The followings are the summary tables of all pharmacology and toxicology studies:

Table 1. GENERAL PHARMACOLOGY STUDIES

Table 2. SAFETY PHARMACOLOGY STUDIES

Table 3. TOXICOLOGY STUDIES

Table 4. SPECIAL TOXICOLOGY STUDIES

(*Tables 5 & 6* are included in the above Overall Summary)

Table 7. REPRODUCTIVE TOXICITY STUDIES

Table 8. GENETIC TOXICOLOGY

Table 9. Effects of AF0150 on Hemodynamics in Rabbits Pretreated with Cardiovascular Stress Agents

Table 10. NOAEL of AF0150 from Safety Pharmacology Studies

Table 11. NOAEL of AF0150 from Toxicity Studies

Table 12. NOAEL of AF0150 from Reproductive Toxicity Studies

Table 1. GENERAL PHARMACOLOGY STUDIES
(All non-GLP Studies)

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg)	Main Results
EB-95-08 Vol.009 P077-097 08/95	Dose-response of Doppler to AF0150	Rabbit 6-Bolus 6-Inf	2.5; 5; 10 Bolus/Infusion	Both IV bolus and 10-min infusion of AF0150 induced dose-dependent increases in Doppler signal of carotid artery blood flow.
EB-97-17 Vol.009 P098-107 01/97; 08/98	Dose-Response of Doppler to AF0150 (3 studies)	Rabbit 6-A 1-B 1-C	0.059-5.0 Bolus	Dose-dependent increase in Doppler signal peak and persistence; consistent results seen in 3 different studies (A,B,C).
EB-95-28 Vol.009 P108-122 10/95-04/96	Effects of AF0150 injection dose and mode on Doppler and echocardiography	Swine 6-Dopp 2-2nd H	0.13; 0.66; 1.3; Bolus/Infusion	Increases contrast of fundamental or second harmonic ultrasound imaging of LV cavity and myocardium, with regional heterogeneity in myocardium images. Optimal dose was 0.66 mg/kg for myocardial imaging; no data showed if dose-dependent increase in LV cavity imaging.
EB-95-25 Vol.009 P123-135 10/95	Dose-response of hemodynamic and echocardiography to AF0150	Dog 6	0.03; 0.09; 0.3; 0.6 Bolus	Dose-dependent increase in myocardial image contrast (optimal dose of 0.3 and 0.6 mg/kg) but dose-independent increase in LV cavity image contrast. AF0150 improved myocardial perfusion determination at 0.3 and 0.6 mg/kg.
RMI-97-01 Vol.009 P136-139 01/97	Computer program for Doppler signal analysis	ND	ND	Fortran program ([redacted]) for analysis of Doppler signal enhancement (peak) and signal persistence.
RMI-97-02 Vol.009 P140-144 05/97	Computer program for Doppler signal analysis	ND	ND	Fortran program [redacted] for analysis of the Doppler signal's resistance to ultrasound power (calculation of the signal decrease ratio).

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg)	Main Results
PSM-97-04 Vol.009 P145-146 07/97	Effects of AF050 fill size and reconstituted concentration on echocardiography	Swine 2	160-240 mg/kg/Hr Infusion	Fill sizes (100mg vs. 200mg) had no significant change on myocardium imaging when reconstituted at the same concentration (20 mg/kg). No differences between the two fill sizes at different IV infusion rates. 100mg fill size reconstituted in 10ml (10mg/ml) provided slightly better opacification.
EB-97-16 Vol.009 P147-158 07/97	Effects of AF050 fill size and reconstituted concentration on Doppler signals and ultrasound power resistance	Rabbit 4	1.0 Bolus	Fill sizes and reconstituted concentration of AF0150 showed similar effects on Doppler signal (peak, persistence and resistance to ultrasound power).
EB-98-14 Vol.009 P159-171 04-09/98	Effects of AF0150 reconstituted with pretreated water (tension and temperature) on Doppler signal	Rabbit 3	0.2, 1.0 Bolus	AF0150 reconstituted in pretreated SWFI (with gas tension and temperatures) had no significant effect on Doppler signal enhancement.
EB-98-17 Vol.009 P172-183 02-03/98	Effects of AF0150 reconstitution conditions (time and vial inversion) on Doppler signal	Rabbit 4	1.0	Optimal efficacy within the first 30 min following reconstitution, and within the first 60 seconds after vial inversion
RE-99-47 Vol.009 P184-205 ND	Effects of different AF0150 microbubble size on Doppler signal	Rabbit 2/3	Not specified	Small microbubbles (<3 um) contributed more to the peak Doppler signal, whereas large microbubbles (>3um) contributed more to Doppler signal persistence
RE-99-46 Vol.009 P213-222 03/99	Effects of ultrasound power on AF0150 microbubble size *in vitro*	N/A	0.056 mg/ml (*In vitro*)	Ultrasound power (6 min exposure) had no significant effect on the size of AF0150 microbubbles in 6% albumin saline solution in a simulated vascular system. Microbubble counts (both small and large) decreased with increasing ultrasound power (time and mechanical index).

