61*
6	0.27	0.79	*0.52*	0.35	0.77	*0.42*	0.25	0.86	*0.61*
Apical 2-chamber segments									
7	0.81	0.91	***0.10****	0.86	0.89	***0.03****	0.75	0.94	*0.19*
8	0.86	0.94	*0.08*	0.88	0.93	***0.05****	0.84	0.95	*0.11*
9	0.14	0.55	*0.41*	0.53	0.70	***0.17****	0.23	0.39	***0.16****
10	0.15	0.53	*0.38*	0.33	0.63	*0.30*	0.15	0.29	***0.14****
11	0.25	0.73	*0.48*	0.43	0.78	*0.35*	0.26	0.80	*0.54*
12	0.21	0.71	*0.50*	0.35	0.74	*0.39*	0.21	0.76	*0.55*
Apical long axis segments									
13	0.31	0.73	*0.42*	0.39	0.70	*0.31*	0.37	0.83	*0.46*
14	0.29	0.73	*0.44*	0.42	0.73	*0.31*	0.32	0.81	*0.49*
15	0.55	0.77	*0.22*	0.63	0.76	***0.13****	0.47	0.79	*0.32*
16	0.60	0.77	*0.17*	0.69	0.76	***0.07****	0.59	0.80	*0.21*
PROTOCOL *IMUS-008* (N = 203)									
Apical 4-chamber segments									
1	0.25	0.70	*0.41*	0.31	0.67	*0.36*	0.22	0.88	*0.66*
2	0.46	0.77	*0.31*	0.53	0.80	*0.27*	0.48	0.93	*0.45*
3	0.21	0.54	*0.33*	0.39	0.65	*0.26*	0.37	0.84	*0.47*
4	0.13	0.51	*0.38*	0.22	0.53	*0.31*	0.24	0.83	*0.59*
5	0.11	0.65	*0.54*	0.13	0.59	*0.46*	0.12	0.87	*0.75*
6	0.05	0.58	*0.53*	0.09	0.57	*0.48*	0.12	0.86	*0.74*
Apical 2-chamber segments									
7	0.34	0.70	*0.36*	0.11	0.60	*0.49*	0.57	0.92	*0.35*
8	0.51	0.76	*0.25*	0.48	0.70	*0.22*	0.70	0.94	*0.24*
9	0.19	0.45	*0.26*	0.30	0.44	***0.14****	0.30	0.81	*0.51*
10	0.10	0.38	*0.28*	0.16	0.39	*0.23*	0.22	0.79	*0.57*
11	0.16	0.57	*0.41*	0.14	0.47	*0.33*	0.24	0.85	*0.61*
12	0.10	0.51	*0.41*	0.07	0.46	*0.39*	0.15	0.83	*0.68*
Apical long axis segments									
13	0.10	0.52	*0.42*	0.07	0.47	*0.40*	0.34	0.89	*0.55*
14	0.18	0.61	*0.43*	0.12	0.54	*0.42*	0.36	0.89	*0.53*
15	0.28	0.53	*0.25*	0.26	0.52	*0.26*	0.43	0.83	*0.40*
16	0.16	0.40	*0.24*	0.30	0.59	*0.29*	0.47	0.83	*0.36*

Source: Modified version of FDA statistical review – Table 3.11, p 17 (IMUS-007) and Table 3.11, p 18 (IMUS-008), as well as Volume 44 p 99 (IMUS-007 – Table VII.11.) and p 101 (IMUS-008 – Table VIII.13.).

* P-value ***not* significant** at the 0.05 level; thus, there is no statistically significant benefit with the use of AF0150.

† Proportion of images rated as optimal by both baseline and AF0150

‡ Proportion of images rated as optimal by AF0150 only

Tabular results for fundamental gated mode, similar to the fundamental continuous mode table above, is illustrated in the Appendix A.

Regarding the fundamental **gated mode** for all three readers, the proportion of images that were optimal at baseline, becoming sub-optimal after AFO150, was less than 0.18 for 4-chamber view, less than 0.19 for the 2-chamber view, and less than 0.19 for the long axis view in Study IMUS-007. In Study IMUS-008, the proportion was less than 0.13 for 4-chamber view, less than 0.14 for the 2-chamber view, and less than 0.15 for the long axis view.

For each segment, the proportion of AFO150 enhanced segments rated as optimal with gated imaging is less than with continuous imaging. In particular, for each segment, a larger proportion of AFO150 enhanced segments was rated as sub-optimal than was rated as optimal with gated imaging. Table 33 presents the AFO150 enhanced segments for each reader that were rated as sub-optimal more often than were rated as optimal with gated imaging (proportion of AFO150 enhanced segments rated as sub-optimal > 0.50 for each segment). Study IMUS-008 is more problematic than study IMUS-007 because two of the three readers rated all AFO150 enhanced segments as sub-optimal. This pattern is probably due to the fact that there is less AFO150 in the blood pool by the time the gated images are acquired. Per protocol, the gated images are acquired after the continuous images are acquired. Thus, an adequate assessment of the effect of AFO150 on endocardial border delineation is not possible with this data.

TABLE 33: EBD – AF0150-Enhanced Segments
Rated as Sub-optimal More Than as Optimal (Gated mode in 3 apical views)

Reader 1	Reader 2	Reader 3
PROTOCOL *IMUS-007* (N = 206)		
Apical 4-chamber segments		
3, 4		3, 4
Apical 2-chamber segments		
9, 10	10	9, 10, 11, 12
Apical long axis segments		
15		15
PROTOCOL *IMUS-008* (N = 203)		
Apical 4-chamber segments		
1, 2, 3, 4, 5, 6	1, 3, 4, 5, 6	
Apical 2-chamber segments		
7, 8, 9, 10, 11, 12	7, 8, 9, 10, 11, 12	10
Apical long axis segments		
13, 14 ,15, 16	13, 14 ,15, 16	

Subset analyses based upon age, race, and gender are tabulated below for both efficacy studies. No subset appears to benefit to a greater degree with the use of AF0150 as a contrast; all subsets had statistically significant improvement in delineation of the endocardial borders. An analysis not performed by the sponsor is an analysis in which efficacy is assessed based upon the number of poorly visualized segments delineated at baseline. For example, the number of poorly visualized segments could have been divided into 2 to 4 images, 5 to 6 images, and 7 to 9 images. Such an analysis may isolate a subset that may benefit from AF0150 administration.

TABLE 34(a) – PROTOCOL *IMUS-007*: EBD – Image Improvement
Subset Analyses – Mean Scores (continuous mode in 3 apical view, *N = 206*)

Variables	Reader 1			Reader 2			Reader 3		
	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
Age									
< 65 years (N =140)	20.9	32.0	*11.1*	25.9	31.2	*5.3*	19.1	29.0	*9.9*
≥ 65 years (N = 66)	22.5	31.6	*9.1*	26.9	31.0	*4.1*	20.9	29.0	*8.1*
Gender									
Male (N = 129)	21.3	31.4	*10.1*	26.7	31.3	*4.6*	19.8	29.1	*9.4*
Female (N = 77)	21.6	32.7	*11.1*	25.5	310	*5.5*	19.5	28.8	*9.3*
Race									
White (N = 174)	21.3	31.8	*10.6*	25.9	30.9	*5.0*	19.4	29.3	*9.9*
Non-White (N = 32)	22.2	32.2	*10.0*	28.0	32.5	*4.6*	21.2	27.7	*6.5*

Source: Modification of data from Volume 90 pp 307 – 313.

TABLE 34(b) – PROTOCOL *IMUS-008*: EBD – Image Improvement
Subset Analyses – Mean Scores (continuous mode in 3 apical view, *N = 203*)

Variables	Reader 1			Reader 2			Reader 3		
	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
Age									
< 65 years (N = 112)	14.6	27.5	*12.9*	14.0	23.8	*9.8*	21.0	29.9	*8.9*
≥ 65 years (N = 91)	16.4	24.6	*8.2*	14.7	22.5	*7.7*	21.5	29.7	*8.2*
Gender									
Male (N = 138)	15.5	27.3	*11.8*	14.7	24.4	*9.6*	21.5	30.4	*8.9*
Female (N = 65)	15.2	23.9	*8.7*	13.5	20.8	*7.3*	20.6	28.4	*7.8*
Race									
White (N = 166)	15.0	25.6	*10.6*	14.0	23.0	*9.0*	21.0	29.9	*8.9*
Non-White (N = 37)	17.4	28.9	*11.5*	15.9	24.2	*8.3*	22.0	29.1	*7.1*

Source: Modification of data from Volume 107 pp 295 – 301.

In conclusion, there is significant improvement in delineating the endocardial border when using AF0150 as a contrast agent in fundamental continuous 2-D echocardiography, especially for those segments with the greatest frequency of poor visualization. This was proven in both studies by evaluating for 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. The mean change scores seem less for gated than for continuous mode but had a similar trend toward statistically significant improvement.

Ejection Fraction

Demonstration of improved assessment of ejection fraction was the subsequent primary endpoint; contingent upon improvement of EF assessment is the improvement of delineating the endocardial border, which had been demonstrated. Ejection fraction (EF) as evaluated by contrast (AF0150) echocardiogram was first compared to non-contrast echo, then compared with the EF assessment by the gold standard RVG / MUGA. The analysis was stratified by the % EF as determined by RVG. Further analyses were performed upon subsets based upon age, race, and gender.

EF categories, using continuous mode echocardiography for both baseline (n-ECHO) and c (contrast)-ECHO, for each reader are listed below and compared with the gold standard RVG. What is notable for IMUS-008 study is that 188 (93%) of the 203 subjects were evaluable for efficacy (15 patients had no RVG to begin with) and that one additional subject did not have the RVG available; ultimately ECHO and RVG data could be retrieved for 187 subjects (92%).

