AI-700 (Perflubutane Polymer Microspheres) For Injectable Suspension Imaging Agent for the Detection of Coronary Artery Disease (Imagify™)

BRIEFING DOCUMENT

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
^{99m}Tc	technetium-99m
ACC	American College of Cardiology
AE	adverse event
AHA	American Heart Association
ANC	absolute neutrophil count
ANGIO	coronary angiogram or coronary angiography
aPTT	activated partial thromboplastin time
ASE	American Society of Echocardiography
AUC	area under the curve
AWQ	acoustic window quality
BARI	Bypass Angioplasty Research Investigation
BMI	body mass index
BNA	brief neurological assessment
bolus	intravenous injection ≤10 minutes duration
BP	blood pressure
bpm	beats per minute
CAD	coronary artery disease
CABG	coronary artery bypass graft
CHF	congestive heart failure
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	C-Reactive Protein
CSR	clinical study report
DIPY	dipyridamole
DMO	data management organization
DBP	diastolic blood pressure
ECG	electrocardiogram
ECHO	echocardiogram or echocardiography
FDA	US Food and Drug Administration
FEV_1	forced expiratory volume in one second
GJS	global jeopardy score
HD	human dose
HR	heart rate
hr	hour(s)
ICANL	Intersocietal Commission for the Accreditation of Nuclear Medicine Laboratories
IND	Investigational New Drug Application
IV	intravenous

Abbreviation	Definition
kg	kilogram(s)
LV	left ventricle
LVG	left ventriculography
LVO	left ventricular opacification
MCE	myocardial contrast enhancement
MCS	minimum criteria for success
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
MI	myocardial infarction
min	minutes
MITT	Modified Intent-to-Treat
mL	milliliter(s)
mmHg	millimeters of mercury
mROC	multi-reader receiver operating characteristic
ms	milliseconds
n	number of patients
NDA	New Drug Application
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NSTEMI	non-ST elevation myocardial infarction
OR	odds ratio
PAC	premature atrial contraction
PAP	pulmonary artery pressure
PBO	placebo
PD	pharmacodynamic
PH	pulmonary hypertension
PIM	pulmonary intravascular macrophages
PK	pharmacokinetic
PR	PR interval (on ECG)
PVC	premature ventricular contraction
QRS	QRS complex interval (on ECG)
QT	QT interval (on ECG)
QTcB	QT interval corrected using Bazett's formula
QTcF	QT interval corrected using Fridericia's formula
RCE	rest contrast echocardiogram
RES	reticuloendothelial system
RR	relative risk
RWMA	regional wall motion abnormality

Abbreviation	Definition
SAE	serious adverse event
SaO_2	arterial oxygen saturation
SAP	statistical analysis plan
SBP	systolic blood pressure
SCE	stress contrast echocardiogram
SD	standard deviation
sestamibi	technetium-99m labeled sestamibi
SPECT	single-photon emission computed tomography
US-ECG	electrocardiogram recorded by ultrasound machine
WBC	white blood cell
WMA	wall motion abnormality

1. EXECUTIVE SUMMARY

1.1 Overview

This document summarizes the available data regarding the effects of AI-700 (Imagify™), an echocardiographic imaging agent for the detection of coronary artery disease (CAD). Acusphere Inc submitted a US New Drug Application (NDA) for AI-700 to the US Food and Drug Administration (FDA) in April 2008 (NDA 22-349).

AI-700 is a lyophilized preparation of porous, synthetic, biodegradable polymer microspheres containing perflubutane gas. The microspheres are composed of poly-(D,L-lactide-co-glycolide) [PLGA] and a phospholipid. AI-700 microspheres are engineered to have a mechanically strong polymer structure[1] and to persist for several minutes in the blood. The gas–filled microspheres are effective reflectors of ultrasound energy and enhance ultrasound images of any structure containing blood.

Studies have demonstrated that AI-700 can withstand the force of ultrasound imaging, thus offering the longer visualization times to assess both myocardial perfusion and wall motion.[1] Myocardial perfusion and myocardial wall motion imaging are essential for the non-invasive diagnosis of coronary artery disease (CAD).[2-4] In ischemic heart disease, decreased relative blood flow (primarily during stress), caused by coronary stenoses, results in detectable myocardial perfusion abnormalities.[5] Wall motion abnormalities (WMAs) arise as CAD progresses and produces functional impairment of myocardial tissue. Therefore, myocardial perfusion abnormalities are an earlier marker for CAD.[5]

Conventional stress echocardiography (ECHO) relies on detecting WMAs for the diagnosis of CAD. This method requires the patient to have an adequate acoustic window (i.e., a body habitus that allows ECHO imaging of the heart).[6] Without adequate acoustic window quality (AWQ), images may be difficult or impossible to evaluate. Ultrasound contrast agents are known to improve ECHO image quality[7] and are currently approved only for the visualization of the endocardial border at rest,[8, 9] which is useful in detecting WMAs.[10] These ultrasound contrast agents are microbubbles of gas encapsulated in thin shells that are vulnerable to destruction during ultrasound imaging and consequently do not provide reliable simultaneous real-time imaging of both perfusion and wall motion.[2, 3, 11-13]

1.2 Product Overview

AI-700 is an echocardiographic imaging agent for evaluation of myocardial perfusion and wall motion during rest and stress. AI-700 is intended for use in stable chest pain patients as a screening tool to stratify patients for referral to coronary angiography (ANGIO). AI-700 has the following proposed indication:

Imagify (AI-700) *is an ultrasound imaging agent indicated for patients with stable chest pain being evaluated for inducible ischemia for the detection of coronary artery disease based on an assessment of myocardial perfusion and wall motion. Imagify echocardiography is accomplished with rest and stress techniques.*

1.3 Clinical Development Program

The NDA summarizes data obtained from 11 studies that included all 1,241 subjects who received either AI-700 or placebo (Table 1). Of these, 1,194 were subjects who received any amount of AI-700. All human exposure to AI-700 was performed under the Investigational New Drug Application (IND 57,288). A total of 911 (76.3%) of 1,194 subjects were patients with suspected CAD and recent history of chest pain who were to receive the intended total dose of 0.080 milliliters/kilogram (mL/kg) AI-700 administered as 2 intravenous (IV) injections (each of 0.040 mL/kg) during same-day rest and dipyridamole stress ECHO imaging sessions (pilot Phase 3 Study AI-700-23 and pivotal Phase 3 Studies AI-700-32 and AI-700-33).

Table 1: Overview of AI-700 Clinical Studies

Study Number	Study Phase	Design	Study Population N = 1241[a] (AI-700/placebo) (1194/47)	Location Number of Sites (n)
AI-700-01	1	Single-blind, placebo-controlled, parallel-group safety study of rising single IV doses	n = 48; Healthy (33/15)	US Single Center
AI-700-02	1	Single-blind, placebo-controlled, parallel-group safety & myocardial imaging study of rising single IV doses	n = 28; Healthy (20/8)	US Single Center
AI-700-04	1	Single-blind, placebo-controlled, PK & safety study with rechallenge	n = 12; Healthy (10/2)	US Single Center
AI-700-05	1	Double blind, placebo-controlled, crossover safety & PK study	n = 18; CHF (8) & Moderate COPD (10) (18/0)	US Multicenter (3)
AI-700-06	1	Randomized, single-blind, active-control safety study	n = 10; Healthy (10/0)	US Single Center
AI-700-20	2	2-stage paired comparison, safety & ECHO imaging study of a single IV dose. Truth was confirmed MI or clinical history of no CAD. Placebo control was pre-contrast imaging	n = 53; Healthy (18) & post-MI CAD pts (35) (53/0)	US Multicenter (4)
AI-700-21	2	Placebo-controlled, 3-stage, safety & ECHO imaging study. Placebo control was cohort of non-contrast patients who received normal saline.	n = 122; Healthy (25) & stable chest pain CAD pts (75) (100/22)	US Multicenter (9)
AI-700-23	3	Pilot, open-label, dual-injection (rest/stress) safety study	n=133; stable chest pain CAD pts (123/0)	International Multicenter (35)
AI-700-32	3	Pivotal, open-label, dual-injection (rest/stress), safety & ECHO imaging study. Non-inferiority analysis of ECHO to SPECT.	n = 321; stable chest pain CAD pts (321/0)	International Multicenter (17)
AI-700-33	3	Pivotal, open-label, dual-injection (rest/stress), safety & ECHO imaging study. Non-inferiority analysis of ECHO to SPECT.	n = 457; stable chest pain CAD pts (457/0)	International Multicenter (11)
AI-700-34	3	Exploratory, open-label, dual-injection, ECHO imaging settings & safety study	n = 39; Healthy (14) & Stable Cardiac pts (25) (39/0)	US & UK Multicenter (4)

[a] Population total includes all subjects and patients enrolled in Phase 1, Phase 2, and Phase 3 studies.
CAD = coronary artery disease, ECHO = echocardiography, Healthy = healthy volunteers, IV = intravenous, MI = myocardial infarction, pts = patients, SPECT = single photon emission computed tomography.

1.4 Clinical Pharmacology

In nonclinical studies, AI-700 microspheres were taken up by macrophages of the reticuloendothelial system (RES). Microspheres remained within RES macrophages until the polymer was hydrolyzed into water-soluble fragments and metabolized.[14] In humans, perflubutane gas was rapidly cleared (half-life <4 minutes) from the vascular space (Study AI-700-04).

The dose of AI-700 required to produce effective myocardial contrast enhancement (MCE) is patient- and imaging technique-dependent. In pre-Phase 3 clinical studies, doses as low as 0.014 mL/kg AI-700 produced prolonged MCE (>5 minutes).[15] Pivotal Phase 3 studies AI-700-32 and -33 demonstrated that the dose of 0.040 mL/kg is an adequate dose to perform a diagnostic stress ECHO with AI-700.

1.5 Efficacy Summary

1.5.1 Overview of Efficacy Studies

All 4 efficacy studies (Studies AI-700-20, -21, -32, and -33) included adults with stable chest pain who were clinically indicated for diagnostic imaging of myocardial perfusion and/or wall motion. Primary efficacy analyses involved comparison of independent blinded results of AI-700 ECHO imaging (3 readers) to an independent truth standard. The two pivotal Phase 3 studies (AI-700-32 and -33), also used a comparative standard [single-photon emission computed tomography (SPECT)].

In Study AI-700-20, subjects underwent rest imaging. In Studies AI-700-21, -32, and -33, suspected CAD patients underwent rest and dipyridamole stress imaging.

Pivotal Phase 3 Studies AI-700-32 and -33 enrolled patients typically referred for noninvasive testing. Patients in AI-700-32 were being screened to determine whether their chest pain was due to ischemia. Patients in AI-700-33 were referred for ANGIO or already had ANGIO but required functional testing to assess significance of coronary lesions. Patients in both trials were drawn from 9 countries in a variety of care settings including private, public, academic, and Veterans Administration hospitals. Thus, the conclusions drawn from the 2 pivotal trials are widely applicable. They provide evidence to support the use of AI-700 as a potential alternative to gated SPECT for the detection of CAD[16, 17] and for risk stratification of patients who may require further evaluation with the more invasive procedure, ANGIO.

Because all the patients in the pivotal studies were clinically indicated for a stress test (either SPECT or ECHO), the patient population represents the intended use population for AI-700.

1.5.2 Phase 2 Efficacy Results

Both Phase 2 efficacy studies (Study AI-700-20 and Study AI-700-21, Section 4.4) suggest that AI-700 ECHO has improved diagnostic performance versus non-contrast ECHO.[15, 18, 19]

In Study AI-700-20, when imaging was optimized (n=25), agreement with SPECT (measured with kappa statistics) was greater for AI-700 ECHO imaging with both perfusion and wall motion than it was for AI-700 ECHO imaging with wall motion alone, or for optimized non-contrast ECHO.[20]

In Study AI-700-21, cumulative AI-700 doses of both 0.03 and 0.11 mL/kg met the study primary endpoint of clinically relevant increases (≥10%) in sensitivity and specificity compared with placebo when using SPECT outcome as a surrogate measure for disease/no disease.[15, 18]

1.5.3 Phase 3 Pivotal Studies

Patients with suspected CAD and recent history of chest pain were enrolled in pivotal Phase 3 studies AI-700-32 and AI-700-33. These patients were to receive the intended total dose of 0.080 milliliters/kilogram (mL/kg) AI-700 administered as 2 IV injections (each of 0.040 mL/kg) during same-day rest and dipyridamole stress ECHO imaging sessions.

1.5.3.1 Statistical Methods (Hierarchical Analysis)

The pivotal Phase 3 studies were designed to compare the accuracy of AI-700 ECHO to that of SPECT for the diagnosis of CAD in patients with recent history of chest pain. In AI-700-32 and AI-700-33 the performance of 3 ECHO readers was compared a SPECT comparator, which was either the study SPECT reader (AI-700-32) or the median-performing SPECT Reader from 3 study SPECT readers (AI-700-33). The ratio [i.e., relative risk (RR) ratio] of accuracy of AI-700 ECHO to the accuracy of SPECT was compared using non-inferiority testing (95% CI of RR>0.83). Similar methods were used for sensitivity and specificity. CAD was defined by ANGIO/left ventriculogram (LVG), when available, or 90-day clinical outcome, or review of ECG and SPECT. See Appendices 1 and 2 for detailed descriptions of efficacy parameters and statistical methods used for hypothesis testing.

Accuracy measures how often information obtained with the diagnostic modality being evaluated represents truth as measured by a gold standard. Unlike the diagnostic statistics of sensitivity and specificity, calculations of accuracy incorporate data from all patients in the efficacy population. In contrast, sensitivity and specificity are calculated using a subset of patients and are more highly influenced by the reader's bias -the tendency to over-call or under-call disease Accuracy provides clinical utility for patients who have an intermediate (~50%) pre-test probability of disease. In addition, the diagnostic utility of myocardial perfusion imaging is best realized when disease prevalence is approximately 50%,[21] the rate observed in Studies AI-700-32 and AI-700-33.

Because accuracy is an objective measure of diagnostic performance, both studies were designed specifying accuracy as the principal primary endpoint to be tested as the first tier in a hiearchical structure. Each study was designed to test the non-inferiority of accuracy for AI-700 ECHO compared with SPECT perfusion imaging. Therefore, the entire Type I error rate (alpha of 0.05) was allocated to testing non-inferiority of accuracy. To assess sensitivity

and specificity, the studies used a statistical gating mechanism. If the data showed non-inferior accuracy, non-inferiority of sensitivity and specificity were to be evaluated. Success for each endpoint was based on at least 2 of 3 ECHO readers demonstrating non-inferiority as compared with SPECT. Success on second tier endpoints (sensitivity and specificity) was not required for the studies to be considered successful.

1.5.3.2 Modifications to the Study Design

FDA approved SPECT agents for the proposed indication on the basis of point estimates for disease detection. The original AI-700 Phase 3 study design proposed by the Sponsor relied on estimation of diagnostic performance of AI-700 for disease detection against the truth standard of ANGIO in one study and against SPECT with 90-day clinical outcome in the other. However, based on a mandate from FDA, the Sponsor agreed to collect SPECT data in the ANGIO study and to use SPECT as a comparator rather than a truth standard in the other study. After the studies began, FDA modified its position and asked that both studies be changed to non-inferiority comparisons with SPECT (with ANGIO serving as the truth standard, when available), based on the proposed indication. Subsequent FDA-requested revisions, including restrictions on eligible SPECT imaging methodology, resulted in exclusion of 92 patients from the modified intent to treat (MITT) population (Section 4.5.1). These modifications had little effect on estimates of diagnostic performance parameters and did not affect the efficacy conclusions, as demonstrated by many sensitivity analyses (i.e., repetition of the primary efficacy analysis on several subpopulations and expanded populations, Appendix 9).

1.5.3.3 Heterogeneous Truth Definition

For detection of CAD, percent stenosis as determined by quantitative coronary ANGIO, whenever available, was used as truth in the pivotal trials. Disease by ANGIO was defined as ≥70% stenosis of a major coronary epicardial artery. Most patients (73.7%) in the pivotal Phase 3 program had ANGIO as the truth standard [119/285 patients (41.8%) in Study AI-700-32 and 369/377 patients (97.9%) in Study AI-700-33]. When ANGIO was unavailable, patients with a confirmed history of myocardial infarction (MI) were considered positive for CAD, as were patients with a subsequently confirmed MI. For all other patients, a cardiologist certified to interpret SPECT determined CAD status based on SPECT results in conjunction with relevant clinical and 12-lead electrocardiogram (ECG) data.

1.5.3.4 Quality of SPECT Comparator

SPECT perfusion imaging quality control was achieved by selecting an independent central Core Lab that was the accreditation certification laboratory for the Intersocietal Commission for the Accreditation of Nuclear Medicine Laboratories (ICANL). The Core Lab provided acquisition guidelines to investigational sites following the recommendations of the Imaging Guidelines for Nuclear Cardiology Procedures, Part 2, American Society of Nuclear Cardiology.[22] Unlike patients with unreadableAI-700 ECHO imaging (MITT population patients considered discordant to the truth standard), patients with poor quality SPECT

studies deemed uninterpretable by the Core Lab (n=4) were removed from the MITT population, i.e., from primary efficacy analyses.

SPECT blinded readers were expert nuclear cardiologists chosen from medical teaching institutions not used as patient enrollment centers. All SPECT readers received the same training developed with assistance from the SPECT Core Lab.

For these reasons, the Sponsor believes that the diagnostic performance of SPECT obtained in Studies AI-700-32 and AI-700-33 represents the optimized performance of SPECT in the patient population being evaluated.

1.5.4 Phase 3 Efficacy Results

1.5.4.1 Primary Efficacy Results

Success for each endpoint was based on at least 2 of 3 ECHO readers demonstrating non-inferiority as compared with SPECT. Results of the non-inferiority analyses for each pivotal Phase 3 efficacy study are summarized below and detailed in Section 4.5.6:

- AI-700-32
  - Non-inferior accuracy (3 of 3 readers)
    - Non-inferior sensitivity (1 of 3 readers)
    - Superior specificity (2 of 3 readers)
    - Non-inferior specificity (3 of 3 readers)
- AI-700-33
  - Non-inferior accuracy (3 of 3 readers)
    - Superior sensitivity (3 of 3 readers)
    - Non-inferior specificity (1 of 3 readers)

In both studies, the accuracy (the principal primary endpoint) of all AI-700 ECHO readers was non-inferior to SPECT perfusion imaging. Values for accuracy were similar for all 6 ECHO readers (range 66-71%) and for all 4 SPECT readers (range 66-70%). Sensitivity and specificity, however, varied by reader. ECHO reader sensitivity ranged from 50-77%, and specificity ranged from 55-88%. Similarly, SPECT reader sensitivity ranged from 57-80%, and specificity ranged from 50-78%.[16]

Acusphere attributes the fact that only 1 of 3 blinded readers met the non-inferiority endpoints of sensitivity in Study AI-700-32 and specificity in Study AI-700-33 to reader subjectivity rather than the quality of information obtained from the images. Tradeoffs between sensitivity and specificity were observed in both studies for both SPECT readers and AI-700 ECHO readers.

The inability to achieve consistent results for sensitivity and specificity is neither unexpected nor compelling evidence of inferior diagnostic performance for AI-700 ECHO as compared to SPECT for 2 reasons:

1. *Test and comparator modalities demonstrated clinically equivalent accuracy.* In the context of studies with approximately 50% disease prevalence (e.g., Studies AI-700-32 and -33) and the non-inferior accuracy of the test modality (AI-700 ECHO), demonstrating non-inferiority for both components of accuracy (sensitivity and specificity) is difficult because of the inverse correlation between sensitivity and specificity and the constraints of the non-inferiority margin.

2. *Variability among SPECT readers for sensitivity and specificity was greater than the difference allowed to demonstrate non-inferiority between the test (AI-700 ECHO) and the comparator (SPECT).* The difference between the highest and lowest sensitivities of the 3 SPECT readers in AI-700-33 was more than 20%; the same was true for specificity. These differences are larger than the differences allowed by the non-inferiority test criteria, which require that differences between AI-700 ECHO and SPECT readers must be <5% to demonstrate non-inferiority (Appendix 2)**.**

1.5.4.2 Other Efficacy Results

Additional noteworthy efficacy results from the pivotal Phase 3 trials:

- All 6 ECHO readers and 3 of 4 SPECT readers detected the more serious and more severe multi-vessel disease, as determined by ANGIO, with ≥70% sensitivity.
- When disease localization was required for correct diagnosis of CAD defined by ANGIO, ECHO readers showed less reduction in sensitivity than did SPECT readers (3-16 % vs. 26-31%).
- AI-700 ECHO diagnostic performance was robust and consistent across all levels of Baseline non-contrast ECHO AWQ. The ECHO readers considered more than 99% of all images in both studies were considered evaluable, despite 27% of patients having poor AWQ.
- More than 80% of patients had MCE >5 minutes (Section 3.1.2).

1.5.4.3 Integrated Analysis of AI-700 Compared with SPECT

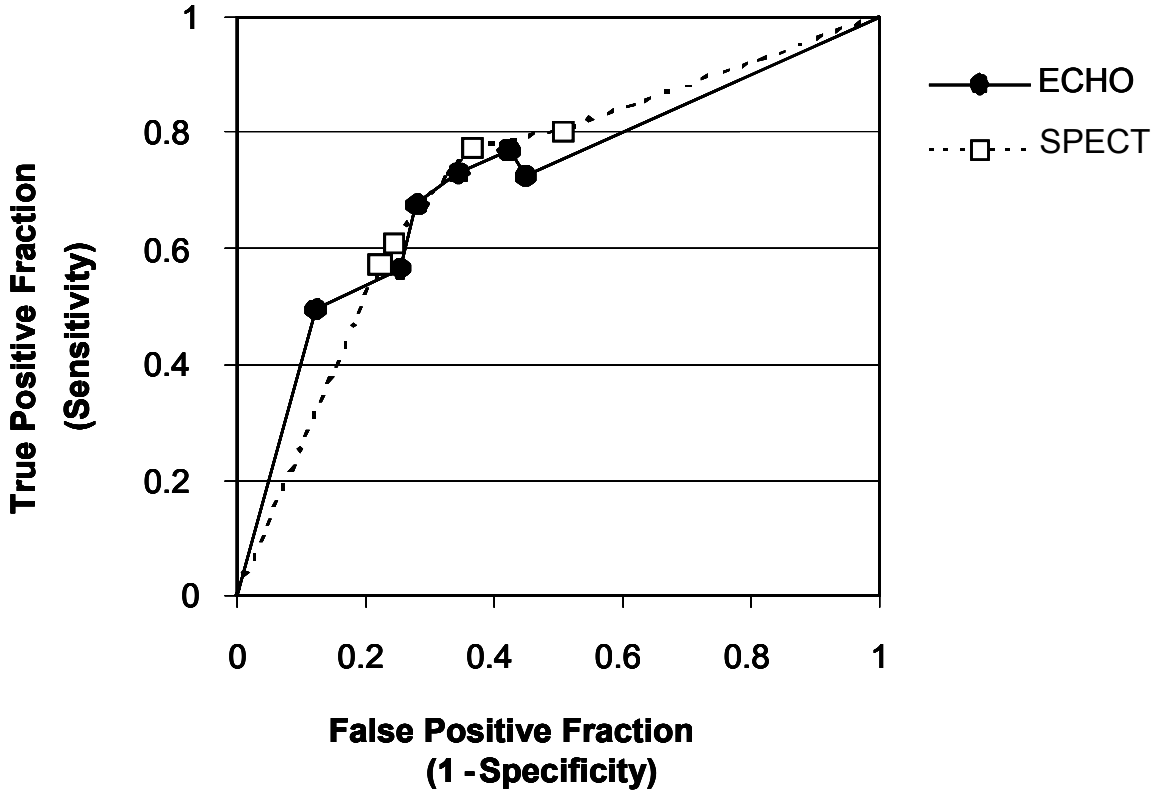
When the pivotal Phase 3 trial results were integrated, several analyses support that AI-700 ECHO and SPECT have similar ability to diagnose CAD. Study AI-700-33 was conducted with 3 SPECT readers with primary analysis performed using the median SPECT Reader as the comparator. Study AI-700-32 had a single SPECT reader. An integrated analysis was performed where all ECHO readers were compared to all SPECT within a study (3 pairs in AI-700-32 and 9 pairs in AI-700-33) and evaluated for superiority. Superiority, unlike non-inferiority, is not based on a subjective non-inferiority margin. For all pairwise comparisons of accuracy, the 95% CI for the ratio of ECHO accuracy to that of SPECT

included 1.0, indicating that neither reader demonstrated superiority over the other. In contrast, pairwise comparisons showed heterogeneous sensitivity and specificity. For sensitivity, AI-700 ECHO was more often superior to SPECT than vice versa (6 versus 4 pairs), but for specificity, no difference was observed (4 versus 4 pairs).

The performance of ECHO and SPECT modalities apart from individual reader variability was evaluated with multi-reader receiver operating characteristic (mROC) curves. The curves of sensitivity versus 1-specificity were similar in shape for AI-700 ECHO and SPECT with equal areas under the curve (0.72). Thus when both Phase 3 studies are considered collectively, the diagnostic performance of the 2 modalities is similar (Figure 1). The mROC curves also illustrate that these variations in reader bias (the position along the mROC curve) are similar for both SPECT and ECHO, thereby supporting the concept that both ECHO and SPECT readers make the same degree of trade-offs between sensitivity and specificity.

The ability of AI-700 ECHO to detect ischemia was evaluated in a pooled analysis of AI-700-32 and -33 consisting of those patients without prior history of MI (n=462) and consequently enriched for those with ischemia. AI-700 ECHO accuracy, sensitivity, and specificity was 69.2%, 63.7%, and 75.1%, respectively, compared to 65.3%, 63.2%, and 67.6%, respectively, for SPECT (Section 4.5.12). Thus, this analysis provides support for the ability of AI-700 ECHO to detect ischemic disease.

Figure 1: Multi-Reader ROC Curve for ECHO and SPECT Readers (Phase 3 Efficacy Studies)



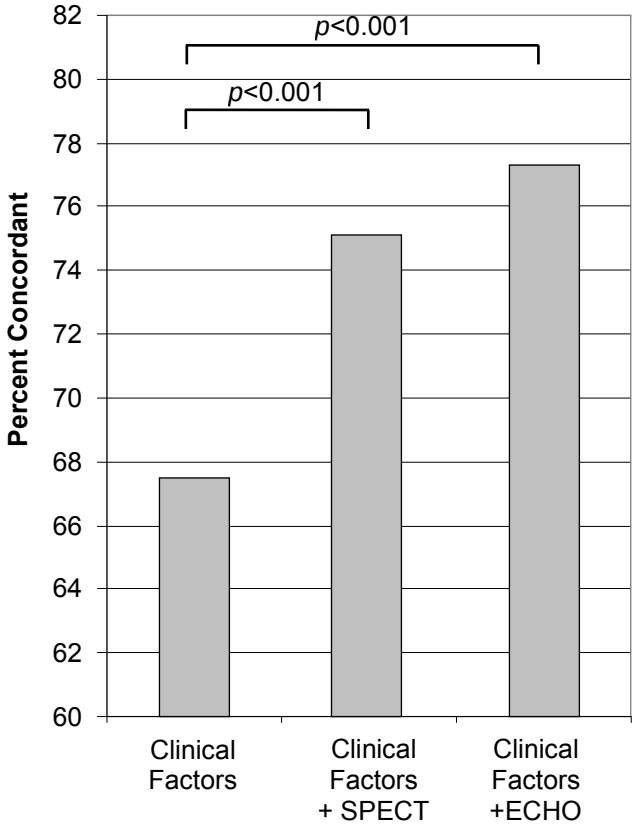
1.5.4.4 Clinical Utility of AI-700 ECHO: Increased Diagnostic Value

In its intended clinical use, AI-700 ECHO imaging information will be integrated with other clinical variables to screen patients to identify those at elevated risk of CAD who would benefit most from further and more invasive evaluation with ANGIO.

In pooled multivariate logistic regression analysis of Studies AI-700-32 and AI-700-33, positive AI-700 ECHO was a clear, significant marker of CAD even after adjustment for clinical factors [age, gender, angina symptoms (atypical, typical and unstable), hypertension, diabetes, and smoking history] (Section 4.5.11.3). The odds ratio (OR) was 5.3 (95% CI: 3.7, 7.5; p<0.001), which was higher than any other factor. Similar associations were observed for all 6 individual ECHO readers (OR range: 3.3 to 5.6; p<0.001) and 4 individual SPECT readers (OR range: 3.6 to 4.9; p<0.001).[23]

The relative predictive value was evaluated for the following models: clinical factors; clinical factors+SPECT; and clinical factors+ECHO. Comparison of the percent concordance statistic (derived from a comparison of predicted disease probability and observed disease) showed a higher percent concordance for models with clinical factors plus either ECHO or SPECT (p-value <0.001, Figure 2).

Figure 2: Relative Predictive Value of 3 Logistic Regression Models



1.5.4.5 Phase 3 Efficacy Conclusions

When the pivotal studies are considered collectively, several analyses demonstrate that AI-700 ECHO and SPECT are clinically equivalent for the diagnosis of CAD:

1. Non-inferiority of ECHO to SPECT for accuracy (principal primary endpoint).
2. Comparable values for sensitivity and specificity.
3. Identical area under the mROC curves.

Lastly, AI-700 ECHO is a statistically significant and independent predictor of CAD, and provides added clinical value in the triaging of patients for ANGIO.

1.6 Safety Summary

1.6.1 Nonclinical Studies of Clinical Relevance

Standard genotoxicity, reprotoxicity, local irritation, and single and repeat dose toxicology studies were conducted. Additional nonclinical studies evaluated cardiovascular/cardiopulmonary safety of AI-700. Appendix 3 contains a summary of all nonclinical safety pharmacology studies and toxicology studies. Single and repeat dose toxicology studies in rats and primates demonstrated no observable adverse effects of AI-700 at high multiples (>10x) of the human dose (HD). No clinically significant findings were observed regarding local IV irritation, genotoxicity, or reproductive/developmental toxicity. Notable nonclinical cardiovascular/cardiopulmonary safety findings are summarized. Doses are described relative to the HD (2 doses of 0.04 mL/kg, i.e., 0.08 mL/kg).

1.6.1.1 Cardiovascular Safety

The cardiovascular safety of an IV injection of AI-700 was evaluated in anesthetized rhesus monkeys and conscious cynomolgus monkeys. Administration of the highest dose (14X the HD) of AI-700 caused an approximate 15 to 25% decrease in mean arterial pressure (MAP) that peaked 5 to 15 minutes after dose administration. This decrease was not associated with a reflex tachycardia, was within normal range, and returned towards pre-dose values prior to dosing with the next dose of AI-700 or by the end of the monitoring period. There were no other pharmacological effects attributed to AI-700 at any dose.

In anesthetized rhesus monkeys undergoing closed-chest AI-700 ECHO imaging, AI-700 doses of 2X the HD were used to study cardiopulmonary safety during high mechanical index imaging. In this study, premature ventricular contractions (PVCs) were observed during destructive pulses (mechanical index ≥ 1.2), and were resolved upon removal of the ultrasound probe. These results are similar to results observed in human studies. No other AI-700-related changes were observed.

A study of cynomolgus monkeys demonstrated that AI-700 above the HD, in combination with moderate to high mechanical index, with and without dipyridamole stress, had no significant effect on structure or physiological function of the cardiopulmonary system.

1.6.1.2 Cerebrovascular Safety

Acute injection of AI-700 into Sprague-Dawley rats through a catheter directed cranially in the carotid artery was used to assess potential brain toxicity associated with direct exposure of AI-700 *via* the arterial circulation. The study was conducted to investigate the potential effects in patients having atrial/septal defects of the heart. A single intracarotid injection of AI-700 at 1.4X the HD did not result in any AI-700-related signs of toxicological significance, changes in hematology or clinical chemistry, or gross or microscopic changes in the brain or heart.

1.6.1.3 Cardiopulmonary Safety

The effect of AI-700 on pulmonary function was evaluated in 2 anesthetized animal models: dogs with acute elevated pulmonary artery pressure (PAP), and pigs.

Anesthetized spontaneously breathing adult mongrel dogs with Sephadex™-induced severe or moderate pulmonary hypertension (PH) were used to assess PAP/resistance, blood gases, and respiratory and cardiac functions following AI-700 administration. Following the Sephadex™-induced PH, all cohorts of dogs showed increases in mean pulmonary artery pressure (mPAP), with the maximum effect usually observed within 5 minutes of AI-700 dosing. This study demonstrated a dose-dependent effect of AI-700 on cardiopulmonary function at dosages of approximately 1.3X and 2.5X the HD.

Administration of AI-700 according to the clinical dosing regimen (2 sequential IV injections with a 1 hour interval between injections) to pigs in a dose range of up to 1X the HD was evaluated. PAP increased by 3 to 16 millimeters of mercury (mmHg) in a dose-dependent pattern. Acute decreases in systemic blood pressure and compensatory increases in heart rate were observed in a non-dose dependent fashion. Decreased blood pressure recovered quickly when dosing was stopped. Indomethacin blocked all blood pressure, heart rate, and PAP changes, suggesting that the observed changes after AI-700 were thromboxane mediated.[24]

Pigs were studied because, unlike humans and other animals used in AI-700 cardiovascular studies, pigs have resident pulmonary intravascular macrophages (PIM). These PIM are involved in the clearance of intravascular particles and have been associated with acute increases in PAP after administration of other ultrasound contrast agents.[25, 24, 26, 27]

AI-700 was generally well tolerated at doses much higher than the clinical dose. Nonclinical safety data suggest that the target organs for toxicity are those of the cardiopulmonary system.

1.6.2 Clinical Safety Findings and Conclusions

1.6.2.1 Summary of Adverse Events

In total, 652 (72%) of 911 chest pain patients undergoing rest and stress imaging in Studies AI-700-23, -32, and 33 reported at least one adverse event (AE). Most AEs were mild in

intensity, of short duration, and resolved without treatment or residual effects. The majority of AEs occurred while patients were under the effects of the pharmacologic stress agent, dipyridamole, and were consistent with its known AE profile.

1.6.2.2 Serious Adverse Events

No deaths or life-threatening serious adverse events (SAEs) were reported.

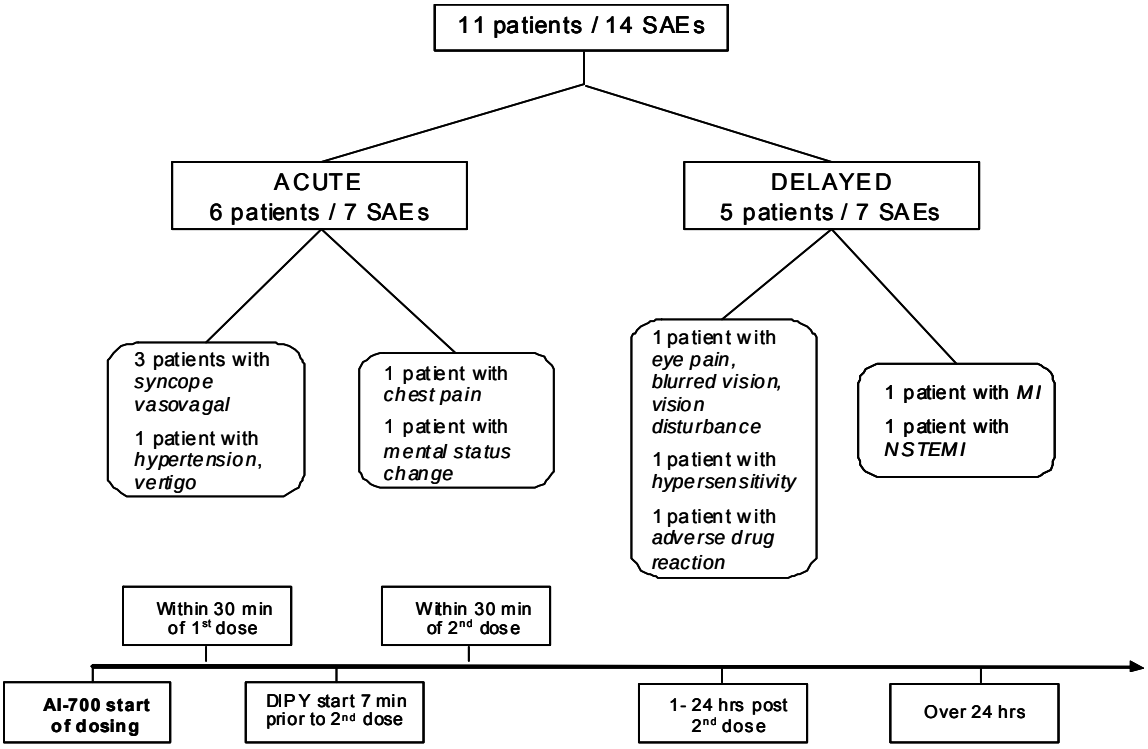
Eleven patients (<1% of subjects receiving AI-700) experienced 14 non-life-threatening SAEs. All reported SAEs occurred in Studies AI-700-23, -32, and -33 (Figure 3).

Syncope vasovagal (without loss of consciousness) was the only SAE with a temporal relationship to AI-700 dosing (onset within 30 minutes of dosing) that was reported more than once (3 patients experienced individual episodes). These AEs differed from other episodes of hypotension in that they were always accompanied by rapid decreases in heart rate or AV block. Symptoms resolved quickly after treatment with IV atropine for bradycardia.

At least 24 hours after AI-700 dosing, 2 additional patients had SAEs that were considered important cardiac events: 1 MI and 1 non-ST elevation myocardial infarction (NSTEMI). Both patients had multiple vessel disease and both complained of recent chest pain at Baseline. During AI-700 stress imaging, both patients had signs of induced ischemia. Both MIs were considered unrelated to AI-700.

The NSTEMI resulted in post-MI bradycardia requiring a pacemaker; all other SAEs resolved without residual effects.

Figure 3: Display of Acute and Delayed Serious Adverse Events by Onset Time



1.6.2.3 Discontinuation of AI-700

One healthy volunteer and 16 CAD patients had AEs that resulted in discontinuation of AI-700. The most common AEs resulting in discontinuation of AI-700 were hypotension (5 patients) and syncope vasovagal (3 patients). For 3 of these 5 patients, hypotension occurred during rest; in all 3 patients, systolic blood pressure (SBP) remained ≥100 mmHg. All patients with hypotension or vasovagal episodes remained conscious.

1.6.2.4 Most Frequent Cardiovascular and Respiratory Adverse Events

Hypotension was the most frequently reported vital sign AE. A total of 38 (4%) of 911 patients in studies AI-700-23, -32 and -33 had 41 hypotensive AEs. Twelve patients had a total of 12 hypotensive AEs at rest; 26 patients had a total of 29 hypotensive AEs at stress (after dipyridamole and prior to Discharge). Thirteen (1.4%) patients required treatment with standard medical care. All hypotensive AEs were temporary and resolved without residual effects.

In Studies AI-700-23, -32 and -33, the most common respiratory AEs were dyspnea (43 AEs in 40 patients, 4.4%) and throat tightness (10 AEs in 10 patients, 1.1%); all other respiratory AEs were reported in ≤1% of patients. Four dyspnea AEs occurred during rest; in all 4 cases, SaO_2 remained ≥90%, the AEs were mild in intensity, and resolved without treatment.

Because respiratory AEs commonly observed with AI-700 ECHO are also common for patients undergoing dipyridamole stress, treatment is readily available in the clinical setting for administration immediately following AE onset.

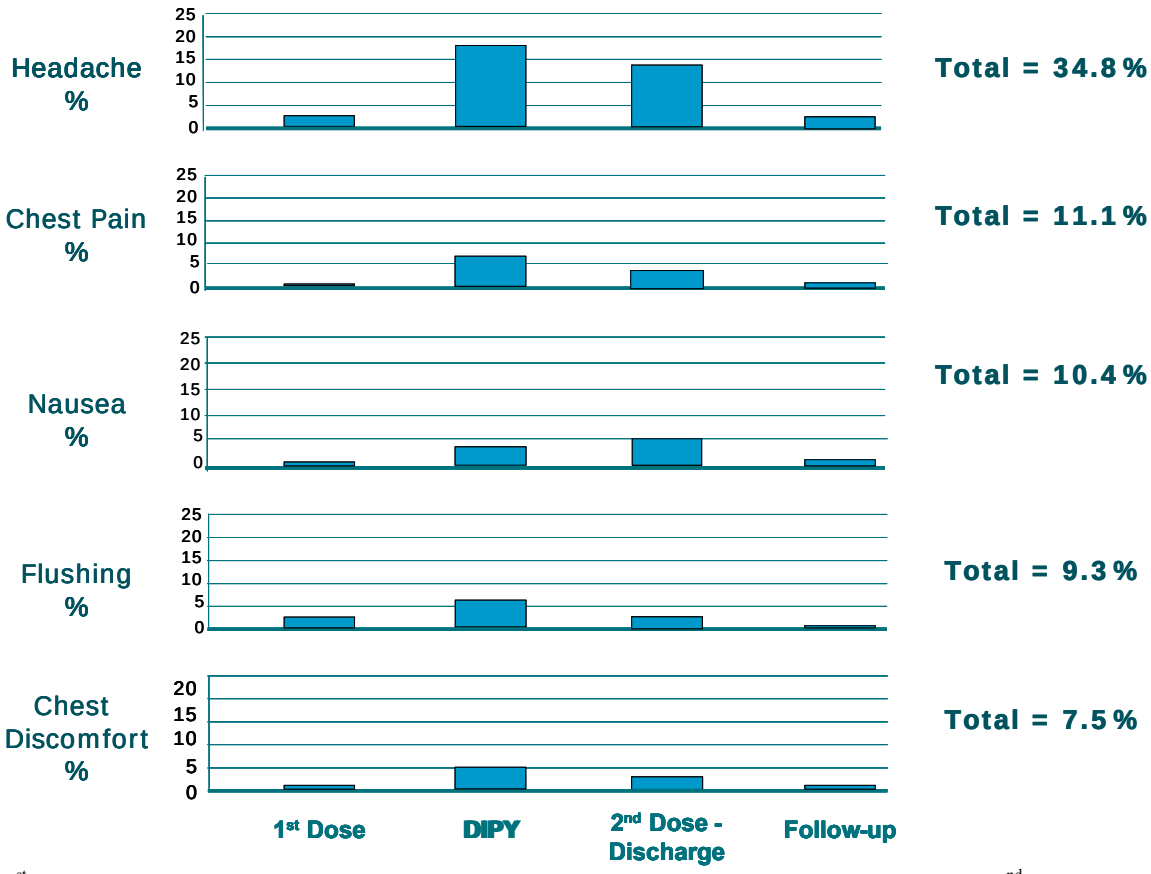
1.6.2.5 Common Adverse Events and Time of Onset

The most common AEs observed in Studies AI-700-23, -32, and 33 were headache, chest pain, nausea, flushing, and chest discomfort (Figure 4).

By time of onset, 105 patients of 911 patients (11.5%) in Studies AI-700-23, -32, and 33 had a reported AE after the first dose of AI-700 and prior to dipyridamole dosing, 391 patients (43.7%) after dipyridamole dosing and prior to the second dose of AI-700, 402 patients (45.0%) after the second dose of AI-700 and prior to Discharge, and 151 patients (16.6%) from Discharge through Follow-up. AE rates were similar to those observed in 22 CAD patients who received 2 doses of placebo and dipyridamole in Study AI-700-21 (9%, 50%, and 23% of patients with reported AEs after first dose of placebo and prior to dipyridamole, after dipyridamole dosing and prior to the second dose of placebo, and after the second dose of placebo, respectively). .

Onset time of the 5 most common AEs observed in these patients is presented graphically in Figure 4. This temporal relationship to administration of dipyridamole and the time of peak pharmacological effect of the stressor suggest that many of the most common AEs were, in part, due to the pharmacological effects of dipyridamole (which induces ischemia).

Figure 4: Number and Percent of Patients with the Five Most Common Adverse Events by Onset Time (Studies AI-700 -23, -32, and -33)



1st Dose = bolus injection of 0.04 mL/kg AI-700 for rest ECHO; DIPY = dipyridamole administration; 2nd Dose = bolus injection of 0.04 mL/kg AI-700 after dipyridamole administration for stress ECHO.

1.6.2.6 Treatment of Adverse Events

AEs requiring treatment were reported in 172 (19%) of 911 patients from Studies AI-700-23, -32, and -33. Concomitant medications most commonly administered on the same day as AI-700 were paracetamol (acetaminophen), given to 49 (5.4%) patients; aminophylline/theophylline, given to 32 (3.5%) patients; and glyceryl trinitrate, given to 20 (2.2%) patients.

1.6.2.7 Dose-Dependence of Adverse Events

In Studies AI-700-20 and -21, in which a single dose of AI-700 (0.014, 0.046, 0.054, or 0.081mL/kg) was administered, there was a dose-dependent increase in AE rates for CAD patients (16%, 18%, 36%, and 42%, respectively according to dose group). The total AE rate for patients receiving placebo was lower (9%). The higher AE rates in patients receiving AI-700 were driven by dose-dependent increases in flushing (0%, 5%, 8%, and 17%, respectively according to dose group). No dose-dependent trends relating to the intensity of AEs were observed in any subject or CAD patient receiving AI-700.

1.6.2.8 Clinical Laboratory Findings

Clinical laboratory results across all studies showed no significant trends in clinical chemistries or hematology labs, with the exception of post-dose fluctuations in white blood cell (WBC) and neutrophil counts which were dose- and time-dependent. These changes were not associated with fever or active infection. WBC changes were primarily driven by fluctuations in neutrophil counts and are probably related to the activation of complement.

In healthy volunteers, increases in activated complement C3a levels were observed within 10 minutes of AI-700 dosing (Study AI-700-04). Increases in activated complement were temporally related to decreases in WBC counts. Both C3a and WBC/differential counts returned towards Baseline levels from 30 minutes to 2 hours post-dose. While no further increase in C3a was observed, WBC and neutrophil counts increased above Baseline levels at 2-8 hours post dose. The delayed transitory increase followed by a return to normal a few days later has been consistently reported in other AI-700 clinical trials. Negative results from skin prick test upon rechallenge with AI-700, a lack of tryptase release (a surrogate marker for histamine), and a lack of AEs relating to hypersensitivity or allergy suggest that AI-700 has low allergic potential. Delayed transient elevations (24 hours post dose) of C-reactive protein (CRP) were observed but remained an order of magnitude below clinical levels associated with symptomatic inflammatory responses (i.e., 10 mg/dL).

1.6.2.9 ECG Findings

Overall, ECG data revealed no proarrhythmic effects due to QT prolongations, or increases in ischemic risk due to AI-700 administration. An increase in multifocal PVCs was observed in Phase 2 studies (AI-700-20 and -21) during AI-700 ECHO imaging when high mechanical index (>1.0) imaging techniques were used. These PVCs stopped when the probe was removed from the chest or when the mechanical index was decreased (≤1.0). This is a well-documented phenomenon that has been reported for other ultrasound contrast agents.[8, 9] Use of mechanical index >1.0 is no longer permitted with ECHO imaging agents. During Phase 3 studies, patient undergoing AI-700 ECHO imaging with MI≤1.0 demonstrated no increased PVC rates regardless of imaging mode.

1.6.2.10 Respiratory Findings

Transient decreases in arterial oxygen saturation (SaO_2) were defined as a post-dose decrease of ≥5% from Baseline and <90% SaO_2. These transient decreases were observed in 23 of 778 patients (3%) in Studies AI-700-32 and -33. Most decreases were isolated and asymptomatic events. Mean SaO_2 values remained within 1% of Baseline at all timepoints, with a nadir occurring at 7-15 minutes post dose. The delayed onset and brief and transient nature of SaO_2 decreases do not support a mechanism of direct obstruction of pulmonary vasculature by particulate matter that comprises AI-700.

Patients with moderate chronic obstructive pulmonary disease (COPD; Study AI-700-05) showed asymptomatic, transient decreases in the forced expiratory volume in 1 second

(FEV_1) following AI-700. Mean decreases at all post-dose timepoints were 7-14% greater following AI-700 administration as compared with placebo. The greatest difference was observed at 15-20 minutes following the second test article dose (mean FEV_1 decrease of 22% for AI-700 versus 8% for placebo). Two patients had clinically significant decreases of FEV_1>15% post dose. One of these patients had a concomitant SaO_2 decrease to 88% (compared with 92% at Baseline). Decreases in SaO_2 were temporary and returned to >90%. All spirometry measurements returned to Baseline values by the 24-hour Follow-up visit.

1.6.2.11 Vital Signs Findings

Heart Rate

The only notable finding in healthy volunteers was an increase in heart rate observed in the highest AI-700 dose group (0.108 mL/kg): mean heart rate was 60.1 beats per minute (bpm) at Baseline, 75.3 bpm at 5 minutes, and 76.7 bpm at10 minutes post dose. As predicted, in all stress imaging studies, a transient elevation in heart rate was noted after dipyridamole stress imaging (Studies AI-700-21, -23, -32, -33). For most patients, increased heart rate (approximately 10-20 bpm) was reversed at 10 minutes post-dose, due to administration of protocol mandated aminophylline.

Blood Pressure

In healthy volunteers and in CHF/COPD patients (Study AI-700-05), mean SBP and diastolic blood pressure (DBP) values remained within the normal range (and within 6% of Baseline) at all timepoints in all dose groups. In CAD patients in Phase 2 dose ranging studies (AI-700-20 and -21), mean SBP and DBP remained within the normal range and within 7% of Baseline at all timepoints. No notable dose-dependent trends were observed. In Studies AI-700-32 and -33, mean change from Baseline SBP (136 mmHg) ranged from a decrease of 9 mmHg (6%) to an increase of 0.5 mmHg (1%) at Discharge. Mean change from Baseline DBP (77 mmHg) ranged from a decrease of 5.5 mmHg (7%) to an increase of 0.1 mmHg (<1%) at 60 minutes after the second AI-700 dose. In both imaging sessions (rest and stress), the nadir for both SBP and DBP occurred between 5-10 minutes post-dose.