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg)	Main Results
EB-97-04 Vol.009 P223-236 ND	Effects of ultrasound power on AF0150-induced Doppler signal of carotid arterial blood flow	Rabbit 7/6	1.0 Bolus	Application of ultrasound power to the heart (closed chest) decreased Doppler signal with increasing power levels; the continuous UP decreased the signal more than the triggered UP. The Doppler signals returned to control level following the release of the UP burst in the lower UP levels (lower than 0 dB). Doppler signal persistence decreased by 13 seconds (compared with no UP application) with no difference between the continuous and triggered UP.
EB-97-20-Amended Vol.009 P237-247 ND	Effects of ultrasound power and frequency on AF0150 Doppler signal	Rabbit 3	1.0 Bolus	The Doppler signals of carotid artery blood flow decreased with increasing ultrasound power levels (0, -3, and –6 dB) at all tested probe frequencies (2.5, 3.5 and 4 MHz). At a given UP level, application of the higher ultrasound transmission frequency resulted in a lower decrease in the Signal Decrease Ratio.
EB-98-18 Vol.009 P248-259 09-10/98	LV cavity imaging using [redacted]	Swine 2	0.125; 0.25 Bolus	Image attenuation decreased with increasing MI settings (0.1-1.4); peak videointensity slightly affected; persistence of videointensity signal greatly reduced at high MI settings (>0.4).
EB-98-19 Vol.009 P260-269 07/98	LV cavity imaging using [redacted]	Swine 2	0.125 Bolus	Reproduced EB-98-18's results.
EB-98-20 Vol.009 P270-282 05-06/98	LV cavity imaging using [redacted] with different imaging frame rate	Swine 3	0.125; 0.25 Bolus	Reproduced EB-98-18's results. Plus, increasing frame rate decreased videointensity signal.
EB-98-16 Vol.009 P283-292 05/98	Effects of pressure-pretreated AF0150 solution on Doppler signal	Rabbit 1	1.0 Bolus	External pressure application to reconstituted AF0150 tends to decrease the Doppler signal (both peak and persistence) with pressure level-dependence. At the high pressure (1090 mmHg), the peak Doppler signal significantly decreased.

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg)	Main Results
EB-98-22 Vol.009 P293-307 11-12/98	Effect hypertension on AF0150-induced Doppler signal and cardiac imaging	Rabbit 7 Swine 4	0.2; 1.0(rabbits); 0.25(Swine) Bolus	High arterial blood pressure and high left ventricle pressure induced by phenylephrine had no effect on Doppler signal and LV cavity image.
EB-98-15 Vol.009 P308-322 01-03/98	Effects of high O2 inhalation on AF0150-induced Doppler and cardiac imaging	Swine 4	0.125; 0.25 Bolus	Inhalation of high O2 (more than 50%) air decreases ultrasound signals (Doppler flow signals and echocardiographic images) induced by AF0150 without significant AF0150 dose-dependence.

Table 2. SAFETY PHARMACOLOGY STUDIES (NOAELs listed in Table 10)

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
Cardiovascular Safety					
EB-95-19 Vol009 P324-334 Oct/94; Jul/95	Effects on HR, MAP and blood cell. (anesthetized; carotid catheterization; up to 1-hr post dosing observation)	Rabbit 3 Control 24 AF0150	20 (52) IV bolus	No changes in HR and MAP. Transient decrease in platelet and WBC (lasting 30 minutes post dosing) Control study was not conducted concurrently with AF0150 treatment study.	No
EB-97-13 Vol. 009 p335-348 ND	Effects on Hemolysis, Hematology and Hemodynamics under ultrasound application, (anesthetized, up to 4-hour post dosing observation)	Dogs 4 males	20 (86) IV infusion (1mg/kg/min, 20 min)	No significant effects on heart rate and artery blood pressure; Hemolysis: <1%, no difference from pre dosing. Transient decrease in platelet (back to baseline within 4 hours) and WBC counts (back to baseline within 2 hours)	No
EB-95-25 Vol.009 P123-135 Oct/95	Dose-response of hemodynamic and echocardiography. (anesthetized, 1-min post dosing observation)	Dog 6	0.03, 0.09, 0.3, 0.6 (-2.6) IV bolus	No change on hemodynamic parameters (systemic artery pressure, pulmonary artery pressure, heart rate, cardiac output) in anesthetized dogs pre- and 1 minute post doing with sustained application of ultrasound power.	No
EB-95-27 Vol.009 P349-362 11/95-03/96	Effects on hemodynamics (HR, BP, CO, PAP), PO2 and Echocardiography. (anesthetized, up to 10-min post dosing observation)	Dog 4	0.4, 0.8, 1.2, 1.6 (-6.9) IV bolus	No significant changes in HR, BP, PAP and CO after AF0150 injection up to 10 min compared to baseline (20-25 min before dosing). Decrease in HR, BP and CO and increase in PAP at baseline with increase in AF0150 doses. No change on arterial PaO2 at pre-dosing and 3-5 min post dosing.	No

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
EB-98-05 Vol009 P363-374 ND	Effects of AF0150 on the Hemodynamics under adenosine-induced CV stress. (anesthetized, up to 1-hr post dosing observation)	Rabbit 4-5/group	2, 20 (5.2, 52) IV bolus	Adenosine decreased MAP (by 18%) and increased HR (by 30%). AF0150 had no significant effects on MAP and HR with the adenosine pretreatment. No effects on arterial blood gas (PaO2, PaCO2, pH, base excess). No changes on hematology (RBC, WBC, Hb, HCT, platelets) at 60 min post dosing.	No
EB-98-06 Vol.009 P375-389 ND	Effects of AF0150 on the Hemodynamics under Dipyridamole-induced CV stress (anesthetized, up to 1-hr post dosing observation)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	Dipyridamole decreased MAP by 18% and increased HR by 22% at 10 min post dosing. AF0150 had no effect on MAP, but transiently (20 min) potentiated tachycardia by 18% at both doses with dipyridamole pretreatment. No effects on arterial blood gas and blood cells.	No
EB-98-07 Vol.009 P390-398 12/97-01/98	Effects of AF0150 on the Hemodynamics under Arbutamine-induced CV stress (Anesthetized)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	Arbutamine increased HR by 32% (about 10 min) without change on MAP. AF0150 had no effects on HR and MAP in arbutamine-pretreated animals.	No
EB-98-08 Vol.009 P399-408 08/98	Effects of AF0150 on the Hemodynamics under Dobutamine-induced CV stress (Anesthetized)	Rabbit 4/group	2, 20 (5.2, 52) IV bolus	Dobutamine increased HR by 32% without effect on MAP. Coadministration of AF0150 had no effect on HR and MAP at 2 mg/kg but slightly increased HR and MAP at 20 mg/kg; No remarkable changes in blood gas and blood cells after dobutamine infusion alone and in combination with AF0150.	No