TABLE 35 – PHASE 3 PROTOCOLS
Correlation of (# Subjects with) ECHO-Assessed EF's vs. RVG Results

	IMUS-007; N = 206				IMUS-008; N = 203			
	Continuous Mode		*Gated* Mode		*Continuous* Mode		*Gated* Mode	
	n-ECHO N (%)	c-ECHO N (%)	n-ECHO N (%)	c-ECHO N (%)	n-ECHO N (%)	c-ECHO N (%)	n-ECHO N (%)	c-ECHO N (%)
Reader 1								
> RVG category	60 (29%)	72 (35%)	62 (30%)	63 (31%)	35 (17%)	71 (35%)	34 (17%)	53 (26%)
= RVG category	74 (36%)	60 (29%)	76 (37%)	68 (33%)	63 (31%)	66 (32%)	52 (26%)	66 (32%)
< RVG category	56 (27%)	59 (29%)	51 (25%)	60 (29%)	89 (44%)	50 (25%)	98 (48%)	67 (33%)
Not available	16 (8%)	15 (7%)	17 (8%)	15 (7%)	16 (8%)	16 (8%)	19 (9%)	17 (8%)
Reader 2								
> RVG category	57 (28%)	83 (40%)	62 (30%)	65 (32%)	43 (21%)	75 (37%)	37 (18%)	53 (26%)
= RVG category	75 (36%)	55 (27%)	75 (36%)	67 (32%)	62 (30%)	70 (34%)	51 (25%)	68 (33%)
< RVG category	58 (28%)	53 (26%)	52 (25%)	59 (29%)	82 (40%)	42 (21%)	96 (47%)	65 (32%)
Not available	16 (8%)	15 (7%)	17 (8%)	15 (7%)	16 (8%)	16 (8%)	19 (9%)	17 (8%)
Reader 3								
> RVG category	61 (30%)	72 (35%)	62 (30%)	61 (30%)	43 (21%)	71 (35%)	36 (18%)	53 (26%)
= RVG category	74 (36%)	59 (29%)	71 (34%)	65 (32%)	60 (30%)	68 (33%)	53 (26%)	66 (32%)
< RVG category	55 (27%)	60 (29%)	57 (28%)	65 (32%)	84 (41%)	48 (24%)	95 (47%)	67 (33%)
ot available	16 (8%)	15 (7%)	16 (8%)	15 (7%)	16 (8%)	16 (8%)	19 (9%)	17 (8%)

* Source: Data for IMUS-007-USA: Volume 90 pp 298 – 303; data for IMUS-008-USA: Volume 107 pp 286 - 291

Based upon the above data, when evaluating the individual blinded readers for improvement of determining EF and then comparing with the RVG, there is no improvement in accuracy using AF0150-contrasted echocardiograms (c-ECHO) versus non-contrast (baseline, n-ECHO) echocardiograms. Indeed, when looking at both studies, approximately one-third of the subjects had c-ECHOs with EF readings consistent with EF determination from RVG readings. There does not appear to be an EF category that would have an improvement in accuracy post-AF0150; the best EF category (> 65%) had mostly underestimated EF readings and all other categories (55%-65%; 45%-54%, 35%-44%; 25%-34%; and < 25%) had predominantly over-estimated EF readings both pre- and post-AF0150. Finally, subset analyses based upon age, gender, and race demonstrated no difference in accuracy between n-ECHO and c-ECHO, even when sub-categorizing based upon the EF categories, as was noted in the data submitted by the sponsor (IMUS-007: Volume 90 pp 323 – 340; Volume 108 pp 008 – 025). All the data recorded in the tables above are from both fundamental continuous and gated modes of 2-D echocardiograms. For IMUS-007, all data demonstrated more instances where baseline n-ECHOs and RVG agreed over c-ECHOs and RVG agreement.

Further investigation of the data demonstate that subset analyses (age, race, and gender) also confirmed no improvement in assessing the EF when using AF0150 contrast for echocardiography. Statistically significant data (bolded and italicized) were noted in the readings of Reader 2, where the p-value (from the McNemar's test) was significant at the 0.05 level. However, the statistical significance was in demonstrating better EF assessment in the baseline n-ECHO over the c-ECHO. For IMUS-008, most of the data demonstrated improvement in assessment of the AF0150-contrasted ECHO (with RVG-agreement) when compared with the baseline non-contrasted ECHO. However, there are no data that approaches the statistical significant level of $p = 0.05$. This data augments the fact that AF0150-contrast enhancement does not improve the echocardiologists' ability to accurately assess EF.

TABLE 36 – PROTOCOL IMUS-007: EF Subset Analysis

"Category 2" Results vs. "Category 3" Results Per Each Reader (Fund. Continuous Mode)

IMUS-007 (N = 206) EF Efficacy N = 191			Reader 1		Reader 2		Reader 3	
			Cat. 2	Cat. 3	Cat. 2	Cat. 3	Cat. 2	Cat. 3
Total †		N = 191	31 (16%)	45 (24%)	***27 (14%)***	***47 (25%)***	28 (15%)	43 (22%)
Age (yrs)	**< 65**	N = 132	23 (17%)	30 (23%)	22 (17%)	32 (24%)	21 (16%)	29 (22%)
	≥ 65	N = 59	8 (14%)	15 (25%)	***5 (8%)***	***15 (25%)***	7 (12%)	14 (24%)
Gender	**Male**	N = 118	19 (16%)	28 (24%)	***16 (14%)***	***30 (25%)***	18 (15%)	27 (23%)
	Female	N = 73	12 (16%)	17 (23%)	11 (15%)	17 (23%)	10 (14%)	16 (22%)
Race	**White**	N = 162	26 (16%)	36 (22%)	21 (13%)	38 (23%)	25 (15%)	34 (21%)
	Non-white	N = 29	5 (17%)	9 (31%)	6 (21%)	9 (31%)	3 (10%)	9 (31%)

Source: Volume 90 pp 317 – 322. ***Bolded italic data*** are data demonstrating statistical significance.
† Total score based upon the "No Change" Scenario.
Category 2. Baseline **pre**-contrast ECHO & RVG ***dis***agree, AND **post**-contrast ECHO & RVG agree.
Category 3. Baseline **pre**-contrast ECHO & RVG agree, AND **post**-contrast ECHO & RVG ***dis***agree.

TABLE 37 – PROTOCOL IMUS-008: EF Subset Analysis

"Category 2" Results vs. "Category 3" Results Per Each Reader (Fund. Continuous Mode)

IMUS-008 (N = 203) EF Efficacy N = 188			Reader 1		Reader 2		Reader 3	
			Cat. 2	Cat. 3	Cat. 2	Cat. 3	Cat. 2	Cat. 3
Total †		N = 188	45 (24%)	42 (22%)	48 (25%)	40 (21%)	46 (24%)	38 (20%)
Age (yrs)	**< 65**	N = 103	28 (27%)	20 (19%)	28 (27%)	21 (20%)	27 (26%)	19 (18%)
	≥ 65	N = 85	17 (20%)	22 (26%)	20 (23%)	19 (22%)	19 (22%)	19 (22%)
Gender	**Male**	N = 128	28 (22%)	29 (23%)	32 (25%)	27 (21%)	30 (23%)	25 (19%)
	Female	N = 60	17 (28%)	13 (22%)	16 (27%)	13 (22%)	16 (27%)	13 (22%)
Race	**White**	N = 166	Not done.	Not done.	Not done.	Not done.	Not done.	Not done.
	Non-white	N = 22	Not done.	Not done.	Not done.	Not done.	Not done.	Not done.

Source: Volume 107 p 292 and Volume 108 pp 004 – 007. ***Bolded italic data*** are data with statistical significance.
† Total score based upon the "No Change" Scenario.
Category 2. Baseline **pre**-contrast ECHO & RVG ***dis***agree, AND **post**-contrast ECHO & RVG agree.
Category 3. Baseline **pre**-contrast ECHO & RVG agree, AND **post**-contrast ECHO & RVG ***dis***agree.

In conclusion, in spite of a demonstrated improvement in visualizing the endocardial borders, this did not translate into an improvement of assessing the EF. In fact, in many cases, the EF "guessed" using no contrast at baseline was closer in agreement with the gold-standard for determining EF – the RVG – than with the EF determination after use of AF0150 for echocardiograms. Again, as in the case of EBD, no patient subset was noted to benefit to greater degree with AF0150 contrast, and fundamental gated ECHO study results were congruent with the fundamental continuous studies. Subsets suggested later that could have been analyzed would be patients with 2 to 4 poorly visualized segments, versus 5 to 9 poorly visualized segments, versus > 9 poorly visualized segments (based upon the "Confirmatory" echocardiograms).

An additional analysis using agreement based on echocardiographic EF's within ± 5% of the RVG-assessed EF was performed; no significant difference was noted (seen on page 19 of the Agency's statistical review).

Segmental Wall Motion

Data was derived from Vol. 90 p 342 (IMUS-007) and Vol. 108 pp 320 – 321 (IMUS-008). In general, the results demonstrate a statistically significant improvement (*p*-values < 0.01) in the ability to assess the wall motion of different segments noted on different views. The next table depicts each blinded reader's assessment of segmental wall motion (SWM) based upon the relationship of the SWM scores to an EBD score, discussed earlier in this review. The *mean number of poorly-visualized segments* (those with an SWM score of 0, equivalent to an EBD score of 0) for both baseline (n-ECHO) and AF0150-treated (c-ECHO) results, and the differences in the mean are tabulated below.

TABLE 38 – PHASE 3 STUDIES: Segmental Wall Motion (Continuous Mode) Mean Change from Baseline Score (# segments NOT visualized)

Reader 1			Reader 2			Reader 3		
Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
IMUS-007: SWM Efficacy N = 205 †								
8.7	3.7	*-5.0**	6.0	3.3	*-2.8**	8.5	3.9	*-4.6**
IMUS-008: SWM Efficacy N = 202 †								
11.9	6.8	*-5.1**	11.5	6.9	*-4.6**	10.5	2.2	*-8.4**

Source: For IMUS-007 – Volume 90, p 342; for IMUS-008 – Volume 108, p 027.
* The *P*-value < 0.01.
† Based upon the "no-change" scenario

Study IMUS-008 has demonstrated a greater improvement in SWM scores than IMUS-007; however, one must take into account that the blinded readers in IMUS-008 felt that a greater number of baseline (n-) ECHO's were poorly visualized. (See "Summary of Segments Not Visualized on the Confirmatory Non-Contrast Echocardiogram and the Baseline Echocardiogram", where the blinded readers' assessments of the baseline ECHO's were compared with the results given for the qualifying ECHO's.) These data again may introduce bias into the results.

TABLE 39 – IMUS-008: MRI Data for Segmental Wall Motion % Segments AGREEing With MRI Results (N = 26)

Reader 1			Reader 2			Reader 3		
Baseline	AF0150	Differ.	Baseline	AF0150	Differ.	Baseline	AF0150	Differ.
12%	41%	*29**	14%	39%	*25**	18%	62%	*44**

Source: Volume 108, p 028 (Table 2.45.1 – MRI efficacy population data)

* The *P*-value < 0.0001 for "Within-Subject Number of Segment Not Visualized"; from ANOVA model, with effect for site.

For IMUS-008, the scores for both n-ECHO and c-ECHO were evaluated in comparison to MRI for a subgroup (N =26) of the subjects. It is not known whether the subset chosen was randomly chosen or if these subjects had the poorest visualized baseline ECHO's, nor is it known why the sponsors chose to evaluate 26 subjects. However, a review of the individual patients involved in the MRI study (Volume 114 pp 173 – 176) and their respective EF data (Volume 114 pp 004 – 014) reveals the subjects chosen had a broad range of EF's at baseline ECHO; therefore this may have been a random selection. Because 2 individuals had untagged MRI data missing, the tagged MRI data was used. As demonstrated in the table above, there was a statistically significantly ($p = 0.01$) higher agreement of c-ECHO SWM scores with MRI when compared with n-ECHO SWM score agreement. This was noted for all 3 readers.