Respiratory Rate and Body Temperature

The only notable change in respiratory rate was a transient 10% increase following dipyridamole administration and the second dose of AI-700, at 5 minutes post-dose. No clinically notable changes or trends in body temperature were observed.

1.6.2.12 Safety Conclusions

AI-700 dipyridamole stress ECHO has a similar risk profile to other pharmacological stress studies in patients undergoing a cardiac imaging procedure for CAD detection.[28-30] Although short lived symptomatic decreases in blood pressure and changes in oxygen saturation were occasionally observed, these were transient and usually mild in intensity. If treatment was required, these AEs were promptly managed by standard medical care with no apparent

life-threatening risk in stable cardiac patients. Lack of overall life-threatening events and the reversible nature of AEs observed following administration of AI-700 indicate that the drug is tolerated in the setting of controlled ischemia induced by dipyridamole stress.

1.7 Benefit/Risk Assessment

1.7.1 Clinical Utility of AI-700 Stress ECHO

Optimal care of patients with suspected ischemic heart disease requires triage based on a comprehensive evaluation of cardiac anatomy and physiology. The recent emphasis on intervention and aggressive pharmacologic management has intensified the need to obtain accurate diagnostic assessment of both coronary anatomy and cardiac function (e.g., regional myocardial perfusion and wall motion). Angiography, the gold standard for determination of CAD, is costly, is associated with significant morbidity and mortality, and is therefore only appropriate for patients with high pretest probability of disease.

The non-invasive stress imaging procedures of SPECT and non-contrast ECHO are the standards of care[31] for determining which patients are referred for ANGIO. SPECT is considered the more sensitive procedure because it is a direct measurement of perfusion and can reveal CAD at an earlier stage. However, SPECT is more expensive and time-consuming than ECHO and exposes patients to ionizing radiation. Non-contrast ECHO imaging has significant positive clinical, logistical, and economic attributes, but has been unable to directly assess perfusion.

AI-700 ECHO has shown the following results:

1. Clinical equivalence to gated quantitative ^{99m}Tc labeled SPECT imaging (Studies AI-700-32 and AI-700-33) for the detection of CAD in stable chest pain patients:
    a. Same reader trade-offs between sensitivity and specificity as SPECT (identical area under the curves for mROC);
    b. Increasing sensitivity for detecting CAD with increasing severity of disease similar to that seen with SPECT.
2. Independent predictor of the presence of CAD (multivariate regression analyses);[23]

These data suggest that AI-700 ECHO should allow cardiologists to obtain the clinical benefit of direct perfusion and wall motion information while retaining the cost, convenience, and availability advantages of ECHO without exposing patients to ionizing radiation.

1.7.2 Risks of AI-700 Stress ECHO

Patients may be administered AI-700 on, at most, only a few occasions during their lifetime and under close medical supervision, in a setting where inducing controlled ischemia is the goal and appropriate management of ischemia and its consequences are anticipated.

Although AI-700 stress ECHO has diagnostic performance similar to SPECT without risks due to radiation exposure, it has been associated with dose-dependent increases in transient,

non-serious, treatable AEs. The most clinically relevant of these are dyspnea and hypotension, which were observed prior to dipyridamole administration in patients with recent chest pain. These AEs are usually self-limiting, occur shortly after dosing, and are short-lived. The mean SBP decrease was <10 mmHg. The mean SaO_2 decrease was <1%. The nadir for both SBP decrease and SaO_2 decrease was approximately 5-15 minutes post AI-700 dose.

When AI-700 stress ECHO using dipyridamole is performed, rates of the most common AEs are similar to those of SPECT using dipyridamole.[29] Because these are also common AEs for patients undergoing stress ECHO, standard treatments exist for their management.[32]

AEs of dyspnea and/or hypotension requiring treatment resolved with standard care (i.e., oxygen for decreased SaO_2, fluid/atropine for hypotension/vasovagal) regardless of whether the AE occurred before or after dipyridamole administration. In addition, aminophylline/theophylline derivatives were effective in reversing dipyridamole-induced AEs. Because most AEs of decreased SaO_2 and/or hypotension had onset times within a few minutes of AI-700 administration, and all patients were in a hospital/clinical setting, management of these AEs was initiated promptly.

Across all AI-700 clinical trials, the risk of an SAE was <1%. The types of SAEs and the temporal relationship of most SAEs to AI-700 do not suggest an underlying mechanism. In addition, the lack of mortality or occurrence of any life-threatening SAE suggests that AI-700 is tolerated in the intended patient population (stable patients with recent chest pain who can undergo a dipyridamole stress test). These patients are at lower risk for CAD than patients with acute myocardial infarction or patients with unstable angina.[31] These higher risk patients have not been studied in AI-700 studies because dipyridamole stress imaging is contraindicated for such patients.[32] Moreover, dipyridamole stress imaging is also contraindicated for patients with compromised pulmonary function (wheezing asthmatics).[32] These patients may also be at added risk of having a pulmonary AE following administration of AI-700.

1.8 Risk Minimization Action Plan

A number of safety mitigation strategies should be considered when performing AI-700 rest/stress imaging procedures.

Premature ventricular contractions (PVCs), common in CAD patients, have been demonstrated to increase during stress imaging procedures. High mechanical index cardiac imaging with ultrasound contrast agents has been shown to induce PVCs.[33] Recent regulatory oversight restricting high mechanical index imaging during contrast ultrasound imaging has been effective in eliminating PVC induction. As a precaution to prevent or minimize the occurrence of PVCs, the mechanical index setting should be ≤1.0 during AI-700 administration.

Baseline ECG and vital sign measurements should be taken prior to AI-700 dosing and longitudinal assessments should be made after dosing, including cutaneous oxygen saturation for patients at risk for hypoxia. Given that risk of acute MI and death are elevated in patients during any stress testing, resuscitation equipment and trained personnel should be readily available.[34] As with other ultrasound contrast agents, the potential exists for acute anaphylactoid reactions,[35] such as hypotension and dyspnea. Standard treatment for these reactions (IV fluids, steroids, bronchodilators, and antihistamines) should be readily available.[36, 37]

Aminophylline is an effective antidote for the bronchoconstrictive and vasodilatory effects of adenosine stressors (dipyridamole and adenosine),[32] and therefore can be administered to reverse AEs that occur after administration of dipyridamole. Because the AEs commonly observed with AI-700 are also common for patients undergoing stress ECHO, standard treatments already exist for their management. Furthermore, given that the onset of the vast majority of AEs was at or near the time of dosing, patients who require treatment are likely to still be in the hospital/clinical setting. If necessary, management of these AEs can therefore be initiated promptly.

1.9 Conclusions

The diagnostic performance of AI-700 stress ECHO in stable chest pain patients has been demonstrated to produce results that are clinically equivalent to those of quantitative stress ^{99m}Tc SPECT myocardial perfusion imaging. Furthermore, the safety profile of AI-700 ECHO plus pharmacologic stress appears to be comparable with SPECT plus pharmacological stress and with non-contrast ECHO plus pharmacological stress. Although some additional short-lived symptomatic decreases in blood pressure and oxygen saturation were observed after AI-700 dosing during rest, if necessary, they were promptly managed by standard medical care.

The potential to conveniently obtain both real-time perfusion and wall motion information without exposure to ionizing radiation is an advantage uniquely offered by AI-700 ECHO. These findings, coupled with data indicating that AI-700 stress ECHO provides added diagnostic value over clinical parameters, support the conclusion that AI-700 ECHO has a favorable risk-benefit profile and will be an effective clinical tool in the triage of patients for the more invasive procedure of ANGIO.

1.10 Phase 4

To continue the understanding of the AI-700 clinical risk benefit profile, a risk management plan is proposed for conduct after launch of AI-700 (Section 6.5). This plan will have a minimum of 2 components: 1) Safety Surveillance to determine the rate of acute serious adverse experiences in clinical practice, and 2) AI-700 ECHO Proficiency Training Plan to ensure that healthcare providers understand how to perform and interpret AI-700 stress ECHO imaging studies.

2. BACKGROUND

2.1 Disease Background and Existing Diagnostic Procedures

Coronary artery disease continues to be the single largest killer of Americans (and others in the western hemisphere), with almost 0.6 million deaths annually in the United States.[38] Optimal care of patients with suspected ischemic heart disease requires triage based on a comprehensive evaluation of cardiac anatomy and physiology. The recent emphasis on intervention and aggressive pharmacologic management has intensified the need to obtain accurate diagnostic assessment of both coronary anatomy and cardiac function (e.g., regional myocardial perfusion and wall motion). Angiography, the gold standard for determination of CAD, is costly, is associated with significant morbidity and mortality, and is therefore only appropriate for patients with high pretest probability of disease. Although ANGIO can depict epicardial arterial patency, it is an indirect technique for predicting regional perfusion and coronary flow reserve, and has been reported to have less prognostic value than myocardial perfusion imaging.[5, 21]

The non-invasive stress imaging procedures of SPECT (approximately 7.6 million per year in the US) and non-contrast ECHO (approximately 3 million per year in the US)[39] are the standards of care[31] for determining which patients are referred for ANGIO. They therefore function as gatekeepers. SPECT relies fundamentally on myocardial perfusion information, and ECHO relies exclusively on functional information (regional and global wall motion). SPECT is considered the more sensitive procedure, because its direct measurement of perfusion can reveal CAD at an earlier stage. ECHO imaging, which has significant positive clinical, logistical, and economic attributes, has been unable to directly assess perfusion and therefore has had more limited clinical application. The greater utilization of SPECT compared with stress ECHO may be attributable to multiple factors: maturity of the technology, reimbursement for the procedure, and the ability of SPECT to evaluate perfusion directly.

2.2 Medical Need

An ultrasound contrast agent for perfusion imaging should be safe, easy to use, and have acoustic properties that allow for the simultaneous assessment of perfusion and wall motion.[10] This last characteristic is important because there is diagnostic synergy between wall motion and perfusion imaging.[2-4] Decreased relative blood flow (primarily during stress) caused by coronary stenoses results in detectable myocardial perfusion abnormalities.[5] As CAD progresses, ischemia produces functional impairment of myocardial tissue that results in WMAs. Therefore, myocardial perfusion abnormalities precede WMAs in the ischemic cascade and are an earlier marker for CAD.[5]

Conventional stress ECHO relies on detecting WMAs for the diagnosis of CAD. However, this method depends on the patient having an adequate acoustic window[6] and often produces images that are difficult or impossible to evaluate. Ultrasound contrast agents are known to improve ECHO image quality.[7] Commercially available ultrasound contrast agents

(Optison® and Definity®) are approved only for the visualization of the endocardial border at rest,[8, 9] which facilitates detection of WMAs.[15] However, these agents are microbubbles of gas encapsulated in thin shells that are vulnerable to destruction during ultrasound imaging and consequently do not provide reliable simultaneous imaging of both perfusion and wall motion.[2, 3, 11-13]

2.3 Coupling of Perfusion and Wall Motion

Myocardial perfusion imaging provides enhanced diagnostic value over that of wall motion.[40] Clinical studies have repeatedly demonstrated that imaging of wall motion and perfusion separately results in lower accuracy than combined reading of both.[2-4] This has been recognized with both SPECT perfusion imaging which has shown improvements in performance with the addition of gated images[41, 42] as well as with ECHO wall motion imaging, which has shown improvement with the addition of myocardial contrast. [2-4]

The higher resolution of ECHO imaging as compared to SPECT imaging[43] allows for the differentiation of endocardial tissue from epicardial tissue. This is important because the subendocardium shows signs of ischemia before the endo- or epicardium. Perfusion defects are initially visualized as subendocardial dark lines (lack of opacification of the inner layer of myocardium in the presence of AI-700) that are present only at stress. After prolonged ischemia or complete vessel occlusion, necrosis occurs and moves progressively across the wall from the endocardium and may ultimately involve the full transmural thickness of the wall. Figure 5 shows how the enhancement of the blood pool in the left ventricle adjacent to the dark subendocardial line increases the contrast of the subendocardial defect thus providing maximum detection.

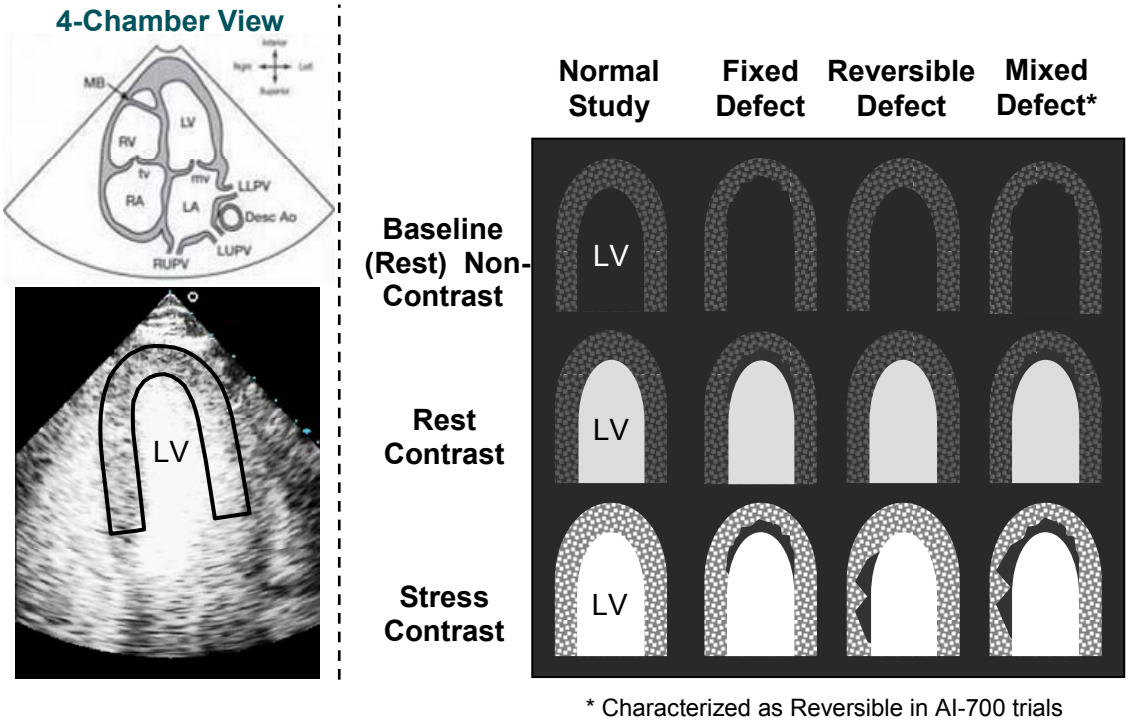
The type of defect (reversible or fixed) and the extent of a defect during rest and stress imaging are known to be strong prognostic markers of major coronary events (MI and deaths).[44-46] Thus the clinical triaging of a patient for potential surgical intervention requires the assessment of the severity/extent as well as the type of defect. This type of assessment requires injection of an imaging agent (SPECT or ECHO) during 2 separate imaging sessions, once during rest and later during cardiac stress. A state of controlled ischemia is induced during stress imaging. There are 2 general types of stress procedures, exercise and pharmacological stress. The most common pharmacological stressors used for perfusion imaging are adenosine agonists, such as dipyridamole. These agents produce negative dromotropic, chronotropic, and inotropic effects on the heart and produce vasodilation in the periphery,[47, 48] thereby increasing blood flow in normal coronary arteries and the regions subtended by those arteries. The opposite effect (decreased blood flow) is observed in stenotic arteries as an indirect result of coronary steal.[48-50] This region of decreased blood flow relative to increased normal blood flow elsewhere is visualized as an inducible (ischemic) defect during a perfusion imaging study.

An inducible/reversible defect is present if there is any evidence of an increase in extent or severity of the defect between the rest and stress session or if the defect is observed only

during the stress session. Reversible defects may be visualized as abnormalities in perfusion alone (earlier stage, smaller extent), or more commonly as abnormalities in both perfusion and wall motion (later stage, larger extent). Patients diagnosed with reversible defects on ECHO have salvageable myocardial tissue that could benefit from being surgically revascularized.

A fixed defect is one in which there is no indication of inducible/reversible ischemia. (i.e., the defect is the same in both rest and stress studies). Fixed defects are usually visualized as defects in both perfusion and wall motion. Typically, these patients are treated medically rather than being referred for ANGIO.

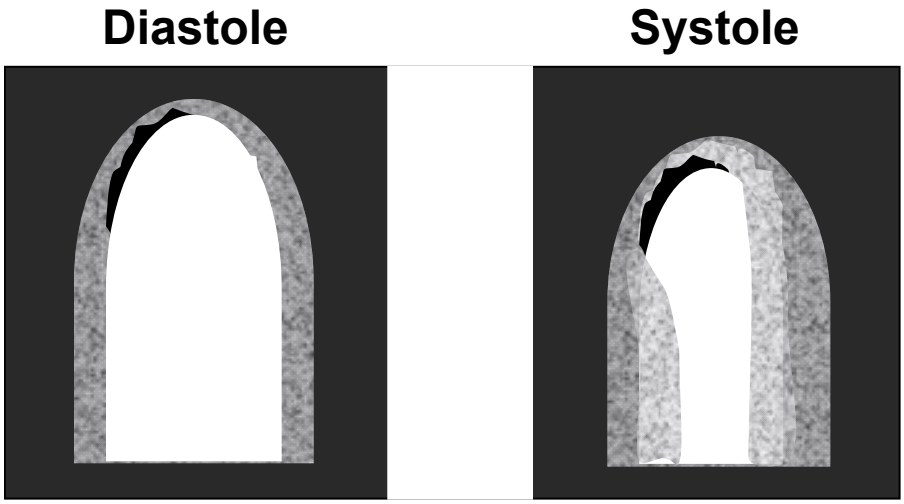
Figure 5: Defect Appearance with AI-700 ECHO



Because perfusion and wall motion cannot be completely and objectively separated, even with extensive image manipulation, Phase 3 trials of AI-700 were not designed to examine wall motion separately from perfusion as would be required to establish the relative roles of these 2 components. It is impossible to assess perfusion alone even by displaying only still frames, because perfusion defects are often accompanied by a lack of wall thickening (a characteristic related to wall motion) that is still visible (Figure 6). The ECHO blinded readers therefore did not discriminate between perfusion and wall motion abnormalities. Moreover, for the comparator arm of the trial, the SPECT readers also used gated images to review wall motion and thickening, consistent with routine clinical practice for SPECT, which integrates the evaluation of perfusion and (resting) wall motion and thickening.

An alternative approach to separating the roles of perfusion and wall motion would have been an assessment of these characteristics at different times and under separate imaging conditions – one optimized to display only perfusion characteristics and a second optimized to display wall motion alone. This would have introduced other variables, because the image planes being evaluated may not be identical (different positioning of the ultrasound transducer by the sonographer) and the degree of stress may be different at the different imaging times. This approach was therefore rejected.

Figure 6: Wall Thickening on Still Frames (AI-700 ECHO)



Ultimately whether or not the added diagnostic value from AI-700 comes from detection of an inducible perfusion defect and/or a previously undetected WMA has no effect on triaging of the patient. Evidence and expert opinion from the American College of Cardiology (ACC) and the American Heart Association (AHA) support that both non-contrast stress ECHO detection of wall motion and stress SPECT detection of perfusion are appropriate and effective diagnostic tools. Both are procedures of choice for the diagnosis of obstructive CAD in patients with chronic stable angina and both are included in Class 1 recommendations.[31]

2.4 Current Diagnostic Imaging Modalities

2.4.1 Coronary Angiography

ANGIO has long been the definitive tool for CAD diagnosis.[21] However, it has an estimated procedural mortality of 1 in 1,000 patients,[51, 52] and poses an additional risk of fatal cancer development in as high as 1 in 2,000 patients due to the ionizing radiation involved.[53] Patients who undergo ANGIO are also at risk for contrast-induced nephropathy.[54] Additionally, procedural complications during ANGIO can occur as well (event rate of 0.81%, no deaths) in 72-hour period following ANGIO).[52, 55]

Appropriate utilization of ANGIO is essential to avoid exposing patients to unnecessary morbidity and mortality. This is the basis for using gatekeeper stress imaging procedures to risk stratify patients prior to ANGIO. The use of gatekeepers reduces procedural complications and hospital costs, particularly when studying patients with stable chest pain and intermediate risk of CAD.[56]

2.4.2 SPECT

The use of SPECT has grown dramatically over the past 20 years with the introduction of new radiopharmaceutical imaging agents, pharmacologic stress agents, and sophisticated imaging equipment and software.[57] Many studies have proven not only the diagnostic utility of SPECT in determining clinically significant CAD, but also its prognostic utility.[58] However, there are a number of non-trivial issues relating to SPECT to consider:

1. Safety: radiation exposure from a single SPECT procedure has been estimated to increase patients' lifetime fatal cancer risk by between 1 in 2,000 and 1 in 10,000 depending on age, sex, and other risk factors.[52, 59] Many patients may undergo multiple procedures, because CAD is a chronic disease. Medical staff and caregivers may also receive radiation exposure from handling and the proximity to radiopharmaceuticals, patients, and waste.
2. Availability: the installed equipment base for SPECT is ~11,000 units.[60] Due to space, radiation shielding, and complex licensure requirements, SPECT is less likely than ECHO to ever be routinely available to patients in smaller cardiology practices.
3. Procedure time: SPECT is a relatively lengthy procedure,[61] involving hours of patient and technical staff time.
4. Functional information: SPECT is primarily a perfusion technique that, if ECG-gated, can evaluate resting, but not stress wall motion and thickening.[57, 61]
5. Processing requirements: SPECT images utilize 3D reconstructions of time-averaged imaging data that require off-line computer analysis and software quantification.
6. Resolution: the resolution for SPECT imaging is ~1cm,[62] which is similar to the thickness of the myocardium. This results in partial volume effect artifacts, which can overestimate small defects.
7. Comfort: SPECT imaging requires patients to remain immobile for up to 20 minutes at a time, to minimize motion artifacts.
8. ^{99m}Tc supply interruptions: molybdenum-99 for ^{99m}Tc generators for the western hemisphere is produced at one reactor site in North America which periodically is forced to halt production, creating scheduling delays for nuclear labs.

Despite these issues, SPECT is currently the preferred gatekeeper, which provides a significant advantage over ECHO, as it can provide the most valuable information–perfusion.

2.4.3 Stress ECHO

Stress ECHO has advantages over SPECT, including:

1. No exposure of patients or staff to ionizing radiation, and no need to store radioactive supplies and waste;
2. With an installed equipment base of 20,000, many hospitals and cardiologists' offices already have ultrasound technology,[63]
3. Faster procedure times (30-60 minutes);
4. Comprehensive anatomic and functional information in real-time for both rest and stress;
5. Equipment has higher intrinsic resolution and is more portable;
6. Cost is $600 less per procedure than SPECT.[64]

But stress ECHO also has limitations, including:

1. Limited AWQ, which may result in a patient's imaging data being of non-diagnostic quality, resulting in the need for contrast ECHO;[6, 65]
2. No contrast agents have been approved to improve image quality for stress imaging. The 2 approved ultrasound contrast agents do improve image quality at rest, but their safety and efficacy during stress conditions have not been established.[8, 9]
3. No perfusion information is possible without use of contrast agents, and no contrast agents have been approved for ECHO perfusion. Although a number of mostly single-center studies have demonstrated that perfusion information can be obtained with currently marketed contrast agents,[2, 66, 67] no data exist from well-controlled, blinded multicenter studies demonstrating similar diagnostic performance of stress ECHO and SPECT in chest pain patients.

As a result of these drawbacks, stress ECHO procedures have grown at only a modest rate, and are used far less often than SPECT procedures.[39] The lack of perfusion information, which is the key benefit of SPECT, is a current limitation in the value of non-contrast ECHO.

2.4.4 ECHO Contrast Imaging Agents

Currently marketed ECHO contrast agents (Optison® and Definity®) are not approved by FDA for stress imaging.[8, 9] Both agents are indicated for use in patients with suboptimal ECHOs for left ventricular opacification (LVO), and to improve delineation of the left ventricular endocardial borders during resting imaging. Currently, an estimated 10-25% of ECHO imaging studies are suboptimal, and could potentially benefit from the use of contrast.[6] Common uses include patients in intensive care units who are intubated and cannot comply with body positioning requirements for optimal imaging, and patients undergoing stress imaging, an off-label use since the safety and efficacy have not been established.[8, 9]

Optison® and Definity® differ physically from AI-700 in terms of design, composition, and size. AI-700 is distinguished from these agents by its polymer shell and structure, perflubutane gas, and size uniformity with the lowest percentage of particles >10 μm and narrowest diameter range. Compared with these microsphere agents containing perflutren, AI-700 has a mechanically stronger structure that does not expand or rupture during ultrasound imaging.[1]

2.4.5 Adverse Events in Perflutren-Containing Microspheres

Perflutren-containing microspheres (Optison® and Definity®) have black box warnings related to reports of serious cardiopulmonary reactions. Use of these agents is contraindicated in patients with the following:[8, 9]

- Right-to-left, bi-directional, or transient right-to-left cardiac shunts;
- Hypersensitivity to perflutren,[8, 9]
- Hypersensitivity to blood, blood products, or albumin (Optison®[8]

Warnings are issued for use of these agents in patients with the following:

- Pulmonary hypertension or unstable cardiopulmonary conditions such as:
    - Acute MI or acute coronary artery syndrome;
    - Worsening or unstable CHF;
    - Serious ventricular arrhythmias;
    - Respiratory failure, includes patients receiving mechanical ventilation,.

As the contraindications and warnings imply, cardiopulmonary events are the primary concern. Definity® has an additional warning for QTc prolongation and is not to be administered by intra-arterial injection.[9] Optison® and Definity® product labels indicate that ECG, vital signs, and cutaneous oxygen saturation need to be monitored in patients with pulmonary hypertension or unstable cardiopulmonary conditions during and for 30 minutes following administration of perflutren-containing microspheres. In the absence of these underlying conditions, patients are to be observed closely during and following administration of perflutren-containing microspheres.[8, 9]

3. CLINICAL PHARMACOLOGY

3.1 Pharmacokinetics and Pharmacodynamics

Two clinical studies evaluated pharmacokinetics (PK) and 5 clinical studies provided data for evaluation of pharmacodynamics (PD) (Table 2).

Table 2: Clinical Studies Providing Pharmacokinetic and Pharmacodynamic Data

Study	Clinical Population	Methodology
Pharmacokinetics		
AI-700-04	Healthy volunteers	Measurement of perflubutane in blood and expired air, with 2-compartment PK analysis, following 2 AI-700 doses of 0.08 mL/kg or placebo[1] IV.
AI-700-05	Patients with CHF and/or COPD	Measurement of perflubutane in blood following a single AI-700 dose of 0.04 mL/kg IV.
Pharmacodynamics		
AI-700-02	Healthy volunteers	Measurement of LVO and MCE following single AI-700 doses ranged from 0.014 mL/kg to 0.081 mL/kg IV.
AI-700-21	Patients with suspected or known CAD and healthy volunteers	Measurement of LVO and MCE following AI-700 dosing: Pilot Phase: 2 AI-700 doses of ≤0.054 mL/kg IV. Bolus Phase: placebo[1] or 2 doses of AI-700 (0.014 or 0.054 mL/kg IV). Ancillary Phase: 2 AI-700 doses – 0.046 mL/kg IV (Session I) and 0.035 mL/kg IV (Session II).
AI-700-32 and AI-700-33	Patients with suspected or known CAD	Measurement of LVO and MCE following 2 AI-700 doses of 0.04 mL/kg IV (rest and stress imaging).
AI-700-34	Patients with prior MI and healthy volunteers	Measurement of LVO and MCE following 2 AI-700 doses of 0.04 mL/kg IV during each of 2 sessions.

CAD = coronary artery disease; CHF = congestive heart failure; COPD = chronic obstructive pulmonary disease; IV = intravenous; LVO = left ventricular opacification; MCE = myocardial contrast enhancement; MI = myocardial infarction; PK = pharmacokinetics
[1] placebo = normal saline.

3.1.1 Pharmacokinetics

Pharmacokinetics of perflubutane gas was evaluated in nonclinical and clinical studies. Microsphere pharmacokinetics and distribution were investigated in nonclinical studies. No assay is available for detection of AI-700 microspheres in humans.

- Pharmacokinetics depends in part upon the size of the microspheres, which have a mean diameter of approximately 2.3 µm.[1] This small size allows for their transpulmonary passage. Nonclinical studies demonstrate that AI-700 microspheres are cleared from the vascular space and taken up by cells of the reticular endothelial system (RES). Microspheres remain within RES macrophages until the polymer is hydrolyzed into water-soluble fragments and eventually metabolized. The insoluble perflubutane rapidly leaks out of the microsphere and is eliminated via the lungs.

- Pharmacokinetics studies in rats showed that, after IV administration of AI-700, 70-77% of the perflubutane was eliminated in expired air by 3 hours after dosing. In addition, 50-85% of palladium-labeled microspheres were cleared from the circulation within 5-7 minutes after completion of dosing.[14] By 24 hours post-dose, the recovered palladium-labeled microspheres were mostly localized in organs of the RES.

Human PK studies also demonstrated rapid clearance of perflubutane. A 2-compartment simultaneous fit of data for blood and expired air showed that, following IV injection, perflubutane was eliminated via the lungs, with a mean terminal elimination half-life of approximately 4 minutes and a longer, rate-limiting distribution half-life of approximately 84 minutes. Nearly half of the administered dose was cleared by 10 minutes after completion of dosing, and almost 75% within 1 hour in healthy volunteers (Study AI-700-04). In patients with chronic obstructive pulmonary disease (COPD) and/or congestive heart failure (CHF), clearance of perflubutane was so rapid that very few samples showed measurable amounts of gas in the blood after completion of dosing (Study AI-700-05).

3.1.2 Pharmacodynamics

AI-700 microspheres have a composition and structure that results in mechanical strength sufficient to withstand the destructive effects of ultrasound during imaging, enabling long and effective myocardial contrast enhancement (MCE) with low visual attenuation (Studies AI-700-21, -32, -33, and -34).[15, 68] AI-700 can be used with high mechanical indices (up to 1.0), thus increasing the acoustic energy of the imaging beam which enables deep penetration of the acoustic signal, resulting in successful imaging of almost all patients, including those with suboptimal AWQs. Because MCE is a marker of myocardial perfusion, and perfusion is needed to maximize sensitivity for evaluation in CAD, MCE is the parameter that best represents the PD of AI-700. Data supporting the choice of AI-700 dose for the Phase 3 studies were acquired from the PD evaluation of AI-700 imaging characteristics in early studies (Table 2).

Based upon results of these Phase 1 and 2 studies, an IV dose of 0.040 mL/kg AI-700 was selected as the dose needed to provide sufficient MCE in most patients. This dose was used in Phase 3 trials and was given during each imaging session, for a total dose of 0.080 mL/kg (two 0.040 mL/kg injections). Each AI-700 dose was injected over 1-10 minutes, a rate that would allow for optimization of MCE. In pivotal Phase 3 studies (AI-700-32 and -33), a dose of 0.040 mL/kg produced MCE of diagnostic quality in >99% of patients and MCE of ≥5 minutes in 80% of patients, demonstrating that the dose of 0.040 mL/kg is an adequate dose to perform a diagnostic stress ECHO with AI-700.

The protocols for AI-700 dosing in Phase 3 trials and the recommendations for clinical use specified that AI-700 should be administered at a rate sufficient to produce MCE, commencing with approximately 33% of the total volume per minute for approximately 30 seconds (i.e., approximately 1/6th of the total volume should be given during the first

30 seconds of dosing). Once MCE is observed, the health care provider should visually evaluate image enhancement and attenuation (if present) in real-time, and adjust the rate of administration as necessary.

3.2 Imaging

Imaging parameters used for each AI-700 study are described in Appendix 4. During the development of AI-700, multiple imaging modes (including fundamental, Doppler, harmonic, and triggered.) and multiple imaging platforms were evaluated for their ability to produce MCE with simultaneous wall motion imaging during real time (>25 Hz) imaging[15] (Appendix 5).

During Phase 2 studies (AI-700-02 and -20), imaging was performed using imaging methods that were also standard methods for non-contrast ECHO. Due to the low contrast sensitivity of these techniques, the amount of contrast agent needed to produce MCE was greater than in subsequent trials, by which time low power (mechanical index =0.3), real time imaging techniques had been developed specifically for contrast imaging. Because AI-700 produced prolonged MCE, extra imaging modes were added to compliment this focus on real-time low mechanical index imaging during Phase 3 trials (Appendix 5). A subsequent Phase 3 study (AI-700-34) has also demonstrated sustained MCE and simultaneous wall motion in real time on major equipment platforms currently on the market, using standard commercially available software.[68]

4. EFFICACY

4.1 AI-700 Clinical Efficacy Program

4.1.1 Clinical Program Rationale

Chest pain is often the initial symptom of ischemic heart disease.[31] Stress ECHO wall motion or stress SPECT perfusion imaging are the recommended initial tests for diagnosing CAD in patients with chest pain.[31] In their most recent Class I recommendations the ACC/AHA consider both stress ECHO and stress SPECT the most appropriate diagnostic methods in the management of patients with chronic stable angina who have an intermediate pretest probability of CAD.[31] Nearly all patients enrolled in Studies AI-700-32 and AI-700-33 had intermediate pretest probability of CAD. The AI-700 Phase 3 program was designed to determine if AI-700 stress ECHO had diagnostic characteristics non-inferior to those of stress SPECT imaging and to evaluate the safety in the stable chest pain population.

Patients enrolled in Studies AI-700-32 and -33 represent patients typically referred for noninvasive imaging. Study AI-700-32 included patients who were scheduled for a stress SPECT to determine if their chest pain was due to ischemia. Study AI-700-33 included patients already referred for ANGIO or patients with a recent ANGIO who required functional testing to assess the significance of coronary lesions. Patients triaged for ANGIO usually have a high pretest probability of CAD,[31, 70] but this trial excluded the highest risk patients (those who were unstable, had elevation in cardiac enzymes, or were unable to undergo a stress study). Therefore, the AI-700-33 study population had a lower risk of CAD than traditional ANGIO patients, and thus also represents the clinical target population for AI-700 ECHO. The disease prevalence for the 2 studies was approximately 50% (44% in Study AI-700-32; 58% in Study AI-700-33) as determined by the truth standard.

The primary objective of the AI-700 clinical development program was to demonstrate the safety and efficacy of AI-700-enhanced cardiac ultrasound in stable chest pain patients being evaluated for inducible ischemia. The proposed indication for AI-700 is:

Imagify™ (AI-700) *is an ultrasound imaging agent indicated for patients with stable chest pain being evaluated for inducible ischemia for the detection of coronary artery disease based on an assessment of myocardial perfusion and wall motion. Imagify™ echocardiography is accomplished with rest and stress techniques.*

4.1.2 Efficacy Studies of Contrast Agents or Stress Perfusion Agents: A Historical Perspective of Registration Trials

The final AI-700 Phase 3 efficacy trial designs and sample sizes reflect changes resulting from a dialog with FDA, whose requirements for NDA submission continued to change after the trials had begun. While these in-process changes inevitably resulted in more complex data analyses, AI-700 trials nonetheless compare favorably with the registration trials for the most recently approved products in similar categories: Myoview® (a nuclear myocardial perfusion imaging agent), Definity® (an ultrasound contrast agent), and Lexiscan® (a

vasodilator pharmacologic stress agent).[9, 71, 72] All of these products were approved during the development of AI-700. Review of their package inserts shows the following:

Myoview: The myocardial perfusion efficacy approval was based on 2 trials with a total of 252 patients (with ischemic heart disease or atypical chest pain) that compared Myoview to a truth standard of "final clinical diagnosis", including ANGIO in 72% of subjects. The endpoint "percentage of correct diagnoses" is mathematically equal to agreement or accuracy, and varied from 63.6% to 75.9% depending on reader, trial, and whether the defect was fixed or reversible. These results were considered comparable to the already-approved thallium imaging (range: 68.9% to 77.7%) in the same patients, but it is unclear whether a formal non-inferiority analysis was performed. The exercise stress indication based on these trials was later expanded to include pharmacologic stress based on an additional 2 studies, with 3 blinded reads, in a total of 57 subjects.[71]

Definity: Efficacy was based on 4 trials with a total of 208 Definity patients (disease status unknown, but entry criteria required suboptimal echocardiograms) who were compared to 41 patients with placebo. Ejection fraction measurements were evaluated in comparison to MRI and were assessed in blinded reads by 3 independent radiologists. Other endpoints were based on visual comparisons of whether the left ventricle was more opacified and whether greater length of endocardium was measurable when using Definity than when using placebo. Significant changes in mean endocardial border length from Baseline were evaluated using paired t-test ($p<0.05$). Clinically relevant endpoints such as wall motion assessment and ejection fraction measurement were reported but data were equivocal. The resulting approved indication was for opacifying the left ventricular chamber and improving the delineation of the left ventricular endocardial border in patients with suboptimal echocardiograms.[9]

Lexiscan: Efficacy as a pharmacologic stressor was determined in 2 trials with an efficacy population of 1871 patients (with known or suspected CAD) who received an initial nuclear stress scan with Adenoscan and then were randomized to received a second stress scan with either Adenoscan or Lexiscan. Only 93% of enrolled patients had images that were "valid for the primary efficacy evaluation". Agreement (based on 3 strata of reversible perfusion defects ranging from normal to large) between 2 successive Adenoscan studies approximately 1 week apart was 61- 64%, whereas the agreement between the Lexiscan study and an Adenoscan study done approximately 1 week previously was 62 - 63%.[72] No truth standard, e.g., ANGIO, was used in these trials.

All these registration trials used blinded reading of images. While this provides significantly greater objectivity, the numerical values in these large, multicenter, registration trials are significantly lower (agreement rates of 61 - 78%) than are generally reported in literature studies. However, studies submitted for publication are more typically single site, with smaller patient numbers, potentially inadequate blinding, and subject to bias (i.e., only the readable images are included in interpretation and only positive studies tend to be submitted for publication).

The Phase 3 efficacy trials (AI-700-32 and -33) compare favorably with these other trials in clinical utility of patient population, rigor of design, truth standard and sample size. The AI-700 studies were most similar in design to that of Myoview.[71] AI-700 Phase 3 efficacy studies enrolled chest pain patients and had ANGIO as a truth standard in approximately 75% of patients. The AI-700 studies had important advantages compared to Myoview pivotal studies: AI-700 studies had a larger efficacy population (662 *vs.* 252 patients, respectively) and had formal null-hypothesis testing using a non-inferiority approach with accuracy as the principal primary endpoint. The AI-700 program was smaller in size than the Lexiscan Phase 3 program. However, unlike the Lexiscan program (7% of patients excluded from analysis), the efficacy analysis included all patients who received any dose of AI-700 and had a truth and comparative standard. Although results among trials cannot be directly compared, the accuracy results of AI-700 (range: 66-71% in Phase 3 studies), are similar in range to accuracy/agreement results demonstrated in Myoview and Lexiscan Phase 3 programs.[71, 72]

4.1.3 AI-700 ECHO Blinded Reader Training

For all AI-700 clinical trials, ECHO readers were selected from institutions that were not participating in patient enrollment. ECHO readers had previous experience with contrast imaging and experience in performing and interpreting rest and stress ECHO studies.

For Phase 3 Studies AI-700-32 and AI-700-33, the 6 ECHO readers (3 different readers per trial) were trained using images acquired from prior AI-700 clinical studies. ECHO readers were provided with personal reading stations consisting of computers, monitors, and video tape players. Independent study observers were present during all reading sessions and ensured that blinded reads were performed independently and without interference.

Blinded reader training focused on teaching each reader to recognize specific diagnostic features and to identify artifacts using an ECHO Reader Training Manual. Defects indicative of CAD were defined as wall motion/wall thickening abnormalities and/or perfusion abnormalities on AI-700 images. Readers did not score wall motion and perfusion defects separately. Each myocardial segment was scored as having no defect, fixed defect, reversible defect, or as not visualizable. Pre-contrast images were not provided to blinded readers for diagnostic assessment, but were provided separately for blinded categorization of each patient's AWQ, a measure of ultrasound image quality. AWQ is often impacted by obesity, lung disease, chest surgery, or other variables.[6, 73] Patients were rated as having good, average, or poor AWQ.

Visual interpretation of AI-700 MCE relied on echocardiographic pattern recognition based on the intensity of MCE within the subendocardium and epicardium, and on observation of changes over time in contrast replenishment following contrast destruction. A defect was considered present when a relative decrease in MCE was seen in 1 region compared with another adjacent region with similar image quality. Wall motion and wall thickening were assessed as in standard stress ECHO.[7, 74] The determination of whether a segment was abnormal was also based on an integrated assessment of all views of the heart (apical 2, 3,

and 4 chamber) obtained during the imaging session. The type of defect (fixed, reversible) was determined by comparing rest and stress results. Phase 3 blinded readers were instructed to sequentially address a set of questions developed specifically for AI-700 imaging:

1. Is the wall segment normal? Does the specific wall being evaluated show homogenous/transmural myocardial contrast filling and wall motion? Does the image brighten to the same intensity immediately following the destruction sequence? If contrast attenuation is present, are there faint, obscured subendocardial dark lines, (hypoperfusion) defects, or wall motion abnormalities?

2. Is there evidence of a reversible/inducible defect? Are there subendocardial dark lines or only epicardial opacification noted during stress?

3. Is there evidence of both reversible and fixed defects or evidence of defect extension during stress? (If so, blinded readers were instructed to interpret the defect as reversible.)

Following unblinding of Study AI-700-32, blinded readers were debriefed and queried about their methods with regard to defect detection during AI-700-32 image reads. Both correctly interpreted (versus truth) cases and incorrectly interpreted cases were discussed during debriefing sessions. This review revealed that 2 of 3 readers had been reluctant to call a study abnormal unless the defect was large. This type of reading bias, a tendency to under-call disease, results in high specificity but is offset by lower sensitivity. This is different from SPECT readers who typically over-call disease (call any observed defect abnormal), resulting in high sensitivity offset by lower specificity.[56] To prevent Study AI-700-33 ECHO readers from continuing this conservative bias, the ECHO reads, which were underway, were terminated without breaking the study blind. Previously, it had been demonstrated that AI-700 ECHO readers could be taught to increase sensitivity at the expense of specificity without decreasing accuracy.[75] Study AI-700-33 ECHO readers were retrained according to this objective. At no time during the blinded read of Study AI-700-33 were the readers (or Sponsor) provided with results from the blinded reads performed prior to retraining.

4.2 AI-700 Efficacy Study Populations and Baseline Characteristics

Study populations and characteristics varied across the efficacy studies (including preliminary and pivotal studies, see Appendix 6 for details). In Phase 2 Studies AI-700-20 and -21, healthy volunteers were included in some analyses of efficacy. Healthy volunteers (mean age 33 years for both studies) were younger than CAD patients, and per eligibility criteria were in good health. Males comprised 33% of healthy volunteers in Study AI-700-20, and 68% of healthy volunteers in Study AI-700-21. The majority of healthy volunteers in both studies were White (72% in Study AI-700-20 and 68% in Study AI-700-21).

Demographic information for patients with suspected or previously diagnosed CAD who participated in Phase 2 and 3 efficacy studies is presented (Table 3). The majority of patients were male. Mean age ranged from 61-63 years. CAD patients in Study AI-700-20 were stable post-MI patients, while patients in the remaining 3 efficacy studies were stable and had a recent history of chest pain.

In Studies AI-700-32 and -33, of the 778 enrolled patients, 48.1% were European (Germany, Italy, France, Belgium, Spain, and the United Kingdom), 46.7% were North American (United States and Canada), and 5.3% were Australian.

Table 3: Demographic and Baseline Characteristics in AI-700 Efficacy Studies – All Known or Suspected CAD Patients Enrolled

Characteristic	AI-700-20 (N=35)	AI-700-21 (N=97)	AI-700-32 (N=321)	AI-700-33 (N=457)
Age (years)				
n	35	97	321	457
Mean (±SD)	63 (7.2)	61 (10.9)	61.1 (9.9)	62.4 (9.6)
Min, Max	52, 77	39, 79	33, 81	34, 83
Sex n (%)				
Male	31 (88.6)	63 (64.9)	216 (67.3)	366 (80.1)
Female	4 (11.4)	34 (35.1)	105 (32.7)	91 (19.9)
Race/Ethnicity n (%)				
Asian	0	4 (4.1)	62 (19.3)	6 (1.3)
Black	6 (17.1)	21 (21.6)	51 (15.9)	33 (7.2)
White	29 (82.9)	65 (67.0)	195 (60.7)	359 (78.6)
Other	0	3 (3.1)	13 (4.0)	59 (12.9)
Ethnicity				
Hispanic	0	4 (4.1)	12 (3.7)	72 (15.8)
Weight (kg)				
n	35	97	321	457
Mean (±SD)	88.8 (17.4)	87 (18.8)	80.7 (16.7)	85.0 (16.5)
Min, Max	58, 120	50, 141	50, 143	41, 150
Height (cm)				
n	35	97	321	456
Mean (±SD)	172.2 (10.5)	170.1 (11.4)	168.7 (10.3)	171.5 (9.6)
Min, Max	141, 190	133, 197	142, 198	131, 203

SD = standard deviation

4.3 Comparison of Primary Efficacy Results Across All Studies

Table 4 lists the 4 efficacy studies. All 4 studies enrolled patients with known or suspected CAD and all patients underwent AI-700 ECHO imaging. All efficacy trials featured primary efficacy analyses based on results from blinded reads of AI-700 ECHO images for the detection of CAD. Primary efficacy parameters varied among studies but always involved SPECT perfusion imaging as either a disease endpoint (truth) or a comparator. Primary efficacy parameters included 1 or more of the following: agreement (kappa statistics), accuracy, sensitivity, and/or specificity.

Table 4: AI-700 Efficacy Studies Enrolling Known or Suspected CAD Patients

Study	CAD Patients Enrolled/ Efficacy Population	Patients	Primary Efficacy Para-meters	Compari-sons	Truth	Statistical objective	Primary Efficacy Conclusions
AI-700-20 (Phase 2)	53 / 47	- CAD (Prior MI and positive SPECT) - Healthy volunteers	Agreement	Pre-contrast to early post contrast ECHO	SPECT	Kappa statistic >0.60	Kappa (≥0.6) for 1 of 3 readers Kappa (≥0.6) for 3 of 3 readers in optimized subgroup
AI-700-21 (Phase 2)	97 / 97	Known or suspected CAD	Sensitivity and specificity	Placebo ECHO	SPECT perfusion and ANGIO	Greater sensitivity and specificity of AI-700 ECHO compared to placebo	Sensitivity and specificity greater than placebo (2 of 3 dose groups)
AI-700-32 (Pivotal Phase 3)	321 / 285	Known or suspected CAD with chest pain	Accuracy, sensitivity, and specificity	SPECT	ANGIO if avail-able, or SPECT, ECG, and medical history	Non-inferior-ity of ECHO to SPECT (relative risk ratio; one-sided alpha=0.025)	Non-inferior accuracy (3 of 3 readers) Non-inferior sensitivity (1 of 3 readers) Superior specificity (2 of 3 readers)
AI-700-33 (Pivotal Phase 3)	457 / 377	Known or suspected CAD with chest pain	Accuracy, sensitivity, and specificity	SPECT	ANGIO if avail-able, or SPECT, ECG, and medical history	Non-inferior-ity of ECHO to SPECT (relative risk ratio; one-sided alpha=0.025)	Non-inferior accuracy (3 of 3 readers) Superior sensitivity (3 of 3 readers) Non-inferior specificity (1 of 3 readers)

Differences in reader performance were observed across studies (Table 5). In Study AI-700-20, the blinded readers read with both higher sensitivity and specificity than in the pivotal Phase 3 Efficacy Studies (AI-700-32 and AI-700-33) or in Study AI-700-21.[15] This may be attributed to the differences in the patient populations. Two factors may explain these discrepancies — disease prevalence and disease severity.

Patients in Studies AI-700-21, AI-700-32, and AI-700-33 represented a continuum of pre-test probability of disease, similar to the proposed clinical population of patients, with a CAD prevalence of approximately 50%. In contrast, the AI-700-20 study population did not represent a continuum of disease but comprised 2 distinct populations —young healthy volunteers and a larger number of patients with confirmed prior MI. Average CAD prevalence in Study AI-700-20 was therefore much higher (74%). Higher disease prevalence often increases observed sensitivity and may have, in part, accounted for estimates of sensitivity typically above 80% in this study.

In preliminary efficacy studies, AI-700 ECHO performance was evaluated by comparison to another diagnostic modality, SPECT. Percent agreement between the 2 modalities is presented in Table 5. Mathematically, percent agreement is identical to accuracy, a similar statistic evaluated in the pivotal efficacy studies. Since the validity (accuracy) of the SPECT diagnosis was not verified by a truth standard the term accuracy is reserved for analysis of agreement between AI-700 ECHO and truth.