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
EB-98-13 Vol.009 P409-421 03-04/98	Effects of AF0150 on Pulmonary Artery Pressure in the U46619-induced Pulmonary Hypertension (anesthetized, up to 10-min post dosing observation)	Rabbit 11 3 control 4 mild 4 moderate	1, 4, 10 (2.6, 10.4, 26) IV bolus to all groups	No remarkable effects on mean systolic PAP, MAP and HR in normal rabbits and U46619-induced pulmonary hypertension (both mild and moderate). MAP baseline increased in both mild and moderate hypertensive rabbits. No remarkable effects on PaO2, PaCO2 and pHa in control and pulmonary hypertensive rabbits. However, base excess was 73% less in the mild hypertensive rabbits and 47% less in the moderate hypertensive rabbits at 10 minutes after 10 mg/kg AF0150.	No
BS-97-09 Vol.010 P016-028 05/97	Effect of AF0150 on the 99mTC-Sestamibi (MIBI) cardiac imaging (anesthetized, 30-min post dosing observation)	Rabbit 32 4 control 6 ischemia	0.5, 2.0 (1.3, 5.2)	No remarkable effects on HR and MAP in rabbits with normal myocardial perfusion and experimental ischemia in the presence or the absence of vasodilator dipyridamole before and after MIBI imaging. No changes on the area at risk, area of infact, MIBI tissue content and MIBI tissue washout in both normal and ischemic rabbits.	No
IMUS-016-TOX Vol.022 P182-286 Oct/95	Hemodynamics in conscious monkeys (non-anesthetized, up to 1-hr post dosing observation)	Cynomolgus monkeys 4/group	0, 10, 20, 40 (0-104) IV bolus, all doses/animal	No significant changes on hemodynamic parameters (SAP, SAP, MAP, PAP, LVP) from 2-60 min post dosing. However, PAP was highly variable, and tends to slightly increase as compared to the baseline (pre dosing). Arterial blood gas analysis was not observed in this study. ECG report was unremarkable, including QTc interval No changes on hematology	Yes
IMUS-035-TOX Vol.023 p001-197 Jan/98	Toxicity study with concurrent application of high power ultrasound (anesthetized, up to 24-hr post dosing observation)	Dogs Male 3/group	0, 20 (0, 86) IV infusion	AF0150 with high power ultrasound application over heart (closed-chest) had no significant effects on clinical signs, hematology, blood chemistry and pathology in dog during 24-hour observation. Increase in uncorrected QT intervals in AF0150-treated animals as compared to pre dosing. QTc seems not to be significantly different.	Yes

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
Neurological and Behavioral Toxicity					
PSM-98-01 Vol 009 P323-323 Sec/ 98	Neurotoxicity following intracarotid injection (abstract only)	Rat 6 (1 control, 5 AF0150)	0.125; 2.0; 4.0; 8.0; 16.0 (0.16-20)	No marketed signs of motor or proprioceptive deficits, ataxia, paresis, paralysis, head tilt, circling or seizures, and behavioral changes at 6 and 24 hours post does.	No
IMUS-042-TOX Vol.024 p001-358 Feb/99	acute toxicity following intra-arterial injection (right-to-left shunt evaluation) (Functional Observational Battery; Spontaneous Locomotor Activity). (8-day post dosing observation	Rats 5/sex/group	0, 4, 16 (0-20) carotid artery catheterization	Multifocal infarction and ischemic lesions in the kidneys, brain, testes and other organs in some animals from both control and AF0150 groups. Neurological signs were consistent with the brain ischemic pathology. AF0150-treated animals tended to have a slightly higher incidence of ischemic pathology than the saline control animals, particularly kidneys and brain infarction.	Yes
IMUS-043-TOX Vol.025 p001-038 Feb/99	Gross behavioral and physiological evaluation using "Primary Observation Test" (Irwin Test).(2.5-hr post dosing observation)	Rats Male 6/group	0, 4, 40, 100 (0-130) IV bolus	No significant gross behavioral or physiological effects. The positive control animals treated with chlorpromazine showed moderate to severe effects on behavior and physiology.	Yes
Renal Toxicity					
IMUS-044-TOX Vol.025 p038-080 Feb/99	Effects on Renal Function in Saline-Loaded Rats; with 24-hour post dose observation	Rats Male 8/group	0, 4, 40, 100 (0-130)	Significant decrease in urinary volume, pH, and urinary Na+, K+ and Cl- excretion at the first 3 hours post dosing at all dose levels (without dose-dependency). **NOAEL** <4 mg/kg (HED: 0.65 mg/kg; HDM: 5-fold PCD)	Yes
Gastrointestinal Toxicity					
IMUS-045-TOX Vol.025 p081-116 Feb/99	Effects on the Gastrointestinal Transit of a Charcoal Meal; with 30-minute observation	Rats Male 8/group	0, 4, 40, 100 (0-130)	No significant effects on gastrointestinal transit of charcoal meal. Positive control (morphine) completely inhibited charcoal emptying from stomach.	Yes

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
Others					
IMUS-046-TOX Vol.025 p117-156 March/99	Effects on Respiration Rate and Body Temperature; with 15-min post dose observation	Rats Male 8/group	0, 4, 40, 100 (0-130)	No significant effects on respiration rate. Slight decrease in core body temperature at 100 mg/kg at 15 minutes post dosing. **NOAEL** = 40 mg/kg (HED: 6.5 mg/kg; HDM: 52-fold PCD)	Yes

APPEARS THIS WAY
ON ORIGINAL

Table 3. TOXICOLOGY STUDIES (NOAELs listed in Table 11)