In conclusion, there appears to be a statistically significant improvement in assessing segmental wall motion when using AF0150 as a contrast agent in echocardiography when compared with baseline non-contrast poorly visualized echocardiograms. It was also determined that there was a statistically higher percentage of segments in the AF0150-contrasted echocardiograms in agreement with MRI studies in comparison with non-contrasted baseline echocardiograms' agreement with MRI studies. However, the MRI study population was too small to draw conclusions; the data is testimonial and is without an objective standard of truth.

SUPPORTING EFFICACY DATA

Duration of Attenuation (DOA) and Duration of Useful Contrast Enhancement (DUCE)

DOA (defined as the time from the 1st appearance of the contrast bolus to the time when the attenuation subsided to the level of the mitral valve) and DUCE (defined as the time that the contrast provided clinically useful enhancement of the endocardial borders without the effect of attenuation) at the dose of 0.125 mg/kg for both studies are tabulated below.

TABLE 40: PHASE 3 STUDIES – Supporting Efficacy Data

Duration of Attenuation Data	IMUS-007	IMUS-008
Number of Subjects	N = 176	N = 193
Mean ± Standard Deviation (minutes)	0.63 ± 1.40	0.80 ± 0.56
Range (minutes)	[redacted]	
Duration of Useful Contrast Enhancement	**IMUS-007**	**IMUS-008**
Number of Subjects	N = 197	N = 200
Mean ± Standard Deviation (minutes)	2.68 ± 1.69	2.58 ± 1.45
Range (minutes)	[redacted]	

Source: Modification of Tables VII.42 (Vol. 44 p 135 for DOA) and VII.44 (Vol. 44 p 136 for DUCE)

In both IMUS-007 and IMUS-008, the mean duration of attenuation was 0.63 minutes (37.8 seconds) and 0.80 minutes (48 seconds) respectively, and a mean duration of contrast enhancement of 2.68 minutes (2 minutes 40.8 seconds) and 2.58 minutes (2 minutes 34.8 seconds) respectively. These results, when taking into account the standard deviations, are similar. Also, results are similar to the dose-response findings in IMUS-018 (which enrolled subjects with EF ≤ 40% at doses of 0.125, 0.25, and 0.5 mg/kg). Below is tabulated a comparison of the DUCE's for all trials where subjects with EF≤ 40% received the proposed clinical dose of 0.125 mg/kg.

TABLE 41 - Duration of Useful Contrast Enhancement: Subjects with EF ≤ 40%

Statistics	IMUS-007	IMUS-008	IMUS-018
Number of subjects (0.125 mg/kg dose)	N = 22	N = 21	N = 18
Mean ± SD (minutes)	1.93 ± 1.58	2.84 ± 1.23	1.22 ± 0.92
Range (minutes)	[redacted]		

Source: From Table VII.46 in Volume 44 p 137.

The mean DUCE for subjects with ejection fractions ≤ 40% in IMUS-007, IMUS-008, and IMUS-018 were, respectively, 1.93 minutes (1 minute 55.8 seconds), 2.84 minutes (2 minutes 50.4 seconds), and 1.22 minutes (1 minute 13.2 seconds). When taking into account the standard deviations, the data is similar.

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EFFICACY CONCLUSIONS

In conclusion, significant improvement in delineating the endocardial border 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. Subset analyses based upon age, race, and gender strengthened those conclusions, although not one particular subset demonstrated greater improvement using AF0150. AF0150 contrast does not improve the ability for the echocardiologists to accurately assess EF, in spite of the improvement in visualizing the endocardial border. Subset analyses did not bring out any population subset that could benefit from use of this product. Thus, the only primary endpoint that has proven efficacy is an improved delineation of the endocardial borders, with no patient subset demonstrating greater efficacy. A secondary endpoint, involving the assessment of segmental wall motion, appeared to have a statistically significant improvement in assessment when using AF0150 as a contrast agent in echocardiography when compared with baseline non-contrast poorly visualized echocardiograms. There was also a statistically higher percentage of segments in the AF0150-contrasted echocardiograms in agreement with MRI studies in comparison 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 standard of truth (objective) and therefore unreliable.

Of note, IMUS-008 was remarkable for the possibility for the introduction of bias, due to readings of the blinded reviewers of the baseline n-ECHOs having poorer visualization of baseline studies when compared with qualifying studies. One would expect the blinded reviewers, being academic physicians, to have a greater confidence in review of the echocardiograms when compared to the (qualifying) results / readings of the community physicians. Nevertheless, the end results were similar to the IMUS-007 study.

Finally, the anatomic endpoint of EBD has been assumed to be a surrogate for the 2 functional endpoints of EF and SWM. Two values are needed for calculating the EF: the end-systolic (ES) and end-diastolic (ED) readings derived from the fundamental gated mode of 2-dimentional echocardiography. Although the sponsor provided EF data *per se*, a database is needed for EBD at ES and ED to help correlate with EF. Improved estimations of ES and ED volumes as a result

of improved EBD should lead to better estimations for EF. Therefore, at this point with this product, it is the view of the clinical reviewer that EBD cannot be useful as a surrogate for EF prediction; however, re-reads of EBD at ES and ED and a blinded EF calculation are recommended.

From the understanding of the clinical reviewer, previously approved ultrasound contrast (microbubble) agents had necessitated demonstration of ES and ED measurements to help in calculating the EF. This is due to the reader's ability to take the points of maximum ventricular volume (ED) and maximum ventricular contraction (ES) into consideration in estimating the volume of blood ejected from the ventricle. The clinical review believes the Agency requires such readings to be recorded and included into this submission, to maintain consistency of all microbubble ultrasound contrast agents.

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VII. INTEGRATED SAFETY SUMMARY

All of the following studies were evaluated for safety. These studies are grouped below in tabular form based upon (1) the dosage, and (2) administration of AF0150.

TABLE 42: 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 IMUS – 008 IMUS – 001	213 232 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
		4.0 mg/kg	IMUS – 012	10
	IV Infusion	4.0 mg/kg	IMUS – 001	4
		80 mg	IMUS – 003	2
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 IMUS – 007	20 81

Source: Volume 44, p 152 (Table VIII.3.), with data referenced to Section 8.XIII.A, Table 1.0.

The total numbers of patients in each dose-group category are as follows:

AF0150 Protocol Types	No. of Subjects
Single dose, 0.125 mg/kg	457
Single dose, other dosages	48
Multiple doses	103
All dose groups	608

DEMOGRAPHICS

Below is a table illustrating the demographic characteristics of subjects in all the studies, delineated by treatment group. (A table illustrating the combined demographics for subjects enrolled in all Integrated Safety and 120-day update studies is **Table 66**, in the **Labeling Section** of this review.) Following that table, separate evaluations of (1) the demographics of the pivotal protocols (Protocols IMUS-007-USA and IMUS-008-USA), (2) the prominent cardio-vascular and

pulmonary medical history and (3) prominent non-cardiopulmonary medical history of the patients enrolled into the pivotal studies.

TABLE 43: INTEGRATED SAFETY SUMMARY
DEMOGRAPHICS FOR SUBJECTS ENROLLED IN ALL STUDIES

Characteristic	Treatment Group				
	AF0150 Dose Group				Saline
	Single doses 0.125 mg/kg	Other doses	Multiple Doses *	All Doses	
	N = 457	N = 48	N = 103	N = 608	N = 101
Age (years)					
• < 65	284 (62%)	45 (94%)	64 (62%)	393 (65%)	72 (71%)
• 65 – 80	155 (34%)	3 (6%)	36 (35%)	194 (32%)	26 (26%)
• > 80	18 (4%)	0	3 (3%)	21 (3%)	3 (3%)
Gender					
• Male	300 (66%)	24 (50%)	74 (72%)	398 (65%)	73 (72%)
• Female	157 (34%)	24 (50%)	29 (28%)	210 (35%)	28 (28%)
Race					
• White	383 (84%)	38 (79%)	77 (75%)	498 (82%)	83 (82%)
• Black	59 (13%)	6 (12%)	17 (16%)	82 (13%)	9 (9%)
• Asian	5 (1%)	1 (2%)	4 (4%)	10 (2%)	1 (1%)
• Other	10 (2%)	3 (6%)	5 (5%)	18 (3%)	8 (8%)
Ejection Fraction (%) by RVG					
• < 50	88 (19%)	0	0	88 (14%)	0
• ≥ 50	291 (64%)	0	0	291 (48%)	0
• unknown	78 (17%)	48 (100%)	103 (100%)	229 (38%)	101 (100%)
Diagnostic History					
• Coronary Artery Disease	178 (39%)	0	29 (28%)	207 (34%)	26 (26%)
• Hypertension	264 (58%)	0	47 (46%)	311 (51%)	47 (46%)
• Hyperlipidemia	104 (23%)	0	18 (17%)	122 (20%)	14 (14%)
• Hypercholesterolemia	92 (20%)	0	8 (8%)	100 (16%)	18 (17%)
• Chronic Obstructive Pulm. Disease	97 (21%)	1 (2%)	13 (13%)	111 (18%)	15 (15%)
• Drug Allergies	156 (34%)	7 (15%)	30 (29%)	193 (32%)	15 (15%)

Source: Volume 51 pp 056 – 058 (Table 2.1)
* See Table 42 (above) for the trials and number of patients involved in the "Multiple Doses" group.

The "multiple doses" group had subjects who had (1) recent myocardial infarctions in IMUS-002, (2) liver or kidney lesion in IMUS-003, and (3) left ventricular dysfunction with EF's between 20% and 40% in IMUS-018; the final "multiple dose" study, IMUS-012, had normal volunteers. Thus, the smaller percentage of patients with critical diagnostic history under the "multiple doses" group must be tempered by the fact that normal volunteers from IMUS-012 are included in the group; this information can camouflage the fact that *that smaller percentage of patients actually have prognostically worse diagnoses, particularly regarding cardiovascular disease.*

Pivotal Protocols IMUS-007 and IMUS-008

For Protocol IMUS-007, more men were enrolled (68%); in addition, men (80%) constituted a significantly greater percentage of patients randomized to the saline arm. The percentage of Caucasians enrolled (83%) and randomized to the AF0150 arm (84%) was significantly higher than African-Americans (11% for both enrollment and AF0150 randomization) and other races. A significant percentage of enrolled patients were < 65 years of age (67%); this was the same percentage for randomization of this age group. Therefore, it appears the characteristic patients enrolled into this study were Caucasian males < 65 years of age. Protocol IMUS-008-USA has virtually the same demographic results.