Table 5: Diagnostic Performance of AI-700 ECHO Across Studies

AI-700 Trial Number	AI-700 Dose (mL/kg)	Disease Endpoint	Disease Prevalence	ECHO Reader	Accuracy/ Agreement (%)	Sensitivity (%)	Specificity (%)
AI-700-20[1]	0.05 or 0.08	SPECT plus history	74%	Reader 1	79	81	75
				Reader 2	87	87	88
				Reader 3	77	68	94
AI-700-21[2]	2 x 0.054	SPECT	46%	Majority Read	79	62	93
	2 x 0.014		32%	Majority Read	76	63	82
	0.046 & 0.035		27%	Majority Read	73	33	88
AI-700-32	2 x 0.04	ANGIO or SPECT, ECG, history	44%	Reader 1	66	77	58
				Reader 2	67	57	75
				Reader 3	71	50	88
AI-700-33	2 x 0.04	ANGIO or SPECT, ECG, history	58%	Reader 1	66	73	55
				Reader 2	70	68	72
				Reader 3	70	73	66

1 For the Study AI-700-20 efficacy population, AI-700 ECHO imaging was performed during rest only and a comparison was made between the early post-contrast ECHO and SPECT.
2 For Study AI-700-21, primary efficacy was based on a majority blind read.
In preliminary efficacy studies (AI-700-20 and -21, AI-700 ECHO performance was evaluated by comparison to disease defined by SPECT. Since the comparison was to another diagnostic test percent agreement (rather than accuracy) between the 2 modalities is presented.
In pivotal efficacy studies (AI-700-32 and -33), accuracy of ECHO was determined using a truth standard.

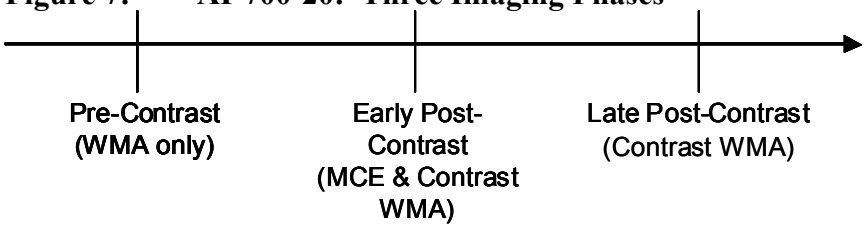
4.4 Phase 2 Efficacy Studies

Two Phase 2 dose-response intra-subject comparison studies of AI-700 ECHO to SPECT (AI-700-20 and AI-700-21) were conducted to determine if AI-700 ECHO could detect myocardial perfusion defects as defined by SPECT imaging. All ECHO and SPECT images were read by independent blinded readers. In both studies, AI-700 ECHO imaging was performed in real time (i.e., frame rates >20 Hz, which allowed for the simultaneous assessment of both perfusion and wall motion).

Study AI-700-20 was performed under resting conditions and therefore only detected the presence of fixed defects (i.e., rest defects indicative of prior MI). In addition, Study AI-700-20 images that were collected at different times during the AI-700 contrast ECHO exam were evaluated for diagnostic utility. The rationale was based on the observation that

under study conditions, MCE, which is critical for perfusion assessment and also allows wall motion assessment, persisted for 2 minutes from the start of AI-700 injection, whereas LVO, which only allows contrast wall motion evaluation, persisted longer. Blinded readers interpreted AI-700 ECHO images from a single patient multiple times (Figure 7): by separate imaging phases representing pre-contrast wall motion, early post-contrast (0-2 minutes after AI-700 administration), and late post contrast (2-5 minutes after AI-700 administration)] and as an integrated blinded read of all post-contrast images.

Figure 7: AI-700-20: Three Imaging Phases



The Efficacy Population in Study AI-700-20 included 47 of 53 subjects.

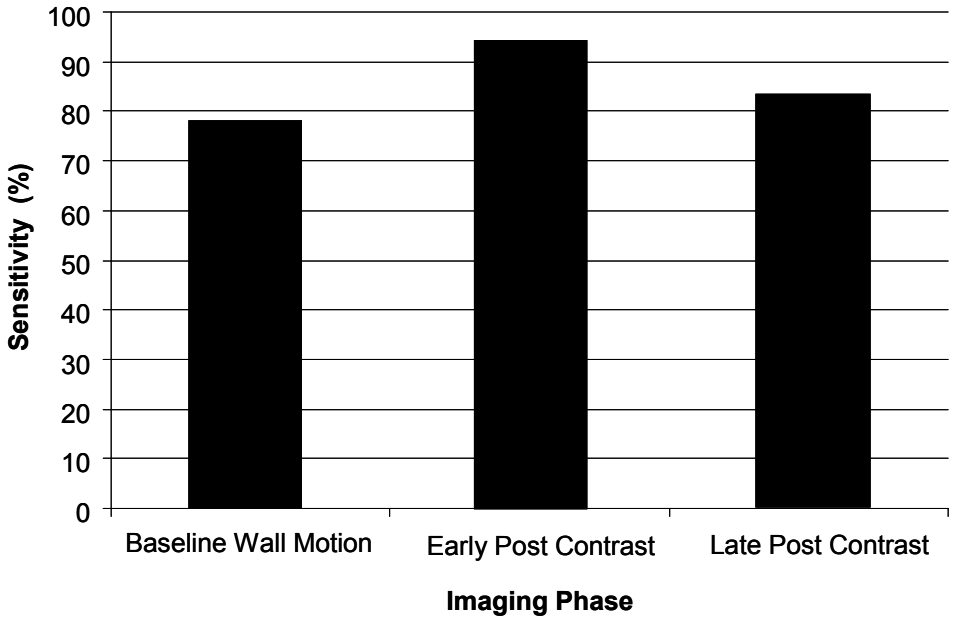
The primary efficacy analysis was based on an AI-700-20 Efficacy Population that included 47 of 53 subjects and compared AI-700 ECHO results at each of the 3 different imaging timepoints vs. the medical history of the subject (history of MI or healthy), with MI being confirmed by fixed defect on historical SPECT. Since different imaging methods and different doses were used, a simple dose-response relationship was not obtained. Imaging performance was dependent on the combination of imaging mode and dose used. Prior to the blind read, images were divided into 2 cohorts, Optimized (25 of 47 subjects) and Suboptimal (22 of 47 subjects), based on imaging method and AI-700 dose. When considering images from the Optimized Cohort (0.08 mL/kg AI-700 and standard harmonic imaging or 0.054 mL/kg AI-700 and low power imaging [mechanical index <0.3]), the majority blind read agreement in the Optimized Cohort was 92%, compared with 85% for the entire efficacy population.[19]

Suboptimal images resulted in lower overall agreement for the early post-contrast phase (77% majority blind read agreement). A potential concern is that poorly performed contrast studies may be detrimental to patient care due to reliance on post-contrast results in preference to results obtained during the pre-contrast wall motion portion of the study. It can be hypothesized that the poorer statistical results obtained from the suboptimal contrast images in Study AI-700-20 are an artifact of the protocol-driven blinded read process. This trend would probably not be observed in clinical practice because pre- and post-contrast images would be interpreted together. This hypothesis was tested in a separate blinded read of the suboptimal imaging studies (using the same readers and case report form). The objective of this ancillary blinded read was to determine the diagnostic performance of these studies when all images (baseline wall motion, early post-contrast, and late post-contrast) were read as a single imaging session. In this analysis, the Suboptimal imaging group had improved agreement, increasing from 77% during early post–contrast to 92% when all images were

read in combination. This suggests that when ECHO studies are evaluated using all available images, readers give the greatest weight to the best images.

The results of Study AI-700-20 also suggest that perfusion and wall motion together provide more diagnostic information than either perfusion or wall motion alone. Majority blind read sensitivity of ECHO (with SPECT as the truth standard) was highest in the Optimized Cohort during the early post-contrast imaging phase as compared with the late post-contrast and before contrast phases, with sensitivities of 92%, 84% and 78%, respectively. This improved performance of early post-contrast imaging over late post-contrast imaging and before-contrast imaging was attributed to the added diagnostic value of MCE and contrast WMA over either WMA alone or contrast WMA (Figure 8).

Figure 8: Study AI-700-20: Sensitivity of ECHO (with SPECT as Truth) by Imaging Phase (Optimized Cohort)



Optimized Cohort: n = 25 patients

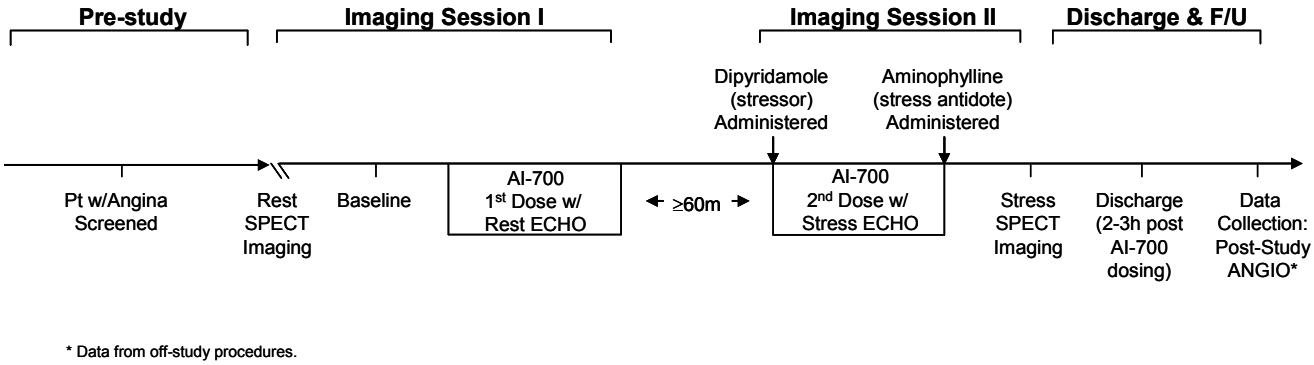
Pre-Contrast: baseline (non-contrast) wall motion;

Early Post-Contrast: MCE and contrast wall motion;

Late Post Contrast: contrast wall motion

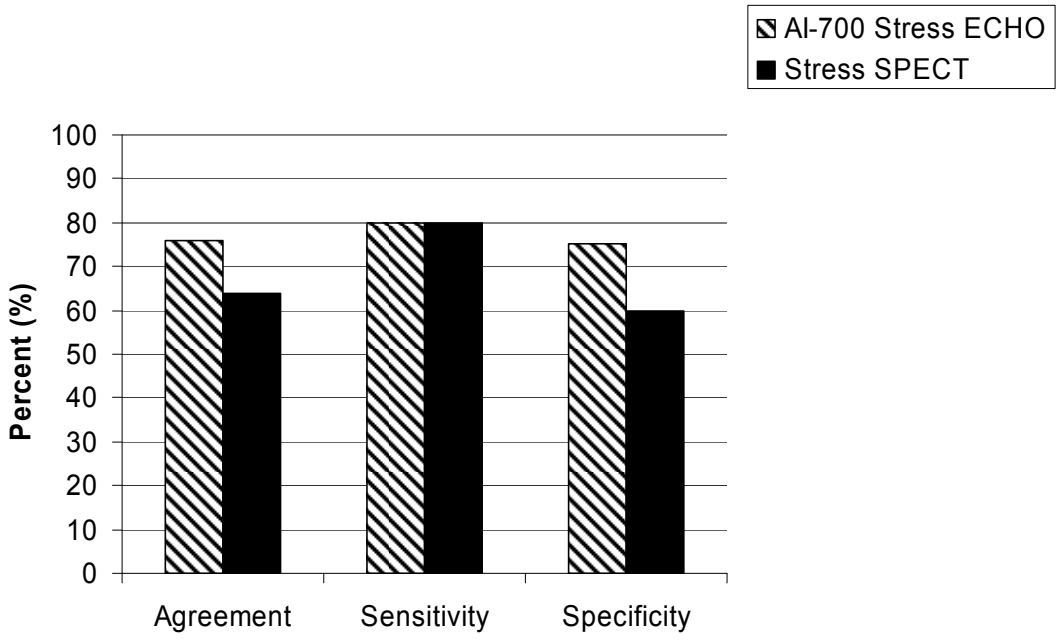
In Study AI-700-21 (Figure 9), all patients with chronic stable angina or other prior diagnostic findings of CAD had both rest and dipyridamole-induced stress ECHO and SPECT on a single day.

Figure 9: Rest / Stress Study Schema



In AI-700-21, diagnostic performance of AI-700 ECHO for defect detection was compared to diagnostic performance of SPECT. Results from the randomized cohorts (n=97 CAD patients) support that AI-700 ECHO has more diagnostic value than non-contrast ECHO (majority blind read agreement with SPECT for doses of AI-700 ranged from 73-78% for different doses of AI-700, as compared with 68% for non-contrast stress ECHO). A more objective evaluation of the performance of AI-700 ECHO was made in a subset of enrolled patients (n=25) with ANGIO evaluations. The angiographic criterion of disease was ≥70% diameter stenosis for a major epicardial artery. Majority blind read agreement between AI-700 stress ECHO and ANGIO and between stress SPECT and ANGIO is presented in Figure 10.

Figure 10: Study AI-700-21: Diagnostic Performance of Stress ECHO and Stress SPECT with ANGIO as Truth



n = 25 patients with ANGIO
Multiple AI-700 dose levels were used in Study AI-700-21

The results of the Phase 2 trials support:

1. AI-700 ECHO has better diagnostic performance than non-contrast ECHO (Studies AI-700-20 and AI-700-21).
2. AI-700 perfusion imaging in conjunction with non-contrast and contrast-enhanced wall motion imaging has incremental diagnostic value over contrast wall motion imaging alone (AI-700-20).
3. Using ANGIO as the truth standard, AI-700 stress ECHO has comparable diagnostic performance to stress SPECT perfusion imaging in patients being evaluated for inducible ischemia (AI-700-21).

4.5 Phase 3 Pivotal Efficacy Studies

Following a review of imaging data from Study AI-700-21, an AI-700 dose of 0.080 mL/kg, administered as 2 injections of 0.040 mL/kg was selected for rest and stress myocardial ECHO imaging (Phase 3 studies). Phase 3 studies were undertaken to evaluate the safety and diagnostic utility of AI-700 in stable patients with recent history of chest pain and suspected CAD. Two large, multicenter clinical trials (Studies AI-700-32 and AI-700-33) were conducted upon completion of a pilot study (Study AI-700-23) which examined the feasibility of the proposed clinical and imaging methodologies. Similar in design to Study AI-700-21, these Phase 3 studies included a rest AI-700 ECHO and a dipyridamole pharmacologic stress AI-700 ECHO, a comparator arm (SPECT), and used ANGIO (when available) as truth in the majority of patients (74%). The angiographic criteria of disease were similar to those used in AI-700-21 (≥70% diameter stenosis for a major epicardial artery).

4.5.1 Impact of Design Change on Study Conduct

Based on study designs used to obtain approvals for SPECT agents for this indication, the 2 pivotal efficacy studies were originally designed to evaluate point estimates of sensitivity and specificity for disease detection (defined by either ANGIO or SPECT). Key study design changes were mandated by FDA and implemented by the Sponsor before and during the in-life phase of both pivotal studies (Appendix 7). Important design changes that impacted the MITT Populations are presented in Table 6. The requested change to a non-inferiority design and revised stress SPECT requirements for both studies resulted in exclusion of 92 enrolled patients from the primary analyses.

Table 6: Key Study Design Changes and Impact on MITT Population

Design Modification	Impact on Study
Change to Non-inferiority • SPECT required in both studies • Positive SPECT blinded read no longer truth standard for Study AI-700-32	31 previously-enrolled patients without SPECT in Study AI-700-33 excluded from MITT Population 27 patients with evidence of CAD without ANGIO excluded from MITT Population
Vasodilatory stress required for SPECT	34 previously enrolled patients with exercise or dobutamine stress in Study AI-700-33 excluded from MITT Population
Original SPECT blinded read of 3 myocardial regions changed to a read of 13 myocardial segments	Required re-training and re-reading of SPECT readers in both studies

4.5.2 Endpoints and Hierarchical Analysis

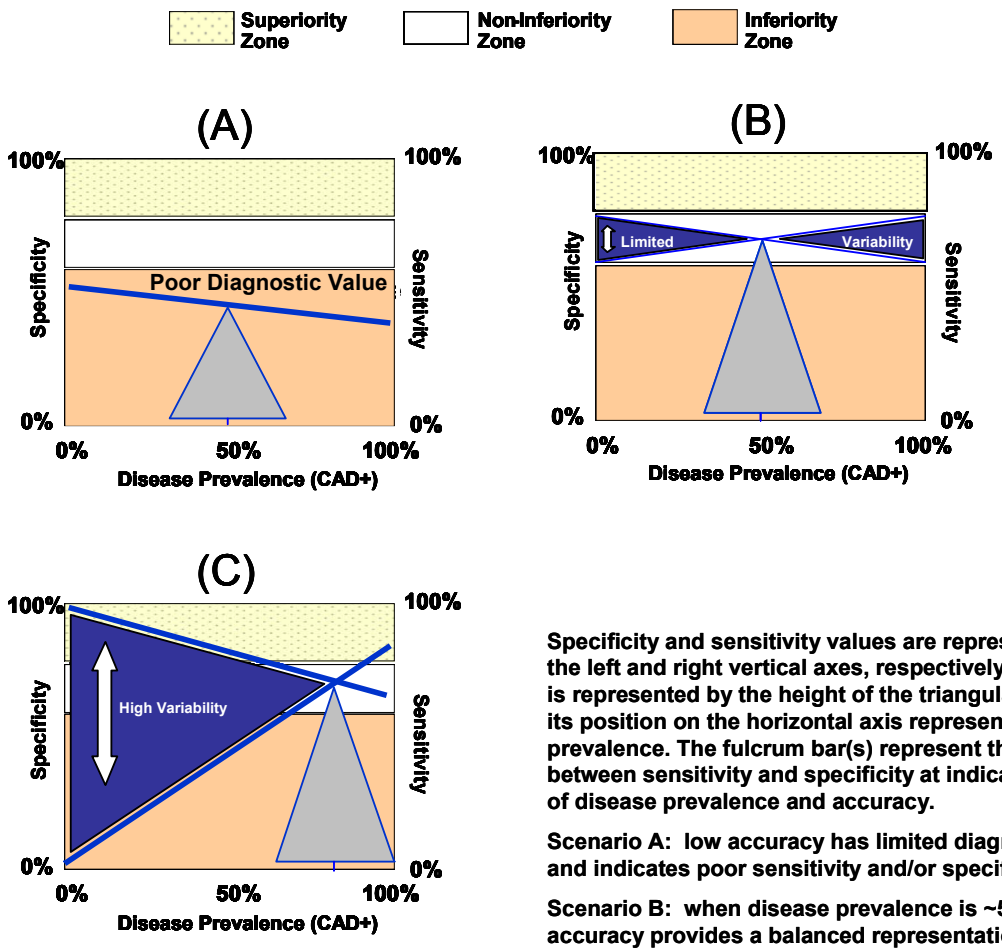
In their final form, the Phase 3 pivotal studies were designed to compare the ability of AI-700 ECHO to the ability of SPECT to diagnose CAD in patients with recent history of chest pain using ANGIO as the truth standard when available. The diagnostic performance (measured as accuracy, sensitivity, and specificity) of AI-700 ECHO to SPECT was compared using non-inferiority testing (see Appendix 2).

Accuracy measures how often information obtained with the diagnostic modality being evaluated represents truth as measured against a gold standard. Unlike the other diagnostic statistics (sensitivity, specificity, positive and negative predictive values), calculations of accuracy incorporate data from all patients in the efficacy population. Sensitivity and specificity s are often less reliable assessments of diagnostic performance because of their susceptibility to reader bias. An untrained reader can read with 100% sensitivity or 100% specificity by calling all patients either diseased or non-diseased, respectively, but reading with 100% accuracy requires skill in image interpretation. Accuracy provides the most clinical utility for patients who have an intermediate (~50%) pre-test probability of disease. In addition, using myocardial perfusion imaging to triage patients for ANGIO has the highest clinical value when disease prevalence is 50%[21] (as was observed in Studies AI-700-32 and AI-700-33).

Because accuracy incorporates data from all patients in the efficacy populations, both studies were designed with a statistical gating requirement specifying accuracy as the principal primary endpoint to which all the Type I error rate was assigned. Each study needed to achieve non-inferiority of accuracy for AI-700 ECHO compared with SPECT perfusion imaging prior to evaluation of sensitivity and specificity. An endpoint was met if at least 2 of 3 ECHO readers were non-inferior to the SPECT reader(s).

Figure 11 displays various scenarios that illustrate the effect of accuracy and disease prevalence on sensitivity and specificity. When accuracy is low (inferiority zone), a diagnostic tool has limited utility (Panel A). When disease prevalence is close to 50% and the percent accuracy is clinically acceptable (non-inferiority zone), accuracy provides a balanced representation of both sensitivity and specificity (Panel B). Alternatively, when disease prevalence is very high (as shown in Panel C) high accuracy requires high sensitivity but provides no understanding of the performance of specificity. Conversely, if disease prevalence is very low, high accuracy requires high specificity, but provides no understanding of sensitivity.

Figure 11: Effect of Accuracy and CAD Prevalence on Sensitivity and Specificity



Specificity and sensitivity values are represented on the left and right vertical axes, respectively. Accuracy is represented by the height of the triangular fulcrum; its position on the horizontal axis represents disease prevalence. The fulcrum bar(s) represent the balance between sensitivity and specificity at indicated levels of disease prevalence and accuracy.

Scenario A: low accuracy has limited diagnostic utility and indicates poor sensitivity and/or specificity.

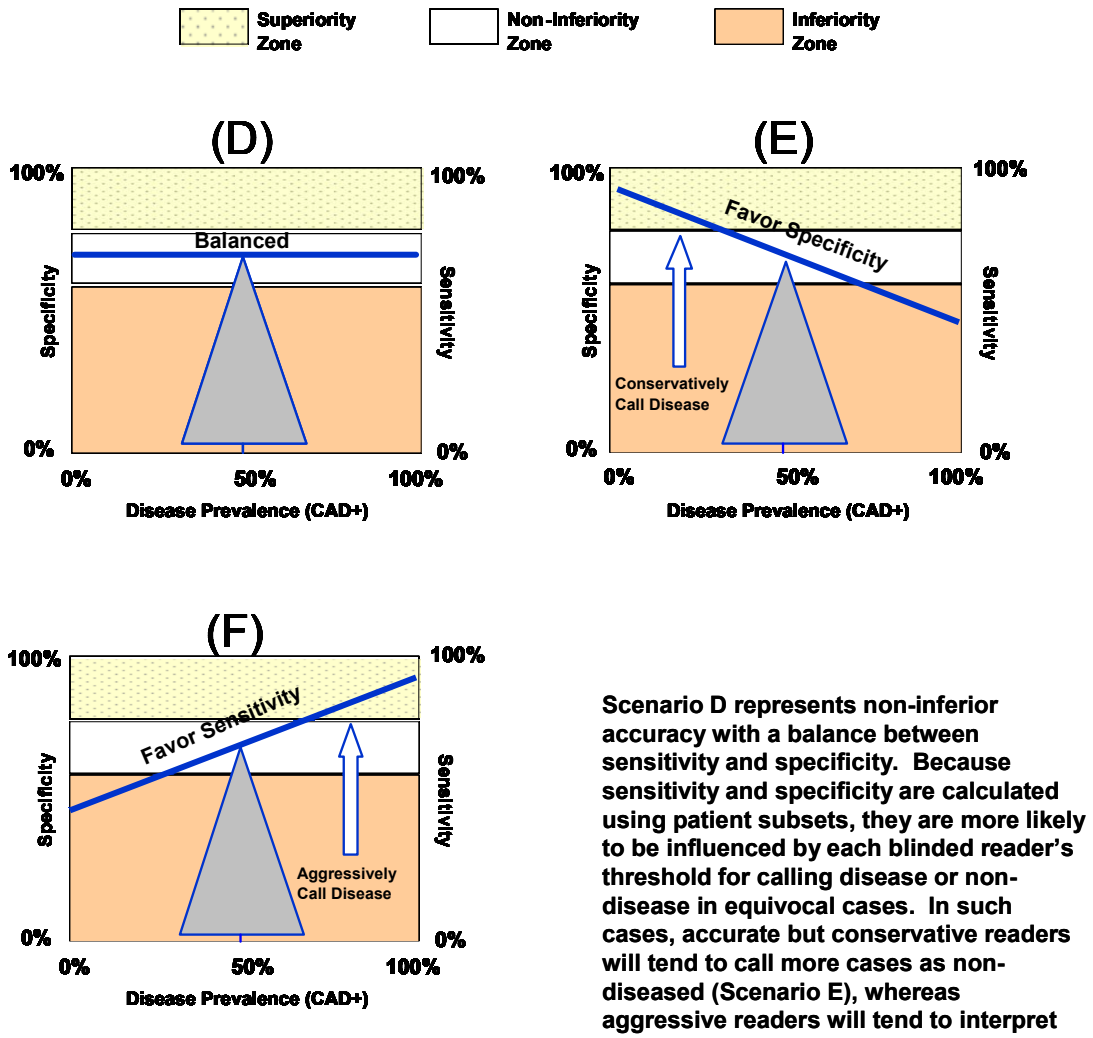
Scenario B: when disease prevalence is ~50%, accuracy provides a balanced representation of both sensitivity and specificity.

Scenario C: high accuracy when disease prevalence is very high or low results in good sensitivity or specificity, respectively, but results in a larger tradeoff with the alternate parameter.

If 2 tests are equally accurate in a population with disease prevalence of 50% (Figure 12), non-inferior levels for both sensitivity and specificity are theoretically possible, but the readers for both modalities must have no bias or the same bias (conservative or aggressive) if both parameters are to show non-inferiority (Panel D). It is also possible that either

sensitivity or specificity may show superiority (Panels E and F). Given the relationship between accuracy, sensitivity, and specificity, a reader demonstrating non-inferior accuracy and superior sensitivity or specificity will likely demonstrate inferiority for the alternative parameter.

Figure 12: Impact of Blinded Reader Bias on Sensitivity and Specificity When Accuracy is Non-Inferior



Scenario D represents non-inferior accuracy with a balance between sensitivity and specificity. Because sensitivity and specificity are calculated using patient subsets, they are more likely to be influenced by each blinded reader's threshold for calling disease or non-disease in equivocal cases. In such cases, accurate but conservative readers will tend to call more cases as non-diseased (Scenario E), whereas aggressive readers will tend to interpret more cases as diseased (Scenario F).

4.5.3 Heterogeneous Truth Definition

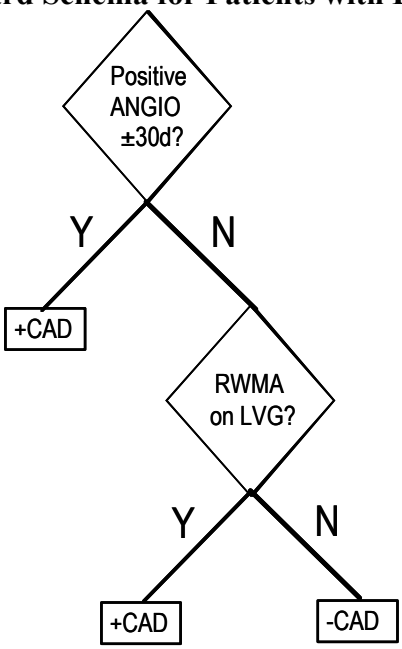
To evaluate the utility of a diagnostic test for detection of CAD, ANGIO is considered the gold standard and is often used as the standard of truth. When possible, ANGIO and, if applicable, left ventriculography (LVG) were used as truth for CAD diagnosis (Figure 13).

For Study AI-700-33, enrolled patients were expected to have evaluable ANGIO/LVG data. For Study AI-700-32, ANGIO data were not required. For both pivotal studies, ANGIO results were assessed by the Angiographic Core Laboratory.

In clinical practice, patients being evaluated for ischemia are often risk-stratified with imaging procedures. Based on imaging results, fewer than 50% of intermediate risk patients evaluated for ischemia go on to have ANGIO.[58, 76, 77] Patients with chest pain who do not go on to ANGIO are diagnosed on the basis of results of negative myocardial stress test and clinical information. A similar evaluation was performed for those patients without ANGIO in Studies AI-700-32 and AI-700-33.

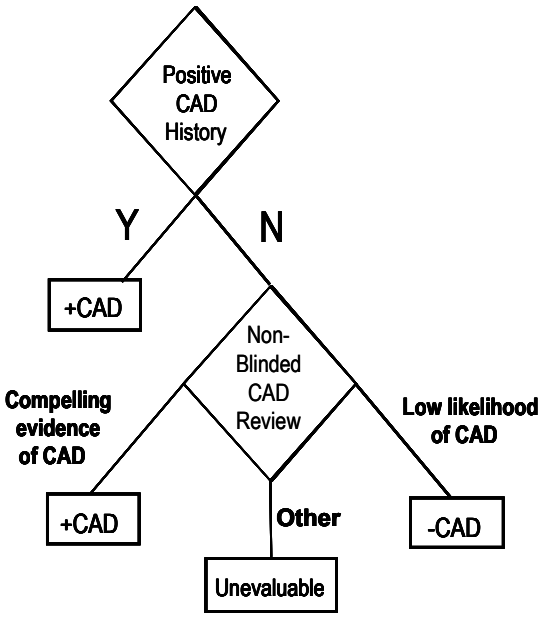
If evaluable ANGIO data were not available, patients with either a confirmed history of MI or a subsequent confirmed MI were considered CAD positive. For all other patients, CAD diagnosis was based on SPECT results in conjunction with relevant clinical and 12-lead ECG data interpreted by a cardiologist certified to interpret SPECT (i.e. non-Blinded CAD Reviewer) (Figure 14).

Figure 13: Truth Standard Schema for Patients with Evaluable ANGIO/LVG



CAD = coronary artery disease; RWMA = regional wall motion abnormality; LVG = left ventriculography.

Figure 14: Truth Standard Schema for Patients without ANGIO



CAD = coronary artery disease.

Although ANGIO correlates well with truth, the correlation is not perfect, and thus ANGIO does not represent absolute truth. Indeed, clinical guidelines caution that ANGIO is not a reliable indicator of functional significance of coronary stenosis.[31] The Sponsor conducted simulations to evaluate the impact of an imperfect truth standard on the observed diagnostic test performance with a population similar to Study AI-700-33. These simulations revealed that when the accuracy of the gold standard is 90% and the true test accuracy is 80%, the observed test accuracy using this imperfect standard would be 74%. Thus, potential disease misclassification resulting from an imperfect truth standard could have a marked effect on the observed performance of a diagnostic test.

Additional issues regarding ANGIO relate to the absolute cut-off value chosen for distinguishing patients with disease from patients without disease. Although ≥70% diameter stenosis is the accepted cut-off for describing a diagnostically significant stenosis in chronic stable angina patients,[61, 78] and was used in these trials, clinically significant stenoses involve other characteristics (length, complexity, presence of thrombus, as well as the possible role of collateral vessels) which were not accounted for in these trials but which can be important in assessing the functional consequence of a coronary stenosis. Additional analyses were performed to evaluate the severity of different stenoses and the extent of myocardial mass subtended by the stenotic artery (Sections 4.5.8.2 and 4.5.9.2).

Discussions of issues in the pivotal studies related to Multiplicity of Endpoints and Non-Inferiority Margin are included in Appendix 8.

4.5.4 CAD Status as Determined by the Truth Standard

The 2 pivotal studies had similar inclusion/exclusion criteria with the exception that all patients with prior coronary artery bypass graft (CABG) were excluded from AI-700-33 whereas only patients with prior CABG within 6 months of dosing were excluded from AI-700-32. For both pivotal studies, CAD presence was assessed using a truth algorithm (see Section 4.5.3). Prevalence of CAD was intermediate in both studies (44% in AI-700-32 and 58% in AI-700-33, Table 7).

Table 7: Coronary Artery Disease Prevalence

Truth	Study AI-700-32 MITT Population (n = 285)	Study AI-700-33 MITT Population (n = 377)
Disease	125 (44%)	220 (58%)
No disease	160 (56%)	157 (42%)

4.5.5 Quality Control for SPECT Comparator

SPECT imaging data were received, inspected, processed, and provided for blinded reading by the Yale Radionuclide Core Laboratory (SPECT Core Lab). This academic and commercial group was chosen because of its experience in supporting both commercially-funded and governmentally-funded clinical trials involving SPECT and because of its highly visible role as the accreditation certification laboratory for the Intersocietal Commission for the Accreditation of Nuclear Medicine Laboratories (ICANL).

SPECT imaging data from Studies AI-700-32 and AI-700-33 were:

- Acquired by investigational sites under imaging guidelines issued by the SPECT Core Lab;
- Inspected by the SPECT Core Lab for photon density ("counts"), patient motion, and suitable imaging parameters prior to release for blinded reading;
- Subject to rejection by the SPECT Core Lab if data were considered uninterpretable based on any of the above-bulleted issues, with subsequent exclusion of the patient from primary efficacy analyses.

Acquisition imaging guidelines were supplied to investigational sites by the SPECT Core Lab in the Yale University Radionuclide (SPECT) Core Laboratory Site Manual. This document follows the recommendations of the Imaging Guidelines for Nuclear Cardiology Procedures, Part 2, American Society of Nuclear Cardiology.[22] Sites without prior ICANL certification were required to submit SPECT imaging data from Pilot Study AI-700-23 for inspection by the SPECT Core Lab to check for proper adherence to the guidelines and suitability for

reconstruction and interpretation. Sites were qualified (allowed to participate in the efficacy studies) only if no significant quality issues were identified by the SPECT Core Lab.

Prior to tomographic reconstruction by the Core Lab, projection images were inspected for an acceptable degree of patient motion (amount not requiring digital correction) and for adequate counts (minimum 100 counts/pixel). Tomographic reconstruction slices were inspected for completeness and suitable image quality and, if considered questionable, were submitted to the Core Lab director for a ruling on suitability for blinded reading. Cases considered unsuitable were communicated to the study's Data Management Organization for exclusion from the MITT and Per-Protocol Populations (n=4 patients for Phase 3 efficacy studies).

SPECT blinded readers were independent expert nuclear cardiologists chosen from medical teaching institutions that were not used as patient enrollment centers. Each SPECT reader reviewed all SPECT data for patients in the MITT Population, without knowledge of patient history, ANGIO, ECHO, or other SPECT readers' assessments. All SPECT readers received the same training developed with participation of the SPECT Core Lab. Each SPECT reader was provided with identical sets of imaging data for each case comprising 1) projection data for a rotating torso display, 2) reconstructed data for a static display of tomographic slices, 3) gated data for 5 standard representative slices demonstrating averaged left ventricular wall motion at rest, and 4) quantitative analysis relative to normal subject databases.

4.5.6 Primary Efficacy Analyses

The efficacy objectives of the pivotal studies were to evaluate the accuracy, sensitivity, and specificity of AI-700-enhanced contrast echocardiography for detecting CAD (via an integrated assessment of perfusion and wall motion) and to demonstrate non-inferiority of the accuracy of AI-700 ECHO to SPECT. Diagnostic statistics are presented in Table 8. Accuracy, sensitivity, and specificity are included for each ECHO blinded reader, and for the (single) SPECT Reader (AI-700-32) and the Median SPECT Reader (AI-700-33). Relative ratios (RR) for a given diagnostic statistic were defined as the ratio of ECHO to SPECT. For the primary efficacy analysis, any AI-700 stress ECHO images that were unavailable or unevaluable for any reason were considered discordant to the truth standard.

In both studies, the accuracy of all AI-700 ECHO readers was non-inferior to SPECT perfusion imaging, with RRs ranging from 0.98 to 1.04. Values for accuracy were similar for all 6 ECHO readers (range 66-71%) and 4 SPECT readers (range 66-70%). Sensitivity and specificity, however, varied by reader with ECHO reader sensitivity, ranging from 50-77%, and specificity ranging from 55-88%. Similarly, sensitivity varied for all SPECT readers, ranging from 57-80%, and specificity ranging from 50-78%. Sensitivity of 4 of 6 ECHO readers was non-inferior to that of the SPECT readers. Moreover, the sensitivity of 3 ECHO readers was also superior to that of the SPECT readers. Specificity of 3 of 6 ECHO readers was non-inferior to that of SPECT with 2 ECHO readers from Study AI-700-32 also demonstrating superior specificity to SPECT.

Table 8: Primary Efficacy Results from AI-700 Studies AI-700-32 and -33

Diagnostic Statistic	AI-700-32				AI-700-33			
	SPECT Reader	ECHO Reader 1	ECHO Reader 2	ECHO Reader 3	Median[1] SPECT Reader	ECHO Reader 1	ECHO Reader 2	ECHO Reader 3
Accuracy	**69.8%**	**66.3%**	**67.0%**	**71.2%**	**67.1%**	**65.5%**	**69.5%**	**70.0%**
RR		0.95	0.96	1.02		0.98	1.04	1.04
95% CI		0.86, 1.04	0.87, 1.06	0.93, 1.12		0.89, 1.07	0.96, 1.12	0.96, 1.13
p-value[2]		0.004*	0.002*	<0.001*		<0.001*	<0.001*	<0.001*
Sensitivity	**77.6%**	**76.8%**	**56.8%**	**49.6%**	**60.9%**	**72.7%**	**67.7%**	**73.2%**
RR		0.99	0.73	0.64		1.19	1.11	1.20
95% CI		0.88, 1.11	0.63, 0.84	0.54, 0.74		1.07, 1.34	1.01, 1.24	1.09, 1.34
p-value[2]		0.002*	0.97	>0.999		<0.001*	<0.001*	<0.001*
p-value for superiority						<0.001	0.020	<0.001
Specificity	**63.8%**	**58.1%**	**75.0%**	**88.1%**	**75.8%**	**55.4%**	**72.0%**	**65.6%**
RR		0.91	1.18	1.38		0.73	0.95	0.87
95% CI		0.78, 1.06	1.04, 1.35	1.24, 1.57		0.62, 0.85	0.84, 1.06	0.76, 0.98
p-value[2]		0.12	<0.001*	<0.001*		0.95	0.013*	0.26
p-value for superiority		0.88	0.006	<0.001				

RR=relative ratio (for each diagnostic statistic, RR was defined as the ratio of ECHO to SPECT); CI = confidence interval.

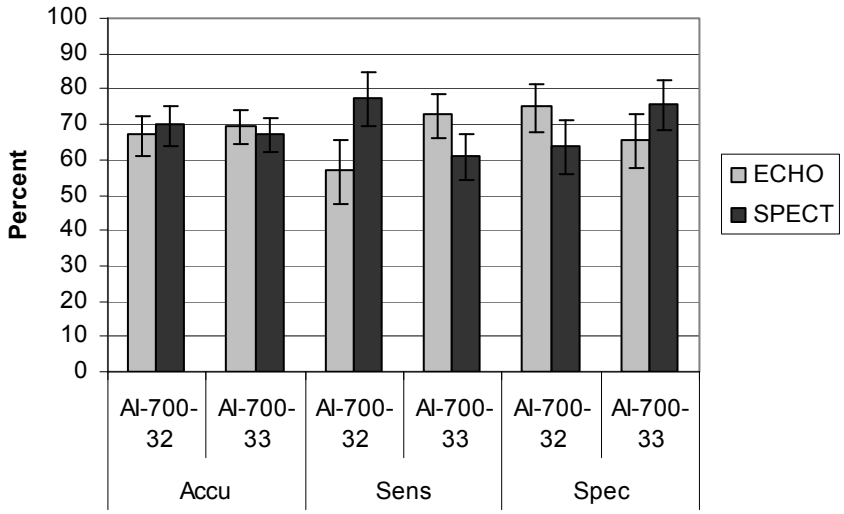
[1] For all Study AI-700-33 primary efficacy analyses, the Median SPECT Reader was SPECT Reader 1. The diagnostic statistics for the remaining SPECT readers were: accuracy 65.8% (SPECT Reader 2) and 67.4% (SPECT Reader 3); sensitivity 57.3% (SPECT Reader 2) and 80.0% (SPECT Reader 3); and specificity 77.7% (SPECT Reader 2) and 49.7% (SPECT Reader 3).

[2] p-values were for non-inferiority tests with a margin of 0.83 and a one-sided alpha; p –values <0.025 were statistically significant.

* p-value indicates ECHO is non-inferior to SPECT

Accuracy, sensitivity, and specificity are illustrated for the median-performing ECHO reader for each Phase 3 efficacy study (Figure 15). Similarly, accuracy, sensitivity and specificity are presented for the single SPECT reader (Study AI-700-32) and the median-performing SPECT reader (Study AI-700-33). In both studies, accuracy estimates are similar for ECHO and SPECT readers, with small confidence intervals. There was more variability among readers for sensitivity and specificity.

Figure 15: Studies AI-700-32 and -33: Diagnostic Performance of Median AI-700 ECHO and SPECT Readers for Primary Efficacy Analysis



Error bars designate 95% confidence intervals.

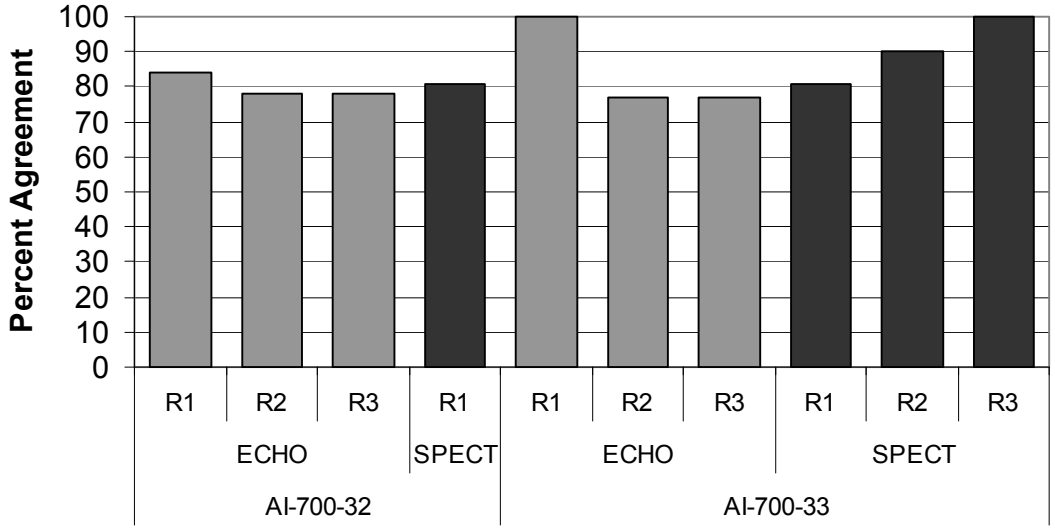
Multiple analyses to test the robustness of efficacy findings were conducted using different study populations (i.e., by repeating the primary efficacy analysis on several subpopulations and expanded populations). The results of these analyses were similar to those of the primary efficacy analyses (Appendix 9).

4.5.7 Inter- and Intra-Reader Variability

Intra-Reader Variability

To assess ECHO intra-reader variability in each Phase 3 study, the blinded readers made independent evaluations of duplicate images from the same patients (Figure 16). Percent agreement ranged from 78.1 to 84.4% in Study AI-700-32, and from 77.4 to 100% in Study AI-700-33, and was statistically significant for each ECHO reader. For SPECT, percent agreement was 80.8% in Study AI-700-32, and ranged from 84.2 to 100% in Study AI-700-33, and was statistically significant for each reader. These data indicate that ECHO readers and SPECT readers had similar reproducibility.

Figure 16: Intra-Reader Agreement in Studies AI-700-32 and -33



Different ECHO readers were used in each study.

Inter-Reader Variability

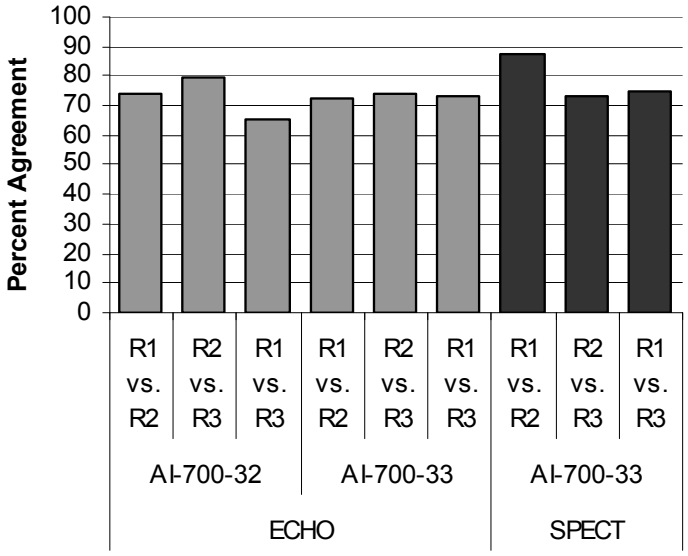
Inter-reader agreement is presented in Figure 17.

In Study AI-700-32, inter-reader agreement among the 3 ECHO readers ranged from 65.6-79.6%, and was statistically significant among all pairs of ECHO readers ($p<0.001$). In Study AI-700-33, percent agreement among the 3 ECHO readers ranged from 72.1-74.0%, and was also statistically significant among all pairs of ECHO readers ($p<0.001$).

Because Study AI-700-32 had a single SPECT reader, there can be no measurement of inter-reader agreement. In Study AI-700-33, percent agreement among the 3 SPECT readers ranged from 72.9-87.5%, and was statistically significant for all pairs of SPECT readers ($p<0.001$).

The findings indicate average inter-reader agreement was not significantly different for AI-700 ECHO readers compared to SPECT readers.

Figure 17: Inter-Reader Agreement in Studies AI-700-32 and -33



Different ECHO readers were used in each study.

4.5.8 Analyses Supportive of Primary Efficacy

4.5.8.1 Association between Defect Detection and Presence of CAD

The association between defects detected with AI-700 ECHO and presence of CAD is presented in Table 9, and between defects detected with SPECT and presence of CAD in Table 10. For both AI-700 ECHO and SPECT, myocardial regions with defects detected by either modality were positively associated with the presence of CAD (odds ratio >1). Patients with defects detected in a single region had greater odds of having a CAD diagnosis compared with those without defects. Increased odds of CAD associated with defect detection were even greater among those patients who had defects detected in more than 1 region compared with those without defects. ECHO readers with the lowest sensitivity (ECHO Reader 3 in Study AI-700-32 and ECHO Reader 2 in Study AI-700-33) had the highest odds ratio for multi-vessel disease, indicating these readers had the greatest ability to discriminate between diseased and non-diseased patients.

The precision of these estimates for both ECHO and SPECT readers varied considerably. Certain cells in the cross-tabulations had very few observations, resulting in wide 95% confidence intervals. For all readers, the 95% CI for the odds ratio excluded one. Thus, for all readers, there was a significant association between defect detection and odds of CAD; the association increased as disease severity increased.

Table 9: Association between Abnormal AI-700 ECHO and Odds of CAD in Studies AI-700-32 and -33

Truth	Odds Ratio (95% CI)					
	Study AI-700-32			Study AI-700-33		
	ECHO R1	ECHO R2	ECHO R3	ECHO R1	ECHO R2	ECHO R3
No defect	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Single region defect	3.8 (2.1, 6.8)	3.2 (1.9, 5.4)	4.8 (2.4, 9.2)	2.6 (1.5, 4.3)	3.8 (2.4, 6.0)	3.7 (2.2, 6.5)
Multiple region defect	6.1 (3.2, 11.8)	13.3 (3.8, 47.2)	20.9 (6.1, 71.3)	4.2 (2.5, 7.1)	74.7 (10.1, 553.4)	6.8 (4.0, 11.6)

Different ECHO readers were used in each study.
R1=reader 1; R2=reader 2, R3=reader 3

Table 10: Association between Abnormal SPECT and Odds of CAD in Studies AI-700-32 and -33

Truth	Odds Ratio (95% CI)			
	Study AI-700-32	Study AI-700-33		
	SPECT	SPECT R1	SPECT R2	SPECT R3
No defect	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Single region defect	4.2 (2.4, 7.5)	4.3 (2.7, 6.8)	3.8 (2.4, 6.2)	2.6 (1.6, 4.3)
Multiple region defect	16.2 (7.0, 37.3)	26.3 (3.5, 199.7)	20.9 (4.9, 89.5)	7.7 (4.2, 14.0)

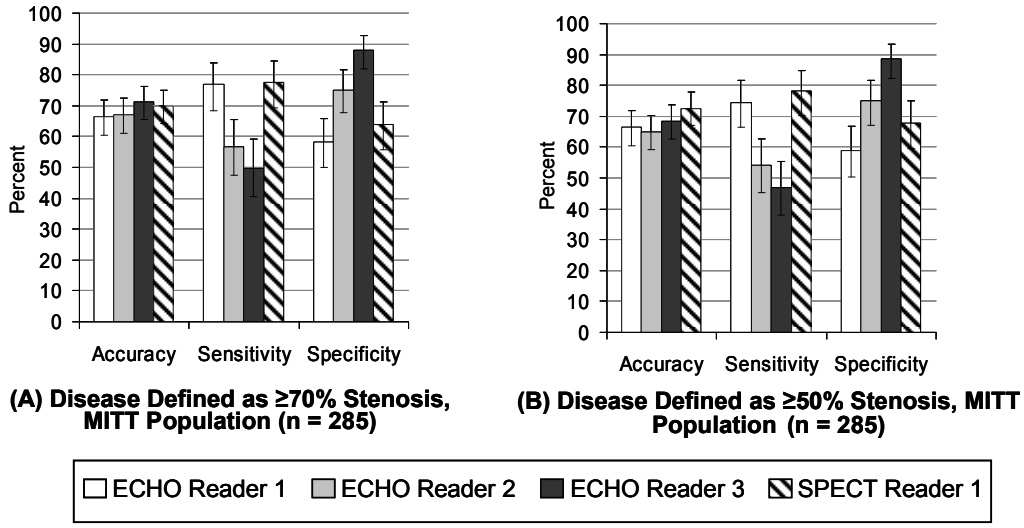
R1=reader 1; R2=reader 2, R3=reader 3

4.5.8.2 Disease Defined by Angiography as ≥50% Diameter Stenosis

For the primary efficacy analyses, a positive ANGIO was defined quantitatively as ≥70% diameter stenosis in any major epicardial artery segment (other than the left main) or RWMA on LVG.[31] The primary efficacy analyses were repeated, using an alternative definition of a positive ANGIO: ≥50% diameter stenosis in any major epicardial artery segment or RWMA on LVG. A ≥50% diameter stenosis cut-off was selected because it is often used in prognostic studies and has been reported in multiple diagnostic studies.[61, 78]

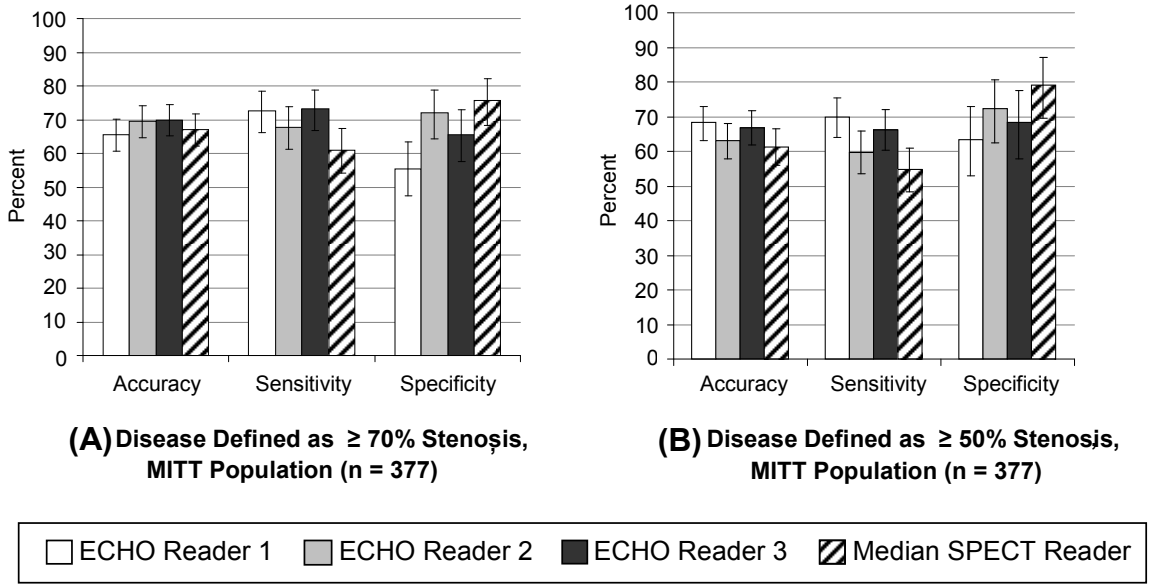
For both Studies AI-700-32 (Figure 18) and AI-700-33 (Figure 19), diagnostic statistics estimates were not appreciably different when ≥50% rather than ≥70% diameter stenosis was used as the criterion for positive CAD on ANGIO.