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
Single Dose Toxicity Studies					
IMUS-037-TOX Vol.010 p179-225 May-June/98	Acute Toxicity Single Dose, IV 14-day observation , termination on day 15	Mice 5/sex/group	0, 200, 400, 800, 1600 (0-1037)	All animal survived to sacrifice day; Transient hypoactivity and dyspnea in some mice at the highest dose. **NOAEL**= 800 mg/kg (HED: 65 mg/kg, HDM: 518-fold); Cecal inflammation in some mice at 400 and 1,600 mg/kg. **NOAEL**= 200 mg/kg (HED: 16 mg/kg; HDM: 259-fold).	Yes
IMUS-010-TOX Vol.010 p226-343 Jul-Aug/95	Acute Toxicity Single dose, IV 14-day observation, termination on day 15	Rats 5/sex/group	0, 200, 400, 800, 1600 (0-2073)	Transient reddening (about 1 hour) of lips, nose, ears, paw and tail. **NOAEL**= 400 mg/kg (HED: 65 mg/kg; HDM: 518-fold). Lower body weight gain at 800 mg/kg in males.	Yes
IMUS-011-TOX Vol.011 p001-276; Vol.012 p001-326 Aug/95	Expanded Acute Toxicity Single dose, IV 14-day observation, Termination on days 2, 8, and 15.	Rats 20/sex/group	0, 50, 200, 400 (0-518)	Vacuolated macrophages in the spleen and mesenteric lymph nodes at all dose groups in a dose-dependent and time-dependent manner. **NOAEL**< 50mg/kg (HED: <8mg/kg; HDM: <65). Slight decrease in platelets, neutrophils, RBC, Hb, HCT, serum total proteins and albumin; and slight increase in BUN, serum creatinine and inorganic phosphorous in some AF0150-treated rats without dose-/time-dependency.	Yes
IMUS-012-TOX Vol.013 p001-399 Aug/96	Expanded Acute Toxicity Single dose, IV 14-day observation, Termination on days 2, 8, and 15	Dogs 6/sex/group	0, 50, 100, 200 (0-864)	Transient (reversible) hemorrhage in liver and gallbladder of some animals at 200 mg/kg (AF0150 > Control) **NOAEL**=100 mg/kg (HED: 54 mg/kg; HDM: 433-fold PCD).	Yes

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
IMUS-039-TOX Vol.014, p001-333 Jun-Jul/98	Expanded Acute Toxicity Single dose, IV 14-day observation	Dogs 5/sex/group	0, 200, 400 (0-1731)	Transient and slight decrease in blood platelets at the dose of 400 mg/kg. **NOAEL**= 200 mg/kg (HED: 108 mg/kg; HDM: 865-fold)	Yes
Multiple Dose Toxicity Studies					
IMUS-013-TOX Vol.015 p001-305 Vol.016 p001-361 Aug-Oct/95	Repeated Dose Toxicity Daily dosing for 17 and 29 days; Termination on days 17, 30 and 44 (15-day recovery after 29-day dosing)	Rats 20/sex/group	0, 50, 200, 400 (0-518)	Macrophage vacuolation in multiple tissues at all dose levels on day 30 and at the end of 15-day recovery period. No NOAEL was established. Eosinophil infiltration in mesenteric lymph nodes and perivascular area in the lungs, with decrease after recovery period. Increase in extramedullary hematopoiesis in the spleen, with slight increase after recovery period. Decreases in blood creatinine, total protein, globulin, AST, ALT, alkaline phosphatase on Day 30.ALT remained decreased after 15-day recovery. **NOAEL** (for all effects)= 50 mg/kg/day (HED: 310 mg/kg/day; HDM: 65-fold).	Yes
IMUS-027-TOX Vol.017 p001-055 June/1995	Repeated Dose Toxicity Daily dosing for 7days; No recovery period	Dogs 3/sex/group	10, No control (43)	Study design was not adequate no control group and no pre-dosing observations.	No

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
IMUS-014-TOX Vol.017 P056-055 Vol.018 P001-269 Sept-Oct/95	Repeated Dose Toxicity Daily dosing for 14 and 28 days; (Termination on days 14, 28 and 42 with 14-day recovery after 28-day dosing)	Dogs 8/sex/group	0, 25, 50, 100 (0-433)	Transient and reversible toxic sings (pale mucous membranes, uncoordinated, hypoactive behavior and vomits), changes on blood chemistry (lower serum protein and cholesterol), and on blood pressure (decreased at 100 mg/kg/day and increased at 50 mg/kg/day). No remarkable macro- and microscopic findings; and no vacuolated macrophages. No effects on cecum. **NOAEL**= 25 mg/kg/day (HED: 14 mg/kg/day; HDM: 108-fold PCD)	Yes
Pharmacokinetics/Toxicokinetics					
IMUS-041-TOX Vol.032 p080-179 Dec-Feb/99	PFH Elimination from Expired Air and Blood	Rats 7/sex (Expired Air) 5/sex (Blood)	20 (26)	PFH elimination from expired air and blood fitted to a two-compartment model. About 90% of the administrated PFH were excreted from lung within first 3 hours post dosing and almost completely eliminated within 48 hours. Blood PFH level decreased by 78% at the first 2 minutes as compared to PFH levels at time 0, and was non-detectable by 24 hours. The terminal elimination half-life of blood PFH was about 88 minutes (based on the pooled data but not individual calculation).	Yes