TABLE 44: PHASE 3 STUDIES – DEMOGRAPHICS

Pivotal Phase 3 Studies: Demographics / Entry Characteristics						
Entry Variable	IMUS-007				IMUS-008	
	Total (AF0150 + Saline)	AF0150		Saline	AF0150	Efficacy
		Safety	Efficacy		Safety	
	N = 294	N = 213	N = 206	N = 81	N = 232	N = 203
Gender, n (%)						
Male	199 (68%)	134 (63%)	129 (63%)	65 (80%)	158 (68%)	138 (68%)
Female	95 (32%)	79 (37%)	77 (37%)	16 (20%)	74 (32%)	65 (32%)
Age, years						
< 65 years	198 (67%)	146 (68%)	140 (68%)	52 (64%)	126 (54%)	112 (55%)
≥ 65 years	96 (33%)	67 (32%)	66 (32%)	29 (36%)	106 (46%)	91 (45%)
Median		57	58	58	62	62
Range	22 – 86	22 – 83	22 – 83	24 – 86	29 – 84	29 – 84
Race						
Caucasian	244 (83%)	180 (84%)	174 (84%)	64 (79%)	191 (82%)	166 (82%)
African-American	31 (11%)	23 (11%)	22 (11%)	8 (10%)	36 (15%)	32 (16%)
Asian	4 (1%)	3 (1%)	3 (2%)	1 (1%)	2 (1%)	2 (1%)
Other	15 (5)	7 (3%)	7 (3%)	8 (10%)	3 (1%)	3 (1%)
Ejection Fraction (general)						
RVG EF < 50%			47 (23%)			41 (20%)
RVG EF ≥ 50%			144 (70%)			147 (72%)
Ejection Fraction (category)						
RVG EF > 65%			29 (14%)			50 (25%)
RVG 55 – 65%			75 (36%)			77 (38%)
RVG 45 – 54%			57 (28%)			32 (16%)
RVG 35 – 44%			18 (9%)			16 (8%)
RVG 25 – 34%			9 (4%)			10 (5%)
RVG < 25%			3 (1%)			3 (1%)
Not Available			15 (7%)			15 (7%)

Medical History

Detailed "medical history" information from the Phase 3 protocols is tabulated below. The only Phase 1 / 2 protocol listing similarly detailed information of cardio-pulmonary and other medical abnormalities was Protocol IMUS-018-USA (Volume 88, pp 058 – 069).

TABLE 45: PHASE 3 STUDIES
DEMOGRAPHICS (Cardiovascular / Pulmonary Disease History)

Entry Variable	IMUS-007			IMUS-008
	Total (AF0150 + Saline) N = 294	AF0150 Safety N = 213	Saline N = 81	AF0150 Safety N = 232
Cardiac Disease				
Hypertension	160 (54%)	113 (53%)	47 (58%)	154 (66%)
Central and peripheral vascular disease				
Cardiac murmurs, undiagnosed; resolved and current	49 (17%)	35 (16%)	14 (17%)	35 (15%)
Edema	21 (7%)	18 (8%)	3 (4%)	34 (15%)
Palpitations, resolved and current	27 (9%)	17 (8%)	10 (12%)	13 (6%)
Chest pain, unspecified, current and resolved	53 (18%)	40 (19%)	13 (16%)	33 (14%)
Peripheral vascular disease, unspecified (resolved and current)	11 (4%)	9 (4%)	2 (2%)	12 (5%)
Heart Failure				
Congestive: resolved + current	35 (12%)	24 (11%)	11 (14%)	40 (17%)
Unspecified	6 (2%)	4 (2%)	2 (2%)	5 (2%)
Ischemic Heart Disease				
Old Myocardial Infarct	13 (4%)	7 (3%)	6 (7%)	35 (15%)
Current "Old" Myocardial Infarct	10 (3%)	10 (5%)	0	3 (1%)
Acute MI, unspecified site	18 (6%)	10 (5%)	8 (10%)	26 (11%)
Angina, resolved	13 (4%)	8 (4%)	5 (6%)	15 (6%)
Angina, "current"	20 (7%)	15 (7%)	5 (6%)	28 (12%)
Atherosclerosis, resolved	4 (1%)	3 (1%)	1 (1%)	1 (0.4%)
Atherosclerosis, "current"	93 (32%)	68 (32%)	25 (31%)	106 (46%)
Cardiomyopathies				
"Hyperkinetic Disease"	5 (2%)	5 (2%)	0	0
"Cardiomegaly"	15 (5%)	12 (6%)	3 (4%)	16 (7%)
Idiopathic Myocarditis	1 (0.3%)	0	1 (1%)	
Atrial Dysrrhythmias				
Atrial Fibrillation	12 (4 %)	8 (4%)	4 (5%)	13 (6%)
Atrial Flutter	1 (0.3%)	0	1 (1%)	2 (1%)
Ventricular Dysrrhythmias				
Ventricular Flutter	1 (0.3%)	1 (1%)	0	0
Ventricular Tachycardia	2 (1%)	1 (1%)	1 (1%)	6 (3%)
Other arrhythmias	14 (5%)	9 (4%)	5 (6%)	14 (6%)
Valvular Disease				
Tricuspid (2° to Chronic Rheumatic Heart Disease)	14 (5%)	14 (7%)	0	7 (3%)
Mitral valve disorders	24 (8%)	22 (10%)	2 (3%)	27 (12%)
Aortic valve disorders	13 (4%)	12 (6%)	1 (1%)	7 (3%)
Pulmonary valve disorders	1 (0.3%)	0	1 (1%)	1 (0.4%)
Pulmonary				
Asthma, unspecified	21 (7%)	16 (7%)	5 (6%)	18 (8%)
Chronic airway obstruction, resolved and current	25 (8%)	20 (9%)	5 (6%)	24 (10%)
Dyspnea & other unspecified respiratory abnormality, resolved and current	64 (22%)	45 (21%)	19 (23%)	36 (15%)
Pneumonia, unspecified organisms (resolved)	12 (4%)	9 (4%)	3 (4%)	13 (6%)
Bronchitis, acute or chronic	10 (3%)	6 (3%)	4 (5%)	15 (6%)

Cardiovascular / Pulmonary Disease History

The most common cardiovascular diseases noted for the patients enrolled in both IMUS-007 and IMUS-008 studies included essential hypertension (54% and 66% respectively), current atherosclerosis (32% and 46% respectively), and congestive heart failure (12% and 17%, respectively). For pulmonary diseases, there was limited involvement, with the most common finding being "non-specific dyspnea" in both studies (22% for IMUS-007, and 15% for IMUS-008).

TABLE 46: PHASE 3 STUDIES
DEMOGRAPHICS (Non-Cardiovascular/Pulmonary Disease History)

Entry Variable	IMUS-007			IMUS-008
	Total (AF0150 + Saline)	AF0150 Safety	Saline	AF0150 Safety
	N = 294	N = 213	N = 81	N = 232
Non-Cardiac (≥ 5% incidence)				
Metabolic				
Pure hypercholesterolemia, current	65 (22%)	48 (22%)	17 (21%)	42 (18%)
Hyperlipidemia (unspecified), current	49 (17%)	35 (16%)	14 (17%)	64 (28%)
Anemia, unspecified, resolved	15 (5%)	12 (6%)	3 (4%)	10 (4%)
Neurological and Psychological				
Tobacco addiction, resolved & current (? Nondependent)	42 (14%)	32 (15%)	10 (12%)	19 (8%)
Depression / dysthymic disorder, resolved and current	28 (9%)	21 (10%)	7 (9%)	28 (12%)
Menopause state	26 (9%)	21 (10%)	5 (6%)	12 (5%)
Dizziness, resolved and current	25 (8%)	17 (8%)	8 (10%)	18 (8%)
Insomnia, resolved and current	11 (4%)	9 (4%)	2 (2%)	17 (7%)
Anxiety (unspecified), current	16 (5%)	13 (6%)	3 (4%)	16 (7%)
Anxiety, unspecified, resolved and current	18 (6%)	15 (7%)	3 (4%)	16 (7%)
Transient cerebral ischemia, resolved and current	10 (3%)	9 (4%)	1 (1%)	16 (7%)
Headache, resolved and current	17 (6%)	13 (6%)	4 (5%)	16 (7%)
Gastrointestinal				
Obesity	20 (7%)	16 (7%)	4 (5%)	37 (16%)
Diverticulosis of colon, current and resolved	7 (2%)	6 (3%)	1 (1%)	12 (5%)
Constipation	10 (3%)	9 (4%)	1 (1%)	13 (6%)
Dyspepsia or otherwise unspec., current and resolved	14 (5%)	10 (5%)	4 (5%)	16 (7%)
Esophageal reflux, current and resolved	17 (6%)	13 (6%)	4 (5%)	22 (10%)
Diaphragmatic hernia, current and resolved	10 (3%)	5 (2%)	5 (6%)	19 (8%)
Genitourinary				
Prostatic hyperplasia, current and resolved	15 (5%)	8 (4%)	7 (9%)	34 (15%)
Special Senses				
Unspecified nasal/sinus diseases (chronic sinusitis)	18 (6%)	15 (7%)	3 (4%)	13 (6%)
Visual Loss (unspecified), current & resolved	15 (5%)	9 (4%)	6 (7%)	22 (9%)
Hearing Loss (unspecifed), current	14 (5%)	11(5%)	3 (4%)	15 (6%)
Endocrinological				
Diabetes mellitus (NIDDM; maturity-onset)	53 (18%)	39 (18%)	14 (17%)	46 (20%)
Hypothyroidism (unspecified), current	17 (6%)	11 (5%)	6 (7%)	21 (9%)
Musculoskeletal				
Scar/skin fibrosis	26 (9%)	15 (7%)	11 (14%)	14 (6%)
Arthropathy / osteoarthrosis, resolved and current	23 (8%)	17 (8%)	6 (7%)	27 (12%)

Non-Cardiovascular / Pulmonary Disease History

The ***non***-cardiovascular diseases of the enrolled patients that clearly traverses both IMUS-007 and IMUS-008 with the highest incidences are pure hypercholesterolemia (22% and 18%, respectively), hyperlipidemia (17% and 28%, respectively), and diabetes mellitus (18% and 20%, respectively).