Figure 18: AI-700-32: Diagnostic Performance Using an Alternative Definition for Positive ANGIO (Disease Defined as ≥50% Diameter Stenosis)



Error bars designate 95% confidence intervals.

Figure 19: AI-700-33: Diagnostic Performance Using an Alternative Definition for Positive ANGIO (Disease Defined as ≥50% Diameter Stenosis)



Error bars designate 95% confidence intervals.

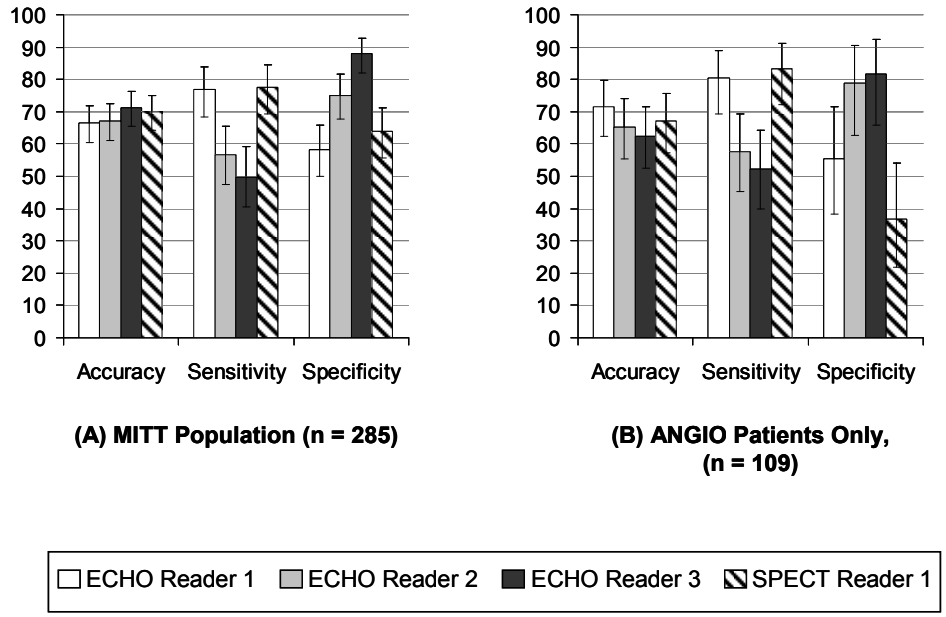
4.5.9 Efficacy Analyses within ANGIO/LVG Subpopulation

4.5.9.1 AI-700 ECHO and SPECT Performance within ANGIO/LVG Subpopulation Compared to MITT Population

Performance was assessed for patients with ANGIO/LVG as the truth standard and compared to performance in the MITT population. Nearly all patients (98%) in the

MITT population of Study AI-700-33 had ANGIO/LVG as the truth standard. Although the ANGIO/LVG subpopulation represented fewer than half of MITT patients in Study AI-700-32, ECHO reader diagnostic performance was similar in the MITT Population and the ANGIO subpopulation (Figure 20). In contrast, specificity of the SPECT reader was much lower in the ANGIO/LVG subpopulation and may reflect either referral bias (fewer patients with negative SPECT studies would go on to ANGIO) or bias in CAD determinations made by the non-blinded CAD reviewer in the absence of ANGIO/LVG assessments.

Figure 20: AI-700-32: Diagnostic Performance of ECHO and SPECT Readers (ANGIO Patients only, Disease Defined as ≥70% Diameter Stenosis)



Error bars designate 95% confidence intervals.

4.5.9.2 AI-700 ECHO and SPECT Performance by Disease Severity within the ANGIO/LVG Subpopulation

The clinical utility of a diagnostic imaging agent for CAD lies not only in its ability to detect myocardial perfusion and function but to determine the extent and severity of disease.[31] In general, the larger and more severe the abnormality, the greater the diagnostic and prognostic significance.[21] Thus, it is clinically more important to detect larger defects with more severe stenoses than smaller/less severe defects. CAD severity is often measured by the diameter stenosis of a major coronary vessel, with the extent of the defect measured by a jeopardy score.

Accordingly, for pivotal Phase 3 efficacy studies, disease severity could only be evaluated in patients who had ANGIO alone or ANGIO/LVG as their truth determination. Two measures of disease severity were analyzed: Global Jeopardy Score (GJS) and number of vessels involved. Percent GJS was derived from the Bypass

Angioplasty Research Investigation (BARI)[79] and is a metric that approximates the portion of myocardium subtended by stenotic vessels.

Reader sensitivity was estimated within subgroups of CAD patients stratified by GJS. In both Phase 3 efficacy studies, ECHO sensitivity increased as disease severity (measured by GJS) increased, regardless of whether disease was defined as ≥ 50% or ≥ 70% diameter stenosis. Similar results were observed among the SPECT readers. For all ECHO readers in each study, sensitivity increased as disease severity increased. All readers read with ≥ 70% sensitivity for lesions with ≥ 30% of the left ventricular mass subtended by the stenotic vessel.

Reader sensitivity was also estimated within subgroups of CAD patients stratified by number of diseased vessels. The population include those in whom the number of diseased vessels could be determined [i.e., CAD status defined by evaluation of either stenosis by ANGIO and/or wall motion by LVG in patients without prior CABG; AI-700-32 (n=89); AI-700-33 (n=353)]. In Study AI-700-32, patients were stratified for single and multi-vessel disease since there were few patients with triple-vessel disease.

For most ECHO and SPECT readers, there was a positive correlation between sensitivity and the number of diseased vessels [Figure 21 (Study AI-700-32) and Figure 22 (Study AI-700-33)]. In multi-vessel disease, sensitivity of 6 ECHO Readers ranged from 70-91%.

Figure 21: AI-700-32: Sensitivity of ECHO and SPECT Readers for Defect Detection in Patients with Disease Severity Defined by Number of Vessels Involved

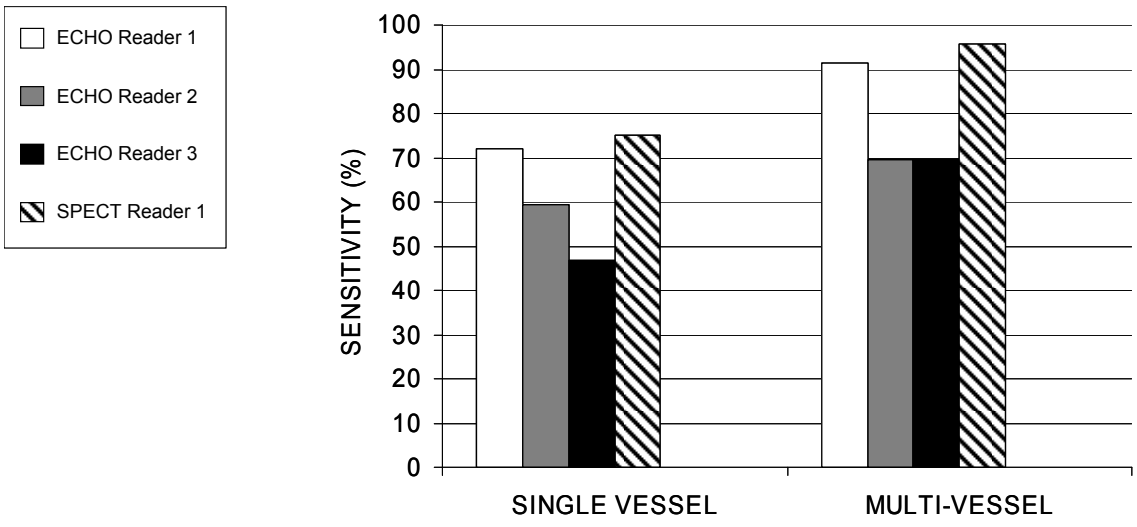
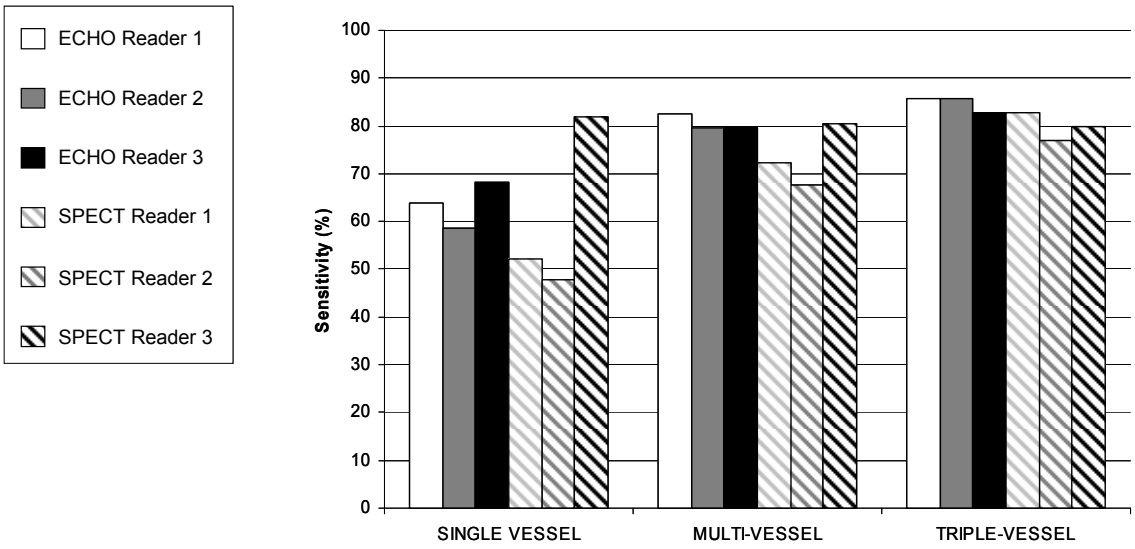


Figure 22: AI-700-33: Sensitivity of ECHO and SPECT Readers for Defect Detection in Patients with Disease Severity Defined by Number of Vessels Involved



4.5.9.3 Disease Diagnosis and Localization in ANGIO/LVG Subpopulation

A third type of analysis performed within the ANGIO/LVG subpopulation was to evaluate the ability of AI-700 ECHO readers to both correctly diagnose and correctly localize defects. The analysis population consisted of patients with localizable disease and was identical to that analyzed by strata of diseased vessels. Sensitivity, the ability to correctly identify disease among patients with disease, was evaluated. Prior estimation of sensitivity concluded correct disease identification if a defect was detected by the reader in a diseased patient. In the present analysis of defect detection and localization, correct disease identification was concluded if a defect was detected by the reader AND correctly localized to at least one region.

Using this stricter criterion, sensitivity of AI-700 ECHO and SPECT was evaluated and compared to primary analysis in the same patient population. As expected, sensitivity of all readers went down. Percent decrease ranged from 3-16% among ECHO readers and 26-31% among SPECT Readers (Table 11). These data suggest that in general, ECHO readers correctly detected and localized disease to a degree no less than SPECT readers. These data also support that aggressive ECHO readers (AI-700-32 ECHO 1 and AI-700-33 ECHO 2 and ECHO 3) were not randomly calling more patients diseased but actually had a lower CAD detection threshold and more correct localization compared to conservative readers.

Table 11: Sensitivity of ECHO and SPECT Readers for Defect Detection and Localization

Study	Reader	Global Detection Sensitivity (%) (95% CI)	Detection and Localization Sensitivity (%) (95% CI)	% Decrease
AI-700-32	ECHO 1	78.4 (64.7, 88.7)	66.7 (52.1, 79.2)	15.0
	ECHO 2	60.8 (46.1, 74.2)	58.8 (44.2, 72.4)	3.3
	ECHO 3	54.9 (40.3, 68.9)	49.0 (34.8, 63.4)	10.8
	SPECT	82.4 (69.1, 91.6)	60.8 (46.1, 74.2)	26.2
AI-700-33	ECHO 1	73.8 (67.1, 79.7)	65.4 (58.4, 71.9)	11.5
	ECHO 2	69.8 (63.0, 76.0)	58.4 (51.3, 65.3)	16.3
	ECHO 3	74.3 (67.7, 80.1)	68.8 (61.9, 75.1)	7.3
	Median SPECT	62.9 (55.8, 69.5)	43.6 (36.6, 50.7)	30.7

[1] Assessed in patients with localizable disease [i.e., CAD defined by evaluation of either stenosis by ANGIO and/or wall motion by LVG in patients without prior CABG; AI 700 32 (n=89); AI 700 33 (n=353)]

4.5.10 Comparisons of Results in Special Populations

Differences in diagnostic performance (accuracy, sensitivity, and specificity) for gender, age and body mass index (BMI) were evaluated in each Phase 3 efficacy study for all ECHO and SPECT blinded readers.

Fewer females were enrolled in the pivotal studies than males, and CAD prevalence was lower in females than in males. All 6 ECHO readers had higher accuracy and specificity in females than in males. The clinical significance of these findings is unclear because most observed differences fell within the confidence intervals of the estimates. Higher specificity and resulting higher accuracy may be due to lower disease prevalence in females.

There was no indication that AI-700 ECHO performance varied by obesity status (obesity defined by BMI as ≥30 kg/m^2). In contrast, all 3 SPECT readers in Study AI-700-33 had lower specificity for obese patients as compared with non-obese patients.

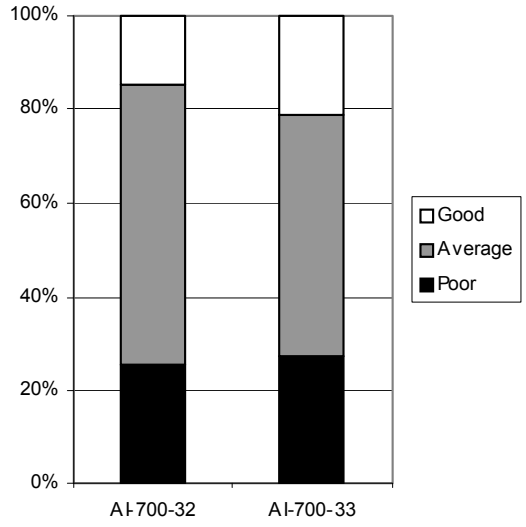
There was a trend for AI-700 ECHO sensitivity to be higher in older patients: 38% in AI-700-32 and 45% in AI-700-33 MITT populations were ≥65 years of age. All 3 AI-700-32 ECHO readers demonstrated higher sensitivity and subsequent higher accuracy in patients ≥65 years compared with <65 years. The clinical significance of these findings is unclear because observed differences fell within the confidence intervals of the estimates and were not observed in Study AI-700-33.

4.5.10.1 Acoustic Window Quality

For imaging with AI-700 to be clinically viable, it must be robust and applicable to most patients rather than only to a subset of patients with good acoustic windows. Moreover the increasing prevalence of conditions associated with poor AWQ (previous chest surgery, COPD, increased age, etc.) increases the importance of consistent performance independent of AWQ. Non-contrast (Baseline) rest images were collected and scored (good, average, or poor) for AWQ by independent ECHO blinded readers for determination of AWQ. Assessments of AWQ were made independent of and blinded to assessments of coronary artery disease (i.e., defect detection). Criteria for AWQ scoring are detailed in the ECHO Blinded Reader Training Manual.

Majority assessment of AWQ from 3 ECHO readers was used to assign patients to an AWQ category (good, average, or poor). For both Studies AI-700-32 and AI-700-33, "average" was the most frequently reported AWQ, and there were more "poor" quality windows than "good" quality windows (Figure 23). For Study AI-700-32, 15% of cases had good AWQ, 60% had average AWQ, and 25% had poor AWQ. For Study AI-700-33, 21% of cases had good AWQ, 52% had average AWQ, and 27% had poor AWQ.

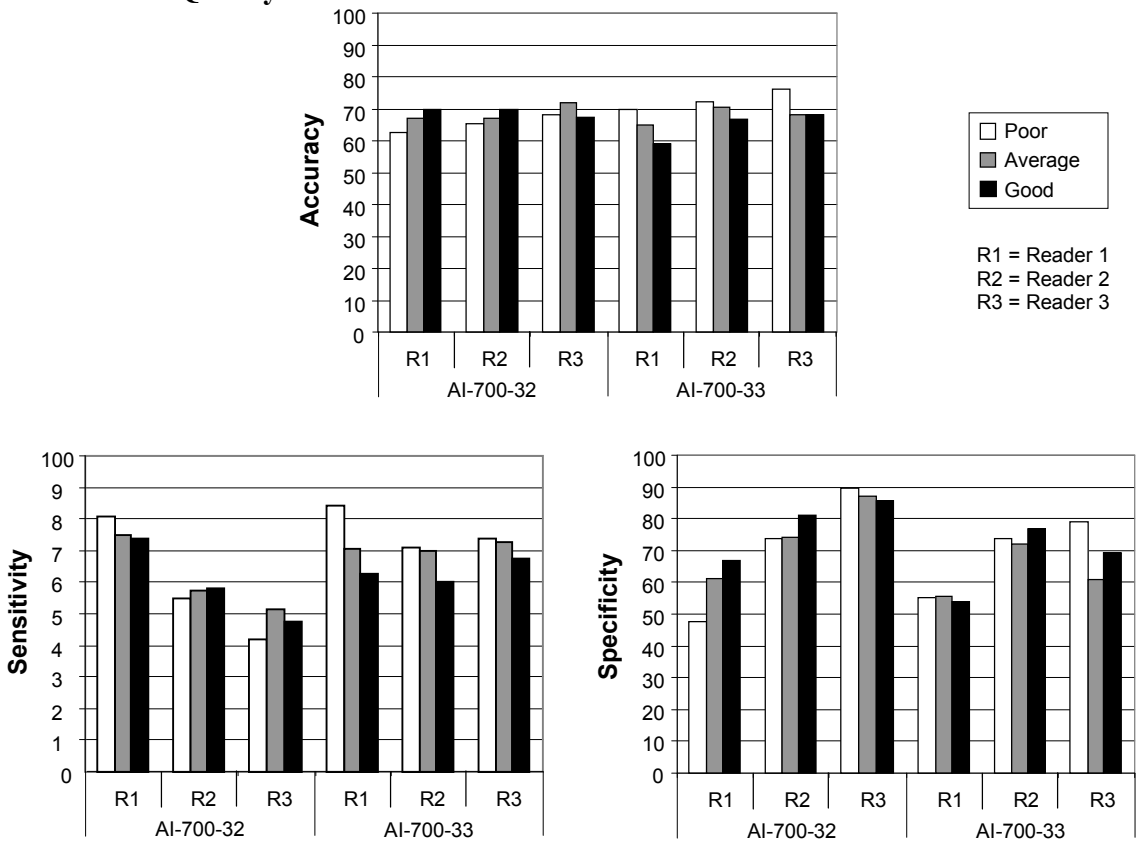
Figure 23: Distribution of Acoustic Window Quality in Studies AI-700-32 and -33



Diagnostic performance of each reader was assessed within strata (Figure 24). No trends in diagnostic performance (measured as accuracy, sensitivity, and specificity) were observed across strata. In other words, the estimates of accuracy, sensitivity, and specificity were similar regardless of AWQ category, supporting that the performance of AI-700 ECHO is consistent, regardless of the patient's AWQ. The similar performance characteristics of AI-700 across different AWQ may be attributed primarily to the fact that AI-700 ECHO imaging can be conducted at higher mechanical indices (≤1.0)[18] than imaging using currently approved contrast agents, which allows better penetration of the

ultrasound beam to facilitate obtaining optimal images, particularly in patients with poor AWQ. Despite the high prevalence of poor AWQ, 99% of all of studies were interpretable by the blinded readers.

Figure 24: Diagnostic Performance of ECHO Readers by Acoustic Window Quality in Studies AI-700-32 and -33



Different ECHO readers were used in each study.

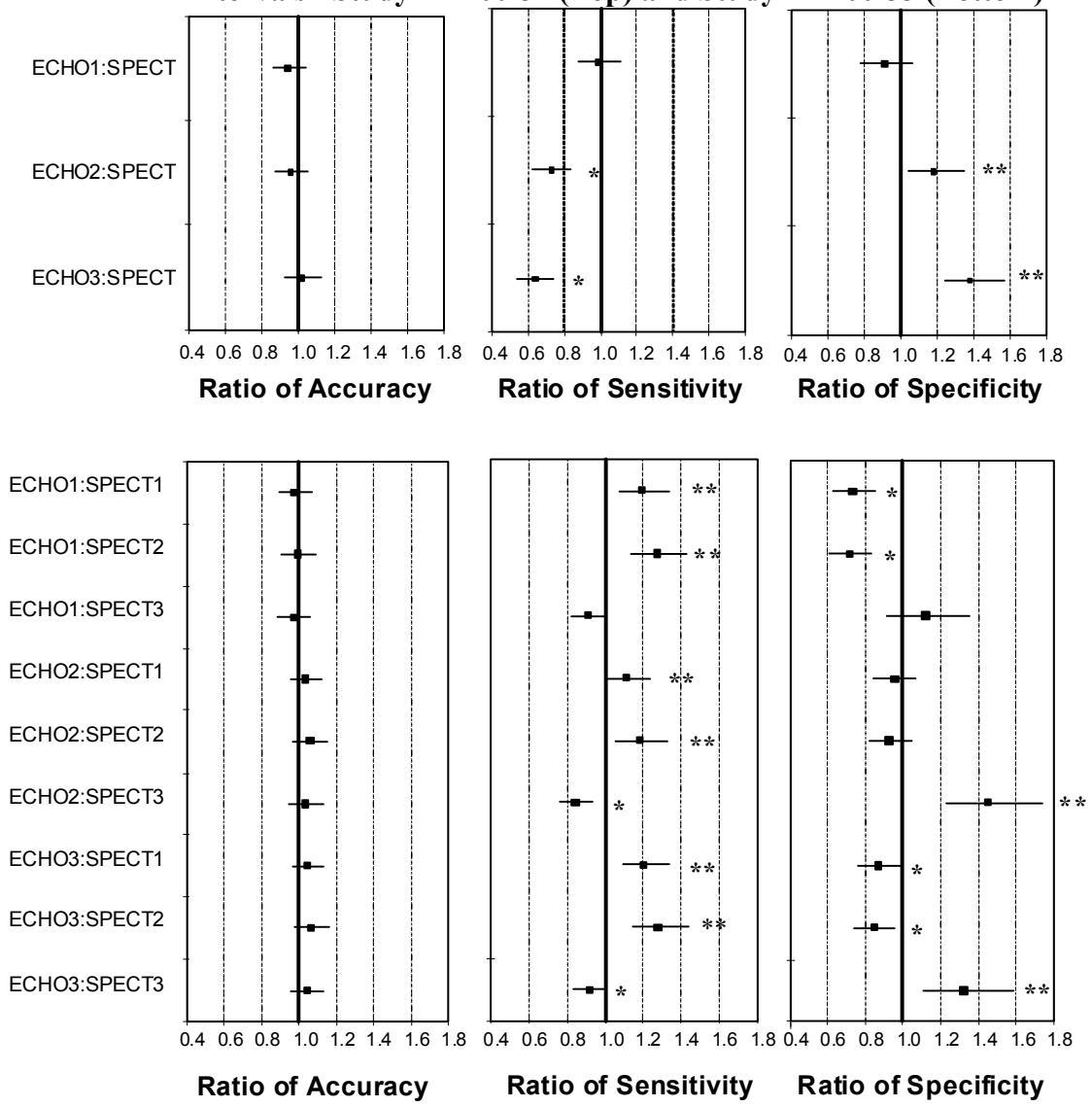
4.5.11 Integrated Efficacy Analyses

4.5.11.1 Pairwise Comparisons of Relative Risk Ratios

Rather than evaluating non-inferiority, which is based on a subjective non-inferiority margin, superiority of all ECHO/SPECT reader pairs within a trial was evaluated for each diagnostic parameter. The null hypothesis of non-superiority is rejected and superiority is concluded if the 95% CI for the RR is greater than and excludes 1.0. If the 95% CI for the RR is <1.0, then superiority of the comparator is concluded. Relative risk ratios of ECHO readers for all primary efficacy parameters were compared with those of SPECT readers. Data for accuracy were homogeneous; for all pairwise comparisons of accuracy, the 95% CI for the ratio includes 1.0, indicating that neither reader demonstrated superiority over the other. In contrast, Figure 25 illustrates the heterogeneity for pairwise comparisons of sensitivity and specificity. For sensitivity, ECHO was more often superior to SPECT than vice versa (6 versus 4 pairs), but no differences were observed for specificity (4 versus 4 pairs). Note that these analyses provide evidence of the theory

in that a reader with superiority in 1 parameter is likely to demonstrate inferiority in the other parameter (Figure 12).

Figure 25: Pairwise Comparisons of Relative Risk Ratios with 95% Confidence Intervals - Study AI-700-32 (Top) and Study AI-700-33 (Bottom)



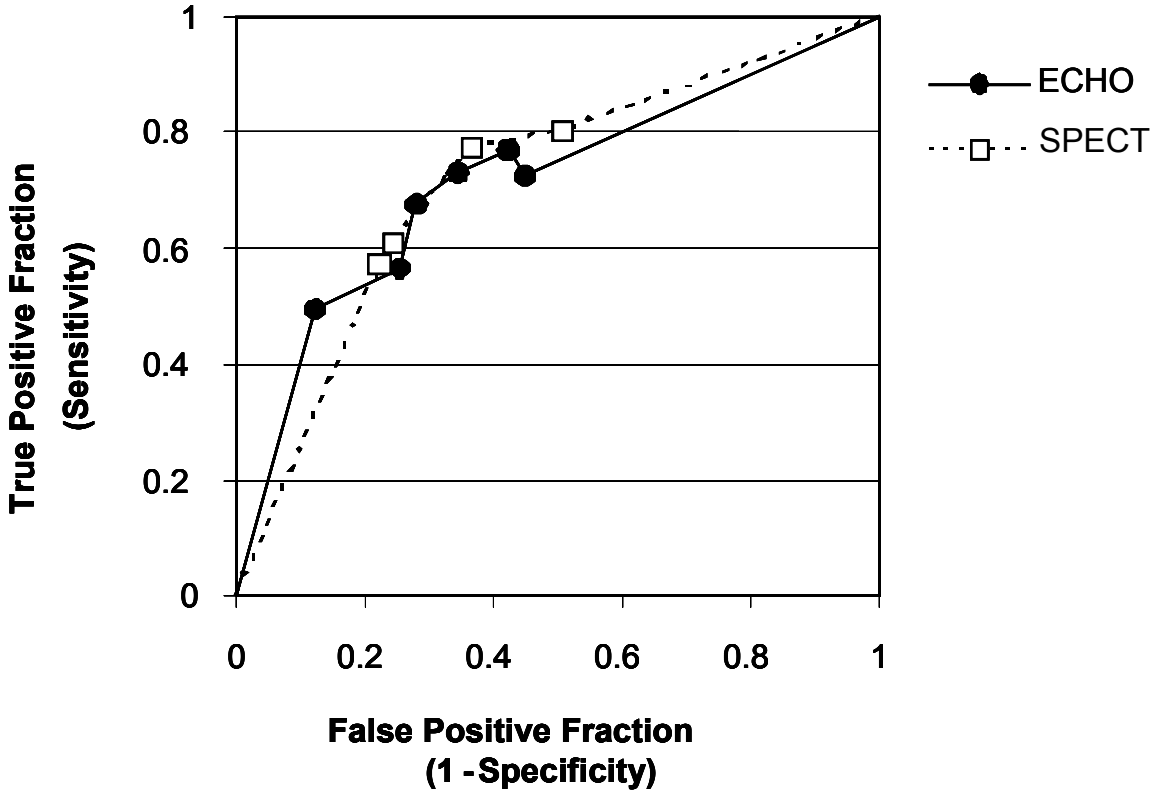
** AI-700 ECHO superior to SPECT
* SPECT superior to AI-700 ECHO

4.5.11.2 Receiver Operating Characteristic (ROC) Curves

To evaluate the performance of ECHO and SPECT modalities apart from individual reader variability, all ECHO and SPECT readers' true positive fraction (sensitivity) versus the false positive fraction (1-specificity) were plotted (Figure 26). The resulting multi-reader receiver operating characteristic curves (mROC) were plotted for each modality. The area under the curve (AUC) from the resulting 2 curves was calculated

using the trapezoidal rule. The shape of the ROC curves for AI-700 ECHO and SPECT perfusion imaging are almost identical, with an equal AUC value of 0.72, thus indicating that, when both Phase 3 studies are considered collectively, the diagnostic performance of the 2 modalities is similar. The ROC curves also illustrate that the variations in reader bias (the position along the ROC curve) is similar for both SPECT and ECHO, thereby supporting the concept that both ECHO and SPECT readers make the same degree of trade-off between sensitivity and specificity.

Figure 26: Multi-Reader ROC Curve for ECHO and SPECT Readers (Phase 3 Efficacy Studies)



4.5.11.3 Clinical Utility of AI-700 ECHO: Increased Diagnostic Value over Clinical Risk Factors

The intended clinical use of AI-700 ECHO is to integrate imaging information along with other clinical variables to screen for those patients at elevated risk of CAD who would benefit most from further more invasive evaluation with ANGIO. In the practice of medicine, the medical history and clinical assessments of the patient, in conjunction with results from cardiac imaging procedures, will be used to select those at elevated risk of CAD for evaluation with ANGIO. To estimate the value of AI-700 ECHO in the context of other clinical factors, multivariate logistic regression models were developed. Clinical factors included in the analysis were those typically used to stratify patients by CAD risk [age (10-yr ordinal categories), gender, and angina symptoms (atypical, typical and unstable)] in addition to other well-characterized risk factors (hypertension and diabetes).

Characteristics of patients with CAD (n=345) and without CAD (n=317) are presented in Table 12. Compared with patients without CAD, patients with CAD were significantly more likely to be older, male, current/former smokers, and to have diabetes. CAD positive patients also had a higher frequency of positive findings on both AI-700 ECHO and SPECT imaging.

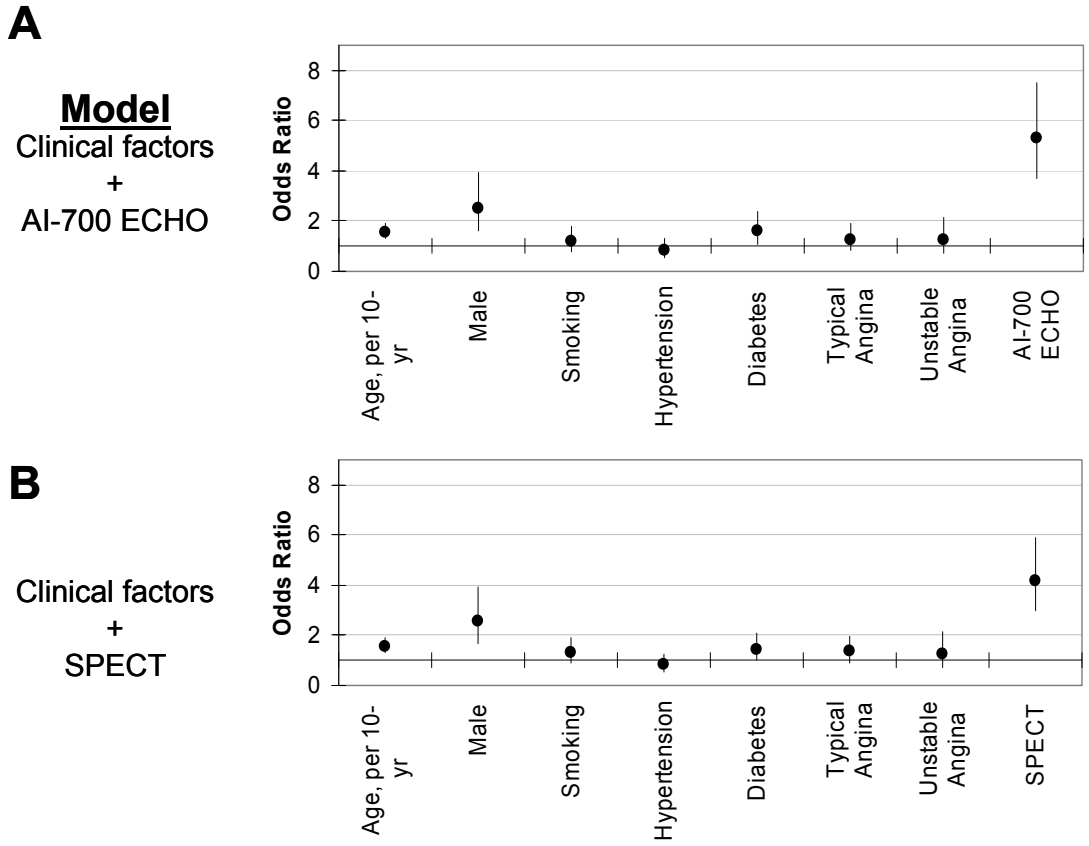
In multivariate analysis adjusted for clinical factors, positive AI-700 ECHO remained a clear, significant marker of CAD [OR (95% CI) = 5.3 (3.7, 7.5); p<0.001] (Figure 27, Panel A). Similar results were observed for SPECT [OR (95% CI) = 4.2 (3.0, 5.9); p<0.001] (Figure 27, Panel B). Association between imaging test results and CAD was evaluated for each AI-700 ECHO and SPECT reader. After adjustment for clinical factors all 6 ECHO readers and all 4 SPECT readers had a clear, significant association with CAD. Each imaging modality remained a significant and independent predictor of CAD.

Table 12: Clinical Characteristics of Patients with and without CAD (MITT Populations)

	Overall	CAD	No CAD	p-value
Age (years)				
N	662	345	317	
Mean (SD)	61.6 (9.8)	63.0 (9.5)	60.1 (9.9)	< 0.001
Median	62	63	60	
Percentiles (25th, 75th)	(55, 69)	(56, 70)	(53, 68)	
Range (min, max)	(33, 83)	(34, 82)	(33, 83)	
Age, 10-year categories, n (%)				
< 50	85 (13)	33 (10)	52 (16)	0.001
50 - ≤ 60	210 (32)	98 (28)	112 (35)	
60 - ≤ 70	240 (36)	133 (39)	107 (34)	
> 70	127 (19)	81 (23)	46 (15)	
Sex, n (%)				
Male	505 (76)	293 (85)	212 (67)	< 0.001
Female	157 (24)	52 (15)	105 (33)	
Smoking status, n (%)				
Current/former smoker	435 (66)	243 (70)	192 (61)	0.008
Never smoked	227 (34)	102 (30)	125 (39)	
Hypertension, n (%)				
Yes	507 (77)	262 (76)	245 (77)	0.680
No	155 (23)	83 (24)	72 (23)	
Diabetes, n(%)				
Yes	205 (31)	125 (36)	80 (25)	0.002
No	457 (69)	220 (64)	237 (75)	
Type of angina, n (%)				
Atypical	201 (30)	93 (27)	108 (34)	0.087
Typical	358 (54)	200 (58)	158 (50)	
Unstable[1]	103 (16)	52 (15)	51 (16)	
Defect noted from ECHO, n (%)				
Yes	320 (48)	235 (68)	85 (27)	< 0.001
No	342 (52)	110 (32)	232 (73)	
Defect noted from SPECT, n (%)				
Yes	337 (51)	236 (68)	101 (32)	< 0.001
No	325 (49)	109 (32)	216 (68)	

Note: P-value for continuous and categorical variables obtained from Kruskal-Wallis and chi-square tests, respectively.
[1] Includes patients with a history of resting chest pain

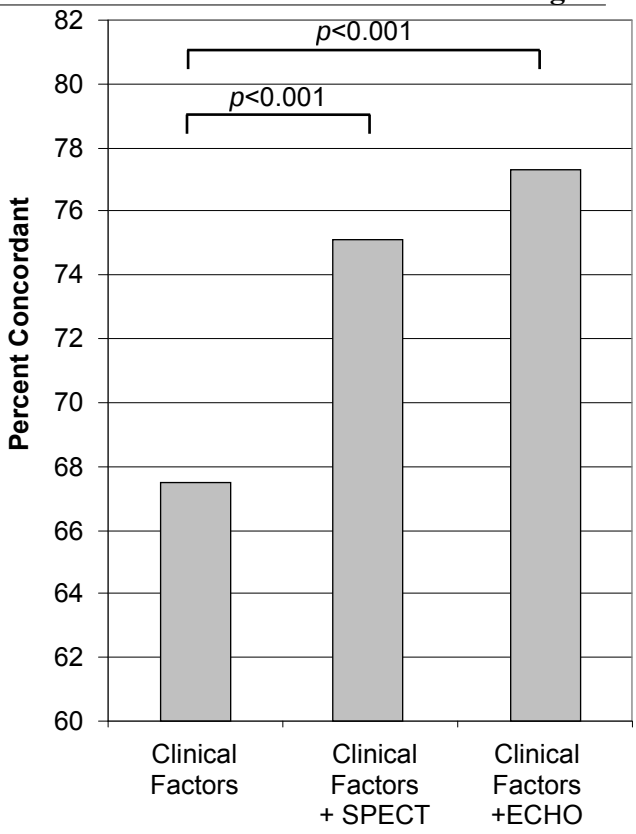
Figure 27: Multivariate Logistic Regression Analysis of Clinical and Imaging Factors



Each multivariate panel presents OR estimates derived from a single model including all the covariates indicated. Error bars designate 95% confidence intervals.

The relative predictive value was evaluated for the following models: clinical factors; clinical factors+SPECT; and clinical factors+ECHO. Comparison of the percent concordance statistic (derived from the probability predicted for each observation) revealed that percent concordance was higher for models with clinical factors and either ECHO or SPECT (p-value <0.001, Figure 28).

Figure 28: Relative Predictive Value of 3 Logistic Regression Models



4.5.12 Pooled Efficacy Analyses

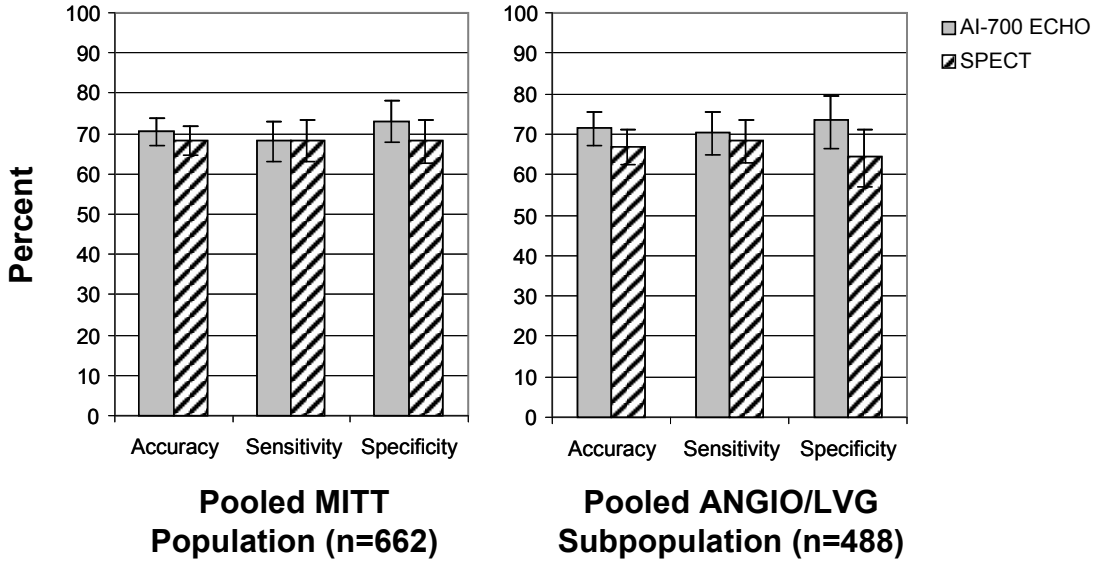
In preparation for this meeting, pooled analysis of Studies AI-700-32 and AI-700-33 was conducted to summarize the diagnostic performance of AI-700 ECHO and SPECT in a single large population representing the intended use population. These analyses were not part of the original NDA submission but are provided here to give an overall assessment of the ability of AI-700 ECHO (majority assessment of 3 readers) to detect CAD (including ischemic disease) when compared to SPECT [SPECT reader (AI-700-32) and majority assessment of 3 readers (AI-700-33)]. Similar to other efficacy analysis, accuracy, sensitivity, and specificity were compared. A second pooled analysis was performed on a clinically important subpopulation, namely, patients without prior history of MI who are likely to have ischemic disease.

4.5.12.1 Diagnostic Performance of ECHO in Pooled Analysis of AI-700-32 and AI-700-33

In pooled analysis of the MITT populations (n=662, 285 from AI-700-32 and 377 from AI-700-33), AI-700 ECHO accuracy, sensitivity, and specificity were 70.5%, 68.1%, and 73.2%, respectively, compared to 68.3%, 68.4%, and 68.1%, respectively, for SPECT (Figure 29).

In pooled analysis of the subpopulation of patients with ANGIO/LVG assessments (n=488), AI-700 ECHO accuracy, sensitivity, and specificity were 71.5%, 70.3%, and 73.4%, respectively, compared to 66.8%, 68.3%, and 64.4%, respectively, for SPECT (Figure 29). This analysis further supports that AI-700 ECHO and SPECT have comparable diagnostic performance when data from Studies AI-700-32 and -33 are pooled. The conclusion is the same when only patients with a homogeneous truth standard (ANGIO/LVG) are considered.

Figure 29: Diagnostic Performance of ECHO in Pooled Analysis of Studies AI-700-32 and AI-700-33

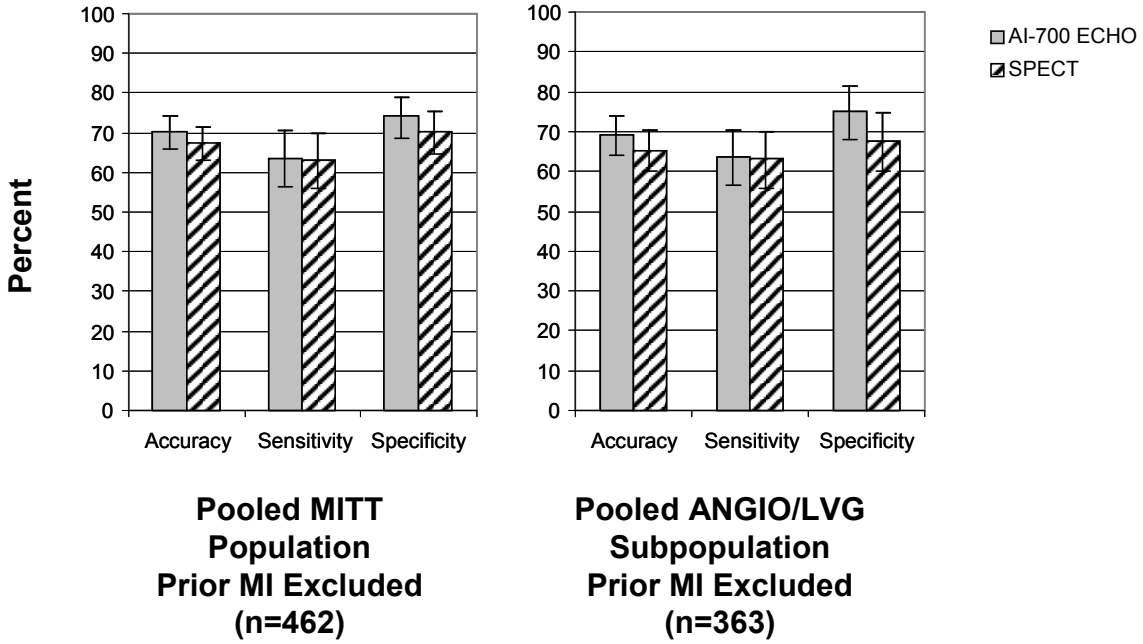


4.5.12.2 Diagnostic Performance of AI-700 ECHO in Populations Enriched for Patients with Inducible Ischemia

To evaluate the performance of AI-700 ECHO to detect inducible ischemia, additional pooled analyses were performed in populations excluding patients with history of prior MI. This resulting subpopulation is likely enriched for patients without resting wall motion abnormalities that had a stress inducible abnormality as the only indication of disease. Similar to Section 4.5.12.1, the analysis was performed in 2 populations: the MITT population excluding patients with prior MI and in patients who had ANGIO/LVG excluding those with prior MI. In the MITT populations excluding patients with prior MI (n=462), AI-700 ECHO accuracy, sensitivity and specificity was 69.2%, 63.7%, and 75.1%, respectively, compared to 65.3%, 63.2%, and 67.6%, respectively, for SPECT (Figure 30).

When analysis was restricted to patients with ANGIO/LVG data (n=363), AI-700 ECHO accuracy, sensitivity and specificity was 70.1%, 63.7% and 74.2%, respectively, compared to 67.5%, 63.2%, and 70.2%, respectively, for SPECT (Figure 30). These data strongly support that AI-700 ECHO is comparable to SPECT for the detection of ischemic disease.

Figure 30: Diagnostic Performance of AI-700 ECHO in Populations Enriched for Patients with Inducible Ischemia



4.5.12.3 Conclusions from Integrated and Pooled Efficacy Analyses

In conclusion, the pooled / comprehensive Phase 3 efficacy analysis indicate that stress AI-700 ECHO has clinically equivalent diagnostic performance as quantitative stress ^{99m}Tc SPECT myocardial perfusion imaging for detection of CAD in chronic stable angina patients. In addition, AI-700 ECHO is a strong predictor of CAD (stronger than all clinical factors tested). AI-700 ECHO is an independent predictor of CAD (i.e., information provided by AI-700 ECHO is not redundant with clinical risk factors).

4.6 Overall Efficacy Conclusions and Discussion

Phase 2 and 3 studies of AI-700 have demonstrated the diagnostic capabilities of this ultrasound contrast agent in detecting coronary artery disease.

Phase 2 studies support that AI-700 ECHO has greater diagnostic ability compared with non-contrast ECHO imaging in patients with known or suspected CAD.

Phase 3 Efficacy Studies AI-700-32 and AI-700-33 indicate that in patients with recent history of chest pain and suspected inducible ischemia, AI-700 rest and stress ECHO has clinically equivalent diagnostic performance as compared with SPECT imaging, with non-inferior accuracy. Variations in sensitivity and specificity were observed and attributed to variations in specific reader bias rather than to characteristics of the imaging modalities. In addition, AI-700 ECHO has been demonstrated to be an effective diagnostic tool in patients regardless of the underlying non-contrast ECHO acoustic window quality. Images were interpretable in 99% of patients.

When Studies AI-700-32 and AI-700-33 are considered collectively, analyses support that AI-700 ECHO and SPECT have similar ability to diagnose CAD. Both modalities had similar ranges of values. The areas under the multi-reader ROC curves were identical. The observed inter- and intra-reader agreements were similar for each modality. Six of 6 (100%) ECHO readers and 3 of 4 (75%) SPECT readers were able to detect the more important and more severe multi-vessel disease with ≥70% sensitivity. AI-700 ECHO and SPECT can predict the presence of CAD, and each modality can provide independent incremental value beyond those provided by clinical factors and the alternative modality.

In conclusion, the data considered in totality support that AI-700 ECHO is clinically equivalent to SPECT for diagnosis of CAD.