Table 4. SPECIAL TOXICOLOGY STUDIES

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
Local Tolerance					
IMUS-028-TOX Vol.018 p270-336 Feb/97	local irritation with a single intravenous, perivenous, intra-arterial injection (1, 4, 15 days post dosing)	Rabbits 3/sex/group	0, 2, 20 (0-52)	Perivenous, but not intravenous or intra-arterial, injection resulted in slight local irritation (erythema), mostly seen at 4 hours post injecting. **NOAEL** < 2 mg/kg (HED: 0.65 mg/kg, HDM: 5-fold)	Yes
IMUS-038-TOX Vol.018 p337-392 May-Jun/98	local tolerance with a single intramuscular injection (1, 4, 8, 15 days post dosing)	Rabbits 3/timepoint	0, 1 (0-2.6)	No significant local irritation	Yes
Immunotoxicology					
IMUS-021-TOX Vol.019 p001-051 Nov/96	Antigenicity Study: Active Systemic Anaphylaxis (ASA); Passive Cutaneous Anaphylaxis (PCA)	Guinea Pigs 5 male/group	20 (for IV) 2 (for SC) ±FCA (35)	No significant active systemic anaphylaxis and passive cutaneous anaphylaxis. One of 5 animals given AF0150 (not with FCA) had retching, which was not associated with clinical sign, lack of similar reaction in the other treated animals and negative PCA reaction. **NOAEL**= 20 mg/kg	Yes
IMUS-029-TOX Vol.019 p052-129 Jan-Feb/97	Dermal Sensitization	Guinea pig 20-AF0150 10-Control 4-Screen	20 mg/ml to saturate filter papers with and without FCA	No significant dermal reactions using the guinea pig maximization test (Magnusson and Kligman Assay). No concurrent positive control (positive control study was conducted separately at different time).	Yes
CMB-96-14 Vol.101 P010-015 March-Jun/96	TNF-α production by blood cells *in vitro*	Human Rat blood	0.5 mg/ml	AF0150 did not increase plasma TNF level when incubated with rat whole blood (data from human blood incubation with AF0150 were not detected). Positive controls (LPS and zymosan) greatly increased TNF levels in both rat and human blood.	No

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg) (HDM)	Main Results	GLP
BC-95-17-Vol.010 P001-009 ND	*In vitro* Activation of Complement (C3) (Amended)	Human plasma	0.5 mg/ml	AF0150 slightly increased C3a (507±295 ng/ml) when incubated *in vitro* with human plasma, as compared to saline (151±26mg/ml). The difference was not statistically significant due to large SD.	No
Single Dose Acute Toxicity Studies with Cardiac Stress					
IMUS-018-TOX Vol.019 p130-384 Oct/96	Acute toxicity study with adenosine pretreatment. 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	AF0150 had no significant toxicity in the adenosine-pretreated rabbits. However, adenosine treatment alone did not induce any pharmacological effects.	Yes
IMUS-019-TOX Vol.020 p001-257 Oc/96	Acute toxicity study with dipyridamole pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	AF0150 had no significant toxicity in the dipyridamole-pretreated rabbits. Dipyridamole treatment alone did not induce any pharmacological effects.	Yes
IMUS-036-TOX Vol.022 p001-181 March/98	Acute toxicity study with arbutamine pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	AF0150 had no significant toxicity in the Arbutamine-pretreated rabbits; Arbutamine treatment alone did not induce any pharmacological effects.	Yes
IMUS-020-TOX Vol.021 p001-257 Oct-Nov/96	Acute toxicity study with dobutamine pretreatment, 14-day observation	Rabbits 5/sex/group	0, 2, 20 (0-52) Single dose IV bolus	AF0150 had no significant toxicity in the Dobutamine-pretreated rabbits. Dobutamine treatment alone did not induce any pharmacological effects.	Yes
Microbubble Size Profiles					
AC-00-08 Fax Submission April/2000	Bubble Size and Distribution at 0, 30 and 60 minutes Post-reconstitution	N/A	N/A	No significant changes on sizes (3-10 um, >10um, total) and corresponding counts of AF0150 microbubbles at 30 and 60 min post reconstitution as compared to right after reconstitution, except that total counts/ml at 60 minutes post reconstitution statistically decreased.	No
Dosage Conversion Fax Submission April/2000	Conversion of AF0150 dose from mg/kg to bubbles/kg	N/A	N/A	Conversion of mg/kg in all GLP studies to bubbles/kg by multiplying 4.9x108/mg AF0150.	N/A

Table 7. REPRODUCTIVE TOXICITY STUDIES (NOAELs listed in Table 12)

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg/day) (HDM)	Main Results	GLP
IMUS-022-TOX Vol.025, p170-279; Vol.026 p001-328 Jan, 1999	Fertility and early embryonic development (Segment I)	Rats 25/Sex	0, 50, 100, 200 (0, 65, 123, 260) IV Injection at 2 (female) and 4 (males) weeks pre-mating till gestation day 7; Sacrifice on gestation day 13	No changes on reproductive performance (mating index, fertility index, pre-coital interval, and female estrous cycle) and male spermatogenesis (testicular and epididymal sperm counts, sperm production rate, sperm motility and morphology). Female necropsy on gestation day 13 showed that AF0150 had no significant effects on pre- and post-implantation losses, number of viable embryos, implantation sites and corpora lutea. No maternal toxicity was observed in the 200 mg/kg/day group.	Yes
IMUS-023-TOX Vol.027 p001-350 Sept, 1998	Teratology (Segment II)	Rats 25 Female	0, 50, 100, 200 (0, 65, 123, 260) IV injection on gestation day 6-17; Sacrifice on gestation day 20	No significant changes on fetal development, viability, body weights, sex ratio, corpora lutea number, implantation sites, postimplantation loss; No external, visceral and skeletal malformations or variations. No maternal toxicity was observed in the 200 mg/kg/day group.	Yes
IMUS-024-TOX Vol.031 p001-337 Sept. 1998	Teratology (Segment II)	Rabbits 22 Female	0, 50, 100, 200 (0, 130, 260, 518) IV injection on gestation day 7-20; Sacrifice on gestation day 29	Slight increase in fetal malformation incidence in the high dose groups, particularly in the 200 mg/kg/day group. Malformations included external, visceral (soft tissue) and skeletal anomalies. The **NOAEL**: 50 mg/kg/day (130-fold HDM) One animal had spontaneous abortion in the 50 mg/kg/day group on gestation day 27. No changes on post-implantation loss, live litter size, mean fetal body weights, fetal sex ratios, fetal developmental variation, the mean number of corpora lutea and implantation sites. No maternal toxicity (clinical signs, body weigh, food consumption, necropsy). **NOAEL**= 200mg/kg/day.	Yes