For the dosage being sought for approval – 0.125 mg/kg x 1 dose – Studies IMUS 001 (normal volunteers were randomized to receive either AF0150 or saline), IMUS-007 and IMUS-008 were analyzed. Safety data were analyzed for various subsets of the populations for the Phase 3 studies; the sample size was 445 (in all Phase 3 studies) out of 608 subjects who received AF0150 in all Phase 1, 2, and 3 trials submitted in this NDA, as per Agency recommendations. The categories the Agency recommended were as tabulated below, addressing all patients who received the recommended dosage of 0.125 mg/kg of AF0150, versus the subjects randomized in the Phase 3 study to receive saline as a control. Notable in the following table is that the baseline EF's are not recorded for the saline group; although the saline group were not evaluated for efficacy, having EF data for the saline population could help during the evaluation for AE's.

TABLE 47 – INTEGRATED SAFETY (using Proposed Clinical Dose)

CATEGORIES	CATEGORY SUBSET	POPULATION SUBSET	AF0150 0.125 mg/kg (N = 457)	AF0150 PHASE 3 (N = 445)	SALINE PHASE 3 (N = 81)
Demographics	**Age**	< 65 years	284 (62%)	272 (61%)	52 (64%)
		≥ 65 years and ≤80 years	155 (34%)	155 (35%)	26 (32%)
		> 80 years	18 (4%)	18 (4%)	3 (4%)
	Gender	Male	300 (66%)	292 (66%)	65 (80%)
		Female	157 (34%)	153 (34%)	16 (20%)
	Race	Caucasian	383 (84%)	371 (83%)	64 (79%)
		Non-Caucasian	74 (16%)	74 (17%)	17 (21%)
	BSA	< 2.0 m^2	225 (49%)	215 (48%)	29 (36%)
		≥ 2.0 m^2	232 (51%)	230 (52%)	52 (64%)
Diagnoses	**Cardiac Disease**	EF < 50%	88 (19%)	88 (20%)	0
		EF ≥ 50%	291 (64%)	291 (65%)	0
		Unknown	78 (17%)	66 (15%)	81(100%)
	Concomitant Medical Illnesses	Hypertension	264 (58%)	264 (59%)	47 (58%)
		Coronary Artery Disease	178 (37%)	178 (40%)	26 (32%)
		Hyperlipidemia	104 (23%)	104 (23%)	14 (17%)
		Hypercholesterolemia	92 (20%)	92 (21%)	18 (22%)
		COPD	97 (21%)	97 (22%)	14 (17%)
		Drug Allergy	156 (34%)	153 (34%)	13 (16%)
Concomitant Medications		Plt Aggregate Inhibitors (excluding heparin)	197 (43%)		35 (43%)
		ACE-Inhibitors	149 (33%)		24 (30%)
		Beta-receptor Blockers	117 (26%)		22 (27%)
		HMG-CoA Reductase Inhibitors	141 (31%)		24 (30%)

CLINICAL ADVERSE EVENTS

Two tables illustrating all reported clinical adverse events are below. The 1st table illustrates the incidence of adverse events, delineated as the total, moderate and severe AE's, for all subjects who received the proposed recommended dosage of a single dose of AF0150 at 0.125 mg/kg. (Those incidences are also viewed in terms of overall incidence, incidence in the Phase 3 studies, and incidence within the saline arm of the Phase 3 studies.) The 2nd table illustrates the incidence of adverse events, comparing the incidence of the PCD versus all other doses and modes of administration of AF0150. The incidences are sorted out by body system below, as well as by the severity of the adverse events.

[Refer to Table(s) 65 (a & b) to review the incidence of the adverse events for all studies combined – the ISS and 120-day update together – located in the Labeling Section near the end of this review. In addition, Appendix D illustrates an extensive table demonstrating the incidence of adverse events (also delineated as the total, moderate and severe AE's) for all subjects in the Phase 1 and 2 studies (all that received both PCD and non-PCD). Finally, Appendix E illustrates an extensive table of the Severe and Moderate Adverse Events recorded in all studies (ISS and 120-day update combined).]

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TABLE 48 – INTEGRATED SAFETY (Studies using Proposed Clinical Dose)

Body Systems	AF0150 – 0.125 mg/kg						Saline
	All Single Dose Studies (n = 457)			All Phase 3 Studies (n = 445)			Ph. 3 (n =81)
	Total	Mod.	Severe	Total	Mod.	Severe	Total
All Subjects w/ AE's	**49 (11%)**	**5 (1%)**	**1 (0.2%)**	**48 (11%)**	**5 (1%)**	**1 (0.2%)**	**5 (6%)**
Body as a Whole	**18 (4%)**	**2 (0.4%)**	**0**	**18 (4%)**	**2 (0.4%)**	**0**	**1 (1%)**
Asthenia	4 (1%)	0	0	4 (1%)	0	0	0
Headache	8 (2%)	0	0	8 (2%)	0	0	1 (1%)
Pain	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Chest Pain	1 (0.2%)	1 (0.2%)	0	1 (0.2%)	1 (0.2%)	0	0
Chills	1 (0.2%)	1 (0.2%)	0	1 (0.2%)	1 (0.2%)	0	0
Cardiovascular	**15 (3%)**	**1 (0.2%)**	**1 (0.2%)**	**15 (3%)**	**1 (0.2%)**	**1 (0.2%)**	**0**
Hypertension	5 (1%)	1 (0.2%)	0	5 (1%)	1 (0.2%)	0	0
Hypotension	2 (0.4%)	0	0	2 (0.4%)	0	0	0
Vasodilation	2 (0.4%)	0	0	2 (0.4%)	0	0	0
Supravent. Tachycardia	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Tachycardia	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Angina Pectoris	1 (0.2%)	0	1 (0.2%)	1 (0.2%)	0	1 (0.2%)	
Digestive	**11 (2%)**	**2 (0.4%)**	**0**	**11 (3%)**	**2 (0.4%)**	**0**	**0**
Diarrhea	4 (1%)	0	0	4 (1%)	0	0	0
Nausea	5 (1%)	2 (0.4%)	0	5 (1%)	2 (0.4%)	0	0
Metabolic	**4 (1%)**	**1 (0.2%)**	**0**	**4 (1%)**	**1 (0.2%)**	**0**	**2 (3%)**
Bilirubinemia	0	0	0	0	0	0	1 (1%)
↑ Creatine Phophokinase	3 (1%)	1 (0.2%)	0	3 (1%)	1 (0.2%)	0	0
Hyperglycemia	1 (0.2%)	0	0	1 (0.2%)	0	0	1 (1%)
↑ Lactic Dehydrogenase	0	0	0	0	0	0	1 (1%)
Nervous	**3 (1%)**	**0**	**0**	**3 (1%)**	**0**	**0**	**0**
Dizziness	2 (0.4%)	0	0	2 (0.4%)	0	0	0
Paresthesia	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Special Senses	4 (1%)	0	0	3 (1%)	0	0	0
Taste Perversion	4 (1%)	0	0	3 (1%)	0	0	0
Urogenital	**1 (0.2%)**	**0**	**0**	**1 (0.2%)**	**0**	**0**	**1 (1%)**
Dysuria	0	0	0	0	0	0	1 (1%)
Albuminuria	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Hemato-Lymphatic	**4 (1%)**	**0**	**0**	**4 (1%)**	**0**	**0**	**0**
↑ Fibrinogen	1 (0.2%)	0	0	1 (0.2%)	0	0	0
Leukocytosis	2 (0.4%)	0	0	2 (0.4%)	0	0	0
Thrombocytopenia	1 (0.2%)	0	0	1 (0.2%)	0	0	0

For these trials involving the proposed clinical dose, the most common (≥ 1% incidence) adverse events listed below in the AF0150 group (all studies and Phase 3 studies) include headache (8 subjects; 2%); asthenia (4 subjects; 1%); hypertension (5 subjects; 1%) and increase creatinine phosphokinase (3 subjects; 1%). All adverse events in the saline group (a total of 5 subjects, or 6% of all subjects) were mild and with an incidence rate of < 2%.

TABLE 49: INTEGRATED SAFETY SUMMARY
Adverse Events Reported in > 1% of Subjects in All Studies by Treatment Group

Body System	AF0150 Treatment Group				Saline
	0.125 mg/kg Single Dose (N = 457)	Other Single Doses (N = 48)	Multiple Doses (N = 103)	All Doses (N = 608)	(N = 101)
Body as a Whole	**18 (4%)**	**6 (12%)**	**18 (17%)**	**42 (7%)**	**2 (2%)**
Chest Pain	1 (0.2%)	0	5 (5%)	6 (1%)	0
Fever	0	1 (2%)	1 (1%)	2 (0.3%)	0
Headache	8 (2%)	5 (10%)	6 (6%)	19 (3%)	2 (2%)
Pain	1 (0.2%)	1 (2%)	4 (4%)	6 (1%)	0
Cardiovascular	**15 (3%)**	**1 (2%)**	**13 (13%)**	**29 (5%)**	**2 (2%)**
Atrial Fibrillation	0	0	3 (3%)	3 (0.5%)	0
Hypertension	5 (1%)	0	2 (2%)	7 (1%)	1 (1%)
Hypotension	2 (0.4%)	0	3 (3%)	5 (0.8%)	0
Palpitation	0	0	2 (2%)	2 (0.3%)	0
Tachycardia	1 (0.2%)	0	2 (2%)	3 (0.5%)	0
Vasodilation	2 (0.4%)	1 (2%)	0	3 (0.5%)	0
Digestive	**11 (2%)**	**4 (8%)**	**10 (10%)**	**25 (4%)**	**1 (1%)**
Constipation	0	0	3 (3%)	3 (0.5%)	0
Diarrhea	4 (1%)	1 (2%)	2 (2%)	7 (1%)	1 (1%)
Flatulence	0	0	2 (2%)	2 (0.3%)	0
LFT Abnormalities	0	1 (2%)	0	1 (0.2%)	0
Nausea	5 (1%)	2 (4%)	3 (3%)	10 (2%)	0
Metabolic	**4 (1%)**	**1 (2%)**	**9 (9%)**	**14 (2%)**	**2 (2%)**
"Edema"	0	0	2 (2%)	2 (0.3%)	0
Hyperglycemia	1 (0.2%)	0	2 (2%)	3 (0.5%)	1 (1%)
Hyperlipidemia	0	1 (2%)	0	1 (0.2%)	0
Hypokalemia	0	0	2 (2%)	2 (0.3%)	0
LDH Increased	0	1 (2%)	1 (1%)	2 (0.3%)	1 (1%)
Nervous	**3 (0.7%)**	**1 (2%)**	**5 (5%)**	**9 (1%)**	**0**
Dizziness	2 (0.4%)	1 (2%)	0	3 (0.5%)	0
Respiratory	**0**	**1 (2%)**	**7 (7%)**	**8 (1%)**	**0**
Dyspnea	0	0	2 (2%)	2 (0.3%)	0
Hiccup	0	1 (2%)	0	1 (0.2%)	0
Special Senses	**4 (1%)**	**2 (4%)**	**0**	**6 (1%)**	**3 (3%)**
Conjunctivitis	0	1 (2%)	0	1 (0.2%)	0
Eye pain	0	1 (2%)	0	1 (0.2%)	0
Taste perversion	4 (1%)	0	0	4 (0.7%)	2 (2%)

Source: Volume 44 p 165 (Table VIII.14.) and Section 8.XIII.A, Tables 3.1.1 and 3.2.1
* Subjects I IMUS-018-USA are counted in the "Multiple Doses" Group.