5. SAFETY

5.1 Nonclinical Safety

5.1.1 Nonclinical Toxicity Studies

Standard toxicology studies were conducted including single and repeat dose toxicology studies, genotoxicity studies, reproductive and developmental toxicity studies and local irritation studies. No clinically significant findings were observed regarding local IV irritation, genotoxicity, or reproductive/fertility toxicity. Additionally, studies were designed specifically to evaluate cardiovascular/cardiopulmonary safety of AI-700. Appendix 3 contains a list of all nonclinical safety pharmacology studies and toxicology studies. Notable nonclinical cardiovascular/cardiopulmonary safety findings are summarized. Doses are described relative to the HD of 0.08 mL/kg.

Table 13 summarizes the results of AI-700 single-dose and repeat-dose toxicity studies in rats and monkeys.

Table 13: Margins of Safety: NOAEL in Single- and Repeat-Dose Studies in Rats and Monkeys

Animal	Study Design	Highest Dose Tested (mL/kg)	NOEL (mL/kg)	NOAEL (mL/kg)	Margin of Safety for Human Dose (HD=0.08 mL/kg)
Single-dose studies					
Rat	14 day recovery	2.86	1.71	1.71	21
Monkey	14 day recovery	5.27	0.26	≥5.27[1]	66
Repeat-dose studies					
Animal	**Study Design**	**Highest Dose Tested (mL/kg)**	**NOEL (mL/kg)**	**NOAEL (mL/kg)**	**Margin of Safety for Human Dose (HD=0.08 mL/kg)**
Rat	15 day repeat; 28 day recovery	1.71	Not established	0.86	11
Monkey	14 day repeat; 28 day recovery	1.59	Not established	≥1.59[1]	20

[1] The highest dose tested had no observable adverse effects; NOEL = No observed effect level; NOAEL = No observed adverse effect level; HD = human dose; Margin of Safety based on NOAEL.

Single- and repeat-dose toxicity studies determined no-observed-adverse-effect-levels (NOAELs) in the rat and monkey and demonstrated that IV administration of AI-700 at NOAEL levels is associated only with the reversible presence of spheroid bodies, presumed to be AI-700 microspheres, within macrophages of RES organs. Because this represents a step in the normal process for elimination of particles from blood circulation, the presence of spheroid bodies is not considered adverse. In both single- and repeat-dose studies, mortality and respiratory insufficiency due to pulmonary capillary occlusion by microspheres were observed in rats but not in monkeys. This difference in species tolerance may be explained by the difference in pulmonary vasculature. The monkey more closely approximates the human pulmonary vasculature. Humans have a capillary

diameter of 8.2 μm.[8] Capillary diameter closely correlates with red blood cell diameter: humans have a red blood cell diameter of approximately 8 μm[80], monkeys of: 7.4 μm,[81] and rats of 6.0-6.8 μm.[81]

5.1.2 Nonclinical Cardiovascular and Cardiopulmonary Safety Studies

The cardiovascular safety of an IV injection of AI-700 was evaluated in anesthetized rhesus monkeys and conscious cynomolgus monkeys. Administration of the highest dose (14X the HD) of AI-700 caused an approximate 15 to 25% decrease in mean arterial pressure (MAP) that peaked 5 to 15 minutes after dose administration. This decrease was not associated with a reflex tachycardia, was within normal range, and returned towards pre-dose values prior to dosing with the next dose of AI-700 or by the end of the monitoring period. There were no other pharmacological effects attributed to AI-700 at any dose.

In anesthetized rhesus monkeys undergoing closed-chest AI-700 ECHO imaging, AI-700 doses of 0.16 mL/kg (2X the HD) were used to study cardiopulmonary safety during high mechanical index imaging. High mechanical index imaging of contrast agents has been demonstrated to induce premature ventricular contractions (PVCs).[33] When AI-700 was administered, PVCs were induced when the mechanical index was ≥ 1.2. PVCs resolved upon removal of the ultrasound probe. These results are similar to results observed in human studies. No histological changes were observed, including to changes based on viability stains. No hemodynamic changes or other AI-700-related changes were observed.

Conscious cynomolgus monkeys undergoing closed-chest AI-700 ECHO imaging were evaluated. Multiple mechanical indices were evaluated: moderate (0.5-0.8) and high (1.1-1.4). As in the Phase 3 clinical paradigm, monkeys received 2 equal IV doses of AI-700 or control (0.9% saline). One dose was given before and one dose was given after administration of dipyridamole (0.56 mg/kg IV). The cumulative administered doses corresponded up to 0.40 mL/kg (5X the HD). In this study, multifocal, isolated PVCs were observed only in some animals, and were associated with placement of a Millar® catheter in the left ventricle. No significant effect on tissue structure or physiologic function of the cardiovascular and pulmonary systems was observed as assessed by evaluation of blood pressure, heart rate, coronary contractility (dP/dT), ECG (including QTc), respiratory rate, tidal volume, gum color, capillary refill time, blood gases, and gross and microscopic histological examinations of organs and tissues (including triphenyl tetrazolium chloride and rapid phosphotungstic acid-hematoxylin viability staining of heart tissue). Overall, in these non-human primate studies, AI-700 at or above the HD in combination with moderate to high mechanical index imaging, with and without dipyridamole stress, was shown to have no significant effect on the tissue structure or physiological function of the cardiovascular and pulmonary systems.

The effect of AI-700 on pulmonary function was evaluated in 2 anesthetized animal models: pigs, and dogs with acute elevated pulmonary artery pressure. Pigs were

evaluated because they are a sensitive model for cardiopulmonary effects of particles. This is attributed to their species-specific PIM.[24]

Administration of AI-700 following the clinical dosing regimen (2 sequential IV injections with a 1 hour interval between injections) to pigs in a range of total doses of 0.008 mL/kg/dose (0.1X the HD) to 0.08 mL/kg/dose (1X the HD) was evaluated. No dose-related effects of intravenous AI-700 were observed in arterial blood gases (pH, pO_2, saturated O_2, and HCO_3^-), SpO_2 (as measured via pulse oximetry), or body temperature. Pulmonary arterial pressure (PAP) was increased between 3 and 16 mmHg in a dose-dependent pattern. Acute decreases in systemic blood pressure and compensatory heart rate increases were observed in a non-dose dependent fashion in 5 of 9 animals administered AI-700. Systemic blood pressure recovered quickly when dosing was stopped and was followed by an increase, which was likely due to over-compensation. In the animals that had a blood pressure reduction, heart rate was increased between 12% and 77%. The responses were similar after the first and second AI-700 doses. Indomethacin blocked all blood pressure, heart rate, and PAP changes, suggesting that the observed changes after AI-700 administration were thromboxane mediated. These results are consistent with previous findings for other ultrasound imaging agents (Aerosomes MRX 115[25], Albunex®[24], and Sonovue®[26]). Unlike humans and other animals used in the AI-700 cardiovascular studies, pigs have resident pulmonary intravascular macrophages (PIM). These macrophages are involved in clearance of IV particles and have been associated with acute increases in PAP after administration of all ultrasound contrast agents by a thromboxane-mediated mechanism.

Anesthetized spontaneously breathing adult mongrel dogs with Sephadex™-induced severe or moderate pulmonary hypertension (PH) were used to assess pulmonary artery pressure/resistance, blood gases, and respiratory and cardiac functions. Moderate PH was defined as an increase of 15 mmHg or 75% over baseline mean pulmonary arterial pressure ($mPAP_b$), whichever was achieved first. Severe PH was defined as an increase of 30 mmHg or 150% over $mPAP_b$, whichever was achieved first. Doses of AI-700 were administered as 2 equal IV injections, one hour apart.

Following Sephadex™-induced PH, dogs received either a high dose of AI-700 (0.2 to 0.23 mL/kg total dose; approximately 2.5X the HD), a low dose of AI-700 (0.10 to 0.11 mL/kg total dose; approximately 1.3X the HD), or a control. All cohorts of animals showed increases in mPAP, with the maximum effect usually observed within 5 minutes of AI-700 dosing. The magnitude of the effect was an mPAP increase of 10-14% (3 to 5 mmHg) for the high dose of AI-700 during both moderate and severe PH, and <7% (2 mmHg) at the low dose of AI-700 during severe PH. Oxygen saturation and heart rate showed similar trends with small (<11%) transient decreases at low dose AI-700 with induced severe PH and at high dose AI-700 with induced moderate PH. The administration of high dose AI-700 during severe PH resulted in larger decreases in oxygen saturation and heart rate, which were most pronounced after the second administration of AI-700 and did not completely return to baseline by the end of the

experiment (1-hour post-second dose). In summary, this study demonstrated a dose-dependent effect of AI-700 on cardiopulmonary function at dosages of approximately 1.3X and 2.5X the HD when administered in this model of acute PH.

Acute injection of AI-700 into Sprague-Dawley rats through a catheter directed cranially in the carotid artery was used to assess the potential brain toxicity associated with direct exposure of AI-700 *via* the arterial circulation. The study was conducted to investigate the potential effects in patients having atrial/septal defects of the heart. A single intracarotid injection of AI-700 at 1.4X the HD did not result in any AI-700-related signs of toxicological significance, changes in hematology or clinical chemistry, or gross or microscopic changes in the brain or heart. Historical concerns surrounding the use of ultrasound contrast agents in patients with cardiac shunts were related to earlier microbubble ultrasound contrast agents that underwent significant expansion and contraction when subjected to the acoustic field.[82] AI-700 has not been studied in patients with cardiac shunts; however, AI-700 microspheres are different than earlier perflutren-containing agents (Optison® and Definity®). Differences include having a smaller mean particle size (4 times smaller than the diameter of a capillary), a narrower size distribution, and a more stable polymer structure that does not increase in size under ultrasound.[1] These distinctions, along with these results of this intracarotid administration study in rats, suggest that AI-700 is likely to be tolerated in patients with cardiac shunts.

AI-700 was generally well tolerated at doses much higher than the clinical dose. Nonclinical safety data suggest that the target organs for toxicity are those of the cardiopulmonary system.

5.2 Human Exposure

At least 1 dose of AI-700 was received by 1,046 CAD patients, 18 CHF/COPD patients, and 130 healthy volunteers in the 11 clinical studies of AI-700. Table 14 summarizes the planned doses of AI-700 by subject type, clinical phase of development, study, and total dose. All 1,194 subjects had 1 or 2 days exposure to AI-700, at total doses ranging from 0.014 mL/kg to 0.162 mL/kg. All doses were administered IV.

A total of 47 patients in Studies AI-700-01, AI-700-02, AI-700-04 and AI-700-21 received placebo only. Eighteen subjects in crossover Study AI-700-05 who received AI-700 treatment also received placebo treatment.

As discussed in Section 3.1.2, based upon results of Phase 1 and 2 studies, an IV dose of 0.040 mL/kg AI-700 was selected as the dose needed to provide sufficient MCE in most patients. This dose was used in Phase 3 trials and was given during each imaging session (rest and stress), for a total dose of 0.080 mL/kg at a rate (two 0.040 mL/kg injections), Each dose was injected over 1-10 minutes, a rate that would allow for optimization of MCE, while minimizing attenuation.

The protocol for AI-700 dosing in Phase 3 trials and the recommendations for clinical use specified that AI-700 should be administered at a rate sufficient to produce MCE, commencing with approximately 33% of the total volume per minute for approximately 30 seconds (i.e., approximately $1/6^{th}$ of the total volume should be given during the first 30 seconds of dosing). Once MCE is observed, the health care provider should visually evaluate image enhancement and attenuation (if present) in real-time, and adjust the rate of administration as necessary.

Table 14: AI-700 and Placebo Exposure, by Study Phase and Subject Type (All AI-700 Studies)

		AI-700 Exposure		Number of Subjects								
					Total AI-700 Dose (mL/kg)							
Phase	**Study**	**No. of Injections**	**No. of Days**	**Placebo**	**0.014**	**0.028**	**0.054**	**0.080-0.081**	**0.108**	**0.160-0.162**	**Total**	**Overall**
1 (Healthy Volunteers* and CHF/COPD patients)	AI-700-01[a]	1	1	15	6	0	6	5	16	0	33	48
	AI-700-02[a]	1	1	8	5	0	10	0	5	0	20	28
	AI-700-04[b]	2	2	2	0	0	0	3	0	7	10	12
	AI-700-05[c]											
	COPD/CHF	2	1	18	0	0	0	18	0	0	18	18
	AI-700-06[d]	4	2	0	0	0	0	0	0	10	10	10
2 (Healthy Volunteers and CAD patients)	AI-700-20[a]											
	HV	1	1	0	0	0	2	16	0	0	18	18
	CAD	1	1	0	0	0	11	24	0	0	35	35
	AI-700-21											
	HV[e]	2	1	0	0	0	0	0	25	0	25	25
	CAD	2	1	22	0	25	0	22	28	0	75	97
3 (CAD patients* and healthy volunteers)	AI-700-23	2	1	0	0	0	0	133	0	0	133	133
	AI-700-32	2	1	0	0	0	0	321	0	0	321	321
	AI-700-33	2	1	0	0	0	0	457	0	0	457	457
	AI-700-34											
	HV	2	1	0	0	0	0	14	0	0	14	14
	CAD	2	1	0	0	0	0	25	0	0	25	25
Totals				47**	11	25	29	1025	74	17	1194	1241

* Study Population unless otherwise specified.

** Excludes 18 patients from crossover Study AI-700-05

a Single IV dose of AI-700.

b Single IV dose of AI-700.

c. Crossover study w/placebo as alternate treatment

d Active control study w/2 lots of AI-700.

e 2 healthy volunteers in the AI-700-21 received ≤0.108 mL/kg

5.3 Safety Populations

Synopses of safety results from all AI-700 studies are in Appendix 10. Only changes in safety parameters which may be clinically relevant are reported in this document. Special focus is placed on the intended dose (2 IV injections of 0.04 ml/kg AI-700) and population (patients with chest pain) who underwent rest and dipyridamole stress imaging (Studies AI-700-23, -32, and -33). These patients represent 911 of the 1241 subjects (73.4%) enrolled in AI-700 trials, and 76.3% of the 1194 subjects who received AI-700. Adverse event data from these 3 trials has been reviewed collectively. For other safety parameters (clinical labs, vital signs, etc.), only results from Studies AI-700-32 and -33 are considered collectively. Other safety parameter data from the pilot study (AI-700-23) were not pooled because (1) AI-700-23 was a training trial intended to assess whether the sites could properly obtain necessary safety data according to the protocol and (2) comparison of AI-700-23 safety analyses to analyses of the pivotal studies did not indicate any notable differences in observed responses.

In addition, AE data in dose-ranging studies of healthy volunteers (Studies AI-700-01, -02, -04, -20, and -21) are collapsed. AE data for CAD patients in the dose ranging studies AI-700-20 and -21 are also presented.

Because the safety profiles from Studies AI-700-06 and -34 are consistent with those from other studies, these small studies are not presented in this safety section. Studies in special populations (CHF/COPD patients in AI-700-05) and studies with novel safety evaluations (immunogenicity in AI-700-04) are presented.

5.4 Summary of Adverse Events in Phase 3 Studies

In total, 652 (72%) of 911 patients in Studies AI-700-23, -32, and 33 reported at least one AE (Table 15). A total of 1879 AEs were reported. Most AEs were mild in intensity, of short duration, and resolved without treatment or residual effects.

Table 15: Summary of Treatment-Emergent Adverse Events (Studies AI-700 -23, -32, and -33)

Parameter	Patients (N = 911) n (%)	Adverse Events (N = 1879) n (%)
Adverse events	652 (71.6)	1879
Serious adverse events	11 (1.2)	14 (0.7)
Adverse events requiring treatment	172 (18.9)	284 (15.1)
Adverse events leading to permanent discontinuation of AI-700	14 (1.5)	34 (1.8)
Adverse events resulting in a temporary interruption of AI-700	7 (0.8)	16 (0.9)
Adverse events resulting in an adjustment of the AI-700 dosing regimen	1 (0.1)	1 (0.1)
Adverse events of mild intensity	571 (62.7)	1449 (77.1)
Adverse events of moderate intensity	225 (24.7)	384 (20.4)
Adverse events of severe intensity	30 (3.3)	46 (2.4)
Adverse events related[1] to AI-700	332 (36.4)	748 (39.8)
Adverse events related[2] to dipyridamole	562 (61.7)	1385 (73.7)

[1] Considered by the Investigator to be possibly, probably, or definitely related to AI-700.
[2] Considered by the Investigator to be possibly, probably, or definitely related to dipyridamole.

5.5 Serious Adverse Events

No SAEs were reported in any Phase 1 or Phase 2 clinical studies of AI-700.

A total of 11 (<1%) of 1194 subjects who received any dose of AI-700 experienced 14 non-life threatening, SAEs. All SAEs were reported in Phase 3 studies: 6 were reported in AI-700-33, 4 in AI-700-32, and 1 in AI-700-23. SAEs are listed in Table 16.

Table 16: Serious Adverse Events

Patient Number (Age/Race/Gender)	SAE(s) and Intensity	Timing	Resolution and Treatment
23.16.003 (76-year-old White female)	mild vertigo and moderate hypertension	after the first dose of AI-700	vertigo resolved without treatment or residual effects; hypertension resolved with treatment (IV metoprolol)
32.07.060 (79-year-old White male)	moderate syncope vasovagal	after the first dose of AI-700	treated with atropine and polygeline (for bradycardia and hypotension); resolved without residual effects
32.02.030 (54-year-old Black female)	severe mental status changes (diagnosed as a probable conversion disorder)	after the second dose of AI-700	treated with IV Solu-Cortef, Benadryl, and a normal saline IV bolus (for possible allergic reaction); resolved without residual effects
32.09.015 (47-year-old White male)	hospitalized for moderate eye pain, moderate blurred vision, and moderate visual disturbance	during the Follow-up period	resolved without treatment or residual effects
32.07.018 (63-year-old Arab male)	hospitalized for a moderate adverse drug reaction (symptoms included dizziness, hot flushes, headache, right-sided chest pain, fever, and chills)	during the Follow-up period	headache treated with paracetamol. SAE resolved without residual effects
33.35.008 (69-year-old White male)	severe hypersensitivity reaction to DIPY* or AI-700	after the second dose of AI-700	resolved with treatment (aminophylline, hydrocortisone, chlorpheniramine) without residual effects
33.35.030 (72-year-old White female	severe syncope vasovagal	after the first dose of AI-700	bradycardia treated with atropine. Hypotension treated with IV saline. SAE resolved without residual effects
33.37.008 (59-year-old White female)	moderate syncope vasovagal	after the first dose of AI-700	resolved with treatment (atropine for bradycardia) without residual effects
33.36.011 (76-year-old White male)	severe myocardial infarction	during the Follow-up period	resolved with treatment (heparin, vasopressors) without residual effects
33.34.016 (80-year-old White female)	moderate myocardial infarction (NSTEMI)	during the Follow-up period	NSTEMI treated with heparin, nitroglycerin, ramipril, aspirin, pravastatin, clopidogrel. Resolved with residual effects (post-MI bradycardia required a pacemaker)
33.42.031 (64-year-old White male)	severe chest pain	after the second dose of AI-700	resolved with treatment (nitroglycerin, tirofiban) without residual effects

* dipyridamole

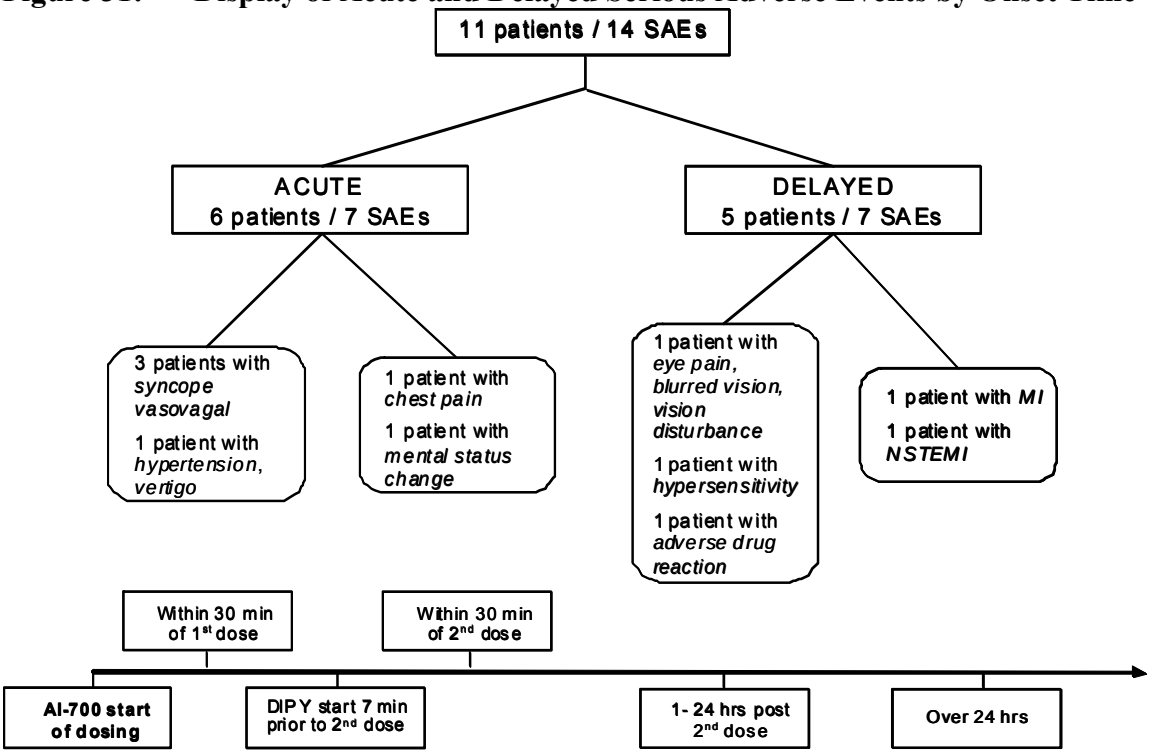
Thirteen of the 14 SAEs resolved without residual effects; the NSTEMI in Patient 33.34.016 resulted in post-MI bradycardia requiring a pacemaker.

Neurological examinations and CT scans in 2 patients (SAEs: mental status change, eye pain, blurred vision, vision disturbances) did not reveal any cerebral damage or evidence of ischemic events.

5.5.1 Onset Time of SAEs

Serious adverse events are presented by time of onset in Figure 31.

Figure 31: Display of Acute and Delayed Serious Adverse Events by Onset Time



Within 30 minutes following initiation of AI-700 dosing, 6 patients experienced 7 SAEs. Following the first dose of AI-700 during rest, 3 patients experienced individual episodes of syncope vasovagal without loss of consciousness, and 1 patient experienced hypertension and vertigo. Within 30 minutes of the second AI-700 dose during stress, 2 patients experienced SAEs: 1 patient experienced a mental status change (considered a possible conversion disorder by the Investigator) and 1 patient experienced chest pain.

One to 18 hours after AI-700 dosing, an additional 3 patients experienced 5 SAEs. One patient had 3 SAEs (eye pain, blurred vision, vision disturbance), 1 patient had hypersensitivity, and 1 patient had an adverse drug event (symptoms included right-sided chest pain, headache, fever, chills, and dizziness).

At least 24 hours after dosing, 2 additional patients had SAEs that were considered important cardiac events: 1 myocardial infarction (MI) without ST segment changes and 1 non-ST elevation myocardial infarction (NSTEMI). Both patients had multiple-vessel disease and both had complained of recent chest pain at Baseline, as documented in medical history and ANGIO findings. Following induction of pharmacological stress, signs of induced ischemia (AEs of chest pain and ECG signs of myocardial ischemia) were observed. Induced ischemia was reversible by aminophylline. In the opinion of both investigators, the MIs were unrelated to AI-700.

5.6 Other Significant Adverse Events

5.6.1 Discontinuation of AI-700 in Healthy Volunteers

Among 130 healthy volunteers, one subject, a 36-year old female healthy volunteer (21.07.904) reported moderate chest pain (Investigator term "warm feeling in chest"), mild heartburn, and moderate tachycardia (Investigator term "tachycardia") during the second rest imaging session. These AEs led to discontinuation of the second dose of AI-700, and resolved without treatment.

5.6.2 Discontinuation of AI-700 in CAD Patients

There were 16 CAD patients who had AEs and were discontinued from AI-700 (Table 17). This includes 2 patients who withdrew consent prior to dipyridamole administration: 32.07.038 (AE of rigors of severe intensity) and 32.07.060 (SAE of syncope vasovagal). These AEs occurred during rest for 14 patients and during stress for 2 patients. The most common AEs resulting in discontinuation of AI-700 were hypotension (5 patients) and syncope vasovagal (3 patients). Vasovagal events differed from hypotension because they were accompanied by decreases in heart rate/AV block and resolved immediately after treatment with atropine.

Respiratory events were reported in 3 patients who discontinued after the first AI-700 dose: Patient 32.16.010 had mild wheezing and moderate cough that were part of a mild allergy-like event; Patient 32.16.021 had tachypnea following an episode of hypotension; and Patient 33.37.046 had decreases in SaO_2/cyanosis [this patient had a history of moderate COPD (FEV_1=61% post bronchodilator)].

Patient 33.37.013 had an AE of ST segment depression accompanied by mild angina during rest. This patient had chest pain prior to the first dose of AI-700, which was treated with nitroglycerin paste. The ST depression occurred at least 45 minutes post-dose and the Investigator considered the AE unrelated to AI-700. The patient did not receive dipyridamole and was discontinued from the study.

Table 17: Adverse Events Resulting in Discontinuation of AI-700 in CAD Patients

Patient Number (Age/Race/Gender)	Adverse Event(s) and Intensity	Timing	Resolution
23.16.003 (76-year-old White female)	mild vertigo*, moderate hypertension*; and moderate headache	after the first dose of AI-700	vertigo and headache resolved without treatment; hypertension resolved with treatment
23.34.003 (68-year-old White male)	moderate hypotension	after the first AI-700 dose	resolved without treatment
32.02.002 (56-year-old Black female)	mild headache and moderate rigors	after the first dose of AI-700	resolved without treatment
32.07.032 (66-year-old Asian female)	mild dizziness, mild chest pain, and moderate hypotension	during the second dose of AI-700	dizziness resolved without treatment; hypotension resolved with treatment
32.07.038 (69-year-old Asian male)	Severe rigors	after the first dose of AI-700	resolved with treatment
32.07.060 (79-year-old White male)	moderate syncope vasovagal*	after the first dose of AI-700	resolved with treatment
32.07.091 (65-year-old Asian female)	mild hypotension	during the second dose of AI-700	resolved with treatment
32.16.010 (64-year-old White female)	mild flushing, mild increased lacrimation, mild wheezing, and moderate coughing	after the first dose of AI-700	flushing/increased lacrimation/wheezing resolved without treatment; coughing resolved with treatment
32.16.021 (68-year-old White male)	severe sweating, severe hypotension, moderate tachypnea, and mild vertigo	after the first dose of AI-700	resolved with treatment
33.35.025 (62-year-old Asian female)	severe hypersensitivity	after the first dose of AI-700	resolved with treatment
33.35.030 (72-year-old White female)	severe syncope vasovagal*	after the first dose of AI-700	resolved with treatment
33.35.035 (61-year-old White female)	moderate allergic reaction	after the first dose of AI-700	resolved with treatment
33.37.008 (59-year-old White female) *	moderate syncope vasovagal*	during the first dose of AI-700	resolved with treatment
33.37.013 (73-year-old White female)	mild angina and moderate ST-segment depression	after the first dose of AI-700	resolved without treatment
33.37.046 (66-year-old White male)	cyanosis & decreased SaO_2	after the first dose of AI-700	resolved with treatment
33.48.047 (73-year-old White female)	weakness, diarrhea, dizziness, feeling hot, 2:1 A-V block, bradycardia, hyperhidrosis, hypotension, and nausea (all severe), and mild QT prolongation	after the first dose of AI-700	resolved without treatment

* Serious adverse event

5.7 Common Adverse Events

5.7.1 Common Adverse Events and Dose-Response in Healthy Volunteers

The most frequently reported AEs among 106 healthy volunteers (Studies AI-700-01, -02, -04, -20, and -21) who received AI-700 were flushing (22 subjects; 21%), headache (13 subjects; 12%), cough (11 subjects; 10%), dizziness (9 subjects; 9%), and dyspnea (8 subjects; 8%). Overall AE rates in subjects who received AI-700 (56%) were similar to placebo (52%). However, examination of AEs by onset time revealed a trend across all AI-700 dose groups, with the majority of AEs (44 of 59 subjects, 75%) occurring within 1 hour post-dose. Among 25 healthy volunteers who received placebo, only 4 (31%) of 13 subjects who experienced AEs had an onset within 1 hour post dose.

In healthy volunteers who received AI-700, dose-dependent increases in flushing, cough, dyspnea, and nausea were observed. Rates of dyspnea and nausea were higher than rates in placebo-treated subjects for the highest AI-700 dose group (0.108mL/kg), which represents a higher than the clinically recommended AI-700 dose (0.080mL/kg).

No dose-dependent trends relating to the intensity of AEs were observed in any healthy volunteers receiving AI-700 (Section 5.7.6).

5.7.2 Dose-Response in CAD Patients (Studies AI-700-20 and -21)

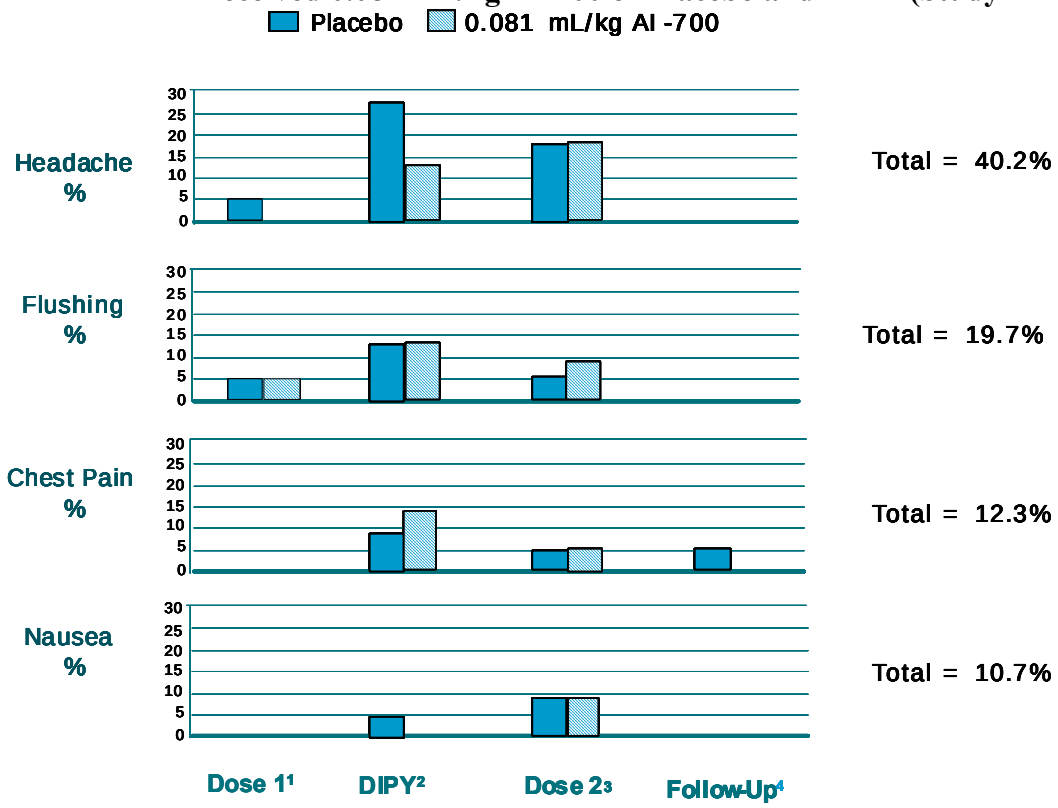
In Studies AI-700-20 and -21, following a single dose of AI-700 (0.014, 0.046, 0.054 and 0.081mL/kg), there was a dose-dependent increase in AE rates for CAD patients (16%, 18%, 36%, and 42%, respectively according to dose group). The total AE rate for patients receiving placebo was lower (9%). The higher AE rates in patients receiving AI-700 were driven by dose-dependent increases in flushing (0%, 5%, 8%, and 17%, respectively according to dose group). No other dose-dependent AEs were observed in patients with CAD.

No dose-dependent trends relating to the intensity of AEs were observed in any CAD patient receiving AI-700 (Section 5.7.6).

5.7.3 Common Adverse Events: Dipyridamole/AI-700 vs. Dipyridamole/Placebo

In the dose-ranging Study AI-700-21, the incidence rates of the most common AEs observed in ≥10% of all CAD patients were compared for the 0.081 mL/kg AI-700 dose group and the placebo group. As shown in Figure 32, similar onset times and incidence rates for these AEs among patients who received dipyridamole with AI-700 and dipyridamole with placebo further support the relationship between the most common AEs and dipyridamole.

Figure 32: Incidence of the Most Common AEs: Observed in ≥10.0% of CAD Patients who Received 0.081 mL/kg AI-700 or Placebo and DIPY (Study AI-700-21)



[1] During or after administration of the first test article dose and before administration of dipyridamole

[2] During or after administration of dipyridamole and before administration of the second test article dose

[3] After administration of the second test dose article and before Discharge

[4] Discharge to Follow-Up

5.7.4 Common Adverse Events in Known/Suspected CAD Patients Undergoing Pharmacologic Stress (Studies AI-700-23, -32, and -33)

Adverse events occurring in ≥1% of patients in Studies AI-700-23, -32, and -33 are presented in Table 18 By time of onset, 12% of patients in Studies AI-700-23, -32, and 33 had a reported AE after the first dose of AI-700 and prior to dipyridamole dosing, 44% after dipyridamole dosing and prior to the second dose of AI-700, 45% after the second dose of AI-700 and prior to Discharge, and 17% from Discharge through Follow-up.

The temporal relationship between the onset of common AEs and the administration of dipyridamole suggested that many of the most common AEs were due in part to the pharmacological effects of dipyridamole.

The 5 most common AEs (Figure 33) were headache, chest pain, nausea, flushing, and chest discomfort.

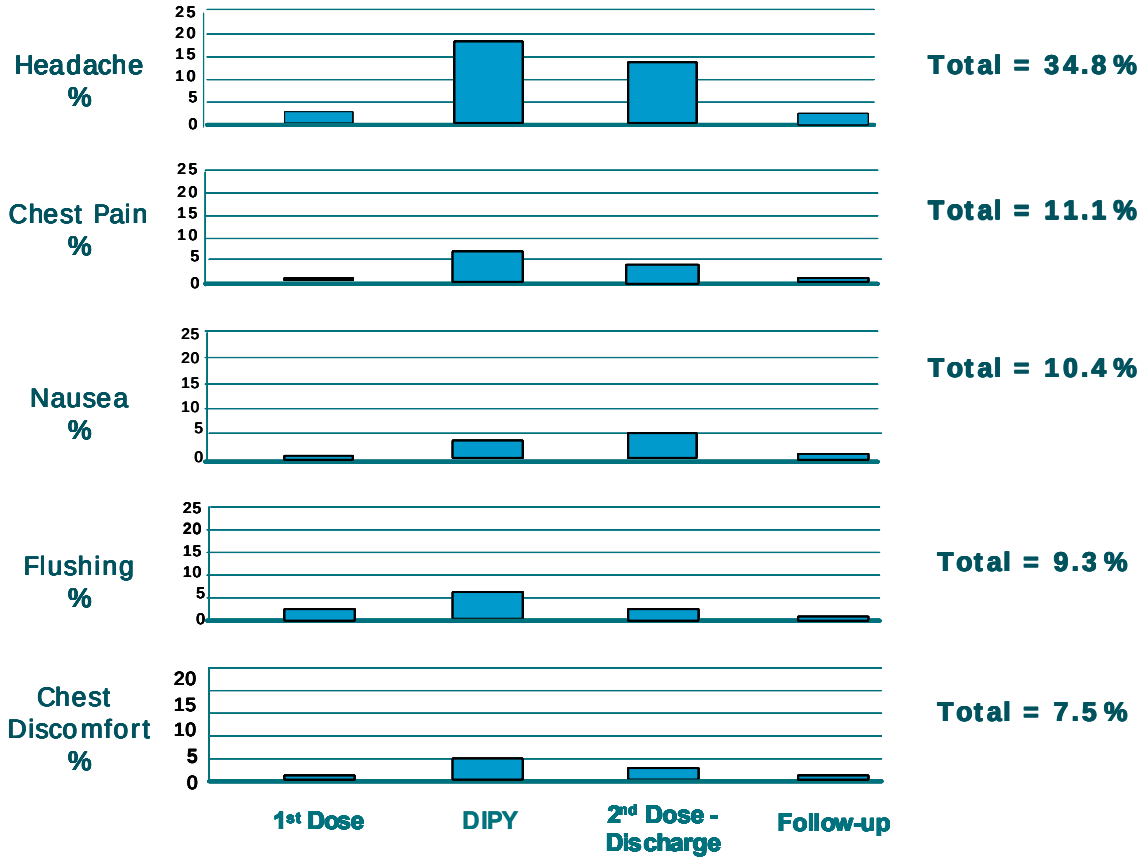
Table 18: Number of Patients with AEs Reported in ≥1% of Patients, by Onset Time (Studies AI-700 -23, -32, and -33)

	Onset Time				
Preferred Term	**1st Dose to DIPY N = 911 n (%)**	**DIPY to 2nd Dose N = 894 n (%)**	**2nd Dose to Discharge N = 894 n (%)**	**Discharge to Follow-up N = 911 n (%)**	**Total N = 911 n (%)**
Number of Patients with ≥1 AE	105 (11.5)	391 (43.7)	402 (45.0)	151 (16.6)	652 (71.6)
Total Number of AEs	179	672	768	260	1879
Headache	27 (3.0)	167 (18.7)	128 (14.3)	28 (3.1)	317 (34.8)
Chest pain	2 (0.2)	60 (6.7)	41 (4.6)	10 (1.1)	101 (11.1)
Nausea	5 (0.5)	34 (3.8)	45 (5.0)	13 (1.4)	95 (10.4)
Flushing	17 (1.9)	52 (5.8)	18 (2.0)	3 (0.3)	85 (9.3)
Chest discomfort	4 (0.4)	45 (5.0)	22 (2.5)	4 (0.4)	68 (7.5)
Leukocytosis[a]	1 (0.1)	0	57 (6.4)	1 (0.1)	59 (6.5)
Dizziness	7 (0.8)	27 (3.0)	16 (1.8)	5 (0.5)	52 (5.7)
Neutrophil count increased[a]	2 (0.2)	0	46 (5.1)	3 (0.3)	51 (5.6)
Feeling hot	6 (0.7)	29 (3.2)	6 (0.7)	4 (0.4)	45 (4.9)
Dyspnea	4 (0.4)	20 (2.2)	16 (1.8)	3 (0.3)	40 (4.4)
Rigors	10 (1.1)	2 (0.2)	16 (1.8)	13 (1.4)	39 (4.3)
ECG ST segment depression	1 (0.1)	18 (2.0)	15 (1.7)	0	33 (3.6)
Hypotension	9 (1.0)	4 (0.4)	18 (2.0)	1 (0.1)	31 (3.4)
Abdominal pain	0	6 (0.7)	11 (1.2)	5 (0.5)	22 (2.4)
Lymphocyte count decreased[a]	2 (0.2)	0	20 (2.2)	0	22 (2.4)
Pain in extremity	1 (0.1)	9 (1.0)	9 (1.0)	1 (0.1)	20 (2.2)
Neck pain	0	12 (1.3)	5 (0.6)	1 (0.1)	18 (2.0)
Pyrexia	1 (0.1)	0	6 (0.7)	12 (1.3)	19 (2.1)
Fatigue	1 (0.1)	2 (0.2)	4 (0.4)	8 (0.9)	15 (1.6)
Hyperhidrosis	5 (0.5)	3 (0.3)	4 (0.4)	5 (0.5)	17 (1.9)
Abdominal pain upper	1 (0.1)	9 (1.0)	5 (0.6)	0	15 (1.6)
Throat tightness	0	7 (0.8)	3 (0.3)	1 (0.1)	10 (1.1)
Dysgeusia	3 (0.3)	6 (0.7)	3 (0.3)	1 (0.1)	13 (1.4)
Monocyte count decreased[a]	1 (0.1)	0	12 (1.3)	0	13 (1.4)
Vomiting	1 (0.1)	3 (0.3)	4 (0.4)	4 (0.4)	12 (1.3)
Hot flush	0	6 (0.7)	5 (0.6)	1 (0.1)	12 (1.3)
Pain in jaw	1 (0.1)	6 (0.7)	6 (0.7)	0	12 (1.3)
Tremor	1 (0.1)	0	9 (1.0)	1 (0.1)	11 (1.2)
Paresthesia	1 (0.1)	6 (0.7)	3 (0.3)	0	10 (1.1)
WBC count increased[a]	0	0	11 (1.2)	0	11 (1.2)
Burning sensation	0	7 (0.8)	3 (0.3)	0	10 (1.1)
Injection site burning	1 (0.1)	7 (0.8)	2 (0.2)	0	10 (1.1)
Angina pectoris	1 (0.1)	4 (0.4)	2 (0.2)	2 (0.2)	9 (1.0)
Myalgia	1 (0.1)	1 (0.1)	0	7 (0.8)	9 (1.0)

[a] Post-dose clinical laboratory samples were collected only at Discharge and Follow-up.

If a patient had more than one occurrence of the same AE at 2 different timepoint categories, that patient was counted only once in the total column. If a patient had more than one occurrence of the same AE within a timepoint category, that patient was only counted once within the timepoint category.

Figure 33: The Number and Percent of Patients with the Five Most Common Adverse Events by Onset Time (Studies AI-700 -23, -32, and -33)



5.7.5 Relationship of Adverse Events in Studies AI-700-23, -32, and -33

The relationships of the 5 most frequently occurring AEs to study drug administration (AI-700 and dipyridamole) as assessed by the Investigator are presented in Table 19. Investigators were more likely to attribute common AEs to dipyridamole than to AI-700.

Table 19: Number of Patients with the 5 Most Common Adverse Events by Relationship to AI-700 and Dipyridamole (Studies AI-700-23, -32 and -33)

		Any Attribution[1]		Definite / Probable Attribution	
MedDRA Preferred Term	All Reports n (%)	AI-700 n (%)	Dipyridamole n (%)	AI-700 n (%)	Dipyridamole n (%)
Headache	317 (34.8)	89 (9.7)	295 (32.4)	2 (0.2)	214 (23.5)
Chest pain	101 (11.1)	22 (2.4)	94 (10.4)	0	77 (8.5)
Nausea	95 (10.4)	31 (3.4)	82 (9.0)	2 (0.2)	52 (5.7)
Flushing	85 (9.3)	26 (2.9)	67 (7.3)	11 (1.2)	55 (6.0)
Chest discomfort	68 (7.5)	11 (1.2)	62 (6.8)	2 (0.2)	40 (4.4)

N= 911 patients; [1] An attribution of definite, probable, or possible

5.7.6 Intensity of Adverse Events

Healthy Volunteers

In the 106 AI-700-treated healthy volunteers (Studies AI-700-01, -02, -04, -20 and -21), 5 subjects (5%) experienced at least 1 event of severe intensity. The only AEs of severe intensity reported more than once were 2 episodes of severe flushing and 2 episodes of dyspnea. An examination of AEs by intensity and dose group did not reveal a dose-dependent increase in the incidence of severe AEs.

CAD Patients in Studies AI-700-20 and -21

In CAD patients receiving AI-700 in Studies AI-700-20 and AI-700-21, most AEs following a single dose of AI-700 were considered by the Investigator to be mild in intensity (37 of 48 events; 77%), with 10 (21%) moderate and 1 (2%) severe in intensity. Of the 110 AI-700-treated patients, 31 (28%) experienced AEs that were considered mild or moderate in intensity. One patient in Study AI-700-21 experienced a severe intensity headache following the first AI-700 dose of 0.054 mL/kg; this AE resolved following treatment. An examination of AEs by intensity and dose group did not reveal a dose-dependent increase in the incidence of severe AEs.

CAD Patients in Phase 3 Studies

Most of the reported AEs in patients in Studies AI-700-23, -32, and -33 (1,833 of 1,879 AEs; 98%) were considered by the Investigator to be of mild or moderate intensity (Table 15). Forty-six AEs were rated severe. Severe AEs were reported by 30 (3%) of the 911 patients, and included chest pain (7 patients; 1%), headache (4 patients; <1%), hyperhidrosis and hypotension (3 patients each; <1%), hypertension, chest discomfort, bradycardia, rigors, hypersensitivity, and tremor (2 patients each; <1%). All other severe AEs occurred in 1 patient each.

Forty-five of the 46 severe AEs resolved without residual effects. The severe AE of injection site burning (verbatim term burning sensation with dipyridamole injection) resolved shortly after completion of the dipyridamole infusion with a residual effect (redness at IV site) that had not resolved at Follow-up.

5.7.7 Adverse Events of Special Interest

5.7.7.1 Adverse Events Requiring Treatment (Studies AI-700-23, -32, and -33)

A total of 172 (19%) of 911 patients in Studies AI-700-23, -32, and -33 experienced 284 AEs that required treatment. Table 20 presents AEs requiring treatment in ≥1% of patients.

Table 20: Most Common Adverse Events Requiring Treatment in ≥1% of Patients (Studies AI-700 -23, -32, and -33)

MedDRA Preferred Term	Patients n (%)
Total	**911 (100)**
Headache	65 (7.1)
Chest pain	24 (2.6)
Nausea	12 (1.3)
Hypotension	12 (1.3)
Dyspnea	10 (1.1)
Rigors	10 (1.1)

Fewer AEs requiring treatment were observed following the first dose of AI-700 (38 of 1879, 2%) than were observed following dipyridamole (73 events, 4%), following dipyridamole and the second AI-700 dose (101 events, 5%), and at Follow-up (72 events, 4%).

Concomitant medications most commonly administered on the same day as AI-700 were paracetamol (acetaminophen), given to 49 (5.4%) patients; aminophylline/theophylline, given to 32 (3.5%) patients; and glyceryl trinitrate, given to 20 (2.2%) patients.

5.7.7.2 Cardiovascular Adverse Events

Vasovagal Events: Table 21 lists the 7 (1%) of 911 patients in Studies AI-700-23, -32, and -33 who had a vasovagal episode reported as an AE (preferred terms: syncope or syncope vasovagal). Three of these 7 vasovagal events were SAEs; all 3 SAEs occurred during rest and led to discontinuation of AI-700 dosing (2 were discontinued by the Investigator and 1 withdrew consent). Major lines of evidence suggest that classical (emotional and orthostatic) vasovagal syncope is not a disease, but rather a manifestation of a non-pathological trait.[83] The 3 vasovagal SAEs differed from other reported hypotensive AEs in that they were accompanied by rapid decreases in heart rate or AV block. All 3 SAEs were quickly resolved following treatment with IV atropine for bradycardia.

Of the remaining 4 vasovagal events, 2 occurred during stress and 2 after Discharge. None of these events were considered related to AI-700. The 2 vasovagal AEs during stress were mild in intensity: one occurred during dipyridamole dosing, with concurrent chest pain, and was treated with normal saline and discontinuation of dipyridamole; the second occurred approximately 2 hours after the second AI-700 dose and did not require treatment.

All 7 patients remained conscious during vasovagal episodes.

Table 21: Patients with Syncope or Syncope Vasovagal Events (Studies AI-700 -23, -32, and -33)

Patient Number	SAE leading to D/C	Imaging Session	Intensity	Age	Gender	CAD Status	Treatment required	Resolution
23.43.001	no	stress	Mild	58	Male	N/E	No	Yes, NRE
32.07.060	yes	rest	Moderate	79	Male	N/E	Yes	Yes, NRE
32.12.001	no	FU	Moderate	66	Male	positive	No	Yes, NRE
33.35.009	no	stress	Mild	68	Female	negative	Yes	Yes, NRE
33.35.030	yes	rest	Severe	72	Female	negative	Yes	Yes, NRE
33.37.008	yes	rest	Moderate	59	Female	positive	Yes	Yes, NRE
33.46.001	no	FU	Moderate	54	Female	positive	Yes	Yes, NRE

D/C = discontinuation; N/E=not evaluated; NRE = no residual effects; FU=Follow-up.

Hypotensive Events: A total of 38 (4%) of 911 patients in Studies AI-700-23, -32, and -33 had 41 hypotensive AEs. Preferred terms were: hypotension (31 patients), decreased blood pressure (6 patients), and systolic blood pressure decreased (1 patient). Hypotensive AEs are presented by time of onset in Table 22.

Table 22: Number of Patients with Hypotensive Adverse Events, by Onset Time (Studies AI-700-23, -32, and -33)

	Onset Time				
Preferred Term	1st Dose to DIPY N = 911 n (%)	DIPY to 2nd Dose N = 894 n (%)	2nd Dose to Discharge N = 894 n (%)	Discharge to Follow-up N = 911 n (%)	Total N = 911 n (%)
Hypotension	9 (1.0)	4 (0.4)	18 (2.0)	1 (0.1)	31 (3.4)
Blood pressure decreased	3 (0.3)	0	4 (0.4)	0	6 (0.7)
Blood pressure systolic decreased	0	0	1 (0.1)	0	1 (0.1)

If a patient had more than one occurrence of the same AE at 2 different timepoint categories, that patient was counted only once in the total column.

None of these hypotensive events were SAEs. However, hypotensive events were the most common cause of discontinuation of dosing, dose adjustment, or dose interruption. Five AEs of hypotension led to discontinuation of dosing (3 during rest and 2 during stress), 1 AE of hypotension during stress led to an adjustment of the dosing regimen, and 1 AE of decreased blood pressure during stress led to a temporary interruption of dosing (Table 23). For 3 of these 7 patients, hypotension occurred during rest; in all 3 patients, SBP remained ≥100 mmHg.