Report # Vol # Page # Study Date	Study Type	Species (no.)	Dose (mg/kg/day) (HDM)	Main Results	GLP
IMUS-025-TOX Vol.028 p001-389; Vol.029 p001-449; Vol.030 p001-434 Sept. 1998	Pre- & Postnatal Development (Segment III)	Rats 25 Females (F0) 25/Sex Offspring (F1) for assessing developm ent	0, 50, 100, 200 (0, 65, 123, 260) IV Injection at gestation day 6 to lactation 20; Sacrifice on lactation day 21. No treatment on offspring.	Decrease in the live birth index in the 200 mg/kg/day group ($p<0.05$ vs. control). Tendency for a decrease in the live litter size and the gestation survival index with a slight increase in stillbirth and total postnatal death (during PND 0-21) in the 200 mg/kg/day group. The **NOAEL** for neonate toxicity: 100 mg/kg/day. No significant difference in the offspring's development (physical, physiology, reproductive performance and fertility) between AF0150-treated and control animals.	Yes

APPEARS THIS WAY
ON ORIGINAL

Table 8. GENETIC TOXICOLOGY

Report # Vol # Page # Study Date	Study Type	Species (no.)	AF0150 Dose	Main Results	GLP
IMUS-015-TOX Vol.031 p338-378 Sept-Nov/1995	Bacterial Reverse Mutation Test (Ame's)	TA98, TA100, TA1535 TA1537 WP2uvrA	0, 1-20 mg/plate (with/without S9)	No significant increase in bacterial reverse mutation in all bacterial strains (cover C-G and A-T point mutation). Positive controls had significant increase in reverse mutation in all bacterial strains. No evidence of significant cytotoxicity at 20 mg/plate in terms of changes on revertant numbers or bacterial background lawn, suggesting that the dose selection may not be sufficient.	Yes
IMUS-031-TOX Vol.032 p001-028 Aug-Sept/1997	Chromosomal Aberration Assay *in vitro*	Human whole Blood culture (lymphocyte)	0, 2-5 mg/ml (with/without S9)	No significant increases in chromosomal aberrations, polypoidy, or endoreduplication in the presence or absence of S9 metabolic activation. Positive controls (mitomycin and cyclophosphamide) significantly increased chromosomal aberrations. Mitotic index decreased by 47% in the 5 mg/ml group.	Yes
IMUS-030-TOX Vol.032 p029-051 Aug/1997	Micronucleus Assay *in vivo*	Mouse 6/sex/group	200-800 mg/kg (-518-fold PCD) Single dose, IV	No significant difference in micronucleated PCEs between AF0150- and saline-treated mice, and no change on PCE:NCE ratio. Positive control (cyclophosphamide) significantly increased micronucleated PCEs in both sexes.	Yes
IMUS-032-TOX Vol.032 p052-079 Aug-Sept/1997	Forward Mutation Assay *in vitro*	L5178Y TK+/- Mouse Lymphoma cell line	0, 1-5 mg/ml (with/without S9)	No significant increase in mutant frequency in the presence and absence of S9 activation. AF0150 at all doses decreased cell growth rate; at 4 and 5 mg/ml the cell growth inhibition by more than 50%. Positive controls (methyl-methanesulfonate and methylcholanthrene) significantly increased mutant frequency by more than 5-fold.	Yes

***Table 9.* Effects of AF0150 on Hemodynamics in Rabbits Pretreated with Cardiovascular Stress Agents**

CV Stress Agents	Pharm Action	Stress Dose*	Stress Responses		AF0150 Treatment			
			MAP	HR	Dose (mg/kg)	Safety Pharm†		Acute Toxicity‡
						MAP/HR	Blood Gas	
Adenosine	Coronary vasodilation ➡Coronary Steal ➡Myocardial ischemia	370	↓18%	↑30%	2, 20	No change	No change	UR
Dipyridamole	Coronary vasodilation ➡Coronary Steal ➡Myocardial ischemia	142	↓18%	↑22%	2, 20	↑ HR# by 18%	No change	UR
Arbutamine	Cardiac positive inotropism (stimulates α- and β-ARs)	0.1	-	↑32%	2, 20	No change	No change	UR
Dobutamine	Cardiac positive inotropism (Stimulates α- and β-ARs)	5,10,20,30,40	-	↑32%	2, 20	No change	No change	UR

MAP: mean arterial blood pressure; HR: heart rate; UR: unremarkable; ↓: decrease and ↑: increase

* IV infusion of CV stress agents at ug/kg/min

† Safety Pharm studies were non-GLP conducted in the anesthetized rabbits (4/group). Animals received each of CV stress agents by IV infusion and, when hemodynamics reached steady-state, were given AF0150 at 2 or 20 mg/kg by IV bolus. (See reviews in Safety Pharmacology Studies section for detail).

‡ Acute Toxicity was determined by single dose GLP studies conducted in rabbits (5/group) treated with AF0150 (2 or 20mg/kg) immediately following IV infusion of adenosine (840ug/kg) or dipyridamole (568ug/kg) or dobutamine (380ug/kg) or arbutamine (0.5ug/kg). The animals were observed for 14 days for clinical signs, hematology, blood chemistry, necropsy and histopathology (heart only). (See reviews in Special Toxicology Studies section for detail).

\# Transiently increased dipyridamole-induced tachycardia at both AF0150 doses. In separate studies propanolol (-adrenergic antagonist) prevented and aminophylline (-adrenergic stimulation) reversed the AF0150-induced tachycardia.