As the sponsor mentioned, the incidence of AE's was highest among the subjects enrolled in the "multiple doses" group (39%), followed by the "other single dose" group (31%), as compared to the single-dosed PCD group (11%). Within the "multiple dose" group, the most frequent AE's reported were headache (6%), chest pain (5%), pain (4%), and atrial fibrillation (3%). The "multiple doses" group had many subjects who had cardiac disease. These patients had (1) recent myocardial infarctions in IMUS-002 (40%), and (2) left ventricular dysfunction with EF's between 20 and 40% in IMUS-018 (17%). The other 2 multidose studies include patients with liver

or kidney lesion in IMUS-003 and Study IMUS-012 which had normal volunteers. The next table (Table 50) concentrates upon the cardio-pulmonary AE's, due to the fact that this particular organ-system had the greatest number (and severity) of AE's with increased morbidity/mortality.

TABLE 50: ISS – Reported Cardio-Pulmonary Adverse Events

Study	ID #	Reported Adverse Event	Severity	Time of Event		Time of AF0150
				Onset	Resolved	
AF0150 0.125 mg/kg (PCD): n = 457						
IMUS-007 (n = 213)	03-063	Tachycardia: Heart rate elevated (22 hours post-AF0150)	Mild	01 / 19 / 99 10:53	01 / 19 / 99 11:30	01 / 18 / 99 12:53
	07-009	Supraventricular Tachycardia	Mild	11 / 20 / 98 2:28	11 / 20 / 98 2:28	11 / 19 / 98 10:52
	09-009✳	Creatinine Phosphokinase ↑, with ↑ CPK-MB mass	Moderate	12 / 02 / 98 11:22	Ongoing	12 / 02 / 98 11:14
	13-003✳	Unstable angina pectoris	Severe	11 / 04 / 98 (? time)	11 / 10 / 98 (? time)	11 / 02 / 98 10:56
IMUS-008 (n = 232)	20-018 †	Creatinine Phosphokinase ↑	Mild	12 / 10 / 98 12:35	12 / 11 / 98 13:35	12 / 10 / 98 11:37
	21-001✳	Chest pain with nausea (concomitantly)	Moderate	05 / 15 / 98 13:00	05 / 15 / 98 13:30	05 / 15 / 98 12:55
	28-001 †	Creatinine Phosphokinase ↑, with ↑ CPK-MB mass	Mild	06 / 04 / 98 (? time)	06 / 05 / 98 (? time)	06 / 04 / 98 16:43
	28-007	Abnormal electrocardiogram	Mild	11 / 18 / 98 13:16	11 / 19 / 98 12:20	11 / 18 / 98 12:22
	28-008	T-wave inversion on ECG post-AF0150	Mild	12 / 16 / 98 16:15	12 / 22 / 98 10:05	12 / 16 / 98 16:09
	30-023	Non-specific ST-T wave change on ECG ("chronic stable")	Mild	12 / 08 / 98 10:38	12 / 08 / 98 11:33	12 / 08 / 98 10:33
AF0150 multiple doses: n = 103						
IMUS-002 (n = 41)	01-002	Bradycardia episode requiring temporary pacemaker	Mild	10 / 09 / 96 (? time)	??????	10 / 16 / 96 15:09
	01-010✳	Concomitant symptoms of atrial fibrillation, dyspnea, and chest pain → cardiac failure	Severe	03 / 12 / 97 20:00	03 / 13 / 97 (? Time)	03 / 12 / 97 16:29
	03-001	Extrasystoles: Marked sinus bradycardia with occasional premature contractions	Mild	02 / 11 / 97 14:07	??????	02 / 04 / 97 16:15
		Heart fluttering → Supraventricular tachycardia	Mild	02 / 13 / 97 8:30	02 / 11 / 97 9:00	
	03-002	Chest pain	Mild	02 / 13 / 97 02:00	??????	02 / 06 / 97 15:51
		Sinus bradycardia	Mild	02/13/97 09:45	???????	

TABLE 50: ISS – Reported Cardio-Pulmonary Adverse Events (continued)

Study	ID #	Reported Adverse Event	Severity	Time of Event		Time of AF0150
				Onset	Resolved	
AF0150 multiple doses: n = 103 (continued)						
IMUS-002 (n = 41) (continued)	03-003	Angina pectoris	Mild	03 / 04 / 97 21:00	03 / 04 / 97 22:45	03 / 04 / 97 10:03
	04-004	Coronary artery disorder (multivessel) → PTCA performed	Moderate	???????	01 / 15 / 97 (? time)	01 / 13 / 97 14:30
		Tachycardia with concomitant hypertension	Mild	01 / 13 / 97 14:36	01 / 13 / 97 14:46	
	04-006	↓ in Heart rate with ↑ in blood pressure	Mild	03 / 10 / 97 (? time)	03 / 10 / 97 (? time)	03 / 10 / 97 13:20
	05-006✳	Atrial fibrillation	Moderate	12 / 20 / 96 15:00	12 / 22 / 96 10:00	12 / 20 / 96 11:57
		Pulmonary edema with (? Concomitant) hypotension	Moderate	12 / 21 / 96 23:00	12 / 25 / 96 (? time)	
		Congestive heart failure (moderate) → cardiogenic shock	Severe	12 / 21 / 96 19:00	12 / 21 / 96 23:00	
	05-009✳	Atrial fibrillation	Mild & Moderate	12 / 24 / 96 01:05	?????	12 / 24 / 96 15:22
		Atypical atrial flutter	Mild	12 / 26 / 96 (? time)	12 / 26 / 96 (? time)	
		Cardiac arrest (asystole x 2 episodes)	Severe	12 / 25 / 96 01:44	12 / 25 / 96 01:47	
	05-010✳	Myocardial infarction, acute (ultimately led to death)	Severe	01 / 01 / 97 20:00	01 / 01 / 97 20:57	12 / 30 / 96 15:04
	06-001	Bradycardia	Mild	02 / 20 / 97 14:51	02 / 20 / 97 16:00	02 / 20 / 97 14:30
IMUS-003 (n = 47) ‡	03-002	Chest pain over right clavicle	Mild	04 / 16 / 97 (? time)	04 / 16 / 97 (? time)	04 / 14 / 97 12:11
	03-003	Tachycardia (with concomitant mild vasodilation / flush)	Moderate	05 / 09 / 97 12:10	05 / 09 / 97 (? time)	05 / 06 / 97 13:20
	01-003	Hypoxia (↓ 0_2 saturation)	Mild	06 / 16 / 97 16:03	06 / 16 / 97 16:22	06 / 16 / 97 15:35
	01-004	Chest pain and concomitant dyspnea	Moderate	11 / 04 / 97 16:00	11 / 05 / 97 16:00	11 / 04 / 97 11:07
	01-005	Chest pain and concomitant cardiac "flutter"/palpitations	Mild	12 / 10 / 97 20:30	12 / 10 / 97 20:30	12 / 09 / 97 10:06

Source: Volume 69 pp 188 – 241 & 242 – 292.
✳ Narratives are provided below, in this review.
† Subject's medical history is summarized in Table 51.
‡ The subjects identified with C-P AE's here all have metastatic disease → ↑ coagulability (Vol. 130 pp 105 – 119).

As illustrated in the table above, the patients experiencing cardiac adverse events are as follows:

AS0150 0.125 mg/kg (PCD): n = 457	10 (2%)
• IMUS-007 (n = 213)	4 (2%)
• IMUS-008 (n = 232)	6 (3%)
AF0150 multiple doses: n = 103	16 (16%)
• IMUS-002 (n = 41)	11 (27%)
• IMUS-003 (n = 47)	5 (11%)

There were more patients experiencing cardiac AE's in the multi-dose groups, where the greatest concentration of subjects having recently acquired cardiac disease were accrued (IMUS-002). At present, it is difficult to determine whether either a recent history of cardiac disease or if multiple dose exposure with AF0150 led to an increased incidence of cardiac AE's. As illustrated in the next sections, it is also noted that the degree of severity of cardiac events is greater in the group of subjects having recently acquired cardiac disease, where the dose of AF0150 may have an additive role in these AE's.

SERIOUS ADVERSE EVENTS

As mentioned within the submission, serious adverse events were reported in no other study but IMUS-002-USA – subjects who experienced a recent myocardial infarction, which included one subject (Subject 05-010) who died and 3 other subjects (Subjects 01-010, 05-006, and 05-009). These subjects are described below; the information was derived from Volume 122 pp 186-188. Missing information includes the dose and amount the subjects received leading to the respective AE's, route of administration, timing of the adverse event to dosing, and vital sign changes.

Subject 05-010 (Death due to myocardial infarction):
This is a 66 year Caucasian female with congestive heart failure (ejection fraction 25%), who was 2 days status-post anterior wall myocardial infarction and percutaneous transluminal coronary angioplasty (PTCA) demonstrating 100% ostial occlusion of the left descending artery. On Study Day 0, the subject received dose 1 -- 0.269 mg/kg of AF0150 -- at 15:04, and dose 2 -- 1.194 mg/kg – at 15:23. Despite a relatively uneventful hospitalization, the patient had a myocardial infarction on Study Day 2 (01/01/97 at 20:00 hrs) and subsequently died on the evening of Study Day 3 due to sudden myocardial rupture.

Subject 01-010 (Severe chest pain, atrial fibrillation, heart failure, and dyspnea)
This subject is an 83 year old Caucasian male with a history significant for coronary artery disease including being status post coronary artery bypass grafting in 1980 for a possible myocardial infarction. This patient was 2 days status-post anterior wall myocardial infarction and PTCA (100% occluded left anterior descending and circumflex coronaries) prior to administration of AF0150. The subject received 0.246 mg/kg for dose 1 (at 16:29) and 1.038 mg/kg for dose 2 (at 16:58). Three and ½ hours after starting AF0150 dosing (at 20:00), the patient had severe

chest pain with atrial fibrillation (treated with digoxin), and heart failure with dyspnea (treated enalapril and furosemide) – all attributed to the underlying disease and resolved the following day (on an unlisted time on Study Day 1). The patient was lost to follow-up and did not complete the study.