All hypotensive AEs were temporary, resolving without residual effects. Treatment for hypotension was less often needed at rest (2 of 12 AEs) than at stress (10 of 29AEs).

Hypotension is an expected AE following administration of dipyridamole and is often associated with reflex tachycardia. Aminophylline is effective for amelioration of dipyridamole-induced hypotension. Administration of IV fluids to expand circulatory volume is standard medical treatments for hypotension.

Table 23: Hypotensive Adverse Events Leading to Discontinuation of AI-700 Dosing, Interruption of Dosing, or Adjustment of Dosing

Patient ID	Adverse Event	Outcome	CAD status	Vital Signs			Treatment Required	Resolution	ECG (BL/ Change)	Concurrent AEs[1]
				SBP (BL / Range concurrent[1] w/AE)	Heart Rate (BL / Range concurrent[1] w/AE)	SaO_2 (BL / Range concurrent[1] w/AE)				
IMAGING SESSION I (REST)										
23.34.003	hypotension	D/C	Negative	120 / 100	64 / 54-56	97 / 95-98	None	Resolved w/o effects	Normal / No Change	None
32.16.021	hypotension	D/C	Negative	118 / 105-121	71 / 51-68	97 / 93-97	promethazine hydrochloride	Resolved w/o effects	Normal / No Change	Flushing, tachypnea, hyperhidrosis, vertigo
33.48.047	hypotension	D/C	Negative	115 / 102-138	76 / 73-88	91 / 95-99	None	Resolved w/o effects	Normal / No Change	Atrioventricular block, diarrhea, dizziness, feeling hot, hyperhidrosis, nausea, bradycardia
IMAGING SESSION II (STRESS)										
32.07.032	hypotension	D/C	Positive	127 / 52-108	75/ 74-86	99 / 96-100	gelofusine	Resolved w/o effects	Normal / No Change	Dizziness, Chest Pain
32.07.091	hypotension	D/C	Negative	151 / 61-105	56 / 65-70	100 / 90-98	atropine, gelofusine, fosinopril	Resolved w/o effects	Normal / No Change	None
32.02.015	hypotension	Dosing regimen adjusted	Negative	117 / 107-109	50 / 61-67	96 / 93-100	aminophylline	Resolved w/o effects	Normal / No Change	Hot flush, Paresthesia
33.49.047	blood pressure decreased	Temporary interruption of dose	Negative	147 / 101-118	62 / 73-80	99 / 90-100	None	Resolved w/o effects	Normal / No Change	Anxiety, headache, nausea, dizziness, rigors

AE=adverse event, BL = Baseline; D/C = Discontinued; w/o = without; PAC = premature atrial contractions; NA = missing truth standard

[1] Concurrent values were those occurring within 30 minutes of the adverse event onset time

The cause of hypotensive AEs during rest is not always known. Many of these patients were taking antihypertensive and angina medication and the timing of these medications relative to dosing may have had an impact.

One hypothesis is that the decrease in blood pressure may be related to activation of complement following administration of AI-700 (Section 5.17). Regardless of the mechanism of these induced blood pressure AEs, the impact on the overall safety profile is believed to be minimal. Treatment for any AEs related to hypotension is readily available in the clinical setting of pharmacological stress ECHO, where stress agent-induced and ischemia-induced hypotension are anticipated events. Blood pressure changes are included in the vital signs section (Section 5.9.2.3).

5.7.7.3 Respiratory Adverse Events (Studies AI-700-23, -32 and -33)

Respiratory AEs were defined as AEs in the respiratory, thoracic, and mediastinal disorders MedDRA system organ class (SOC). A total of 79 (8.7%) of 911 patients in Studies AI-700-23, AI-700-32, and AI-700-33 reported a total of 98 respiratory AEs. The most common respiratory AEs were dyspnea (43 AEs in 40 patients, 4.4%) and throat tightness (10 AEs in 10 patients, 1.1%); all other respiratory AEs were reported in ≤1% of patients (Table 24).

There were 12 patients with respiratory AEs following the first dose of AI-700 and prior to dipyridamole. Four patients had mild AEs of dyspnea during rest; these 4 AEs resolved without treatment. For all 4 patients with dypsnea at rest, SaO_2 remained ≥90%. One patient (33.35.047) had an AE of mild hypoxia during rest. For this patient, the lowest SaO_2 value was 89%, which was reported at 15 minutes post-dose. This AE resolved following treatment with supplemental oxygen.

The majority of patients with respiratory AEs had onset after dipyridamole and before Discharge: 37 patients had onset after dipyridamole and before the second dose of AI-700; 27 patients had onset after the second dose of AI-700 and prior to Discharge. During stress, 37 patients had AEs of dyspnea. These dyspnea AEs were mild or moderate in intensity; 11 patients required treatment (supplemental oxygen, rescue inhalers and aminophylline). One patient with an AE of dypsnea during stress had an SaO_2 value which met outlier criteria (below 90% and ≥5% decrease from Baseline); this patient had an SaO_2 of 88%, compared to a Baseline value of 93%.

Table 24: Patients with Respiratory Adverse Events by Time of Onset (Studies AI-700-23, -32, and -33)

MedDRA Preferred Term	1st Dose to DIPY n (%)	DIPY to 2nd Dose n (%)	2nd Dose to Discharge n (%)	Discharge to Follow-up n (%)	Total n (%)
Respiratory, thoracic, and mediastinal disorders	**12 (1.3)**	**37 (4.1)**	**27 (3.0)**	**12 (1.3)**	**79 (8.7)**
Dyspnea	4 (0.4)	20 (2.2)	16 (1.8)	3 (0.3)	40 (4.4)
Throat tightness	0	7 (0.8)	3 (0.3)	1 (0.1)	10 (1.1)
Cough	4 (0.4)	2 (0.2)	2 (0.2)	0	7 (0.8)
Pharyngolaryngeal pain	0	5 (0.6)	1 (0.1)	1 (0.1)	7 (0.8)
Nasal congestion	1 (0.1)	0	3 (0.3)	0	3 (0.3)
Wheezing	1 (0.1)	0	1 (0.1)	1 (0.1)	3 (0.3)
Bronchospasm	0	0	2 (0.2)	0	2 (0.2)
Hypoxia	1 (0.1)	0	1 (0.1)	0	2 (0.2)
Sinus congestion	2 (0.2)	0	1 (0.1)	0	2 (0.2)
Tachypnea	1 (0.1)	1 (0.1)	0	0	2 (0.2)
Breath sounds decreased	0	0	0	1 (0.1)	1 (0.1)
Choking sensation	0	1 (0.1)	0	0	1 (0.1)
Dry throat	0	0	1 (0.1)	0	1 (0.1)
Epistaxis	0	0	0	1 (0.1)	1 (0.1)
Hyperventilation	0	0	0	1 (0.1)	1 (0.1)
Lung crepitation	0	0	0	1 (0.1)	1 (0.1)
Nasal passage irritation	0	1 (0.1)	0	0	1 (0.1)
Pharynx discomfort	0	1 (0.1)	0	0	1 (0.1)
Pulmonary congestion	1 (0.1)	0	0	0	1 (0.1)
Pulmonary edema	0	0	0	1 (0.1	1 (0.1)
Rhinitis	0	0	0	1 (0.1)	1 (0.1)
Rhinorrhoea	0	0	0	1 (0.1)	1 (0.1)
Sneezing	0	0	0	1 (0.1)	1 (0.1)
Throat irritation	0	0	1 (0.1)	0	1 (0.1)

Note that the total number of patients for all preferred AE terms within the respiratory SOC may be greater than the number of patients within that SOC, as patients are counted once within each SOC. Similarly, if a patient had more than one occurrence of the same AE at 2 different timepoint categories, that patient would be counted only once in the total column. If a patient had more than one occurrence of the same AE within a timepoint category, that patient would only be counted once within the timepoint category.

5.7.7.4 Hematologic Abnormalities Reported as Adverse Events

The majority of laboratory abnormalities reported as AEs in the 911 patients in Studies AI-700-23, -32, and -33 were hematologic abnormalities. Hematologic abnormalities reported as AEs are summarized in Table 25, overall and by intensity. All hematologic abnormalities reported as AEs were assessed by the Investigators as mild or moderate in intensity; none were rated severe. The most common hematologic abnormalities reported as AEs were leukocytosis (59 patients; 7%) and neutrophil count increased (51 patients; 6%). Laboratory sampling timepoints are specified in Table 26.

For all patients with hematologic abnormalities reported as AEs at Discharge who also had hematology data at Follow-up, the abnormal laboratory values that had precipitated the AE had returned to Baseline or near-Baseline values by Follow-up.

Table 25: Hematologic Abnormalities Reported as Adverse Events, Overall and by Intensity (Studies AI-700 -23. -32 and -33)

	Highest Intensity			
MedDRA Preferred Term	**Mild n (%)**	**Moderate n (%)**	**Severe n (%)**	**Total n (%)**
Leukocytosis	34 (3.7)	25 (2.7)	0	59 (6.5)
Neutrophil count increased	40 (4.4)	11 (1.2)	0	51 (5.6)
Lymphocyte count decreased	21 (2.3)	1 (0.1)	0	22 (2.4)
Monocyte count decreased	12 (1.3)	1 (0.1)	0	13 (1.4)
White blood cell count increased	7 (0.8)	4 (0.4)	0	11 (1.2)
White blood cell count decreased	6 (0.7)	0	0	6 (0.7)
Platelet count decreased	4 (0.4)	0	0	4 (0.4)
Neutrophil count decreased	3 (0.3)	1 (0.1)	0	4 (0.4)
aPTT prolonged[1]	3 (0.3)	0	0	3 (0.3)
Eosinophil count decreased	3 (0.3)	0	0	3 (0.3)
Neutrophilia	1 (0.1)	2 (0.2)	0	3 (0.3)
Basophil count increased	2 (0.2)	0	0	2 (0.2)
Monocyte count increased	2 (0.2)	0	0	2 (0.2)
Anemia	0	1 (0.1)	0	1 (0.1)
Band neutrophil count increased	1 (0.1)	0	0	1 (0.1)
Hematocrit decreased	1 (0.1)	0	0	1 (0.1)
Hemoglobin decreased	1 (0.1)	0	0	1 (0.1)
Lymphocyte count increased	1 (0.1)	0	0	1 (0.1)
Lymphocytosis	1 (0.1)	0	0	1 (0.1)
Thrombocytopenia	1 (0.1)	0	0	1 (0.1)

[1] The 3 patients with AEs of aPTT (activated partial thromboplastin time) prolonged had been premedicated for ANGIO.

5.8 Clinical Laboratory Findings

Table 26 presents laboratory parameters that were measured in all studies, unless otherwise noted.

Table 26: Clinical Laboratory Parameters Collected and Analyzed, by Collection Timepoint, in All AI-700 Clinical Studies

		01	02	04	20	21	05	06	23	32	33	34
Standard Clinical Chemistry	albumin, ALK, ALT, AST, bicarbonate, BUN, Ca, Cl, CK, Creatinine, GGT, glucose, LDH, Ph, K. Na, bilirubin, cholesterol, protein, triglycerides, uric acid,	BL; 2, 8, 24,48, 120h		BL; 30m; 2, 8, 24, 96h	72 hr	BL; 2h; FU	BL; 24h	BL; 48h	BL; 2-3 hr, 48h	BL;2-3 hr, 72h		
Standard Hematology	HgB, Hct, RBC, MCV, MCH, MCHC, PLT											
Coagulation	PT, aPTT											
CBC	WBC, ANC, ALC, monocytes, eosinophils, basophils											
Urinalysis[2]	pH, specific gravity, protein, glucose, ketone, bilirubin, occult blood, nitrite, urobilinogen, microscopic exam											
Special Laboratory Tests	CRP, C3, C3a, tryptase	ND	ND	BL; 30m; 1, 2, 24h; 2-yr[3]	ND	ND	ND	ND	ND	ND	ND	ND

1 BL = Baseline/pre-dose; all timing is post-dose; ND=not done
2 Urinalysis not performed in Studies AI-700-04, AI-700-06, AI-700-21, and AI-700-34.
3 Clinical labs collected following rechallenge dosing, approximately 2 years after initial dosing in Study AI-700-04.

5.8.1 Serum Chemistry, Coagulation, and Urinalysis

In both healthy volunteers and patients with CAD, changes in laboratory parameters including serum chemistry, coagulation, and urinalysis were minimal and did not appear to be correlated with AI-700 dosing. Most of the changes observed could be explained by Baseline clinical conditions and changes in food intake during and following Discharge. In the dose-ranging studies, laboratory parameter changes in patients and healthy volunteers were not dose-dependent and were similar to changes observed in similar cohorts that received placebo.

Individual clinical laboratory outlier values were observed during the course of AI-700 clinical development; however, these were isolated cases that, upon review of subject medical

history and clinical conditions, did not indicate any clinical trends nor warrant further investigation.

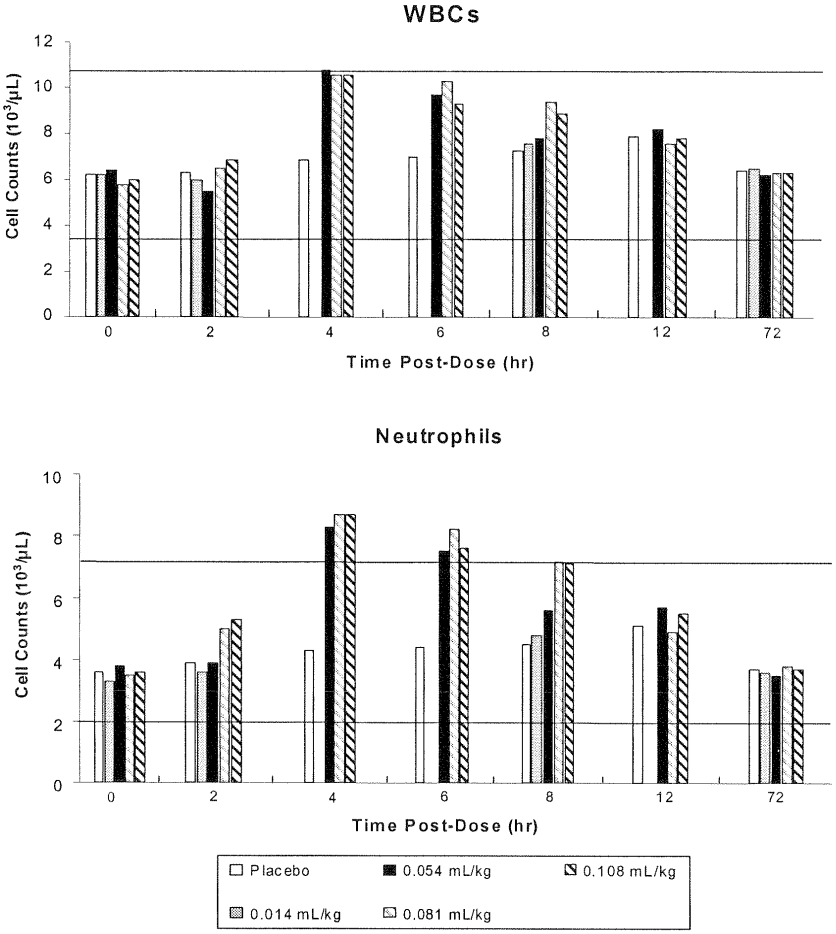
5.8.2 Hematology

Only the hematologic parameters of WBC and neutrophil counts demonstrated clinically relevant fluctuations or trends across studies.

5.8.2.1 Hematology Results in Healthy Volunteers

In the 106 healthy volunteers from Studies AI-700-01, -02, -04, -20, and -21 who received AI-700, mean neutrophil and WBC counts were within normal range at Baseline and Follow-up for all AI-700 dose groups. Dose-dependent increases from Baseline in both parameters were observed, with the highest mean percentage increases from Baseline occurring in the 2 highest AI-700 dose groups. For healthy volunteers in these dose groups that had data available at the 4 and 6-hour post-dose timepoints, mean neutrophil counts increased up to approximately 200% of Baseline values (and were above the normal range, Figure 34). An increase of a lesser magnitude was observed for the higher dose groups for WBC counts (approximately 100%). A gradual return toward Baseline in both parameters was observed from 6 hours post-dose through Follow-up. The trends seen in shifts from Baseline in neutrophil and WBC counts were consistent among healthy volunteers in all dose-ranging studies. No changes in these parameters were reported as AEs.

Figure 34: Mean WBC and Neutrophil Counts Over Time for Healthy Volunteers Following a Single Injection, by Dose Group (Studies AI-700-01, -02, -04, -20, and -21)



Horizontal lines represent normal ranges.
Some timepoints were not collected for all dose groups.

5.8.2.2 Hematology Results in CAD Patients (Studies AI-700-32 and -33)

For CAD patients in Studies AI-700-32 and -33, a similar trend to that observed for healthy volunteers was seen for WBC and neutrophil counts (Table 27). Mean WBC count increased from $6.8\times10^3/\mu L$ at Baseline to $9.1\times10^3/\mu L$ at Discharge, returning to $6.9\times10^3/\mu L$ at Follow-up (WBC normal range: 3.8 to $10.7\times10^3/\mu L$). The increase appears to be primarily related to a transient increase neutrophil count. Neutrophil counts increased from $4.3\times10^3/\mu L$ at Baseline to $7.4\times10^3/\mu L$ at Discharge, above the normal range (1.96 to $7.23\times10^3/\mu L$), before returning to $4.4\times10^3/\mu L$ at Follow-up.

Table 27: Summary of Hematology Parameters Over Time: WBC and Neutrophils (Studies AI-700 -32 and -33)

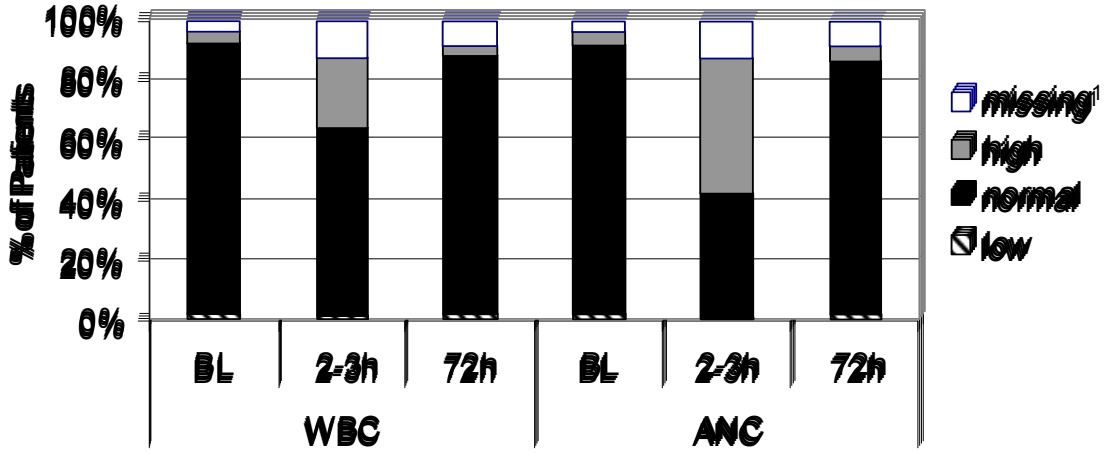
Parameter	Baseline	Discharge	Follow-up
WBC ($\times 10^3/\mu L$)			
n	745	681	715
Mean (SD)	6.8 (1.9)	9.1 (2.8)	6.9 (2.0)
Median	6.5	9.0	6.6
Neutrophils ($\times 10^3/\mu L$)			
n	745	681	715
Mean (SD)	4.3 (1.6)	7.4 (2.6)	4.4 (1.7)
Median	4.0	7.3	4.1

Shift analyses from Baseline to Discharge and from Baseline to Follow-up were conducted for all hematology laboratory parameters. Figure 35 presents the percentage of patients with values that were at, above, or below the normal range or that were missing for WBC and neutrophils.

Transient shifts to above the normal range for both WBC and neutrophils are apparent at 2-3 hours post AI-700 dosing. A total of 161 (26%) of the 631 patients with WBC counts within the normal range at Baseline had WBC values increase to above the normal range at Discharge. The majority of these shifts were to Grade 1 elevations in WBC ($>10.7\times10^3/\mu L$). A total of 18 patients (3%) had shifts from normal or Grade 1 Baseline values to Grade 2 elevated WBC ($>15.0\times10^3/\mu L$) at Discharge. Conversely, 10 (2%) of 631 patients with WBC counts within the normal range at Baseline had WBC values decrease to below the normal range at Discharge. By the Follow-up evaluation, 644 patients (93%) of the 691 patients with data available had WBC counts in the normal range.

A total of 320 (51%) of the 624 patients with neutrophils within the normal range at Baseline had absolute neutrophil count (ANC) increases above the upper limit of normal at Discharge. The majority of these shifts were to Grade 1 ($>7.23\times10^3/\mu/L$). A total of 58 patients had shifts in neutrophils from normal or Grade 1 at Baseline to Grade 2 ($>11.0\times10^3/\mu L$) at Discharge. Recovery was noted at Follow-up, with 603 (93%) of 646 patients who had normal neutrophil at Baseline and an available Follow-up evaluation having ANC values within the normal range. No shifts to Grade 3 at Discharge or Follow-up were seen in WBC or neutrophil counts.

Figure 35: Percent of Patients with WBC and ANC Values Within, Above, or Below the Normal Range, or Missing, by Timepoint (Studies AI-700 -32 and -33)



1 Missing laboratory values were primarily the result of patients being discontinued prior to undergoing scheduled ANGIO/LVG procedures.

5.8.2.3 Immunogenicity of AI-700 (Study AI-700-04)

Study AI-700-04 evaluated the potential immunogenicity of AI-700 following rechallenge as well as standard safety assessments. AI-700 dosing was conducted in 2 stages, approximately 2 years apart. Subjects received either AI-700 or placebo. AI-700 immunogenicity was evaluated by measuring C3a, C3, tryptase, CRP, WBCs, and neutrophils. During Stage 1, 10 subjects received AI-700 and 2 subjects received placebo. During Stage 2, 7 subjects received AI-700 and 2 subjects received placebo.

Prior to rechallenge with AI-700 (Stage 2), subjects received a percutaneous skin prick test (SPT). No subject displayed evidence of a hypersensitivity reaction. There were no noteworthy changes from Baseline to any post-dose timepoint for tryptase (a surrogate marker of histamine release) for either AI-700-treated or placebo-treated subjects. No allergic reactions were reported.

However, a transitory decrease in WBCs was observed 6 minutes post AI-700 dose. This effect was mainly due to a decrease in circulating neutrophils, with the nadir coinciding with the peak of C3a, which increased in all AI-700-treated subjects. This phenomenon was followed by a recovery phase in which neutrophils/total WBC and C3a returned towards Baseline levels, within 30 minutes to 2 hours. During Stage 1, C3a levels were above the normal range (<940 ng/mL) in 2 of 10 subjects (1034 ng/mL and 1430 ng/mL at 6 minutes post dose). These changes were not observed in the placebo-treated subjects. No further changes were observed in C3a levels. Activated C3a assists the RES in clearance of particles from systemic circulation.[84-86]

Neutrophils and WBCs were above Baseline levels 2 hours post-dose, and returned to Baseline levels at approximately 24-hours post dose. CRP, an acute phase protein involved in the innate immune response, increased above normal levels in 9 of 10 subjects at 24 hours;

the highest reported value was 0.92 mg/dL. No laboratory abnormalities were reported as AEs. Neutrophil, WBC, and CRP changes did not appear to be related to signs of infection or to represent a significant inflammatory response.

Based on percutaneous skin prick test and immunological laboratory test findings, AI-700 does not appear to be immunogenic (Section 5.17).

5.9 Vital Signs

Vital signs, including heart rate, respiratory rate, body temperature, and systolic and diastolic blood pressures, were measured at Baseline and at multiple post-dose timepoints during all AI-700 clinical studies. In general, measurements were made at similar timepoints throughout all phases of clinical development.

5.9.1 Heart Rate

For healthy volunteers in the dose-ranging studies (Studies AI-700-01, -02, -04, -20, and -21), mean heart rate was within the normal range (60-100 bpm) at all timepoints. No dose-dependent increases in mean heart rate were observed with single injections of up to 0.081 mL/kg AI-700; the clinically intended dose. A heart rate increase was seen in the 21 subjects who received the highest dose (0.108 mL/kg); this is a higher dose than was used during Phase 3. In healthy volunteers who received the highest AI-700 dose, mean heart rate was 60.1 bpm at Baseline, 75.3 bpm at 5 minutes, and 76.7 bpm at 10 minutes post dose.

In Studies AI-700-23, -32 and -33 (patients received the intended AI-700 dose administered during rest and dipyridamole stress imaging), mean heart rate remained essentially unchanged during rest and showed an acute transient increase of approximately 15 bpm for 10 minutes following AI-700 dosing and during peak pharmacological stress. For most patients, this trend was reversed at 10 minutes post-dose due to the protocol-mandated administration of aminophylline.

5.9.2 Blood Pressure

5.9.2.1 Healthy Volunteers

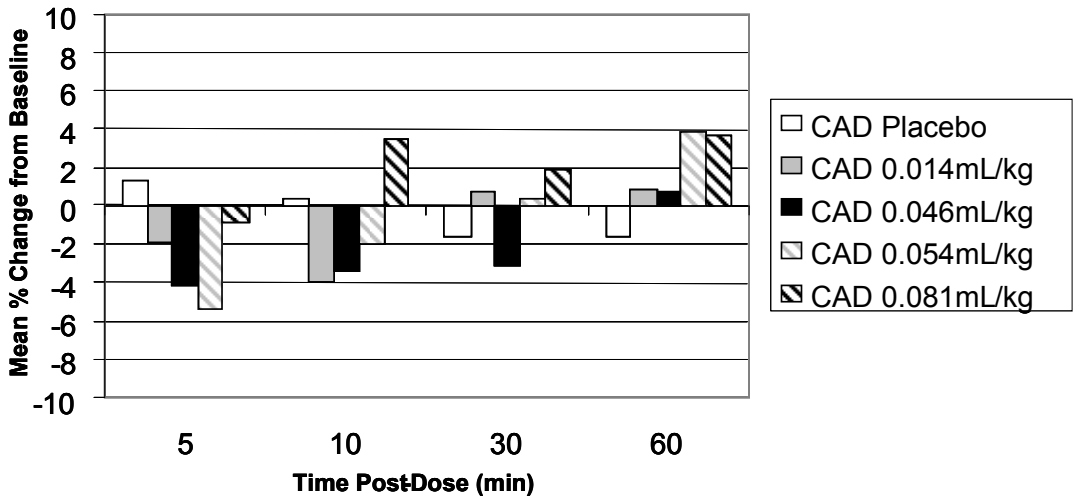
In 106 healthy volunteers (Studies AI-700-01, -02, -04, -20, and -21), mean SBP and DBP values remained within the normal range (90-140 mmHg for SBP and 50-90 mmHg for DBP) at all timepoints in all dose groups. Mean SBP remained within 6% of Baseline values for all dose groups. Changes of a similar magnitude were observed for DBP in all AI-700 dose groups. Hypotension was reported as an AE for 1 healthy volunteer at 20 minutes following a single injection of 0.081 mL/kg AI-700. The subject's lowest blood pressure reading (84/49 mmHg) occurred at that time. In Study AI-700-06, no notable changes from Baseline were observed in mean SBP or SBP after AI-700 administration and no blood pressure abnormalities were reported as AEs.

5.9.2.2 CAD Patients (Studies AI-700-20 and -21) and CHF/COPD Patients

In Study AI-700-05, no notable changes from Baseline in blood pressure were observed in CHF/moderate COPD patients.

In Studies AI-700-20 and -21, no notable changes from Baseline in mean SBP or DBP were observed in CAD patients. For all AI-700 dose groups and placebo, mean SBP remained within 7% of the Baseline value at all post-dose timepoints evaluated. Figure 36 presents mean percent change from Baseline in SBP for all treatment groups in Studies AI-700-20 and -21 following a single injection of AI-700.

Figure 36: Mean Percent Change from Baseline in Systolic Blood Pressure Following a Single Injection in CAD Patients (Studies AI-700-20 and-21)



Blood pressure abnormalities were reported as AEs in 5 of these CAD patients from Studies AI-700-20 and -21. Three patients experienced AEs of decreased blood pressure/hypotension following single injections of 0.054 to 0.108 mL/kg AI-700. One patient in the 0.028 mL/kg dose group and 1 patient in the 0.108 mL/kg dose group (each with prior history of hypertension) had significant elevations in blood pressure reported as AEs following administration of dipyridamole and the second AI-700 dose. All events were considered mild and resolved within 10 minutes of onset.

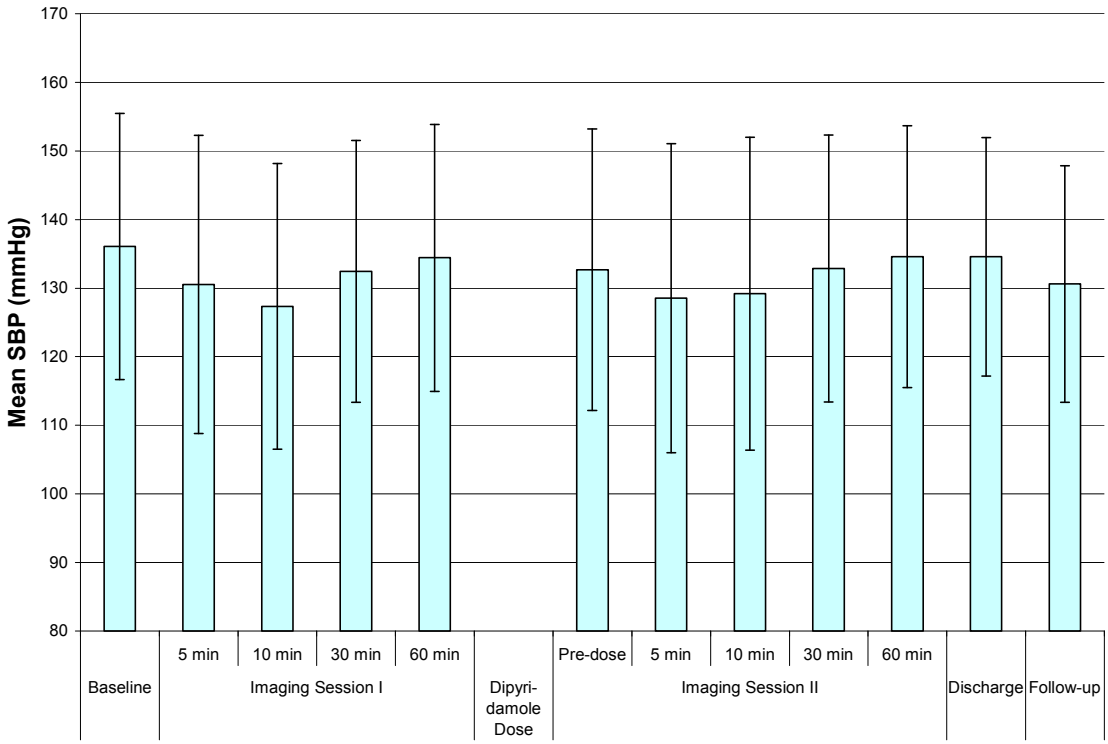
5.9.2.3 CAD Patients (Studies AI-700-23, -32, and -33)

In Study AI-700-23, mean SBP and DBP remained within 5% of Baseline.

In Studies AI-700-32 and -33, mean change from Baseline SBP (136 mmHg) ranged from a decrease of 9 mmHg (6%) to an increase of 0.5 mmHg (1%) at Discharge. Mean change from Baseline DBP (77 mmHg) ranged from a decrease of 5.5 mmHg (7%) to a decrease of 0.1 mmHg (<1%) at 60 minutes following the second AI-700 dose. During rest, the nadir occurred at 10 minutes post-dose for both SBP and DBP. During stress, the nadir occurred at 5 minutes post-dose for both SBP and DBP. Figure 37 presents mean SBP over time.

Blood pressure decreases reported as AEs (hypotension or blood pressure decreased) are presented in Section 5.7.7.2. Hypertensive AEs (preferred terms: blood pressure increased, hypertension, or hypertensive crisis) were reported in 8 (1%) of 911patients. One moderate hypertensive event was reported as SAE during rest (Patient 23.16.03, Section 5.5). No other hypertensive AEs were reported during rest. A severe hypertensive event was reported during stress in Patient 32.02.030 (SAE of mental status change, Section 5.5). A total of 4 patients reported hypertensive AEs post dipyridamole. All hypertensive AEs resolved without residual effects.

Figure 37: Mean Systolic Blood Pressure over Time (Studies AI-700-32 and -33)



Error bars designate ±1 standard deviation.

A categorical outlier analysis for the changes in SBP was conducted to identify patients that met any of the following predefined outlier criteria: out of normal range (defined as 90-200 mmHg), >40 mmHg change from Baseline, or changes of >25% at 5 or 10 minutes post-dose compared to the immediately preceding measurement. Of the 778 patients, 80 (10%) patients met SBP outlier criteria during rest; 63 of these patients for decreases and 24 patients for increases. During stress, 145 (19%) patients met SBP outlier criteria; 97 of these patients for decreases and 76 for increases. Most patients meeting SBP outlier criteria that were decreases did so at 5 and 10 minutes post dose. This corresponded to the time courses of decreases in mean SBP, with nadirs at 5 and 10 minutes post-dose.

See Section 5.7.7.2 for a presentation of AEs of special interest in Studies AI-700-23, -32, and -33, including hypotensive and vasovagal syncope AEs.

5.9.3 Respiratory Rate

No clinically notable changes or trends from Baseline in mean respiratory rate were observed in healthy volunteers in any dose group in the AI-700 dose-ranging studies or in CHF/COPD patients (Study AI-700-05).

Mean respiratory rate in Study AI-700-23 during rest remained within 1 respiration per minute of the baseline rate (16.7 respirations per minute) at all post-dose timepoints. Mean respiratory rate peaked during stress, at 5 minutes following dipyridamole and AI-700 dosing, to 18.1 breaths per minute, representing an increase from Baseline of approximately 10%. Mean respiratory rate returned to near Baseline levels at Follow-up.

No clinically significant trends in mean respiratory rate following AI-700 dosing were observed in patients in Studies AI-700-32 and -33, with the exception of the first timepoint following administration of dipyridamole and AI-700 during peak stress (5 minutes post dose), when respiratory rate was elevated to 17.6 breaths per minute, representing an increase from Baseline of approximately 10%. Respiratory rates remained generally stable at all timepoints post-dosing. Changes in respiratory rate during stress were temporally related to respiratory AEs in some patients, including dyspnea, throat tightness, cough, bronchospasm, and hypoxia. These AEs were primarily observed during peak pharmacological stress and most were considered related to dipyridamole.

5.9.4 Body Temperature

No clinically notable changes or trends from Baseline in mean body temperature were observed in healthy volunteers or CAD patients in any AI-700 study. Pyrexia was reported in 1 healthy volunteer following the second dose of ≤0.054 mL/kg AI-700. Adverse events of pyrexia and body temperature increased were reported in 24 (3%) of 911 patients in Studies AI-700-23, -32, and -33. However, these AEs did not appear to be associated with other signs of infection. The majority of these AEs were mild in intensity, required only symptomatic treatment, and resolved with no residual effects.

5.10 ECG Findings

Table 28 outlines the ECG parameters evaluated and methods used to evaluate those parameters. During AI-700 clinical studies, 12-lead ECGs were used to monitor heart rhythm and ECG interval durations. Evaluations of 12-lead ECGs included multiple automated analyses of 10-second rhythm strips and approximately 3 hours of continuous Holter monitoring (done in a subset of consecutive patients at selected sites). For evaluations of QT interval, 2 corrections were used: Bazett's correction method (QTcB) and Fridericia's correction method (QTcF). The QTcF method was selected as the preferred method, based on results from Study AI-700-21 indicating that dipyridamole produced significant increases in heart rate, which is known to decrease the accuracy of the QTcB correction method.[87, 88] In Studies AI-700-32 and AI-700-33, automated reads were manually over-read since automated reads are known to produce ECG intervals of a misleadingly longer duration,

thereby overestimating the likelihood of prolonged QTc.[88] To obtain a uniform and accurate evaluation, all 12-lead paper ECGs for patients with QTcF increases from Baseline/Stress Baseline of >60 milliseconds (ms) and for patients with QTcF shifts from Baseline to ≥500 ms were provided to the ECG Core Laboratory for manual over-read of source QT values data.

PVCs were evaluated using 3 lead US-ECG tracing which were recorded on S-VHS tape during ECHO imaging.

Table 28: ECG Data Obtained During AI-700 Clinical Development Studies

Study Number	12-Lead ECG						US-ECG
	Heart Rate	ECG Intervals					PVC/PAC
		PR	*QRS*	*QT*	*QTcB*	*QTcF*	
AI-700-01[1]	✓	✓	✓	✓	✓		
AI-700-02[1]	✓	✓	✓	✓	✓		✓
AI-700-05[2]	✓	✓	✓	✓	✓		
AI-700-06[3]	✓	✓	✓	✓			
AI-700-20[3]	✓	✓	✓	✓	✓		✓
AI-700-21[3]	✓	✓	✓	✓	✓		✓
AI-700-23[3]	✓	✓	✓	✓	✓	✓	✓
AI-700-32[4]	✓	✓	✓	✓	✓	✓	✓
AI-700-33[4]	✓	✓	✓	✓	✓	✓	✓
AI-700-34[3]	✓	✓	✓	✓	✓	✓	✓

ECG = electrocardiogram; US-ECG = ultrasound ECG; PVC/PAC = premature ventricular (atrial) contractions.
✓ Performed during study at pre- and post-dose timepoints.
1 Obtained using continuous ECG telemetry.
2 Obtained using continuous Holter 12-lead ECG.
3 Obtained using paper 12-lead ECG only.
4 Obtained using both continuous Holter 12-lead and paper 12-lead ECGs.

5.10.1 12-Lead ECGs

Heart rates obtained from the 12-lead ECGs corroborated the results obtained from the vital signs in healthy volunteers, COPD/CHF patients, and CAD patients.

Overall interval changes on ECG in healthy volunteers, COPD/CHF patients, and CAD patients did not appear to be clinically significant and demonstrated no added pro-arrhythmic risk. No healthy volunteers had ECG changes reported as AEs.

A summary of the 12-lead ECG analysis, focusing on proarrhythmic risk from QTc prolongation and ECG-related ischemic AEs, is provided for CAD patients in Studies AI-700-20, -21, -32 and -33.

CAD Patients in Dose-Ranging Studies AI-700-20 and -21

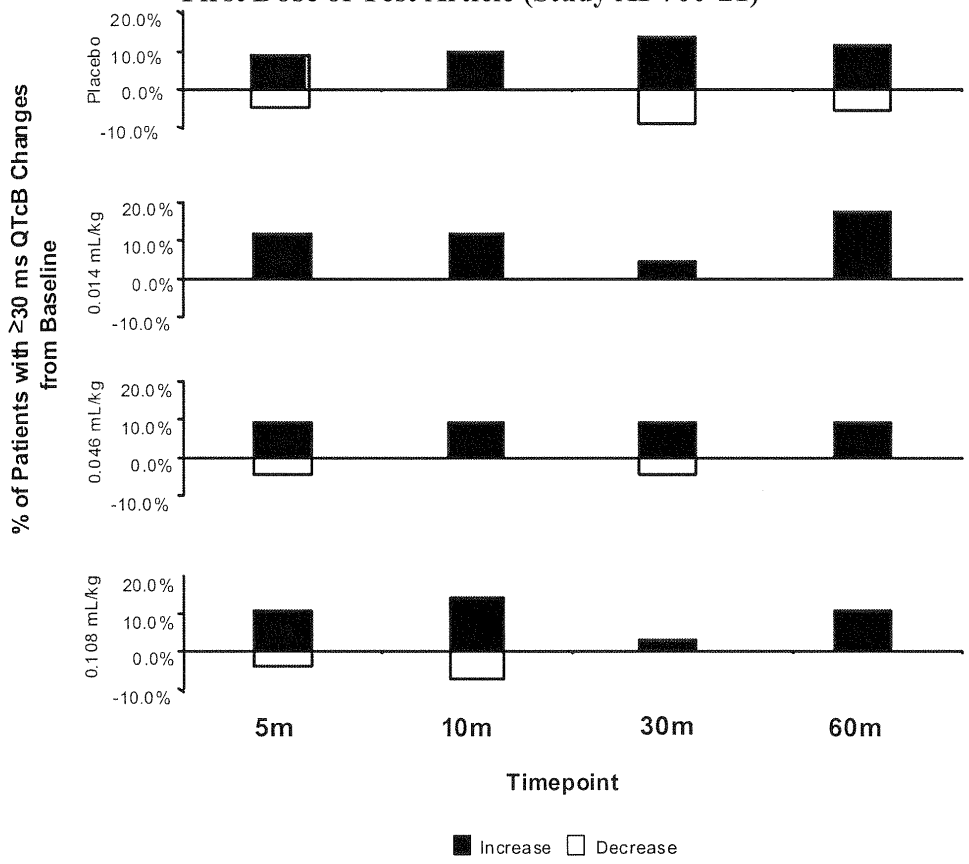
In both studies, in the absence of pharmacologic stress (i.e. excluding timepoints during stress), QTcB results demonstrated a maximum mean increase of 6.4 ms in 110 patients treated with AI-700. A similar mean increase was observed in the 22 patients who received placebo (8.3 ms). Although individual patients in Study AI-700-21 may have had changes in

QTcB values at various isolated timepoints, Figure 38 illustrates that no significant trends over time were observed, and there was no association with dose. Patients who received AI-700 had a similar rate of change compared with those who received placebo: 37 (34%) of 110 CAD patients had QTcB changes from Baseline of >30 ms, compared with 7 (32%) of 22 CAD patients who received placebo.

In these 2 studies, a total of 9 patients had 15 episodes of QTcB intervals >500 ms. Of these, 8 (7%) of 110 patients received AI-700 and 1 (5%) of 22 patients received placebo. A review by the Sponsor indicated that the majority of these increases were likely spurious and not associated with clinically significant changes from Baseline. Cardiac rhythm was normal in 8 of the 9 patients; the exception was 1 patient who received AI-700 and had abnormal cardiac rhythm following dipyridamole.

In AI-700-21, dipyridamole was administered during stress to 75 patients receiving AI-700 and 22 patients receiving placebo. The maximum mean increase among all dose groups was 24 ms, which occurred immediately following dipyridamole administration and at 5 minutes following the second dose of AI-700 (during peak pharmacological stress). Similarly, patients who received placebo had a maximum mean increase of 22 ms during peak pharmacological stress.

Figure 38: Percent of Patients with QTcB Interval Changes >30 ms Following the First Dose of Test Article (Study AI-700-21)



A total of 10 (9%) of 110 CAD patients treated with AI-700 experienced 16 ECG changes on US-ECG and 12-lead paper ECG that were reported as AEs: 15 (94%) were mild and 1 (6%) was moderate in intensity. Nine of the 10 patients were enrolled in AI-700-21. In AI-700-20 and -21, PAC/PVCs were the most frequently observed AEs, reported in 6 of 10 patients who reported ECG-related AEs post AI-700 dose. As demonstrated in the subsequent US-ECG analysis (Section 5.10.2), single PVCs were most often observed during and immediately following high mechanical index (1.1-1.9) destruction pulse imaging. Current regulatory restrictions do not allow contrast imaging at an MI higher than 1.0. The remaining AEs observed during dosing were ischemic in nature, mild in intensity, and occurred during stress. The rate of ECG-related AEs did not appear to be dose-dependent. The overall rate of ECG-related AEs was comparable to placebo-treated CAD patients in Study AI-700-21: 3 of 22 (14%) patients reported 3 ECG-related AEs.

Studies AI-700-23,-32 and -33

In Study AI-700-23, there were no clinically significant trends in ECG findings. The 133 CAD patients in Study AI-700-23 had similar ECG assessments to those of CAD patients enrolled in Studies AI-700-32 and -33.

In Studies AI-700-32 and -33, a total of 778 patients had serial 12-lead paper ECG data collected. A subset of 109 patients also had Holter-12 lead continuous ECG recordings collected. Holter data were analyzed only for QTc interval changes.

Mean changes from Baseline in both PR and QRS intervals were small at all post dose timepoints; maximum changes ranged from a decrease of 3.3 ms to an increase of 2.9 ms (Table 29). Categorical outlier analyses performed for PR and QRS complex intervals did not reveal any clinically significant trends.

Changes in mean QTcF values from Baseline are presented by imaging session. The maximum mean increase in QTcF of 7 ms was observed following dipyridamole and prior to the second dose of AI-700.

Table 29: 12-Lead ECG Heart Rate and Intervals (PR, QRS, QT, QTcF), and Mean Percent Changes from Baseline (Studies AI-700-32 and -33)

	Heart Rate			PR			QRS			QT			QTcF		
Timepoint	Mean (bpm)	SD	Change from Baseline (%)	Mean (ms)	SD	Change from Baseline[1] (%)	Mean (ms)	SD	Change from Baseline[1] (%)	Mean (ms)	SD	Change from Baseline[2] (%)	Mean (ms)	SD	Change from Baseline[2] (%)
Imaging Session I															
(Pre-Dose)	65.0	10.91	N/A	173.8	28.66	N/A	93.3	18.05	N/A	404.4	37.19	N/A	412.6	28.81	N/A
5 minutes	65.6	11.44	1.4	171.8	27.91	-1.1	93.3	18.74	0.2	405.1	37.57	0.3	414.7	29.17	0.6
10 minutes	67.6	11.50	4.4	171.1	27.98	-1.2	92.8	18.73	-0.3	402.3	35.52	-0.4	415.8	27.89	0.9
30 minutes	64.9	11.23	0.3	172.8	28.59	-0.3	93.0	18.81	-0.1	406.6	36.59	0.7	414.6	27.88	0.6
60 minutes	64.8	11.20	0.2	174.5	29.92	0.5	93.1	18.76	0.0	406.3	36.26	0.6	414.1	28.28	0.5
Dipyridamole Stress Administered															
Imaging Session II															
(Pre-Dose)	80.7	14.79	25.4	176.7	29.68	2.0	92.9	18.55	-0.1	383.1	37.43	-5.1	419.5	29.93	1.9
5 minutes	80.4	13.60	25.0	176.3	29.96	1.6	93.3	18.49	0.3	381.6	36.25	-0.1	417.9	30.01	-0.3
10 minutes	76.0	12.93	18.0	172.6	27.69	-0.3	93.3	18.62	0.3	387.6	36.13	1.4	416.6	29.14	-0.6
30 minutes	70.5	12.55	9.1	173.5	28.97	0.1	93.6	18.51	0.6	396.5	36.63	3.8	415.4	28.18	-0.8
60 minutes	70.0	12.37	8.6	173.9	28.47	0.3	93.8	19.11	0.7	395.8	35.58	3.7	413.9	27.54	-1.2
Discharge	73.4	13.63	13.6	170.3	27.74	-1.6	92.9	19.11	0.1	388.1	36.08	-3.6	411.8	27.01	0.2

ms = milliseconds, bpm = beats per minute, SD = standard deviation, N/A=not applicable
[1] Pre- and post-dose Imaging Session I values for HR, PR, and QRS reflect the change from Imaging Session I pre-dose Baseline.
[2] Pre- and post-dose Imaging Session II values for QT and QTc reflect change from Stress Baseline (i.e., following dipyridamole administration and immediately prior to the second AI-700 dose).

A categorical analysis of QTcF values from Studies AI-700-32 and -33 was performed. The number and percentage of patients with QTcF values ≥450, ≥480, and ≥500 ms at any measured timepoint (values identified in FDA Guidance E-14 as clinically relevant changes in QTc interval) are presented in Table 30. The largest numbers of outliers were observed immediately after dipyridamole administration and prior to the second dose of AI-700.

Table 30: 12-Lead ECG: Number of Patients with QTcF Values ≥450 ms, ≥480 ms, and ≥500 ms, by Timepoint (Studies AI-700 -32 and -33)

Time Post Dose[1]	Total N	QTcF ≥450 ms n (%)	QTcF ≥480 ms n (%)	QTcF ≥500 ms n (%)
Baseline	778	74 (9.5)	15 (1.9)	6 (0.8)
Imaging Session I				
5 min	768	76 (9.9)	15 (2.0)	4 (0.5)
10 min	771	92 (11.9)	14 (1.8)	3 (0.4)
30 min	776	80 (10.3)	17 (2.2)	2 (0.3)
60 min	773	82 (10.6)	19 (2.5)	3 (0.4)
Imaging Session II				
Post dipyridamole (prior to 2nd dose of AI-700)	755	109 (14.4)	24 (3.2)	5 (0.7)
5 min	761	102 (13.4)	18 (2.4)	4 (0.5)
10 min	761	94 (12.4)	11 (1.4)	3 (0.4)
30 min	762	81 (10.6)	15 (2.0)	4 (0.5)
60 min	757	72 (9.5)	12 (1.6)	3 (0.4)
Discharge	726	58 (8.0)	8 (1.1)	3 (0.4)
Follow-up	732	50 (6.8)	8 (1.1)	3 (0.4)

[1] Minutes after AI-700 dosing.