Table 10. NOAEL of AF0150 from Safety Pharmacology Studies

Report No.	Study Type	Species	Dose (mg/kg)	NOAEL (mg/kg)			HDM[b] (Fold)
				Adverse Effects above NOAEL	Animal	HED[a]	
Cardiovascular Safety Studies							
EB-95-19	HR, MAP and blood cell.	Rabbit	20	No significant effects on HR and MAP	20	6.5	52
				Transient decrease in platelet and WBC (lasting 30 minutes post dosing)	<20	<6.5	<52
EB-97-13	Hemolysis, Hematology and Hemodynamics with ultrasound application	Dogs	20	No significant effects on HR and MAP	20	11	86
				Transient decrease in platelet (4 hours) and WBC counts (2 hours)	<20	<11	<86
EB-95-25	Dose-response of hemodynamic and echocardiography.	Dog	0.03, 0.09, 0.3, 0.6	No change on ABP, PAP, HR, CO in anesthetized dogs pre- and 1 min post doing with sustained application of ultrasound power.	0.6	0.3	2.6
EB-95-27	Hemodynamics (HR, BP, CO, PAP), PO2 and Echocardiography.	Dog	0.4, 0.8, 1.2, 1.6	No significant changes on HR, BP, PAP and CO. Decrease in HR, BP and CO and increase in PAP at the baseline with AF0150 dose-dependence. No change on arterial PaO2	1.6	0.9	6.9
EB-98-05	Hemodynamics under adenosine-induced CV stress.	Rabbit	2, 20	No significant effects on HR, MAP, arterial blood gas (PaO2, PaCO2, pH, base excess) and hematology (RBC, WBC, Hb, HCT, platelets)	20	6.5	52
EB-98-06	Hemodynamics under Dipyridamole-induced CV stress	Rabbit	2, 20	No significant effect on MAP, No effects on arterial blood gas and blood cells.	20	6.5	52
				Transiently (20 min)-potentiated tachycardia by 18% at both doses with dipyridamole pretreatment.	<2	0.6	<5.2
EB-98-07	Hemodynamics under Arbutamine-induced CV stress	Rabbit	2, 20	No significant effects on HR and MAP in arbutamine-pretreated animals.	20	6.5	52
EB-98-08	Hemodynamics under Dobutamine-induced CV stress	Rabbit	2, 20	Slight increase in HR and MAP at 20 mg/kg	2	0.65	5.2
				No remarkable changes in blood gas and blood cells.	20	6.5	52

Report No.	Study Type	Species	Dose (mg/kg)	NOAEL (mg/kg)			HDM[b] (Fold)
				Adverse Effects above NOAEL	Animal	HED[a]	
EB-98-13	Pulmonary Artery Pressure in the U46619-induced Pulmonary Hypertension	Rabbit	1, 4, 10	No significant effects on PAP, MAP and HR in normal rabbits and U46619-induced pulmonary hypertension; No remarkable effects on PaO2, PaCO2 and pH.	10	3.2	26
				Base excess was 73% less in the mild hypertensive rabbits and 47% less in the moderate hypertensive rabbits at 10 minutes after 10 mg/kg AF0150.	4	1.3	10.4
BS-97-09	Effect on myocardial ischemia and 99mTC-Sestamibi (MIBI) cardiac imaging	Rabbits	0.5, 2.0	No significant effects on HR and MAP in rabbits with normal myocardial perfusion and experimental ischemia in the presence or the absence of vasodilator dipyridamole before and after MIBI imaging. No changes on the area at risk, area of infact, MIBI tissue content and MIBI tissue washout in both normal and ischemic rabbits.	2	0.65	5.5
IMUS-016-TOX	Hemodynamics in conscious monkeys	Monkeys	0, 10, 20, 40	No significant effects on SAP, SAP, MAP, PAP, LVP and PAP (highly variable). ECG report was unremarkable, including QTc interval; No changes on hematology. Arterial blood gas analysis was not done.	40	13	104
IMUS-035-TOX	Toxicity with concurrent high power ultrasound application	Dogs	0, 20	Increase in uncorrected QT intervals. QTc seems no significantly different.	<20	<10.8	<86
				No significant effects on clinical signs, hematology, blood chemistry and pathology with high power ultrasound application over heart (closed-chest).	20	10.8	86
Neurological and Behavioral Toxicity							
PSM-98-01	Neurotoxicity following intracarotid injection (abstract only)	Rats	0.125, 2, 4, 8, 16	No marketable signs of motor or proprioceptive deficits, ataxia, paresis, paralysis, head tilt, circling or seizures, and behavioral changes in 6 hours and after overnight.	16	2.6	20

Report No.	Study Type	Species	Dose (mg/kg)	NOAEL (mg/kg)			HDM[b] (Fold)
				Adverse Effects above NOAEL	Animal	HED[a]	
IMUS-042-TOX	Acute neurotoxicity following intra-arterial injection Observational Battery; Spontaneous Locomotor Activity). (8-day post dosing observation	Rats	0, 4, 16 carotid artery catheterization	Multifocal infarction and ischemic lesions in the kidneys, brain, testes and other organs in some animals from both control and AF0150 groups. Neurological signs were consistent with the brain ischemic pathology. AF0150-treated animals tend to have a slight higher incidence of ischemic pathology than the saline control animals, particularly renal and brain infarction.	<4	<0.65	<5
IMUS-043-TOX	Gross behavioral and physiological effects (Irwin Test). (2.5-hr post dosing observation)	Rats	0, 4, 40, 100	No significant gross behavioral or physiological effects.	100	16.2	130
Renal Toxicity							
IMUS-044-TOX	Renal Function in Saline-Loaded Rats; with 24-hour post dose observation	Rats	0, 4, 40, 100	Significant decrease in urinary volume, pH, and urinary Na+, K+ and Cl- excretion at the first 3 hours post dosing at all dose levels (without no dose-dependence).	<4	0.65	5
Gastrointestinal Toxicity							
IMUS-045-TOX	Gastrointestinal Transit of a Charcoal Meal; with 30-minute observation	Rats	0, 4, 40, 100	No significant effects on gastrointestinal transit of charcoal meal.	100	16.2	130
Others							
IMUS-046-TOX	Respiration Rate and Body Temperature; with 15-min post dose observation	Rats	0, 4, 40, 100	Slight decrease in core body temperature at 100 mg/kg at 15 minutes post dosing.	40	6.5	52
				No significant effects on respiration rate	100	16.2	130

a. HED: Human Equivalent Dose (mg/kg) based on body surface area conversion
b. HDM: Human Dose Multiple (Fold) based on Planned Clinical Dose (PCD) of 0.125 mg/kg and body surface area conversion