Subject 05-006 (Hypotension and cardiogenic shock)
This subject is a 68 year old Caucasian male, 2 days status-post anterior wall myocardial infarction and PTCA (76% - 99% occluded left anterior descending coronary artery) prior to AF0150 administration. On Study Day 0, the patient received 0.264 mg/kg AF0150 for dose 1 at 11:57, followed by dose 2 of 0.746 mg/kg at 12:26. The patient experienced moderate atrial fibrillation 3 hours after beginning dose 1 (at 15:00 hrs; treated with digoxin), which resolved on Study Day 2 (at 10:00). The next day (Study Day 1 at 19:00 hrs), the patient experienced severe cardiogenic shock [hypotension (96/49 mm Hg) and moderate congestive heart failure] with pulmonary edema (occurring at 23:00 hrs and treated with diuretics), which was resolved the same day. On Study Day 2, the patient experienced hyperglycemia and moderate aspiration pneumonia, the latter which is ongoing and not followed up. This subject completed the study.

Subject 05-009 (Severe heart arrest)
This subject is a 72 year old Caucasian male, 1 day status-post right wall myocardial infarction and PTCA (100% occluded right coronary artery) prior to AF0150 administration. On Study Day 0, the subject received dose 1 -- 0.267 mg/kg of AF0150 -- at 15:22 and dose 2 -- 0.928 mg/kg – at 15:36. Prior to AF0150 administration, the patient experienced moderate atrial fibrillation (Study Day 0 at 01:05 hrs). On Study Day 1 (at 01:44 hrs), the patient experienced severe heart arrest (resolved the same day), and on Study Day 2 (at unknown time), the patient had mild atrial flutter (resolving the same day) and pneumonia (ongoing). This subject completed the study.

SEVERE AND MODERATE AE'S

The 6 patients in the Phase 3 studies who experienced moderate and severe adverse events are briefly narrated below:

1. **One subject** in the studies using AF0150 at 0.125 mg/kg (Studies IMUS-007, -008, and –001) had a ***severe adverse event***. No discussion exists in the submission detailing the AE suffered by the single patient. The event was implied in Volume 44 p 173 and in a table in Volume 51 p 118. This information was finally isolated in Volume 97 pp 148 – 150.
   a) **Study #007 Patient #13-003** is a 58 year old white male (? Medical history; EF 55% - 65%) who, 2 days post-AF0150 administration (11/02/98 at 10:56, receiving 0.126 mg/kg AF0150), was noted to have ***angina pectoris*** on 11/04/98 (time unlisted). The event was resolved 11/10/98 (7 days later).
2. **Five subjects** had ***moderate adverse events***. All 5 of those patients were enrolled in two Phase 3 studies: 2 subjects in the IMUS-007 study (out of 213 subjects in the AF0150 arm, or 1%), and 3 subjects in the IMUS-008 study (out of 232 subjects enrolled, or 1%). Information for adverse events in IMUS-008-USA was obtained from Volume 114 pp 183 – 186.
   a) **Study #007 Patient #9009**: 74 year old white male, with a history of Type II diabetes mellitus, aortic and mitral valve disorders, angina pectoris, cardiomegaly, and peripheral vascular disease. During the 1st hour post-AF0150 (administered 0.125 mg/kg on 12/02/98 at 11:14), the subject's CPK-MB was noted at 11:22 to be increased from the baseline of 5.5 ng/mL (normal value is ≤ 5 ng/mL) to 8.9 ng/mL. Because of the normal level of concomitant unfractionated CPK level of 173 ng/mL (normal range is 24 to 195 ng/mL), this was recorded by the investigators as a laboratory abnormality (***"moderate CPK increase"***) that was possibly/probably related to the study drug. At 24 hours post-AF0150, the CPK-MB remained elevated at 8.1 ng/mL; at 15 days post-AF0150, the CPK-

MB was 6.2. No diagnostic assessment (i.e. myocarditis, pericarditis, ischemia, infarction) was recorded in the submission. The adverse event later resolved.

b) **Study # 007 Patient #13010**: 57 year old white male was given 0.127 mg/kg AF0150 on 11/18/98 at 10:30; ***moderate nausea*** began 11/18/98 at 11:00 (30 minutes post-AF0150) and resolved 2100 (lasted 10 hours).
c) **Study # 008 Patient # 21001**: 74 year old white female was given 0.128 mg/kg AF0150 on 05/15/98 at 12:55; ***moderate chest pain*** (began 05/15/98 at 13:00 & resolved on 05/16/98 at 09:00) occurred 5 minutes after AF0150. ***Moderate nausea*** (began 05/16/98 at 09:00 & resolved at 17:00) occurred the next day upon cessation of the chest pain. Although no diagnostic assessment was recorded, one can surmise that the pain was gastro-intestinal in origin.
d) **Study # 008 Patient # 23-010**: 82 year old female was given 0.125 mg/kg AF0150 on 09/15/98 at 10:47; ***moderate hypertension*** began 2 days later on 09/17/98 at 14:15.
e) **Study # 008 Patient # 27021**: 82 year old white male was given 0.127mg/kg AF0150 on 10/15/98 at 15:30; ***moderate chills / rigors*** began 10/15/98 at 16:45 and resolved at 17:45.

Regarding subset analyses for the incidence of adverse events, the following is tabulated (Vol. 44-p.178) for the group of patients enrolled in the Phase 3 studies (using the PCD):

Age	Patients ≥ 65 years of age treated with AF0150 have an increased overall incidence of AE's, particularly noted in those > 80 years of age. This was made very apparent when compared with similar-aged subjects treated with saline.
Gender	Although slightly more females reported AE's than males in both groups, there were no clinically significant trends.
Race	Despite a significantly higher overall incidence of AE's among Caucasians when compared with non-Caucasians, it is difficult to draw definitive conclusions due to the low number of non-Caucasians enrolled (74 subjects in total; 17% of subjects enrolled).
Cardiac Disease	There was a slightly higher incidence of overall AE's in subjects with EF ≥ 50%, but the profile of AE's are no different from subjects with EF < 50%.
Concomitant Medicines	There was actually a slightly higher incidence of overall AE's among subjects who were receiving HMG CoA-reductase inhibitors and platelet-aggregation inhibitors. Nevertheless, there was no difference in the AE profile among all subjects enrolled in the different AF0150 trials.

LABORATORY STUDIES (ADVERSE CHANGES / EVENTS)

The normal ranges for the different laboratory tests/values used for the protocols were provided in Volume 74, pp 326 – 340 (Section 8.XIII.C, Listing 11). As mentioned earlier, no "potentially clinically significant" laboratory values could be found in the submission.

Clinical data summaries are located in Volume 69, in Section 8.XIII.B (Section 8 = Clinical Data; XIII = ISS tables, figures, and listings; B = ISS data summary figures), pp 069-001 through 069-

094. The data contained in this area are box-whisker plots for all laboratory studies. These box-whisker plots contain the median values, the 25 and 75th percentiles, and error bars at the end of which are 1.5 interquartile ranges. Finally, there are "plot symbols" placed where subjects with outlier value measurements for each laboratory parameter were found.

The box-whisker plots demonstrate little-to-no changes in all parameters; those few median values that changed were negligible, many possibly representing acute phase reactions, not major (or minor) enough to cause clinical manifestations. Those negligible changes were noted among patients receiving multiple doses of AF0150.

Clinical laboratory values listed as adverse events (tabulated below) included cardiac enzyme increases (n = 3), electrolyte imbalances (n = 11), blood count changes (n = 3) and coagulation parameters (n = 1). No clinically significant trends were noted (Vol. 44 p 231). An increase in C3a levels had been recorded after AF0150 administration in normal volunteers, but was not associated with any clinically significant changes in vital signs and laboratory values. Such an increase may be as an acute phase reaction.

TABLE 51 – INTEGRATED SAFETY: Reported Clinical Laboratory AE's

PROTOCOL	SUBJECT	MEDICAL HISTORY	PARAMETER(S)	CLINICAL SIGNIFICANCE
AF0150 – 0.125 mg/kg single dose: n = 457				
IMUS-007 (n = 213)	09-009	Cardiomegaly, angina, PVD, diabetes, and cardiac valve disorders	CPK-MB fraction – moderate increase (but normal CPK)	Baseline = 5.5 ng/mL (normal ≤ 5.0); 1 hr post-AF0150 = 8.9; 24 hrs post-AF0150 = 8.1; and 15 days post-AF0150 = 6.2 ng/mL. AE is ongoing. [Total CPK was 173 U/L (normal = 24 - 195).]
IMUS-008 (n = 232)	20-003	Diabetes, DJD and BPH; also HTN, angina, and s/p old MI	Serum glucose – mild increase	Noted 19 hours after AF0150 dosing at unspecified dose mg/dL. Baseline = 184 mg/dL (normal = 68 to 118); 1 hr post-AF0150 = 107. AE resolved spontaneously 5.3 hours later.
	20-018	Hyperlipidemia and diabetes (gestational); pleurisy	CPK – mild increase	Baseline = 109 U/L; 1 hr post-AF0150 = 351; 24 hr post-AF0150 = 116 and was reported as resolved at that time.
	28-001	HTN, angina, status post MI's & cardiac arrests; hyperlipidemia	CPK-MB fraction – mild increase	Baseline = 1.3 ng/mL; 1 hr post-AF0150 = 8.2; 24 hr post-AF0150 = 1.4 ng/mL.
	28-002	HTN, angina, status post MI & arrhythmias; hyperlipidemia; BPH	Decreased platelets – mild	Baseline = 158 x $10^3/\mu L$ (normal = 140 - 450 x $10^3/\mu L$) ; 1 hr post-AF0150 = 120 x $10^3/\mu L$; resolved spontaneously after 13 days.
	30-006	CHF, HTN with CAD, IDDM with retinopathy, and mitral valve disease	Fibrinogen – mild increase	Baseline = 420 mg/dL (normal = 200 to 400); 1 hr post-AF0150 = 538 mg/dL; spontaneously resolved 20 hr later, with 24 hr post-AF0150 = 463 mg/dL.