A categorical analysis of changes from Baseline in QTcF was conducted. Outliers were changes from Baseline of >30 ms, >60 ms, or that increased from <500 ms at Baseline to ≥500 ms after AI-700 dosing. At most timepoints during rest and stress, 5-6% of patients had QTcF increases of >30 ms. The exception was at the timepoint that was post dipyridamole and prior to second AI-700 dose, when 13% of patients had an increase >30ms; this increase was probably due to increased heart rate. Following the manual over-read of source QT values data (at Duke ECG Core Laboratory), 3 patients were determined to have a QTcF change of >60 ms and 1 patient was determined to have a QTcF of 507 ms. For all 4 patients, changes in QTc were influenced by extreme heart rate values and did not appear to be related to AI-700. No clinical symptoms or ECG changes suggestive of ventricular arrhythmias were observed.

QTcF values captured by Holter ECG and by paper 12-lead ECG were analyzed at each minute post dose. Holter data showed limited variability between the Baseline, 5-minute, and 10-minute values recorded by paper 12-lead ECG and a confirmed lack of added pro-arrhythmic risk from QTc prolongation post-AI-700.

Ninety (10%) of 911 patients in Studies AI-700-23, -32 and -33 had 103 ECG changes reported as AEs. Most of these AEs occurred following dipyridamole administration.

All ECG-related AEs resolved without residual effects. Eighty-three of these AEs were considered at least possibly related to dipyridamole; 20 were considered at least possibly related to AI-700.

A total of 53 patients reported 55 ST-T wave changes and other ECG signs of myocardial ischemia (see Table 31, preferred terms: ECG ST segment abnormal, ECG T wave inversion, ECG ST-T change, ECG ST segment depression, ECG ST-T segment depression, ECG signs of myocardial ischemia). Prior to dipyridamole dosing, 1 patient had an AE of ST segment depression. This patient had a concomitant AE of mild angina. The ST depression occurred at least 45 minutes post-dose and the Investigator assessed the event as unrelated to AI-700. The patient did not receive dipyridamole and was discontinued from the study. No other treatment was required. All other ECG related events indicative of ischemia were reported during stress.

Table 31: Patients with ECG-Related Adverse Events Indicative of Ischemia, by Onset Time (Studies AI-700-23, -32, and -33)

Ischemic Adverse Event	Onset Time				Total N = 911 n (%)*
	1st Dose to DIPY N = 911 n (%)*	DIPY to 2nd Dose N = 894 n (%)*	2nd Dose to Discharge N = 894 n (%)*	Discharge to Follow-up N = 911 n (%)*	
ECG related events indicative of ischemia					53 (5.8)
ECG ST segment depression	1(0.1)	18 (2.0)	15 (1.7)	0	33 (3.6)
ECG ST segment abnormal	0	2 (0.2)	5 (0.6)	0	7(0.8)
ECG signs of myocardial ischemia	0	3 (0.3)	3 (0.3)	0	6 (0.7)
ECG T wave inversion	0	2 (0.2)	2 (0.2)	0	4 (0.4)
ECG ST-T change	0	3 (0.3)	0	0	3 (0.3)
ECG ST-T segment depression	0	0	1 (0.1)	0	1 (0.1)

If a patient had more than one occurrence of the same AE at 2 different timepoint categories, that patient would be counted only once in the total column.

The remaining ECG related AEs can be categorized as sinus or ventricular arrhythmia, QT prolongation, AV conduction abnormalities, and other non-specified events (preferred terms: ECG change, ECG abnormal, QRS axis abnormal, ECG T wave abnormal, ECG repolarization abnormality, ECG T wave amplitude decreased). Overall, a total of 9 (1%) of 911 patients reported ECG-related AEs after the first dose of AI-700, but prior to dipyridamole (including 5 patients with sinusal arrhythmia or PVCs, 2 patients with QT prolongation, 1 patient with ST depression, and 1 patient with an AV conduction abnormality).

Twelve (1.2%) of 911 patients had PVCs reported as AEs [preferred terms: ventricular extrasystoles (7 patients), ventricular bigeminy (2 patients), ventricular trigeminy (2 patients), and ventricular fibrillation (1 patient)]. Three of these patients had PVCs reported as AEs (preferred term ventricular extrasystoles) during AI-700 imaging: 1 patient during rest and 3 patients during stress. PVCs were isolated events and

appeared unifocal in morphology, which does not support the concept of contrast-induced PVC generation.

One of 911 patients had sustained ventricular arrhythmia (AE preferred term; ventricular fibrillation). This AE (reported in Patient 33.42.037) occurred during a scheduled coronary angiography procedure performed 24 hours post-dose and so was considered unrelated to AI-700 by the Investigator.

5.10.2 Ultrasound ECGs

Three-lead US-ECG recordings were evaluated for PVCs and premature atrial contractions (PACs). Other cardiac ECG arrhythmias cannot be diagnosed using the US-ECG tracing. The occurrence of PVCs was recorded by time-interval and by imaging mode (destruction pulse imaging, triggered imaging, real-time continuous imaging), and post imaging (Appendix 4 and 5).

Although a single AE of PACs was reported in 1 patient enrolled in Study AI-700-21, PACs were not considered clinically significant; PACs were reported as an AE .

CAD Patients in Dose-Ranging Studies AI-700-20 and -21

In Study AI-700-20, an analysis of PVC occurrence as it related to imaging mode was not conducted; ECG recordings were not always obtained. In Study AI-700-21, an increased rate of PVCs was observed only in patients who received AI-700. This dose-dependent increase was almost exclusively observed during destruction pulse imaging when the mechanical index was set between 1.1-1.9 (Table 32).

Table 32: Number of Patients with an Increase from Baseline Rate of PVCs on US-ECG Following Dual Injection of Test Article, by Imaging Session and Imaging Mode (Study AI-700-21)

	Number of Patients with Increased PVC Rate[1]			
		AI-700 Dose Group (mL/kg)		
Imaging Mode	Placebo (n = 22)	0.014 (n = 25)	0.046 (n = 22)	0.054 (n = 28)
Imaging Session I (Rest)				
Destruction Pulses	0	3 (12%)	3 (14%)	5 (18%)
Triggered Imaging	0	0	0	0
Continuous Imaging	0	0	0	0
Post Imaging	0	0	0	0
TOTAL	0	3 (12%)	3 (14%)	5 (18%)
Imaging Session II (Stress)				
Destruction Pulses	0	1 (4%)	3 (14%)	7 (25%)
Triggered Imaging	0	0	0	1 (4%)
Continuous Imaging	0	0	0	2 (7%)
Post Imaging	0	0	0	2 (7%)
TOTAL	0	1 (4%)	3 (14%)	8 (29%)

1 Subject must have satisfied at least one of 3 criteria: 1) if Baseline rate = 0, then 5 PVCs/minute; 2) if Baseline rate > 0, then the greater of a doubling of the Baseline rate or 5 PVCs/minute; 3) if destruction pulses were used, then 2 PVCs total following destruction pulses.

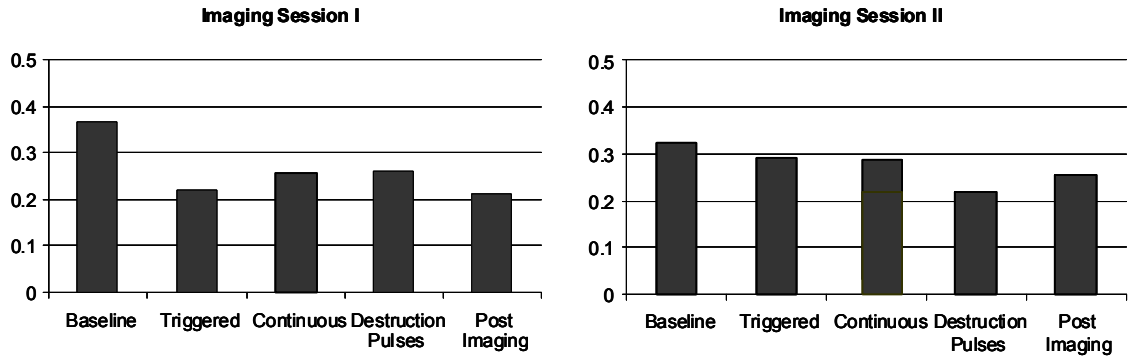
A detailed review of AEs, vital signs, and oxygen saturation data from all subjects with an increased rate of PVCs showed that there were no noteworthy changes in these parameters at the times during which the increased rates of PVCs were recorded.

Studies AI-700-23, -32 and -33

In Study AI-700-23, 2 of 123 CAD patients demonstrated an increase in PVC rates (1 during post-imaging and 1 during triggered contrast imaging). No correlation between PVC rates and imaging mode was observed.

In AI-700-32 and -33, the mechanical index remained ≤1.0 during all imaging modes. The mean rate of PVC occurrence did not exceed the Baseline rate of 0.37 PVCs/minute during either imaging session, regardless of imaging mode. The mean rate of PVCs is presented graphically by imaging session and imaging mode in Figure 39.

Figure 39: Mean Rate of PVCs Observed on Ultrasound ECG (Studies AI-700-32 and -33)



Eighteen (2.3%) of 778 patients had protocol deviations of brief episodes of imaging with mechanical index >1.0 (range: 5-206 sec). During these episodes of imaging with mechanical index >1.0, four patients had PVCs. The rates and morphologies of observed PVCs in these 4 patients were similar to those observed pre-dose, at Baseline, and did not exceed 3 PVCs/minute.

Based on the results of the US-ECG analysis, the Sponsor concluded that AI-700 had no proarrhythmic effect on PVC rates during rest/stress imaging in stable chest pain patients, provided the mechanical index was kept below 1.0.

5.10.3 ECG Conclusions

No healthy volunteers had ECG changes reported as AEs.

Analysis of 12-lead ECGs focused on proarrhythmic risk from QTc prolongation and ischemic changes during AI-700 imaging. No clinically significant differences in ECG parameters (PR, QRS, QT/QTc) that indicate an effect of AI-700 on ECG intervals were observed. In addition, the AEs reported in relation to ECG changes indicative of ischemia were almost exclusively attributed to dipyridamole and did not indicate an increased risk from administration of AI-700.

Analysis of US-ECG focused on proarrhythmic risk from PVC induction during AI-700 imaging. High mechanical index cardiac imaging with ultrasound contrast agents are known to induce PVCs.[33] In pre-Phase 3 studies, and in animal studies with AI-700 using a high mechanical index (>1.0), PVCs were observed and were shown to stop when the mechanical index was lowered or the probe was removed from the chest. In animal studies, high mechanical index PVC induction had no effect on histology or viability of myocardial tissue. During Phase 2 AI-700 trials, a detailed review of AEs, vital signs, and oxygen saturation data from all subjects who had an increase in the rate of PVCs showed that there were no noteworthy temporal associations. All PVCs resolved upon removal of the ultrasound probe from the chest with no residual effects.

Recently, FDA restricted high mechanical index imaging (>1.0) during contrast ultrasound imaging. Data from the Phase 3 studies have demonstrated that when mechanical index does not exceed 1.0, the rate of PVCs does not increase with AI-700 imaging.

In conclusion, the results of extensive ECG evaluations indicate that AI-700 had no added proarrhythmic effects and does not appear to increase ischemic risk in CAD patients being evaluated for ischemia with AI-700 dipyridamole stress ECHO.

5.11 Physical Examination

A physical examination was performed in Studies AI-700-23, AI-700-32, and AI-700-33 at Baseline, Discharge, and at the Follow-up visit. Findings upon physical examination were unremarkable.

5.12 Pulse Oximetry

Measurement of SaO_2 by pulse oximetry was performed in all clinical studies of AI-700. For Studies AI-700-23, -32, and -33, SaO_2 was measured at Baseline, and at 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 20, 30, and 60 ±10 minutes following each AI-700 dose, and at Discharge from the clinical site.

Healthy Volunteers

A review of the SaO_2 data among 106 healthy volunteers (Studies AI-700-01, -02, -04, -20, and -21) showed no clinically significant findings. An asymptomatic SaO_2 decrease below 90% was recorded in 1 subject.

CAD Patients (Studies AI-700-20 and -21)

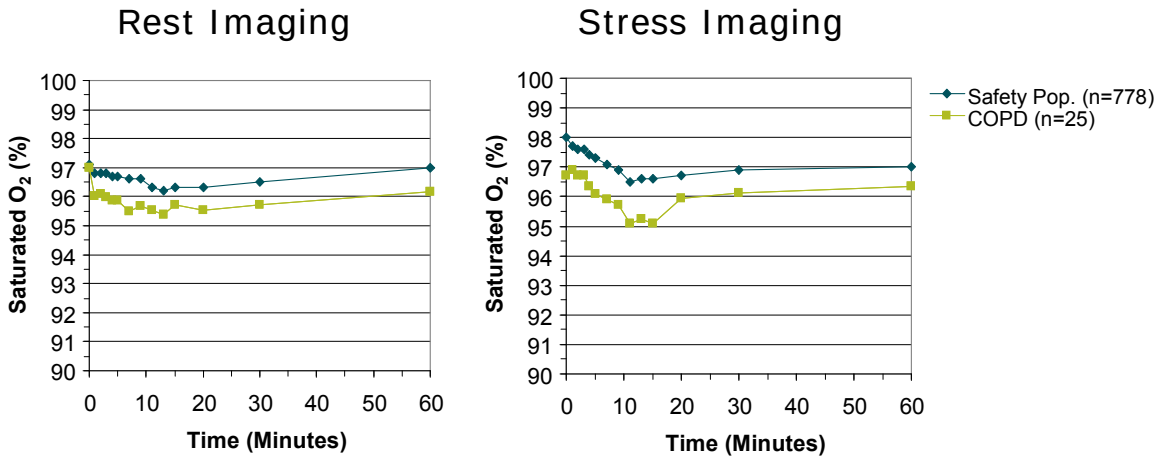
No trends in mean change from Baseline in SaO_2 values were observed among patients treated with either AI-700 or placebo. Decreases in SaO_2 below 90% (range: 84-89%) were recorded after dosing in 10 (9%) of the 110 CAD patients who received AI-700. SaO_2 decreases in all 10 patients were ≥5% below Baseline measurements. In 9 of 10 patients, SaO_2 decreases occurred during rest. This subset of 10 patients had a higher BMI and a lower mean SaO_2 at Baseline as compared with the overall population: 34.2 versus 30.1 and 95% versus 97%, respectively. All decreases in SaO_2 were transient and occurred in patients who received an AI-700 dose higher than the intended single dose (0.04 ml/kg).

Oxygen saturation decreased was reported as an AE in 2 patients; dyspnea was reported as an AE in another 2 patients. All 4 AEs resolved without residual effects.

CAD Patients (Studies AI-700-32 and -33)

In AI-700-32 and -33, mean SaO_2 fluctuated within 1% of the Baseline value at all post-dose timepoints (Figure 40). A similar time course was seen for COPD patients, although mean SaO_2 was lower.

Figure 40: Mean SaO_2 by Pulse Oximetry, By Imaging Session and Timepoint (Studies AI-700 -32 and -33)



Patients in Studies AI-700-32 and AI-700-33 with at least 1 treatment-emergent SaO_2 value indicating a decrease of ≥5% from Baseline to <90% are presented in Table 33. Of the 23 patients (3% of 778 patients) who met this criterion, all but 4 had SaO_2 values ≥87%: 10 experienced a decrease during rest only, 11 experienced a decrease during stress only, and 2 had decreases during both rest and stress. SaO_2 values for all of these patients were ≥90% by Discharge (or the last observation carried forward), with the exception of Patient 33.37.047 for whom a single SaO_2 value <90% (89%) was reported at Discharge. Most decreases were isolated, asymptomatic events predominantly in patients who were current or former smokers. Mean SaO_2 remained within 1% of Baseline at all timepoints, with a nadir occurring 7-15 minutes post dose. The delayed timing of SaO_2 changes along with their transient occurrence does not support the hypothesis of a direct blockage of pulmonary vasculature by AI-700 microspheres.

Respiratory AEs are summarized in Section 5.7.7.3.

Table 33: Patients with Absolute SaO_2 Decreases of ≥5% from Baseline to <90% (Studies AI-700 -32 and -33)

Patient Number	Smoking / Respiratory History	Baseline SaO_2 (%)	Lowest SaO_2 Value(s)			Post-Dose SaO_2 Range[1]	Concurrent AE
			SaO_2 (%)	Post-dose Time(s)	Imaging Session(s)		
32.16.007	--	99	88	1m	II	88-99	Headache
32.16.010	Former	97	89	20m	I	89-99	Cough, Lacrimation, Flushing Wheezing
32.18.008	Former	93	88	11m	I, II	88-96	Hypotension
32.32.004	Former	93	87	13m	II	87-97	Headache, Nausea
33.34.003	Former	97	89	7m	I	89-98	none
33.34.012	-	95	87	11m	I	87-96	none
33.34.016	-	95	89	15m	I	89-97	none
33.35.035	-	99	89	3m	I	89-99	Allergy to contrast
33.35.047	Former	97	89	15m	I	89-95	Hypoxia[2]
33.35.082	Former	98	83	7m	II	83-99	Bronchospasm, nausea, vomiting and hypoxia
33.37.005	-	92	87	13m	II	87-94	none
33.37.008	Former	95	89	20m	I	89-97	Syncope vasovagal
33.37.009	Former	96	89	13, 15m	II	89-94	none
33.37.024	-	94	88	7m	II	88-96	none
33.37.027	Former	92	85	9m	II	85-97	none
33.37.041	Former	93	88	15m	I	88-94	none
33.37.046	Former	95	83	15m	I	83-92	Facial flushing, SaO_2 decrease and cyanosis
33.37.047	-	95	89	2-3h	II	89-95	none
33.37.053	Former	94	89	20m	I,II	89-95	none
33.46.004	Former / Pneumonia 1990	93	88	15m	II	88-98	Dyspnea
33.46.023	Current / Pulmonary nodules	95	88	7, 9m	II	88-95	Musculoskeletal discomfort, headache, SaO_2 decrease, and ventricular extrasystoles
33.48.007	Former smoker	97	89	11, 13m	I	89-95	none
33.48.056	Current smoker	91	82	11m	II	82-94	none

[1] Post-dose range is any time after first AI-700 dose through Discharge.
Patient 33.37.047 had a history of moderate COPD (FEV1=61% post bronchodilator).

Moderate to Severe CHF/Moderate COPD Patients (Study AI-700-05)

To determine whether patients with more severe cardiopulmonary compromise had changes in pulmonary function, Study AI-700-05 study enrolled patients with moderate to severe CHF and patients with moderate COPD. All patients were treated with both AI-700 and placebo in this cross-over study. In CHF patients, mean SaO_2 following AI-700 dosing (2 doses of 0.04 mL/kg, both at rest) was >95% and changed <1% from Baseline at all timepoints. No CHF patient had a decrease in SaO_2 to <90% at any measured timepoint. No notable differences between AI-700 and placebo were seen with regard to mean SaO_2 levels over time in CHF patients. No CHF patient had a decrease in SaO_2 to <90% at any measured timepoint. In moderate COPD patients, the greatest difference between treatments in the mean SaO_2 change from Baseline was seen at 15 minutes and at 20 minutes following the first dose of test article (0.7% decrease for AI-700 versus 0.4% increase for placebo). In the AI-700 COPD cohort, 2 patients had SaO_2 values <90% (minimum values: 88% and 89%; Baseline values: 92% and 94%, respectively). Both patients were asymptomatic, but 1 patient had a concurrent decrease in FEV_1. For both patients, SaO_2 values decreased to <90% within the first 20 minutes post dose; and recovered to >90% thereafter.

Pulse Oximetry Conclusions

Changes in mean SaO_2 were small (1%) and comparable to mean changes seen following placebo treatment. This small decrease was observed even in patients with compromised pulmonary function (moderate to severe CHF patients and moderate COPD patients). Notable decreases in SaO_2 were recorded in individual patients following AI-700 dosing, even in the absence of the pharmacological stressor. The decreases in SaO_2 were usually asymptomatic, transient, and resolved without residual effects, with or without treatment. Oxygen was effective therapy when given. Decreases in SaO_2 were not likely to cause a discontinuation of dosing. The delayed timing of SaO_2 decreases, along with their brief and transient occurrence, does not support the hypothesis of a direct blockage of pulmonary vasculature by AI-700 microspheres. The overall impact on patient safety due to changes in levels of blood oxygenation appears to be minimal and manageable. Because the respiratory AEs commonly observed with AI-700 are also common for patients undergoing stress ECHO (e.g., dyspnea), treatment is readily available in the stress laboratory setting, and can be administered immediately following AE onset.

Nonetheless, based on nonclinical studies (elevated PAP in dogs with PH study) and on respiratory function results in moderate COPD patients, patients with compromised cardiopulmonary systems may be at higher risk of AEs after AI-700. Contraindications in the proposed package labeling include respiratory failure and severe obstructive lung disease such as severe COPD, severe emphysema, or pulmonary emboli may be at higher risk of AEs after AI-700. Patients with bronchoconstrictive or bronchospastic lung disease (e.g., asthma) are contraindicated (Adenoscan[28]) and warned (Lexiscan[72]) against stress diagnostic examinations using these agents; therefore these patients would

be also be contraindicated for AI-700 stress studies (see Appendix 11 for proposed product labeling).

5.13 Brief Neurologic Assessment

A brief neurological assessment (BNA) to assess fine and gross motor movement, strength, sensation, and cranial nerve function was performed in Studies AI-700-23, -32, and -33 at Baseline, at 30 minutes after the first AI-700 dose, at 30 minutes after the second AI-700 dose, and at Discharge. The majority of patients had normal BNA findings at all assessments. One of 911 patients (Patient 32.02.030) had treatment-emergent abnormal findings following dipyridamole administration during stress imaging. Abnormal findings were for fine and gross motor movement and strength (assessed by wiggling toes and squeezing fingers), and for sensation (repeat area being touched). Two CT scans and a brain MRI indicated no changes from the CT scan prior to AI-700 dosing. No neurologic deficit was identified. The SAE resolved without residual effects.

5.14 Concomitant Medications

All concomitant medications were recorded throughout all studies of AI-700, from Screening and Baseline through Follow-Up.

No drug-drug interaction studies of AI-700 have been conducted. The potential for drug-drug interaction between dipyridamole and aminophylline or theophylline used during AI-700 stress imaging has not been evaluated. There are no anticipated drug-drug interactions. AI-700 is not known to bind to any receptors or protein-binding sites.

5.15 Special Populations

The safety of AI-700 use in various demographic sub-populations (age, gender, race/ethnicity, and BMI) and in patients with histories of diabetes and of smoking was evaluated. Some variation in common AE rates were observed for certain populations, but these differences were generally of small magnitude and did not indicate that special preventative measures or mitigation strategies needed to be considered for any special population.

AI-700 has not been studied during pregnancy, during labor and delivery, in nursing mothers, or in a pediatric population.

5.16 Overdose

The consequences of an overdose with AI-700 are not known. Treatment of an overdose should be directed toward support of vital functions and prompt institution of symptomatic therapy.

5.17 Complement Activation

The clearance of particles from the bloodstream has been associated with complement activation. Complement activation following administration of AI-700 was assessed in 12 healthy volunteers (10 AI-700/2 placebo) enrolled in Study AI-700-04, using immunologic laboratory tests (C3a, C3). Increases in C3a were observed within 10 minutes of AI-700 dosing in all 10 subjects. C3a levels exceeded the normal range (<940 ng/ml) for 2 of these subjects; maximum values were 1034 and 1430 ng/ml. These changes were temporally related to a decrease in WBC count. Decreases in WBCs were driven by a decrease in neutrophils. Both C3a and neutrophil/WBC counts returned to Baseline values within 2 hours post dose. A compensatory rebound in neutrophils/total WBC counts above normal range followed at 2-8 hours post dose before levels normalized by Follow-up (72 +/- 48 hrs) (see Section 5.8.2.3).

Complement activation has been reported to be associated with the release of vasoactive substances that can cause systemic hypotension.[89] Although no decreases in blood pressure were observed in Study AI-700-04, decreases in blood pressure were observed in some Phase 3 patients after AI-700 administration but before dipyridamole (see Section 5.9.2.3).

A threshold level of C3a may be required before patients experience clinical symptoms. In asymptomatic patients undergoing dialysis, mean C3a level were twice the highest value reported with AI-700 (2907 ± 372 ng/mL vs 1430 ng/mL). In comparison, a mean level of 8533 ± 157 ng/mL was observed in patients with "first-use" syndrome, in which patients experience chest pain, dyspnea, and hypotension during exposure to new cuprophane-membrane dialyzers.[90]

The clearance of AI-700 microspheres from the bloodstream by RES macrophages is supported by findings in animal studies. Changes in neutrophils, oxygen saturation, and blood pressure in Phase 3 patients observed (especially at or around 10-15 minutes post AI-700 dose), may reflect the normal mechanism of clearance of AI-700 microspheres by the RES. These changes, though real, seldom manifest themselves clinically in humans. In some instances, they may present as AEs of flushing, hypoxia, and decreased blood pressure after the first dose of AI-700, when the clinical picture is not confounded by the much more prominent effects of dipyridamole and induced ischemia (when it occurs).

In order to investigate a possible link between complement activation and decreases in blood pressure, the Sponsor used neutrophil levels in the Phase 3 trials as a surrogate marker of complement. The Sponsor analyzed changes in neutrophils in all patients who met pre-defined outlier criteria for a decrease in SBP within 30 minutes of the first AI-700 dose (60 of 63 patients who met outlier criteria, Section 5.9.2.3). Mean neutrophil levels in the subset of patients who met outlier criteria were consistent with the overall study population and did not demonstrate a larger magnitude of change. These

data do not suggest a relationship between decreases in SBP and higher than expected complement activation.

Overall findings support the activation of complement following AI-700 administration which reflects the normal mechanism of AI-700 clearance from the bloodstream by the RES.

5.18 Safety Conclusions

AI-700 dipyridamole stress ECHO has a similar risk profile to other pharmacologic stress studies in patients undergoing a cardiac imaging procedure for CAD detection.[28-30] Although some short-lived symptomatic decreases in blood pressure and changes in oxygen saturation were occasionally observed prior to stress, these events were promptly managed by standard medical care with no apparent life-threatening risk to stable cardiac patients. Lack of overall life-threatening events and the reversible nature of AEs observed following administration of AI-700 indicate that AI-700 is tolerated in the clinical setting of controlled ischemia induced by dipyridamole stress.

6. BENEFITS AND RISKS

6.1 Introduction

CAD continues to be the single largest killer of Americans (and others in the western hemisphere), with almost 0.6 million deaths in the United States each year.[38] Optimal care of patients with suspected ischemic heart disease requires triage based on a comprehensive evaluation of cardiac anatomy and physiology. The recent emphasis on intervention and aggressive pharmacologic management has intensified the need to obtain accurate diagnostic estimation of both coronary anatomy and cardiac function (e.g., regional myocardial perfusion and wall motion). Angiography, the gold standard for determination of CAD, is costly, is associated with significant morbidity and mortality, and is therefore most appropriate for patient populations with high pretest probability of disease. Although ANGIO can depict epicardial arterial patency, it is an indirect technique for predicting regional perfusion and coronary flow reserve.[5, 21]

The non-invasive stress imaging procedures of SPECT (approximately 7.6 million per year in the US) and non-contrast ECHO (approximately 3 million per year in the US)[39] are the standards of care[31] for determining which patients are referred for ANGIO, and which patients are not. They therefore function as gatekeeping imaging modalities. SPECT relies primarily on myocardial perfusion information, and ECHO relies exclusively on functional information (regional and global wall motion). SPECT is considered the more sensitive procedure, because its direct measurement of perfusion can reveal CAD at an earlier stage. However, SPECT is expensive, time consuming, and exposes patients to ionizing radiation. ECHO imaging, which has significant positive clinical, logistical, and economic attributes, has been unable to directly assess perfusion and therefore had limited its clinical application. The greater utilization of SPECT compared with stress ECHO may be attributable to multiple factors: maturity of the technology, reimbursement for the procedure, and the ability of SPECT to evaluate perfusion directly.

AI-700 stress ECHO would allow cardiologists to obtain the clinical benefit of direct perfusion and wall motion information while retaining the cost, convenience, and availability advantages of ECHO without exposing patients to ionizing radiation.

6.2 AI-700 Benefits

AI-700 stress ECHO can serve as a gatekeeper for ANGIO. In addition, AI-700 will address the drawbacks of the current gatekeeping procedures (SPECT and non-contrast stress ECHO), and has the following benefits:

- Comparable values for accuracy, sensitivity, and specificity as SPECT
- Same reader trade-offs between sensitivity and specificity as SPECT (identical area under the curves for mROC);

- Increasing sensitivity for detecting CAD with increasing severity of disease:
- AI-700 ECHO is a significant and independent predictor of CAD, and provides added clinical value in the triaging of patients for ANGIO.

These benefits should provide clinical utility by giving clinicians an accurate test for risk stratification of chest pain patients, and by offering patients a fast, convenient, widely available, low-cost test - without exposure to ionizing radiation. Improvements in hardware and software for acquisition, analysis, and quantitative analysis of imaging data dramatically increased accuracy in the early years after introduction of SPECT and established the basis for its current diagnostic performance. With experience and refinement of the methods, the accuracy of AI-700 ECHO should increase over time. ,
Risks of Stress Imaging Techniques

SPECT (but not ECHO) has a delayed risk of fatal malignancy which is related to exposure to ionizing radiation. This risk is independent of the short-term procedural risk. The short-term procedural risk is similar for exercise stress ECHO and SPECT, and is due to the stress test itself. This risk is low for exercise stress,[52, 91] but many patients are unable to exercise adequately and require use of pharmacologic stressors (40% of SPECT studies)[92]. Approved pharmacologic SPECT stressors are adenosine, dipyridamole, and regadenoson.[28, 29, 72] Use of these agents carries significant increased risk[28, 29, 72] resulting primarily from induction of a controlled state of ischemia and activation of various adenosine receptors. However, pharmacologic stress also provides significant benefits, including detection of inducible ischemia in patients who could not exercise and would otherwise have to proceed directly to ANGIO. Because stress ECHO imaging allows real-time visualization of wall motion, if life-threatening ischemia develops, the clinician can immediately stop imaging and reverse the effects of the pharmacologic stressor. Other stress imaging techniques rely on ECG and clinical changes, but these appear later in the ischemic cascade.

The comparative risk of AEs associated with different stressors and/or imaging modalities as well as with AI-700 and dipyridamole are listed in Table 34. Similar types of AEs were observed with all pharmacological stress imaging procedures. The rate of AI-700 stress ECHO AEs compared favorably to that of non-contrast pharmacological stress ECHO. In particular, and most importantly, there does not seem to be any potentiation of chest pain associated with the addition of AI-700 to a dipyridamole stress test. Some differences in less clinically important AEs were observed, and include lower incidence of PVCs and palpitations following the addition of AI-700, and a higher rate of headache. The reason for this higher rate of headache is unknown, but headache was reported 10 times more frequently after dipyridamole than after the first dose of AI-700 (during rest).

There were no deaths or life-threatening SAEs in any of the 1,194 subjects who received AI-700. However, the risk of life-threatening events associated with dobutamine stress ECHO was estimated as 1 in 557 patients in an international study of 85,997 patients.[93] This is more than twice the risk associated with dipyridamole stress (1 in 1,294).[93] These results, along with data from Table 34 and Table 35 support that the overall safety profile of AI-700 stress ECHO with dipyridamole is comparable to the safety profile observed when adenosine agonist were used as stressors and appears safer than the standard current pharmacological stress ECHO procedure (dobutamine).

Table 34: The Effect of AI-700 ECHO and/or Pharmacological Stress on the Rate of Adverse Events in Patients being Evaluated for Inducible Ischemia

Adverse Event	AI-700+DIPY Treatment			Pharmacologic Stress Only Studies		
	1st dose to DIPY (n=911) n (%)	DIPY to 2nd dose (n=894) n (%)	Total (n=911) n (%)	IV DIPY Imaging[1] (n=3911) n (%)	Adenosine[2] (n=1421) n (%)	Dobutamine[3] (n=1012) n (%)
Chest pain[4]	7 (0.8)	110 (12.3)	182 (20.0)	770 (19.7)	568 (40.0)	309 (30.5)
Flushing	17 (1.9)	52 (5.8)	85 (9.3)	132 (3.4)	625 (44.0)	104 (10.3)
Headache NOS	27 (3.0)	167 (18.7)	317 (34.8)	476 (12.2)	256 (18.0)	138 (13.6)
Dizziness (exc Vertigo)	7 (0.8)	27 (3.0)	52 (5.7)	460 (11.8)	171 (12.0)	n/a
Dyspnea NOS	4 (0.4)	20 (2.2)	40 (4.4)	100 (2.6)	398 (28.0)	123 (12.2)
Nausea	5 (0.5)	34 (3.8)	95 (10.4)	180 (4.6)	185 (13.0)	81 (8.0)
Ventricular extrasystoles[4]	2 (0.2)	3 (0.3)	11 (1.2)	204 (5.2)	n/a	121 (12.0)
ECG abnormalities /ST-T changes[4]	0	3 (0.3)	4 (0.4)	292 (7.5)	n/a	n/a
Arrhythmia[4]	3 (0.3)	6 (0.7)	18 (2.0)	127 (3.2)[2]	14 (1.0)	71 (7.0)
AV Block[4]	1 (0.1)	3 (0.3)	11 (1.2)	4 (0.1)	89 (6.3)[3]	n/a

Table lists all AEs which occur in at least 5% of patients using any pharmacologic stressor.
n (%) = Number and percentage of subjects with at least one adverse event in the specific category.
DIPY = dipyridamole; n/a = not available

1. From dipyridamole package insert[29]; arrhythmia = patients who experienced tachycardia.
2. From Adenoscan package insert[28]; AV block = patients who experienced first-degree, second-degree, and third-degree AV block.
3. From published literature[30]
4. See Appendix 12 for an explanation of how AEs were tallied for AI-700 patients.

Life-threatening AEs observed in patients undergoing pharmacologic stress imaging with dobutamine or dipyridamole are presented by stressor in Table 35.

Table 35: Life-threatening Adverse Events Observed in Patients Undergoing Pharmacologic Stress Imaging by Stressor

	International Stress ECHO Registry	
Life threatening Adverse Events	**Dobutamine[1]** (N=35,103)	**Dipyridamole[1]** (N=24,599)
Acute MI	11	5
Ventricular tachycardia (sustained)	27	1
Ventricular fibrillation	11 (2 deaths)	2
Cardiac asystole	2	4
Cardiac rupture	5 (3 deaths)	0
Stroke/Transient ischemic attack	3	3
Hypotension/cardiogenic shock	2	4 (1 death)
3rd degree AV block	2	0
TOTAL Rate	**63 (0.18%)**	**19 (0.08%)**

[1] From published literature[93]

6.3 Safety of AI-700

Although AI-700 stress ECHO has diagnostic performance similar to SPECT without risks due to radiation exposure, it has been associated with dose-dependent increases in transient, non-serious, treatable AEs. The most clinically relevant of these are transient episodes of dyspnea and hypotension which have been observed prior to dipyridamole administration in patients with recent chest pain. Notably, AEs of dyspnea and hypotension were of shorter duration, milder intensity, associated with fewer concomitant AEs, and required treatment less frequently than the same AEs occurring after dipyridamole. These AEs appear to be self-limiting. The mean SBP decrease was <10 mmHg. The mean SaO_2 decrease was <1%. The nadir for both SBP decrease and SaO_2 decrease was approximately 5-15 minutes post AI-700 dose.

The cause of these non-serious AEs is unknown though it is speculated that the normal clearance of microspheres from the bloodstream may be responsible (Section 5.8.2.3). When AI-700 stress ECHO using dipyridamole is performed, rates of the most common AEs are similar to those of SPECT using dipyridamole (Table 34). Because these are also common AEs for patients undergoing stress ECHO, standard treatments exist for their management.

Standard treatments for decreases in SaO_2 and/or hypotension were effective in all patients who were treated. Standard treatments included oxygen for decreased SaO_2 and fluid/atropine for hypotension/vasovagal. In addition, aminophylline/theophylline derivative was effective in reversing dipyridamole -induced AEs. Because most AEs of dyspnea and/or hypotension had onset times within a few minutes of AI-700 administration, and all patients were in a hospital/clinical setting, management of these AEs was initiated promptly. As with other ultrasound contrast agents, the potential exists for acute anaphylactoid reactions,[35] such as hypotension and dyspnea. Standard treatment for these reactions (IV fluids, steroids, bronchodilators, and antihistamines) should be readily available for patients in a cardiovascular care setting.[36, 37]

Across all AI-700 clinical trials, the risk of an SAE was <1%. The types of SAEs and the temporal relationship of most SAEs to AI-700 do not suggest an underlying mechanism for SAEs. In addition, the lack of mortality or occurrence of any life-threatening SAE suggests that AI-700 is tolerated in the intended patient population (stable patients with recent chest pain who can undergo a dipyridamole stress test). Although these patients have history of chest pain and have more CAD risk factors than exercise stress ECHO patients, they are at lower risk for CAD than patients with acute myocardial infarction or patients with unstable angina.[31] These higher risk patients have not been studied in AI-700 trials because dipyridamole stress imaging is contraindicated for such patients.[32] Additionally, dipyridamole stress imaging is also contraindicated for patients with compromised pulmonary function (wheezing asthmatics).[32] These patients may also be at added risk of having a pulmonary AE following administration of AI-700.

In Phase 3 clinical trials, the AI-700 dose was fixed and was higher than that required to achieve adequate MCE for many patients, even with the extensive imaging required by the protocol. In clinical practice, there will be fewer mandated image acquisitions and imaging will be tailored to each patient, thus decreasing the time of imaging and amount of AI-700 needed. Patients will likely be dosed to effect, which should result in all patients receiving an individualized minimally efficacious dose.

6.4 Conclusions

The diagnostic performance of AI-700 stress ECHO in stable patients with recent chest pain has been demonstrated to produce results that are clinically equivalent to those of quantitative stress ^{99m}Tc SPECT myocardial perfusion imaging. Furthermore, the safety profile of AI-700 ECHO plus pharmacologic stress appears to be comparable with pharmacological stress imaging using SPECT and with non-contrast ECHO. The potential to conveniently obtain both real-time perfusion and wall motion information without exposure to ionizing radiation is an advantage uniquely offered by AI-700 ECHO. These findings, coupled with data indicating that AI-700 stress ECHO provides added diagnostic value over the combination of clinical parameters and SPECT, support the conclusion that AI-700 ECHO has a favorable risk-benefit profile and will be an

effective clinical tool in the triage of patients for the more invasive procedure of ANGIO.

6.5 Phase 4

Draft Risk Management Plan

To continue understanding the AI-700 clinical risk benefit profile, a risk management plan is proposed post-launch of AI-700. This plan has not yet been agreed upon with FDA, although discussions regarding the value of these proposals have been initiated.

Safety Surveillance

The morbidity and mortality of CAD is high, and this justifies some inherent risk in stress testing. Thus, the safety profile of AI-700 in the clinical setting must be considered in the context of the risk-benefit analysis. The safety of AI-700 following rest and or rest stress imaging has been demonstrated in controlled clinical trials that have enrolled over 1000 subjects, many of whom were patients with significant CAD and other co-morbidities. Although no patients administered AI-700 died or had a life threatening SAE, the potential for low-frequency adverse events may become evident only following exposure of a larger population, and outside of the scope of a controlled clinical study.

Based upon years of experience with the currently approved cardiac ultrasound contrast agents, FDA has recently requested post-marking safety surveillance studies of Optison and Definity to evaluate the potential for acute, contrast agent-induced, serious cardiopulmonary events. A post-marketing safety surveillance study to determine the frequency of SAEs (including short term mortality and morbidity) in patients receiving Imagify in routine clinical use could provide important insight into the rate of SAEs in clinical practice. A formal study is required because post-marketing adverse reactions are reported voluntarily from a population of uncertain size, such that it is not possible to reliably estimate the frequency of these reactions or establish a causal relationship to drug exposure. The Sponsor therefore proposes conducting a Phase 4 open-label, observational study in chronic stable chest pain patients with 2 cohorts run in parallel. Cohort 1 would include patients undergoing an AI-700 stress imaging study and Cohort 2 would include patients undergoing a non-AI-700 stress imaging study.

Primary endpoints would include death or life-threatening cardiopulmonary and/or cerebrovascular events (MI, sustained ventricular tachycardia or fibrillation, stroke/TIA, hypotensive shock, 3 degree AV block) occurring within 24 hours of stress testing. The relative event rates will be adjusted for certain covariates including age, gender, and comorbidity, including presence of coronary disease. The cohort sample size will depend on the incident rate of important SAEs in the comparator arm and the margin of difference being evaluated. The sample size estimate could be based upon the control arm by an independent Data Monitoring Board (DMB). An independent Events Committee would also be responsible for adjudication of all primary endpoints.

AI-700 ECHO Proficiency Training Plan

Based on the experience gained during the Phase 3 clinical trials program, appropriate healthcare provider training is a critical component of the marketing program for AI-700. Cardiologists, cardiac sonographers, and nurses will be provided with comprehensive training on all aspects of AI-700 use. AI-700 distribution will be controlled by the Sponsor such that training will be required for end users with proficiency demonstrated prior to commercial product shipment. Perceptorship programs will be followed by subsequent training using self-teaching tools, case studies, and in-service tutorials.

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APPENDIX 1: PARAMETERS USED TO EVALUATE DIAGNOSTIC PERFORMANCE

Efficacy parameters included operational diagnostic test parameters: accuracy, sensitivity, and specificity. A 95% confidence interval was calculated for all efficacy parameters. Operational diagnostic test parameters are illustrated in Appendix Table 1. Test-to-truth concordance is described by numbers of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). **Accuracy** is the probability of patients correctly diagnosed [(TP+TN)/(TP+TN+FP+FN)]. **Sensitivity** is the probability of patients with disease correctly diagnosed [TP/(TP+FN)]. **Specificity** is the probability of patients without disease correctly diagnosed [TN/(TN+FP)].

Appendix Table 1: Operational Diagnostic Test Parameters

		DIAGNOSTIC TEST Classification	
		Disease	No Disease
TRUTH STANDARD Classification	Disease	TP	FN
	No Disease	FP	TN

TP=true positive, TN=true negative, FP=false positive, FN=false negative

APPENDIX 2: PROSPECTIVE PRIMARY EFFICACY ANALYSIS AND NON-INFERIORITY ANALYSIS

For both pivotal efficacy studies hypothesis testing for the primary efficacy analysis was prospectively defined prior to database lock in the statistical analysis plans. Statistical analysis plans were finalized and submitted to the FDA prior to unblinding of any efficacy data on May 15, 2006 and April 13, 2007 for AI-700-32 and AI-700-33, respectively. Criteria for success and hypotheses tested were identical for both studies. Accuracy was the principle primary efficacy endpoint. A two-step approach was applied to test non-inferiority. If the overall primary efficacy endpoint, accuracy, was met, the second step of the non-inferiority analyses (non-inferiority analyses of specificity and sensitivity) was conducted to evaluate these 2 components of the accuracy endpoint.

Excerpts from the SAPs describing these methods are included. An appendix describing the computational details appeared in both SAPs and is also included here.

1. Excerpt from AI-700-32 Statistical Analysis Plan-Primary Analysis

Non-Inferiority Tests

To evaluate efficacy, sequential non-inferiority tests between ECHO and SPECT will be conducted and will include comparison of accuracy, followed by sensitivity and specificity.

Accuracy will be assessed on a patient-by-patient basis for the MITT Population. Accuracy of AI-700 will be compared to accuracy of SPECT, with accuracy being determined by the truth standard.

- Null hypothesis:
  - ECHO is inferior to SPECT for accuracy of CAD detection (for at least 2 of 3 ECHO readers)
- Alternative hypothesis:
  - ECHO is not inferior to SPECT for accuracy of CAD detection (must be achieved for at least 2 of 3 ECHO readers)

If the lower bound of the 2-sided 95% CI for relative risk ratio is > 0.83 for at least 2 of the 3 AI-700 ECHO Blinded Readers, then the primary efficacy endpoint of accuracy will have been met. (See Appendix A for computational details of relative risk ratio.)

If the null hypothesis criteria are met for accuracy, a second set of primary efficacy analyses will be the sensitivity and specificity on a patient-by-patient basis, for the MITT Population. Sensitivity (specificity) of AI-700 will be compared to sensitivity (specificity) of SPECT versus truth in a non-inferiority analysis[94] based on the relative risk ratio of ECHO to SPECT.

- Null hypothesis:
  - ECHO is inferior to SPECT for sensitivity of CAD detection (for at least 2 of 3 ECHO readers)

OR

  - ECHO is inferior to SPECT for specificity of CAD detection (for at least 2 of 3 ECHO readers)
- Alternative hypothesis:
  - ECHO is not inferior to SPECT for sensitivity of CAD detection (must be achieved for at least 2 of 3 ECHO readers)

AND

  - ECHO is not inferior to SPECT for specificity of CAD detection (must be achieved for at least 2 of 3 ECHO readers)

For both sensitivity and specificity, the primary efficacy endpoint will have been met if ECHO meets the MCS and the lower bound of the 2-sided 95% CI for relative risk ratio is > 0.83, for at least 2 of the 3 AI-700 ECHO Blinded Readers.

Confidence intervals around the relative risk ratio of ECHO to SPECT will be calculated according to the method described in section 4.

For accuracy, sensitivity, and specificity, if the lower bound of the relative risk ratio 95% CI is greater than 0.83 for 2 of 3 AI-700 ECHO blinded readers, then the primary efficacy endpoint of sensitivity (specificity) will have been met.

For accuracy, sensitivity, and specificity, if the lower bound of the two-sided 95% CI for the ratio of ECHO to SPECT is greater than 1, then ECHO could be assumed to be superior to SPECT.

2. Excerpt Number 1 from AI-700-33 Statistical Analysis Plan

Efficacy Analysis

Non-inferiority analysis: Evaluable ANGIO/LVG will serve as the truth standard for judging the ability of AI-700 contrast ECHO to detect CAD, with SPECT imaging as an independent comparator. In the absence of evaluable ANGIO data, the alternative truth standard will be used. This truth algorithm will also be applied to patients who have a history of CABG.

Diagnostic test parameters will include accuracy, sensitivity, specificity, positive predictive value, and negative predictive value,[95] calculated using the values represented schematically in Table 1.

Primary Efficacy Analyses

Non-Inferiority Tests: To evaluate efficacy, sequential non-inferiority tests between ECHO and SPECT will be conducted. They will include a comparison of accuracy, followed by sensitivity and specificity.

Accuracy will be considered the principle primary efficacy endpoint because accuracy, unlike sensitivity and specificity, is calculated using the entire study population (i.e., both diseased and non-diseased patients). In the context of a study like AI-700-33, accuracy is the most objective of the diagnostic parameters because reader bias (the reader's tendency to over-call or under-call disease) cannot increase accuracy but can alter sensitivity and specificity.

A two-step approach will be applied to testing non-inferiority. If the overall primary efficacy endpoint, accuracy, meets the non-inferiority criterion, the second step of the non-inferiority analyses (non-inferiority analyses of specificity and sensitivity) will be conducted to evaluate these 2 components of the accuracy endpoint. If the accuracy endpoint does not meet the non-inferiority criterion, then the second step (non-inferiority analyses of sensitivity and specificity) will not be conducted.

Accuracy will be assessed on a patient-by-patient basis for the MITT Population. The ratio of the accuracy of each AI-700 ECHO Reader to the accuracy of the median SPECT Reader will be calculated.

- Null hypothesis:
  - ECHO is inferior to SPECT for accuracy of CAD detection (for at least 2 of 3 ECHO readers)
- Alternative hypothesis:
  - ECHO is not inferior to SPECT for accuracy of CAD detection (must be achieved for at least 2 of 3 ECHO readers)

Confidence intervals around the relative risk ratio of ECHO to SPECT will be calculated according to the method described in section 4. If the lower bound of the 2-sided 95% CI for relative risk ratio is >0.83 for at least 2 of the 3 AI-700 ECHO Blinded Readers, then the primary efficacy endpoint of accuracy will have been met. (See section 4 for computational details of relative risk ratio.)

If the null hypothesis is rejected for accuracy, a second set of additional primary efficacy analyses will be the sensitivity and specificity on a patient-by-patient basis for the MITT Population. Sensitivity (specificity) of each AI-700 ECHO Reader for the detection of CAD defined by the truth standard will be compared with sensitivity (specificity) of the median SPECT Reader versus truth in a non-inferiority analysis[94] based on the relative risk ratio of ECHO to SPECT.

- Null hypothesis:
  - ECHO is inferior to SPECT for sensitivity (specificity) of CAD detection (for at least 2 of 3 ECHO readers)

- Alternative hypothesis:
    - ECHO is not inferior to SPECT for sensitivity (specificity) of CAD detection (must be achieved for at least 2 of 3 ECHO readers)

For both sensitivity and specificity, the additional primary efficacy endpoints will have been met if the lower bound of the 2-sided 95% CI for relative risk ratio is >0.83 for at least 2 of the 3 AI-700 ECHO Blinded Readers.

For accuracy, sensitivity, and specificity, if the lower bound of the relative risk ratio 95% CI is greater than 0.83 for 2 of 3 AI-700 ECHO blinded readers, then the overall primary efficacy endpoint of accuracy and the additional primary efficacy endpoints of sensitivity and specificity will have been met.

For accuracy, sensitivity, and specificity, a lower bound of the two-sided 95% CI for the ratio of ECHO to SPECT above one will provide statistically significant evidence that ECHO is superior to SPECT.