Table 11. NOAEL of AF0150 from Toxicity Studies

Report No.	Study Type	Species	Adverse Effects	NOAEL (mg/kg)	HED[a] (mg/kg)	HDM[b] (Fold)
IMUS-037-TOX	Standard Acute Toxicity	Mice	Transient hypoactivity and dyspnea	800	65	518
			Cecal inflammation	200	16	130
IMUS-010-TOX	Standard Acute Toxicity	Rats	Transient (1-hr) reddening of the lips, nose, ears, paw and tail.	400	65	518
IMUS-011-TOX	Expanded Acute Toxicity	Rats	Vacuolated macrophages (irreversible) at all dose levels in spleen and lymph notes, etc.	<50	<8	<65
IMUS-012-TOX	Expanded Acute Toxicity	Dogs	Transient liver and gallbladder hemorrhage in some animals (AF0150>Control) at 200mg/kg.	No data from doses of 50 and 100 mg/kg		
IMUS-039-TOX	Expanded Acute Toxicity	Dogs	Transient and slight decrease in blood platelets	200	108	866
IMUS-013-TOX	Multiple Dose 29-day daily and 15-day recovery	Rats	Vacuolated macrophage in multiple tissues at all dose levels	<50	<8	<65
			Eosinophil infiltration in mesenteric lymph nodes and perivascular area in the lungs; Increase in extramedullar hematopoiesis in the spleen.	50	8	65
			Decreases in blood creatinine, total protein, globulin, AST, ALT, alkaline phosphatase on day 30; ALT remained decreased after a 15-day recovery period.	50	8	65
IMUS-014-TOX	Multiple Dose 28-day daily and 15-day recovery	Dogs	Transient and reversible toxic sings (pale mucous membrane, uncoordinated, hypoactive behavior and vomiting), changes on blood chemistry (lower serum protein and cholesterol), and on blood pressure	25	14	108

a. HED: Human Equivalent Dose (mg/kg) based on body surface area conversion
b. HDM: Human Dose Multiple (Fold) based on Planned Clinical Dose (PCD) of 0.125 mg/kg and body surface area conversion

***Table 12.* NOAEL of AF0150 from Reproductive Toxicity Studies**

Report No.	Study Type	Species	Dose	Adverse Effects	NOAEL (mg/kg/day)	HED[a] (mg/kg/day)	HDM[b] (Fold)
IMUS-022 -TOX	Segment I Fertility and early embryonic development	Rats	0, 50, 100, 200 mg/kg/day	↓ Male Fertility Index	100	16	130
				↓ Mean epididymal sperm number	100	16	130
				No tox in female at all doses	200	32	260
				No maternal Toxicity	200	32	260
IMUS-023 -TOX	Segment II Teratogenicity	Rats	0, 50, 100, 200 mg/kg/day	Not remarkable at any dose	200	32	260
				No maternal toxicity	200	32	260
IMUS-024 -TOX	Segment II Teratogenicity	Rabbits	0, 50, 100, 200 mg/kg/day	External malformations (microphthalmia, spina bifida, mandibular agnathia, astomia and an open left eyelid).	50	16	130
				Visceral malformations (hydrocephaly, increased cavitation of lateral ventricles and 3rd ventricles)	100	32	260
				Skeletal malformations (vertebral/rib anomalies, fused sternebrae)	100	32	260
				No maternal toxicity	200	64	520
IMUS-025 -TOX	Segment III Pre-/post-natal development	Rats	0, 50, 100, 200 mg/kg/day	Neonatal toxicity (↓live birth index, ↓ live little size; ↓ gestation survival index; ↑ stillbirth and postnatal death)	100	16	130
				No effects on offspring development (physical, physiological, reproductive performance and fertility)	200	32	260
				No maternal toxicity	200	32	260

c. HED: Human Equivalent Dose based on body surface area conversion
d. HDM: Human Dose Multiple based on Planned Clinical Dose (PCD) of 0.125 mg/kg and body surface area conversion

34. RECOMMENDATION

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

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

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

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

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

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

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

5. Significant renal toxicity of AF0150 was noted in a rat study with saline volume challenge test (Study #IMUS-044-TOX). The NOAEL was less than 4 mg/kg, which is converted to

human equivalent dose of <0.65 mg/kg and human dose multiple of <5 folds. The sponsor needs, **before approval**, to address this issue by correlating this finding with renal observations in clinical trials, particularly in those patients with decreased renal function.

6. AF0150 was studied in animals given pharmacological stress agents, but not administrated a physical stress test (such as treadmill). Neither stress test with AF0150 were evaluated in humans Since it is conceivable that AF0150 will be used with either pharmacological or physical stress tests in a clinical setting, the sponsor needs to comment on this.

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

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

The sponsor's Version:

Table 1. Microbubble Parameters	
Size:	Draft
Number:	

Recommended revision:

Table 1. Microbubble Parameters

Size (*Median diameter:* *μm*)	Number/ml (% of total)
All sizes	5.9-13.7 x 10^8 (100%)
< 3 μm	x 10^8 (%)
3- 10 μm	x 10^8 (%)
> 10 μm	x 10^8 (%)

The blanks need to be filled by the sponsor and confirmed by the chemist.

36. Investigator's Brochure/Informed Consent Review:

37. Signatures

Reviewer: /S/ July 25, 2000

Jin Chen, MD, PhD
Pharmacologist

Date

Team Leader: /S/ 8/8/00

Nakissa Sadrieh, PhD
Pharmacologist

Date

please refer to team leader memo for synopsis

37. CC: list

Original NDA
HFD-160/Division Files
HFD-160/Love/Loewke/Jones/Parker/Salazar/Sadrieh/Chen

38. Appendix: none

39. Draft Date:	**Data Review**	**April 20, 2000**
	Labeling Review	**July 20, 2000**

40. Addendum:

NOT APPLICABLE

NOT APPLICABLE