TABLE 51 – INTEGRATED SAFETY: Reported Clinical Laboratory AE's (continued)

PROTOCOL	SUBJECT	MEDICAL HISTORY	PARAMETER(S)	CLINICAL SIGNIFICANCE
***AF0150 – 0.125 mg/kg single dose*: n = 457 (continued)**				
IMUS-008 (n = 232) continued	30-021	CAD, HTN, aortic valve disorders, hyperlipidemia, and alcoholism	Leukocytosis – mild	Baseline = 7.4 x $10^3/\mu L$ (normal = 4.1 - 12.3 x $10^3/\mu L$); 24 hrs post-AF0150 = 13.9 x $10^3/\mu L$. Along with ↑'ed WBC's, the differential count of PMN's was baseline = 4.91 x $10^3/\mu L$ (normal = 2.03 - 8.36 x $10^3/\mu L$); 24 hr post-AF0150 = 11.4 x $10^3/\mu L$. Finally, % PMN's was baseline = 66.4% (normal = 41% - 77%) and 24 hrs post-AF0150 was 82%. All values spontaneously resolved after 45 days.
	30-028	HTN, CAD, CHF, s/p CABG, restrictive lung disease, PVD, mature onset DM, and retinal hemorrhage, blindness and macular degeneration	Leukocytosis – mild	Baseline = 9.9 x $10^3/\mu L$ (normal = 4.1 - 12.3 x $10^3/\mu L$); 24 hrs post-AF0150 = 13.4 x $10^3/\mu L$. Along with ↑'ed WBC's, the differential count of PMN's was baseline = 6.33 x $10^3/\mu L$ (normal = 2.03 - 8.36 x $10^3/\mu L$); 24 hr post-AF0150 = 8.95 x $10^3/\mu L$. Finally, the differential count of lymphocytes was baseline = 2.85 x $10^3/\mu L$ (normal = 1.02 to 3.36 x $10^3/\mu L$); 24 hr post-AF0150 = 3.64 x $10^3/\mu L$. All values resolved spontaneously after 20 days.
***AF0150 – Multiple doses*: n = 103**				
IMUS-002 (n = 41)	05-006	*No information.*	Hyperglycemia	Baseline = 314 mg/dL (normal = 68 to 118); Day 2 = 158 mg/dL; resolved on Day 5.
	05-007	*No information.*	Hypokalemia	No baseline listed; hypokalemia (unknown value) was recorded on Day 3, resolving the same day.
IMUS-003 (n = 47)	03-001	*No information.*	Hyperlipidemia (elevated triglycerides)	Study Day -3 = 433 mg/dL; Baseline = 385 mg/dL; 1 hr post-AF0150 = 493 mg/dL (same value on Study Day 2). (Normal triglyceride levels = 0 – 226 mg/dL.)
	03-007	Hepatic hemangioma.	Hyperbilirubinemia	Baseline = 1.1 mg/dL; 1 hr post-AF0150 = 1.3 (same value at 4 hr post-AF0150); returned to normal on next day (Study Day 1). (Normal total bilirubin levels = 0 – 1 mg/dL.)
	03-008	Ovarian cancer with hepatic metastases.	SGOT increased	Baseline = 25 UL; 1 hr post-AF0150 = 50 U/L; all other liver enzyme tests were normal (normal range = 0 – 34 IU/L)
	03-009	*No information.*	Decreased serum phosphate	Baseline = 2.3 mg/dL; 4 hrs post-AF0150 = 1.7 mg/dL; returned to normal (range = 2.5 – 4.7 mg/dL) afterwards.
	03-010	*No information.*	Hyperglycemia	Baseline = 98 mg/dL; 4 hr post-AF0150 (non-fasting level) = 141 mg/dL. (Normal range = 70 – 111 mg/dL)
	05-002	*No information.*	Elevated liver enzymes (normal values are – AST: 0 – 34 mg/dL ALT: 0 – 39 mg/dL AlkØ: 40 – 122 IU/L	Baseline AST = 39 U/L; day 1 post-AF0150 = 68 U/L; day 2 post-AF0150 = 44 Baseline ALT = 46 U/L; day 1 post-AF0150 = 61 U/L; day 2 post-AF0150 = 45 U/L Baseline Alk Ø = 183 U/L; day 1 post-AF0150 = 177 U/L; day 3 post-AF0150 = 159 U/L

TABLE 51 – INTEGRATED SAFETY: Reported Clinical Laboratory AE's (continued)

PROTOCOL	SUBJECT	MEDICAL HISTORY	PARAMETER(S)	CLINICAL SIGNIFICANCE
***AF0150 – Multiple doses:* n = 103 (continued)**				
IMUS-018 (n = 18)	02-001	Patient was taking a non-K+ sparing diuretic before and during the study. *No other information.*	Hypokalemia	Baseline = 4.7 mEq/L; 1 hr AF0150 time-point = 3.7 mEq/L ; 24 hr AF0150 time-point = 3 mEq/L; resolved 34 days later. (Normal range = 3.5 – 5.1 mEq/L)
	02-009	*No information.*	Increased LDH	Baseline = 579 IU/L; 24 hr post-AF0150 = 724 IU/L; resolved 20 days later. (Normal range = 97 – 239 IU/L)
Saline: n = 101				
IMUS-007 (n = 81)	07-002		Hyperglycemia	Mild in severity; no other information.
	09-006		Bilirubinemia and LDH increased	Both were mild in severity; no other information.

Source: Modification of Table VIII.59 – Volume 44 p 231. Normal lab values → Volume 74 pp 326 – 340.

Potentially Clinically Significant (PCS) Laboratory Changes

The first table represents potentially clinical significant changes in coagulation values after administration of AF0150 in subjects with both normal and abnormal baseline PT and aPTT. In IMUS-007, there were 14 subjects (6%) with abnormalities post-AF0150, and 3 subjects (4%) with abnormalities post-saline infusion. In IMUS-008, there were 16 subjects (7%) with abnormalities post-AF0150 administration. Finally, for the multi-dosage IMUS-018 study, there were 5 (28%) subjects with abnormalities in post-AF0150 administration.

TABLE 52 – INTEGRATED SAFETY

Subjects with "Potentially Clinical Significant" Values for PT and aPTT* Post-dosing

Protocol	Subject	Parameter (seconds)	Observed Values (seconds) Baseline	1 hour	24 hour	Clinical Significance
Baseline values either normal or missing						
AF0150 0.125 mg/kg single dose						
IMUS-007-USA (N = 213)	03-015	PT	Not available	Not available	**18.7***	Concurrent Medicine
	11-007	PT	Not available	Not available	**19.1***	Not evaluated
		APTT	Not available	Not available	**116***	Not evaluated
	03-002	APTT	Not available	**53.1***	Not available	No
	03-010	APTT	28.6	38.3	**40.8***	No
	04-016	APTT	25.4	26.1	**41.5***	No
	12-007	APTT	Not available	36.6	**223.2***	Concurrent Medicine
	16-007	APTT	28.5	21.3	**44.7***	No
IMUS-008-USA (N = 232)	24-003	APTT	Not available	33.1	**68.1***	Concurrent Medicine
	24-007	APTT	26.5	27.1	**123.6***	Collection error
	24-009	APTT	Not available	27.2	**84.7***	Concurrent Medicine
	27-003	APTT	Not available	**43.8***	26.1	No
	27-007	APTT	30.9	**61.2***	29.9	No
	27-015	APTT	32.6	**40.8***	35.9	No
	27-034	APTT	21.6	**50.5***	19.6	No
	27-037	APTT	26.2	**64.0***	29.2	No
	30-016	APTT	30.1	**43.9***	32.0	Collection error
	30-027	APTT	22.0	Not available	**48.0***	Collection error

TABLE 52 – INTEGRATED SAFETY

Subjects with "Potentially Clinical Significant" Values for PT and aPTT* Post-dosing

Protocol	Subject	Parameter (seconds)	Observed Values (seconds) Baseline	1 hour	24 hour	Clinical Significance
Baseline values either normal or missing						
AF0150 multiple doses						
IMUS-018-USA (N = 18)	01-001	APTT	28.1	**86.1***	25.2	No
	02-011	APTT	32.0	**47.0***	**45.0***	No
Saline						
IMUS-007-USA (N = 81)	10-003	PT	Not available	**21.9***	**18.7***	Subject's Disease
		APTT	Not available	**44.0***	37.0	Subject's Disease
	02-010	APTT	Not available	32.3	**91.1***	Collection error
Baseline values abnormal						
AF0150 0.125 mg/kg single dose						
IMUS-007-USA (N = 213)	05-029	APTT	39.6	39.4	**42.1***	Concurrent medicine
	06-016	PT	15.6	**20.1***	Not available	Unknown
		APTT	37.2	**52.6***	Not available	Unknown
	07-003	APTT	32.7	30.2	44.0*	Concurrent medicine
	08-001	APTT	36.3	30.1	**83.4***	Concurrent medicine
	09-001	PT	16.6	**18.4***	16.7	Concurrent medicine
		APTT	39.4	**60.9***	40.0	Concurrent medicine
	09-005	APTT	37.6	**86.0***	32.0	Study Drug; coll. error
	12-001	APTT	35.3	35.5	**57.3***	Unknown
IMUS-008-USA (N = 232)	22-021	APTT	33.8	**40.7***	Not available	No
	25-007	APTT	37.0	37.5	**44.1***	Unknown
	27-019	APTT	35.9	**40.3***	28.8	No
	27-025	APTT	37.9	**48.0***	**41.3***	No
	28-001	APTT	37.4	**43.3***	33.3	No
	30-007	APTT	36.3	32.6	**40.5***	Concurrent medicine
AF0150 multiple doses						
IMUS-018-USA (N = 18)	02-002	APTT	39.0	**84.0***	39.0	No
	02-008	APTT	40.0	**49.0***	40.0	No
	02-012	APTT	34.0	**43.0***	**60.0***	No
Saline						
IMUS-007-USA	06-001	APTT	34.5	**42.5***	30.9	Unknown

Source: Volume 44, pp 205 – 206 (Tables VIII.45 and VIII.46; bolded numbers with asterisks are PCS values.

* Normal ranges: PT = low normal range of 9 – 11 seconds to high normal range of 12 – 14.5 seconds.
aPTT = low normal range of 20 – 25 seconds to high normal range of 33 – 35 seconds.

The most frequently noticed value that changed is the aPTT. Most episodes of increased aPTT are recorded after the 1st hour post-AF0150, with the aPTT being slightly more prolonged in subjects treated with AF0150 versus saline. Nevertheless, there is no significant difference in single-dose AF0150 versus saline treatment. Multiple AF0150 dosing appears to cause an increase in aPTT in greater numbers of subjects. No explanation for the change in these parameters was suggested. Virtually all subjects had aPTT's returned to normal after 24 hours. None of the coagulation assessments was reported as an adverse event. An additional coagulation factor, fibrinogen, was noted to have potentially clinically significantly changes in some subjects. In IMUS-007 and IMUS-008, there were 5 subjects (2%) for each study with fibrinogen increases post-AF0150, and no subjects with abnormalities post-saline infusion. Because fibrinogen is known to be an acute phase reactant, the increase in fibrinogen levels noted in all subjects may reflect this non-specific reaction to exposure to AF0150. Finally, for the multi-dosage IMUS-002 study, there were 10 (24%) subjects with abnormalities in fibrinogen post-