3. Exerpt Number 2 from AI-700-33 Statistical Analysis Plan

Justification of the Relative Risk Non-Inferiority Analysis and Margins
Acusphere will use a relative risk ratio approach to assess non-inferiority because of its advantages over an absolute difference in the context of this study:

1. As the sensitivity and specificity values decrease, the allowable absolute difference also decreases, thus lowering the potential for ECHO to be declared non-inferior to SPECT.
2. Conversely, as the sensitivity and specificity values increase, the allowable absolute difference also increases, which is acceptable because the point estimates for ECHO would, by default, need to remain clinically relevant values.
3. Primarily owing to referral bias (patients positive for disease using SPECT are referred for ANGIO; patients negative for disease using SPECT are not), the use of SPECT leads to overestimation of sensitivity and underestimation of specificity (i.e., errors of estimation). A relative risk ratio approach compensates for errors of estimation by the use of SPECT as a comparative standard because the relative difference between ECHO and SPECT values that would still result in non-inferiority changes as the magnitude of the values change. A non-inferiority margin defined by a ratio translates into a larger absolute difference when values are higher.

The selection of margins for the non-inferiority analyses was based primarily on clinical grounds. The primary focus was to determine what level of SPECT performance would be considered clinically similar to that of AI-700 ECHO when diagnosing CAD. The resulting margin using the relative risk ratio approach is 0.83 for accuracy, sensitivity, and specificity.

The minimum ECHO values allowed before ECHO is considered non-inferior to SPECT are presented in Appendix Table 2. These numbers were derived assuming a sample size of 126 diseased patients or 142 non-diseased patients, with a one-sided p-value of <0.025 and a nuisance parameter assuming independence between ECHO and SPECT.
When the diagnostic performance of SPECT is low, ECHO values for both sensitivity and specificity must be very similar to and often higher than those of SPECT to conclude non-inferiority using a relative risk approach with a margin of 0.83.. For example, when the SPECT point estimates for sensitivity is below 65%, the ECHO point estimates of sensitivity must be **higher** than SPECT in order for ECHO to be non-inferior to SPECT. Appendix Table 2 shows that for both sensitivity and specificity ranging from 50-95% ECHO would only be considered non-inferior to SPECT if the observed relative risk ratio is >0.90.

Appendix Table 2: Minimum Non-inferiority AI-700 ECHO Point Estimates as Compared to SPECT

	Sensitivity (Margin=0.83)			*Specificity (Margin=0.83)*		
SPECT Value (%)	Minimum ECHO* Value (%)	RR	p-value	Minimum ECHO* Value (%)	RR	p-value
95	86.5	0.91	0.0242	86.1	0.91	0.0237
90	83.1	0.92	0.0250	82.7	0.92	0.0239
85	79.8	0.94	0.0240	79.3	0.93	0.0238
80	76.2	0.95	0.0241	75.6	0.95	0.0248
75	72.6	0.97	0.0243	72.0	0.96	0.0244
70	68.8	0.98	0.0245	68.2	0.97	0.0242
65	65.0	1.00	0.0245	64.4	0.99	0.0242
60	61.8	1.02	0.0248	60.4	1.01	0.0243
55	57.1	1.04	0.0242	56.4	1.03	0.0246
50	52.9	1.06	0.0248	52.3	1.05	0.0241

RR=relative risk ratio
* No value of ECHO below this value is considered non-inferior to SPECT
Note: Table is derived from a simulated non-inferiority analysis using a relative risk ratio approach[6] assuming a sample size of 126 true positive or 142 true negative patients with a one-sided p-value of <0.025 and a nuisance parameter assuming independence between ECHO and SPECT.

The selection of a non-inferiority margin for the AI-700-33 study is based primarily on maintaining consistency with the completed Phase 3 study (AI-700-32).

4. The following appeared as an appendix in both AI-700-32 and -33 Statistical Analysis Plans

Computational Details for a Non-Inferiority Analysis Using the Relative Risk Ratio

The results of a study in which 2 sets of dichotomous responses are measured on each patient can be displayed in a 2-by-2 table in which one response is shown across the columns and the other is shown down the rows. For patients with CAD, the outcome probabilities are classified in Appendix Table 3.

Appendix Table 3: Patients with Coronary Artery Disease

ECHO	SPECT Yes	SPECT No	Total
Yes	p_{11}	p_{10}	P_T
No	p_{01}	p_{00}	$1-P_T$
Total	P_S	$1-P_S$	1

'Yes' indicates agreement of ANGIO to ECHO and SPECT, respectively. For example, for the true positives, p_{11} represents the proportion of both ECHO and SPECT cases which test positive, P_T represents the sensitivity of ECHO relative to ANGIO, and P_S represents the sensitivity of SPECT relative to ANGIO. The agreement of ECHO and SPECT can be determined then, from the resulting 2-by-2 table. A table constructed for the true negatives would present specificities on the margins.

The (sensitivity/specificity) hypotheses are given by:

H_0: PT / PS ≤ RE versus H1: PT / PS > RE,

where R_E corresponds to the non-inferiority parameter (also called δ).

Testing

According to Nam and Blackwelder (2002) [1], the test statistic for the asymptotic test based on the constrained maximum likelihood for large n is given by:

$$Z(R_E) = \frac{\sqrt{n}(\hat{P}_T - R_E \hat{P}_S)}{\sqrt{R_E(\tilde{p}_{10} + \tilde{p}_{01})}},$$

where

$$\tilde{p}_{10} = \frac{-\hat{P}_T + R_E^{\,2}(\hat{P}_S + 2\hat{p}_{10}) + \sqrt{(\hat{P}_T - R_E^{\,2}\hat{P}_S)^2 + 4R_E^{\,2}\hat{p}_{10}\hat{p}_{01}}}{2R_E(R_E+1)}$$

$$\tilde{p}_{01} = R_E\tilde{p}_{10} - (R_E - 1)(1 - \hat{p}_{00})$$

$$\hat{p}_{01} = \frac{n_{01}}{n}, \hat{p}_{10} = \frac{n_{10}}{n}, \hat{P}_T = \frac{n_{10} + n_{11}}{n}, \hat{P}_S = \frac{n_{01} + n_{11}}{n}$$

If $Z(R_E)>1.96$ (1-sided $\alpha=0.025$) then the null hypothesis is rejected, and we conclude that ECHO is non-inferior to SPECT.

The lower bound (R_L) of the 100(1-α)% CI for the ratio must be solved iteratively from the following:

$$-Z_{1-\alpha/2} = \frac{\sqrt{n}(\hat{P}_T - R_L \hat{P}_S)}{\sqrt{R_L(\tilde{p}_{10} + \tilde{p}_{01})}}.$$

If this value of the lower bound is greater than R_E, then ECHO is considered non-inferior to SPECT.

The upper bound can be computed similarly:

$$Z_{1-\alpha/2} = \frac{\sqrt{n}(\hat{P}_T - R_U \hat{P}_S)}{\sqrt{R_U(\tilde{p}_{10} + \tilde{p}_{01})}}.$$

Power/Sample Size

The 2-by-2 table above includes 4 parameters (p_{11}, p_{10}, p_{01}, and p_{00}), but the power and sample size calculations only require 2 parameters, P_S and R_A, (the ratio of ECHO to SPECT under the alternative). A third parameter is defined implicitly since the sum of the 4 parameters is one. The fourth, or nuisance parameter, remains unaccounted for. This parameter must be addressed to fully specify the problem. The ratio p_{11}/P_S was used for sample size calculations. (Any one of the individual cell parameters could have been chosen as well.)

Sample size calculations were obtained using the PASS software package [2]. The power when the true value of the relative risk ratio is R_E can be evaluated exactly using the multinomial distribution. The PASS software uses a normal approximation to the multinomial as follows:

$$\beta(R_A) = \Phi(c_U)$$

where

$$c_U = \frac{z_{1-\alpha}\sqrt{\bar{V}_0(T_0)} - E_1(T_0)}{\sqrt{V_1(T_0)}}$$

$$\bar{V}_0(T_0) = \frac{R_E(\bar{p}_{10} + \bar{p}_{01})}{n}$$

$$E_1(T_0) = (R_A - R_E)P_S$$

$$V_1(T_0) = \frac{(R_A + R_E{}^2)P_S - 2R_E p_{11} - (R_A - R_E)^2 P_S{}^2}{n}$$

$$\overline{p}_{10} = \frac{-P_T + R_E{}^2(P_S + 2p_{10}) + \sqrt{(P_T - R_E{}^2 P_S)^2 + 4R_E{}^2 p_{10} p_{01}}}{2R_E(R_E + 1)}$$

$$\overline{p}_{01} = R_E \overline{p}_{10} - (R_E - 1)(1 - p_{00})$$

Then the approximate sample size is given by:

$$n = \{z_{1-\alpha}\overline{v}_0{}^{\frac{1}{2}} + z_{1-\beta}v_1{}^{\frac{1}{2}}\}^2 / \{(R_A - R_E)P_S\}^2$$

where

$$\overline{v}_0 = R_E(\overline{p}_{10} + \overline{p}_{01}), v_1 = (R_A + R_E{}^2)P_S - 2R_E p_{11} - (R_A - R_E)^2 P_S{}^2 .$$

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APPENDIX 3: NONCLINICAL STUDIES

An overview of nonclinical safety pharmacology and toxicity studies conducted with AI-700 is presented in Appendix Table 4.

Appendix Table 4: Overview of Nonclinical Safety Pharmacology and Toxicity Studies Conducted with AI-700

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Safety Pharmacology					
Cardiopulmonary safety (pilot study)	Intravenous	Yorkshire swine	Single doses: 0.004, 0.01, 0.02, 0.04, 0.08; 0.05X, 0.13X, 0.25X, 0.5X, 1X Two doses separated by 1 hour: Total dose: 0.08 mL; 1X	Single doses: At 0.05X: no significant findings. At 0.13X, 0.25X, 0.5X, and 1X: transient self-limiting increases in pulmonary artery pressure; acute decreases in systemic blood pressure and compensatory heart rate increases in some animals. Two doses separated by 1 hour: At 1X: transient self-limiting increases in pulmonary artery pressure; acute decreases in systemic blood pressure and compensatory heart rate increases in some animals.	Charles River Laboratories BAA00018
Cardiopulmonary safety	Intravenous	Yorkshire swine	Two doses separated by 1 hour Total dose: 0.008, 0.04, 0.08; 0.1X, 0.5X, 1X	All doses: dose dependent transient self-limiting increases in pulmonary artery pressure; acute decreases in systemic blood pressure and compensatory heart rate increases in some animals not correlated to dose.	Charles River Laboratories BAA00016
Cardiovascular safety	Intravenous	Rhesus monkeys (phase 1) Cynomolgus monkeys (phase 2)	0, 0.11, 0.54, 1.1; 0X, 1.4X, 6.8X, 14X	At 1.4X and 6.8X: no significant findings. At 14X: slight, transient decrease in BP; AI-700 was well tolerated in anesthetized and conscious monkeys.	Primedica BAAW-100

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Cardiopulmonary safety with high MI imaging	Intravenous	Rhesus monkeys	0.16; 2X	PVCs were observed when the mechanical index was ≥ 1.2. . PVCs were resolved upon removal of the ultrasound probe No other AI-700-related changes were observed. No histology changes including viability staining. No hemodynamic changes.	Charles River Laboratories BAAW-107
Cardiopulmonary safety with high MI imaging and DIPY stress	Intravenous	Cynomolgus monkeys	0, 0.08, 0.40; 0X, 1X; 5X	AI-700 in combination with moderate to high MI, with and without DIPY stress, had no significant effect on the tissue structure or physiological function of the cardiovascular and pulmonary systems, including ECG parameters such as QTc.	Acusphere 2700-122
Cardiopulmonary safety in pulmonary hypertension model	Intravenous	Dog	0, 0.10-0.11, 0.20-0.23; 0X, 1.3-1.4X, 2.5-2.9X	A dose-dependent effect of AI-700 at 1.3X and 2.5X on cardiopulmonary function was observed when administered in this model of Sephadex™-induced acute pulmonary hypertension.	Acusphere 2700-123

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Acute Toxicity Studies					
Single-dose (with 24-hour observation period)	Intravenous	Sprague-Dawley rats	0, 0.29, 1.71, 2.86; 0X, 3.6X, 21X, 36X	At 36X: Mortality (5/5M, 2/5F) was observed, and the cause of death was respiratory arrest. Histological findings were confined to spheroid bodies in the small pulmonary capillaries of the alveolar septa and at the injection site. AI-700-related changes in animals 48 post-dose included spheroid bodies located primarily in the lungs and injection site and, to a far lesser degree, the livers and spleens of these animals. Spheroid bodies were most remarkable in the lungs and were generally present in multinucleated giant cells located at the alveolar septa of these rats. This microscopic finding was associated with an increase in mean lung weight (absolute and relative) in the 21X males. A NOEL was not established. A NOAEL of 3.6X was established.	Covance 6694-112
Single-dose (with 14-day observation period)	Intravenous	Sprague-Dawley rats	0, 0.29, 1.71, 2.86; 0X, 3.6X, 21X, 36X	At 36X: Mortality (2/5M, 3/5F); ataxia; recumbency; labored breathing; tonic/clonic convulsions (F), immediately after dosing. Histological findings were confined to spheroid bodies in the capillaries and small vessels of the lung, the kidney and at the injection site in animals that died on study. Cause of death was respiratory insufficiency. Spheroid bodies were not found in any surviving animals at termination. Single administration of AI-700 at dose levels of 3.6X and 21X did not produce any treatment-related effects. A NOEL and a NOAEL of 21X were established	Covance 6694-107

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Single-dose (with 14-day observation period)	Intravenous	Cynomolgus monkeys	0, 0.26, 1.98, 5.27; 0, 3.3X, 25X, 66X	At 25X and 66X histological findings were confined to spheroid bodies within the macrophages of the spleen and at the injection site. Accumulation of test material at the injection site may be due to vascular leakage after IV injection or residual material from procedure. No mortality and no changes in body weight or clinical pathology were observed at any dose. A NOEL of 3.3X was established. A NOAEL of 66X was established.	Covance 6694-105
Repeat-dose Toxicity Studies					
2-week repeat-dose with 28-day recovery	Intravenous	Sprague-Dawley rats	0, 0.29, 0.86, 1.71; 0X, 3.6X, 11X, 21X	Terminal sacrifice: At 21X/day: Mortality (3/9 M); labored respiration and/or hypoactivity (M), immediately after dosing; decreased body weight gain (M); cause of death was respiratory insufficiency. At ≤ 21X/day, histological findings were spheroid bodies within the macrophages of the lung, spleen, kidney, liver and at injection site. At ≥11X/day: increased relative and absolute spleen, lung and liver weights. A NOEL was not established. Recovery sacrifice: After 28-day recovery, macrophage/syncytial cells containing spheroid bodies were still present, though to a lesser degree at the injection site. A NOAEL of 11X/day was established.	Covance 6694-108

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
2-week repeat-dose with 28-day recovery	Intravenous	Cynomolgus monkeys	0, 0.26, 0.79, 1.59 mL/kg; 0X, 3.3X, 10X, 20X	Terminal sacrifice: At 20X/day: increased absolute and relative lung weights in females, which correlated with histomorphological findings. At ≤ 20X/day: histological findings were spheroid bodies in the macrophages of the lung, spleen, kidney, liver, and at the injection site (3.3X/day and 20X/day only) in both sexes. A NOEL not established. Recovery sacrifice: NOAEL of 20X/day based on the reversibility of organ weight alterations and all histomorphological changes with the exception of spheroid bodies present in the macrophages at the injection site in a single male.	Covance 6694-106
Genotoxicity Studies					
Mutagenicity in *Salmonella species and Escherichia coli*	*In vitro*	Salmonella typhimurium TA98, TA100, TA1535, TA1537, E. coli WP2uvrA	Dose Range Finding: 0.000238, 0.000357, 0.00119, 0.00238, 0.0119, 0.0238, 0.0357, 0.119, 0.179 mL/mL Mutagenicity Assay: 0, 0.000357, 0.00119, 0.00357, 0.0119, 0.0357, 0.179 mL/mL	AI-700 was not mutagenic at up to 0.179 mL/mL, to four *S. typhimurium* strains (TA98, TA100, TA1535, TA1537) and *E. coli* (WP2*uvr*A) when tested with or without rat liver S-9 metabolic activation.	Covance 19499-0-422OECD

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Chromosomal aberration in CHO cells	*In vitro*	CHO cells	Initial Assay: 0, 0.00488, 0.00975, 0.0195, 0.0391, 0.0781, 0.156 mL/mL Confirmation Assay: 0, 0.000286, 0.000714, 0.00286, 0.00714, 0.0286, 0.0714, 0.143 mL/mL	AI-700 was not mutagenic when evaluated in CHO cells with or without mammalian liver microsomal S-9 activation at concentrations up to 0.143 mL/mL.	Covance 19499-0-437OECD
Mouse micronucleus	Intravenous	Mouse micronucleus assay	Dose Ranging Study: 0.63, 1.25, 2.5, 5.0, 6.5, 7.5, 10.0 mL/kg; 8X, 16X, 31X, 63X, 81X, 94X, 125X Micronucleus Assay: (M): 0, 1.25, 2.5, 5.0 mL/kg; 0X, 16X, 31X, 63X (F): 0, 1.63, 3.25, 6.5 mL/kg; 0X, 20X, 41X, 81X	AI-700 induced clinical signs of toxicity at the high doses but was not cytotoxic to the bone marrow. AI-700 was not mutagenic when evaluated in the mouse micronucleus assay at doses of up to 63X for males and 81X for females.	Covance 19499-0-455OECD

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Reproductive and Development Toxicity Studies					
Fertility	Intravenous (daily: males 28 days prior to placement for mating, during mating and until necropsy; females 14 days prior to placement for mating during mating and up to day 7 of gestation)	Sprague-Dawley rats	0, 0.12-0.13, 0.36-0.38, 0.71-0.75; 0X, 1.5-1.6X, 4.5-4.8X, 8.9-9.4X	Paternal toxicity observed at 9X/day based on body weight and mortality. No effects upon male or female reproduction at any dose level.	CTBR-96796
Range-finding embryo-fetal development	Intravenous (gestation days 6-17)	Sprague-Dawley rats	0, 0.29, 0.88, 1.76; 0X, 3.6X, 11X, 22X	Maternal mortality was observed at 11X/day and 22X/day. Evaluation of developmental toxicity in the 22X/day group was not performed. No developmental toxicity was noted at 3.6X/day and 11X/day.	WIL-365001
Embryo-fetal development	Intravenous (gestation days 6-17)	Sprague-Dawley rats	0, 0.11, 0.34, 0.68; 0X, 1.4X, 4.3X, 8.5X	All doses: No maternal toxicity, no evidence of embryolethality, teratogenicity or fetotoxicity.	CTBR-96784
Range-finding embryo-fetal development	Intravenous (gestation days 7-19)	New Zealand White rabbits	0, 0.03, 0.14, 0.28, 0.42; 0X, 0.38X, 1.8X, 3.5X, 5.3X	5.3X/day: Maternal mortality and increased respiration rate. 3.5X/day: Increased maternal respiration rate. No evidence of fetotoxicity, embryolethality or teratogenicity at any dose.	CTBR-96785

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Range-finding embryo-fetal development	Intravenous (gestation days 7-20)	New Zealand White rabbits	0, 0.13, 0.38, 0.51, 0.64, 0.77; 0X, 1.6X, 4.8X 6.4X, 8X, 9.6X	Maternal mortality at 4.8X/day, 6.4X/day, 8X/day, and 9.6X/day. No maternal toxicity was observed at 1.6X/day. Evaluation of developmental toxicity was not possible due to the limited number of litters available for evaluation.	WIL-365003
Embryo-fetal development	Intravenous (gestation days 7-19)	New Zealand White rabbits	0, 0.07, 0.22-0.23, 0.33-0.34; 0X, 0.9X, 2.8-2.9X, 4.1-4.3X	No maternal toxicity and no evidence of embryolethality, teratogencity or retotoxicity at any dose.	CTBR-96795
Local Tolerance					
Local tolerance (injection site)	Intravenous and Perivascular	New Zealand White rabbit (ear)	0, 1.0 mL IV 0, 0.5 mL PV	All animals survived. <u>IV Injection:</u> No irritation at injection site in any animals. <u>Perivascular Injection</u>: 6/6 animals had erythema, irritation, inflammation and histological evidence of tissue injury at 24 hours post AI-700 dose with regression in progress but not complete by 96 hours post-dose.	Huntingdon 98-1838

Study Type and Duration	Route of Administration	Species and Strain	Doses (mL/kg; X HD)	Major Findings	Study Number
Other Studies					
Antigenicity	Intraperitoneal/ Intravenous/ Intradermal (For ASA, IP AI-700 was used for induction phase and IV AI-700 was for the challenge phase. The intradermal route was used for the PCA).	Guinea pigs	0, 1.0 mL/kg; 0X, 13X	The results of this study indicate that AI-700 induced mild reactions (retching and/or pawing at the nose or face) consistent with active systemic anaphylaxis in 4 of the 10 animals in the group treated with AI-700 throughout the ASA phase. During the passive cutaneous anaphylaxis phase of the study, there were no reactions indicative of passive cutaneous anaphylaxis to the serum from these animals.	Covance 6694-110
Single-dose toxicity (reconstitution procedures)	Intravenous	Sprague-Dawley rats	1.5 to 1.7 mL/kg; 19-21X	Slight hypoactivity and labored or rapid respiration were noted with all reconstitution procedures in some animals immediately after dosing. Two animals died immediately after dosing. Incidences of ataxia and recumbency were also noted. Venting during reconstitution minimized the incidence and duration of the observed clinical signs.	Covance 6694-111
Toxicity to brain	Intracarotid	Albino rats	Escalating Dose Study: 0.0028, 0.028, 0.11, 0.14 mL/kg; 0.035X, 0.35X, 1.4X, 1.8X. Main Study: 0, 0.011, 0.11 mL/kg; 0X, 0.14X, and 1.4X	A maximum tolerated dose of 1.4X was established. At this dose, no outward signs of toxicity, no changes in clinical chemistry values, and no treatment-related macroscopic or microscopic observations were noted.	CTBR-56440

[1]For the ASA (active systemic anaphylaxis) method, AI-700 was administered by the intraperitoneal route for the induction phase and by IV route for the challenge phase. The intradermal route was used for the PCA (passive cutaneous anaphylaxis) method.

APPENDIX 4: IMAGING PARAMETERS FOR ALL AI-700 STUDIES

AI-700-02

Harmonic grayscale or harmonic power Doppler in continuous, triggered, or variable (combination of continuous and triggered) imaging modes were performed in each of 2 contrast imaging sessions. The mechanical index was kept between 0.8-1.1. The imaging frequency was 2.1/4.2 MHz. The frame rates were set at 15Hz for continuous imaging, <1 Hz for triggered imaging, or 1-15Hz for variable imaging.

AI-700-20

Low-power real-time and high-power triggered imaging modes were performed in each of 3 contrast imaging sessions. The mechanical index was kept between 0.1-1.2. The imaging frequency was the highest available setting for the given system. The frame rate was to be set at approximately 15Hz for low-power mode, and <1 Hz for the triggered mode.

AI-700-21

Low-power real-time and harmonic grayscale imaging modes were performed in each of 2 contrast imaging sessions. The mechanical index was kept between 0.2-0.3 for low-power mode, and ≤1.0 for harmonic grayscale mode. For destruction pulse imaging high MI was 1.1 -1.8. The imaging frequency was not specified. The frame rate was not specified.

AI-700-23

Low-power real-time and high-power triggered imaging modes were performed in each of 2 contrast imaging sessions. The mechanical index was kept between 0.2-0.3 for low-power mode with a 20-frame impulse at 1.0, and 0.8-1.0 for triggered mode. The imaging frequency was 2.5 MHz for low-power mode, and 1.3/3.6 MHz for high-power mode. The frame rate was set at approximately 20-30Hz for low-power mode, and <1 Hz for the triggered mode.

AI-700-32

Low-power real-time and high-power triggered imaging modes were performed in each of 2 contrast imaging sessions. The mechanical index was kept between 0.2-0.3 for low-power mode with a 20-frame impulse at 1.0, and 0.8-1.0 for triggered mode. The imaging frequency was 2.5 MHz for low-power mode, and 1.3/3.6 MHz for high-power mode. The frame rate was set at approximately 20-30Hz for low-power mode, and <1 Hz for the triggered mode.

AI-700-33

Low-power real-time and high-power triggered imaging modes were performed in each of 2 contrast imaging sessions. The mechanical index was kept between 0.2-0.3 for low-power mode with a 20-frame impulse at 1.0, and 0.8-1.0 for triggered mode. The imaging frequency was 2.5 MHz for low-power mode, and 1.3/3.6 MHz for high-power mode. The frame rate was set at approximately 20-30 Hz for low-power mode, and <1 Hz for the triggered mode.

APPENDIX 5: IMAGING MODES (PHASE 3 STUDIES)

AI-700 Imaging Overview

During both rest and stress imaging sessions, sequences were acquired after the start of AI-700 injection for each of 3 apical views (4-chamber, 3-chamber, and 2-chamber). Additional sequences were acquired as needed, at the discretion of the Investigator. The first imaging mode used was a low-mechanical index (MI) Power Modulation (Real-time) Imaging Mode (MI≤0.3). This was followed approximately 2-4 minutes later by the Ultraharmonic (Triggered) Imaging Mode (MI≤1.0). All imaging sessions were recorded in their entirety on sVHS videotape and representative images were captured in digital format. Settings were the same for both rest and stress imaging sessions.

Power Modulation (Real-time) Imaging Mode – Low MI

The real-time low MI (0.2-0.3) imaging mode with destruction pulsing was used to visualize AI-700 contrast enhancement of myocardial blood flow and tissue in real-time. This imaging mode allowed visualization of wall motion with high temporal resolution, but with lower special resolution, and replenishment of AI-700 post-destruction pulse.

The wash-in and filling of the left ventricular cavity and myocardium was imaged in real-time at an MI of 0.2 to 0.3. Upon filling, a 20 frame (<1 second) destruction pulse (DP) at an MI of 1.0 was applied and refill of contrast into the myocardium was observed over subsequent beats. Slow refilling following destruction indicates disease in the territory in which it appears.

A 15-beat digital acquisition began 3 beats prior to the DP, and continued for approximately 12 beats following the destruction pulse. During each imaging session, one digital acquisition was required for each of 3 apical views. The mean duration of observation during continuous imaging was 5.3 minutes (SD=2.1) during Rest and 5.1 minutes (SD=2.3) during Stress. The mean duration of observation during destruction pulses was 0.35 minutes (SD=0.14) during Rest and 0.34 minutes (SD=0.15) during Stress.

Ultraharmonic (Triggered) Imaging Mode – High MI

The triggered harmonic (intermittent) imaging mode (mechanical index of ≤1.0) was used to visualize AI-700 contrast enhancement of myocardial blood flow and tissue during the triggered imaging sequence. The high- MI second harmonic imaging mode was used prior to activating the trigger function. For each view, 5 steady heart beats of imaging were required prior to activating the trigger sequence. This mode was also used between views, to get a better plane or to change trigger parameters. Triggered imaging also allowed for a high special resolution of the images, but with low temporal resolution. Typically, it was operated at a frame rate of 30 Hz with a mechanical index of 0.9-1.0. At no time during imaging was the MI to exceed 1.0. Once the trigger function was activated using the AutoBeat Sequence,

the frame rate decreased to between 0.25 and 3 Hz (depending on the patient's heart rate and triggering ratio), assuming that at a heart rate of 60 beats per minute (bpm), 1:1 triggering is 1 Hz. The mean duration of observation during triggered imaging was 4.9 minutes (SD=1.6) during Rest and 4.5 minutes (SD=1.7) during Stress.

The AutoBeat Sequence allows the user to set an automatic trigger sequence. The triggered imaging sequence was preset to acquire and update images at a pulse-to-heartbeat ratio of 1:1 x 4 cycles, 1:2 x 4 cycles, and 1:4 x 3 cycles. Therefore, the total number of pulses was 11 and there were 24 heartbeats per sequence. Delayed refill at 1:1 triggering which subsequently fills normally at higher triggering intervals indicates disease in the territory in which it appears.

When the AutoBeat Sequence was activated, pressing the Acquire key automatically started the trigger sequence. Acquisition stopped automatically at the end of the sequence. The trigger was set to approximately 300 milliseconds (msec) after the R wave on the ECG tracing in order to represent end-systole.

APPENDIX 6: AI-700 EFFICACY STUDIES

A tabular listing of efficacy studies is presented Appendix Table 5.

Appendix Table 5: AI-700 Clinical Efficacy Studies

Study ID	Number of Study Centers and Location(s)	Study Start, Enrollment status, date Total Enrollment / Enrollment goal	Design Control type	Study and Control Drugs Dose, Route, and Regimen	Study Objective	Duration	Gender M/F Mean Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint(s)
AI-700-20	4 investigative sites in the United States.	Initiation Date: 16 December 1999 Completion Date: 19 June 2000 Enrollment: 53 subjects (18 healthy volunteers in the Pilot Stage and 35 CAD patients in the Open Stage). Goal: 16 to 40 healthy volunteers (4 to 10 per site) in the Pilot Stage and 40 to 80 CAD patients (10 to 20 patients per site) in the Open Stage.	Phase 2, open-label, multi-center, single-dose, paired-comparison study. Each subject served as his/her own control. Conducted in 2 stages: Pilot Stage (healthy volunteers) and Open Stage (clinically stable CAD patients who had a perfusion defect localized by SPECT imaging).	Pilot Stage: AI-700 0.081 mL/kg (16 subjects) and AI-700 0.054 mL/kg (2 subjects) Open Stage: AI-700 0.081 mL/kg (24 subjects) and AI-700 0.054 mL/kg (11 subjects) Duration of administration was not to exceed 4 minutes	To evaluate the safety of a single dose of AI-700 administered intravenously to healthy volunteers and patients with coronary artery disease (CAD). To evaluate the utility of using AI-700 as an ultrasound contrast agent for echocardiographic assessment of myocardial perfusion in patients with CAD.	Single dose of AI-700	In the Pilot Stage, 12 subjects were female; 6 were male. Mean age was 33 years (range: 26 to 45 years). In the Open Stage, 31 subjects were male; 4 (were female. Mean age was 63 years (range: 52 to 77 years).	Pilot Stage: Males and females 18 to 50 years of age and considered healthy based on medical history, physical examination and 12-lead ECG findings, vital signs, and clinical laboratory tests. Open Stage: Males and females between 18 and 75 years who were post-MI, had a perfusion defect localized by SPECT within 1 month before Screening, and considered clinically stable.	Statistics of agreement, sensitivity, specificity, positive predictive value, and negative predictive value were computed. If results indicated good or excellent chance-adjusted agreement (Kappa statistic >0.6) between ECHO and SPECT for the detection of perfusion defects by at least 2 of 3 blinded readers, then the primary efficacy objective was met.

Study ID	Number of Study Centers and Location(s)	Study Start, Enrollment status, date Total Enrollment / Enrollment goal	Design Control type	Study and Control Drugs Dose, Route, and Regimen	Study Objective	Duration	Gender M/F Mean Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint(s)
AI-700-21	9 investigative sites in the Unites States.	Initiation Date: 02 October 2000 Completion Date: 27 April 2001 Enrollment: 122 subjects (25 healthy volunteers in the Pilot Stage and 97 CAD patients in the Bolus and Ancillary Stages). Goal: Up to 48 healthy volunteers and 111 patients with suspected or diagnosed CAD, and evidence of inducible myocardial ischemia	Phase 2, multicenter, open-label, blinded-read, placebo-controlled, multiple-dose safety study. Conducted in 3 stages: Pilot Stage (healthy volunteers), Bolus Stage and Ancillary Stage (patients with suspected ischemic heart disease) with each stage comprising 2 imaging sessions.	Pilot stage: 2 injections of ≤ 0.054 mL/kg AI-700, one at each of 2 RCE. Bolus stage: (Groups 2-4) 2 injections of AI-700 or placebo (1RCE, 1 at SCE). Group 2: 0.054 mL/kg AI-700. Group 3: 0.014 mL/kg AI-700. Group 4: 0.054 mL/kg placebo Ancillary stage (Group 5): 2 injections of AI-700 (0.046 mL/kg at RCE and 0.035 mL/kg at SCE).	To evaluate the safety of AI-700 administered intravenously to healthy volunteers at rest and patients with suspected ischemic heart disease at rest and under pharmacologic stress. To evaluate the sensitivity and specificity of AI-700 for detecting myocardial perfusion defects in patients with suspected ischemic heart disease.	2 doses of AI-700 or placebo, one during each of 2 myocardial imaging sessions. Imaging sessions occurred on the same day and were separated by a period of at least 1 hour.	Pilot Stage (healthy volunteers): 17 males (68%), 8 females (32%). Mean age: 33 years (range: 23 to 52 years). Bolus and Ancillary Stages (CAD patients): 63 males (65%), 34 females (35%). Mean age: 61 years (range: 39 to 79 years).	Pilot Stage: Men and women 18 to 50 years of age; healthy (based on medical history, physical examination, vital signs, 12-lead ECG, Exclusion ECHO), and at low risk of CAD. Bolus and Ancillary Stages: Men and women 18 to 80 years of age with suspected or diagnosed CAD, evidence of inducible ischemia, moderate to good ECHO AWQ.	At least one AI-700 dose group was to have higher sensitivity and specificity as compared to placebo. The minimally efficacious dose was to be determined by the results of the primary efficacy analysis and the duration of ECHO contrast enhancement analysis. Compared the sensitivity and specificity of AI-700 SCE versus stress SPECT for detection of myocardial defects when evaluated on a subject-by-subject basis for each of the four patient dose groups in the ITT Population.

Study ID	Number of Study Centers and Location(s)	Study Start, Enrollment status, date Total Enrollment / Enrollment goal	Design Control type	Study and Control Drugs Dose, Route, and Regimen	Study Objective	Duration	Gender M/F Mean Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint(s)
AI-700-32	17 investigative sites in North America (United States, Canada), Europe (United Kingdom, France, Germany), and Australia.	Initiation Date: 12 December 2003. Completion Date: 14 December 2005. Enrollment: 321 patients. Goal: Approximately 400 patients.	Phase 3, international, multicenter, open-label safety and blinded efficacy study.	Patients dosed by body weight; each was to receive 2 separate bolus intravenous injections of 0.04 mL/kg AI-700 of up to 10 minutes duration each, administered during RCE and SCE, for a total AI-700 dose of 0.08 mL/kg.	To determine the accuracy, sensitivity, and specificity of AI-700-enhanced echocardiographic imaging for assessing CAD in patients being evaluated for inducible ischemia. To demonstrate the safety of AI-700 administered intravenously to patients with suspected ischemic heart disease.	Two injections of 0.04 mL/kg AI-700, one during each of 2 myocardial imaging sessions. Imaging sessions occurred on the same day and were separated by a period of at least 1 hour.	216 males (67.3%) and 105 females (32.7%). Mean age: 61 years (range: 33 to 81 years).	Clinically stable men and non-pregnant/ non-lactating women 18 to 80 years of age, who were being evaluated for the presence of inducible ischemia, and were indicated for SPECT perfusion imaging. Patients had a low to intermediate probability of CAD based upon recent history of angina, with or without prior history of MI. As measured by the truth standard, actual CAD prevalence was 44%.	Compared the ability of AI-700 ECHO and SPECT to detect CAD as defined by the truth standard. Truth was assessed by ANGIO/LVG results if available, subsequent history of MI or death during the 90-day follow-up, by prior MI, or by the non-blinded CAD Reviewer. Sequential non-inferiority testing of the ratios (≥ 0.83; alpha=0.025) of AI-700 ECHO to SPECT for accuracy, sensitivity, and specificity. Each of 3 ECHO blinded readers was compared to a SPECT reader. If at least 2 of 3 ECHO readers demonstrated non-inferior accuracy, then sensitivity and specificity were to be assessed separately for non-inferiority.

Study ID	Number of Study Centers and Location(s)	Study Start, Enrollment status, date Total Enrollment / Enrollment goal	Design Control type	Study and Control Drugs Dose, Route, and Regimen	Study Objective	Duration	Gender M/F Mean Age (Range)	Diagnosis Inclusion Criteria	Primary Endpoint(s)
AI-700-33	11 investigative sites in the United States and Europe (England, Italy, Belgium, Spain, and Germany).	Initiation Date: 17 December 2003. Completion Date: 23 May 2006. Enrollment: 457 patients. Goal: 450 patients.	Phase 3, international, multicenter, open-label safety and blinded efficacy study	Patients were dosed by body weight; each was to receive 2 separate bolus intravenous injections of 0.04 mL/kg AI-700 of up to 10 minutes duration each, administered during RCE and SCE, for a total AI-700 dose of 0.08 mL/kg.	To determine the accuracy, sensitivity, and specificity of AI-700-enhanced echocardiographic imaging for assessing CAD in patients being evaluated for inducible ischemia. To demonstrate the safety of AI-700 administered intravenously to patients with suspected ischemic heart disease.	Two injections of 0.04 mL/kg AI-700, one during each of 2 myocardial imaging sessions. Imaging sessions occurred on the same day and were separated by a period of at least 1 hour.	366 males (80.1%) and 91 females (19.9%). Mean age: 62.4 yrs (range: 34 to 83 years).	Clinically stable men and non-pregnant/non-lactating women 18 to 80 years of age, who had recently undergone or were scheduled to undergo ANGIO. Patients were to have had intermediate to high probability of CAD based on recent history of angina, with or without a prior history of MI. As measured by the truth standard, actual CAD prevalence was 58%.	Compared the ability of AI-700 ECHO and SPECT to detect CAD as defined by the truth standard. Truth was assessed by ANGIO/LVG results if available. Sequential non-inferiority testing of the ratios (≥ 0.83; alpha=0.025) of AI-700 ECHO to SPECT for accuracy, sensitivity, and specificity. Each of 3 ECHO readers was compared to the Median SPECT Reader. If at least 2 of 3 ECHO Blinded Readers demonstrated non-inferior accuracy, then sensitivity and specificity were to be assessed separately for non-inferiority.

RCE = rest contrast echocardiography; SCE = stress contrast echocardiography; AWQ=acoustic window quality, MI=myocardial infarction

APPENDIX 7: PRODUCT DEVELOPMENT HISTORY

In developing the program for AI-700, the Sponsor reviewed the approval history for SPECT contrast agents used in this indication and FDA guidance documents. Initially, the focus was on quantifying the ability of AI-700 to detect disease, as defined in one trial (AI-700-33) by the "gold standard" of coronary angiography (ANGIO), despite the imperfect nature of the gold standard. Although ANGIO can depict epicardial arterial patency, it is an indirect technique for predicting regional perfusion and coronary flow reserve, and has less prognostic value than myocardial perfusion imaging.[5, 21] In addition, in a regulatory study, somewhat arbitrary decisions are required on disease definition, e.g. 50% or 70% stenosis. Nevertheless, this approach appeared reasonable based on previous regulatory approvals. The other Phase 3 trial (AI-700-32) was to compare AI-700 with SPECT as a disease reference standard, based on its approval for this indication. Together, the 2 trials would cover the spectrum of higher risk patients scheduled for ANGIO and lower risk patients being diagnosed by SPECT.

However, during early discussions with FDA, it became apparent that potential use of AI-700 to triage patients for ANGIO was leading to the desire to see comparative performance against SPECT and the Sponsor was encouraged to include patients with SPECT results in a trial where ANGIO was being used as the disease reference (AI-700-33) and to minimize using SPECT as a truth standard in the other trial (AI-700-32). The design was amended accordingly.

After the trials started, and with a change in management at the FDA review division, the Sponsor was asked to change the trial design to a non-inferiority analysis of AI-700 ECHO against SPECT, with ANGIO as the disease reference standard for both. This approach presents significant statistical challenges (see Appendix 5) in quantifying and establishing the comparability of 2 diagnostic techniques against an imperfect standard, with multiple endpoints, particularly when there is a reciprocal relationship between 2 of the endpoints being assessed (sensitivity and specificity).

The Sponsor discussed these challenges at pre-NDA meetings and was encouraged to look at markers of clinical utility, encompassing all data from the 2 pivotal studies.

The detailed history of interactions follows.

The AI-700 Phase 3 clinical program consists of a Pilot Phase 3 Study AI-700-23 and two Phase 3 Efficacy Studies (AI-700-32 and AI-700-33).

Originally the 2 efficacy studies were designed as disease detection, point-estimate trials. The original AI-700-32 and AI-700-33 protocols were submitted to FDA on 21 October 2002. Both studies were to enroll patients with known or suspected CAD who were being evaluated for inducible ischemia.

- In Study AI-700-32, all patients were to be clinically indicated for, and to undergo, on-study ^{99m}Tc sestamibi SPECT perfusion imaging. SPECT data with 90-day

clinical outcome were to serve as the truth standard, unless eligible ANGIO/LVG data were available.

- In Study AI-700-33, all patients were to have recently undergone or were scheduled for ANGIO/LVG, for regional defect assessment and localization. ANGIO/LVG data were to serve as the truth standard.

During a conference call on 11 June 2002 with the Division (Dr. P. Love, the Division Director), the Sponsor was encouraged to pursue the target population (stable chest pain patients with suspected CAD) and a population in whom regional angiographic concordance could be obtained. In a submission on 30 August 2002, the Sponsor agreed with the Division recommendation to include sensitivity and specificity as co-primary endpoints in both studies and to increase the trial sample size because the studies were originally powered based on single endpoints. The Sponsor also agreed to collect SPECT in the majority of patients in Study AI-700-33, and agreed that SPECT in Study AI-700-32 would not be used as the truth standard but rather would be used as the comparator to provide further understanding of the performance of AI-700 as an alternative to SPECT.

Amendment 2 protocols for Studies AI-700-32 and AI-700-33 were submitted on 4 November 2003. Study enrollment began in December 2003. At a meeting with the Division (Dr. G. Mills, the new Division Director) on 7 October 2004, the Division requested that both primary study analyses be changed to non-inferiority analyses of AI-700 vs. SPECT, because AI-700 ECHO is intended to be used as an alternative to SPECT.

The Phase 3 protocols were revised further (Amendment 3) and were submitted with the corresponding Statistical Analysis Plans (SAPs) on 2 November 2004, with a request to meet with the Division. Prior to the meeting, the Division sent a fax dated 22 March 2005 with comments concerning the design and analysis of Phase 3 trials. The Division requested that SPECT be added as a comparator arm to show that AI-700 has sensitivity and specificity comparable to SPECT, with the standard of truth being ANGIO with clinical follow-up for patients with lower risk of disease. The Division also asked that a minimum threshold for SPECT performance be established and commented on the splitting of patient populations into 2 risk groups in the 2 pivotal Phase 3 trials.

In the 28 July 2005 submission, the Sponsor summarized the modifications made to the Phase 3 program to reflect the Division's requests:

- Null hypothesis testing would be used rather than point estimation,
- Non-inferiority study design to compare AI-700 with SPECT,
- Include minimum criteria for success (MCS) of SPECT comparator,
- SPECT reads would be re-initiated by blinded readers using segmental analysis (same segments reported for both SPECT and ECHO),

- Designation of true positive for disease would be based on ANGIO (≥70% stenosis in at least 1 major artery or 2 smaller coronary arteries),
- Study AI-700-32 would use a truth schema less reliant on SPECT as 'truth',
- Study AI-700-32 patient population would be enriched to include more patients who have coronary angiography results,
- Study AI-700-33 would include SPECT analysis based on SPECT studies performed using pharmacological stress and 99mTc sestamibi,
- No further exercise stress or dual isotope SPECT would be collected in Study AI-700-33,
- Majority ECHO blinded read scoring would be eliminated; the primary analysis would use the '2 of 3 readers rule'.

In August 2005, the Division responded that the primary efficacy statistical methodology should be based on absolute difference, rather than risk ratio, and that the proposed MCS for SPECT were too low. Revised protocols were submitted [AI-700-32 (Amendment 4) on 04 October 2005, and AI-700-33 (Amendment 5) on 30 December 2005]. In the December submission, the Sponsor informed the Division that the primary efficacy evaluation for each trial would be a 2-sequential-step primary analysis:

1. AI-700 ECHO accuracy is non-inferior for at least 2 of 3 ECHO blinded readers;
2. If met, then the derivatives of accuracy (i.e., sensitivity and specificity) would be evaluated independently for non-inferiority to SPECT and must be met by at least 2 of 3 ECHO readers. In this hierarchical structure, the entire type 1 error rate (alpha of 0.05) was allotted to the first tier endpoint (accuracy), and trial success was based on demonstrating accuracy for 2 of 3 ECHO readers.

To avoid delays in patient enrollment, the protocols were amended to address the agreed upon operational changes to the studies while negotiations continued with the Division regarding the non-inferiority analyses. For this reason, detailed planned statistical analyses for efficacy were removed from the protocols, and were replaced with references to the final statistical analysis plans. The revised SAPs were submitted 15 March 2006, for Study AI-700-32 and on 2 February 2006 for Study AI-700-33. The Division responded in a 2 May 2006 fax, agreeing to the use of a relative risk analysis and questioning the proposed non-inferiority margin and the proposed MCS for SPECT. The Division also made a number of recommendations that were accepted by the Sponsor. Although agreement was not reached on the non-inferiority margin and MCS, it became necessary for the Sponsor to unblind and report the results of Study AI-700-32 due to fiduciary obligations. Therefore, in order to analyze the results of Study AI-700-32, the final SAP was submitted on 16 May 2006, and the Division was informed that the study was being unblinded. The top-line results were submitted to FDA on 5 June 2006.

Following a review of Study AI-700-32 efficacy results, the Sponsor informed FDA on 06 October 2006 that it was amending the conduct of the blinded reads in Study AI-700-33 in 2 respects:

1.) The number of SPECT blinded readers was increased from 1 to 3 to better reflect variability in SPECT reader performance, rather than using a single SPECT reader who was required to meet a subjective MCS, as was the case in Study AI-700-32. This action was justified by the Sponsor in a position paper submitted to FDA on 13 April 2007, which was supported by experts in the fields of contrast ECHO (Michael H. Picard, MD), nuclear cardiac imaging (James Udelson, MD), and biostatistics (Janet Wittes, PhD).

    In addition to detailing controls established to ensure SPECT comparator data quality [e.g., use of an independent SPECT core lab, quantification, gated stress, single isotope (^{99m}Tc)], the position paper discussed problems inherent in selecting SPECT MCS based upon historical study data. Suitable historical studies indicative of SPECT performance would be difficult to identify, and would require 1) similar study populations based upon AI-700 study eligibility criteria and disease prevalence, and 2) similar AI-700 study efficacy criteria, including definition of disease and blinded read methodology. The paper concluded that the criteria recommended by the Division did not meet these requirements and that the risk of rejecting trial data using unsubstantiated MCS values was not justified.

2.) Based upon the performance of the ECHO blinded readers in Study AI-700-32, the Study AI-700-33 blinded read was prematurely terminated to allow re-training of the Study AI-700-33 ECHO readers. The objective of this retraining was to improve reader sensitivity, without impacting reader accuracy. Following completion of the retraining, the blinded read was re-initiated for the entire image set. At no time during the study was the Sponsor unblinded to any blinded read results. Details on the retraining process were provided in the AI-700-33 ECHO Blinded Reader Re-training Manual, submitted to FDA on 6 October 2006.

    The final SAP (Revision 2) for Study AI-700-33 was included in the April 2007 submission. Further discussion of the remaining unresolved issues was postponed until after the results of Study AI-700-33 became available. On April 30, 2007, the Sponsor submitted top-line results of Study AI-700-33 and informed FDA of its intent to seek pre-NDA meetings to discuss plans for the submission of the NDA for AI-700.

Pre-NDA discussions with the division's new management (Dr. D. Rieves, the current Division Director) focused on clinical utility of the agent. The Sponsor was encouraged to perform the additional analyses that are part of the NDA and have been presented in this briefing document in Section 4.5.11.3.

APPENDIX 8: ADDITIONAL STATISTICAL CONSIDERATIONS

Non-Inferiority Margin

Ideally, defining an appropriate non-inferiority margin should use both clinical and statistical criteria. For diagnostic tests, few clinical guidelines are available to inform selection of an appropriate margin. The endpoints of the two Phase 3 studies were the diagnostic parameters of accuracy, sensitivity and specificity. Unlike other clinical endpoints, such as laboratory test results or time to cancer recurrence, diagnostic parameters are more meaningful for a population than for a single patient and thus physicians cannot provide insight based on their practice experience. In addition, ECHO readers made assessments completely blinded to all patient data. Such blinding does not reflect clinical practice.

Acceptable values for diagnostic tests are often based on published literature resulting from research reflecting heterogeneous study methods, disease definitions, and patient populations. This heterogeneity likely contributes to the wide range of values reported in the literature. Moreover, values reported in the literature are often biased because positive favorable studies are more likely to be published than negative studies. Many of the studies are done in a manner or in a population that is not relevant to the setting of a clinical trial blinded read. Some studies are in single-centers where blinding is incomplete, so that readers have knowledge of the pre-test probability of disease in the patient population. This is further complicated by the fact that the Sponsor is unaware of any intra-subject non-inferiority trials published which compared the diagnostic performance of SPECT perfusion imaging with another myocardial perfusion procedure.

Referral bias in the comparator (SPECT) is another potential limitation of literature-based criteria. Patients with positive SPECT are more likely to be sent for ANGIO, which contributes to the definition of truth. Accordingly, patients with negative SPECT results may be under-represented in the study population, resulting in fewer true and false negative patients, which, in turn, may inflate estimates of sensitivity and deflate estimates of specificity.

Thus, even with careful consideration, any specified non-inferiority margin will be arbitrary and based on limited relevant clinical and statistical information.

In the pivotal efficacy studies, AI-700-32 and AI-700-33, the relative risk ratios for accuracy, sensitivity and specificity of AI-700 ECHO to SPECT was evaluated in non-inferiority analyses, each with a margin of 0.83. Given the non-inferiority relative risk ratio design, the margin defines the lower bound of the 95% confidence interval (not the point estimate) for the ratio of ECHO to SPECT. Thus, the point estimate for the ratio of ECHO to SPECT must be markedly greater than 0.83 to conclude non-inferiority.

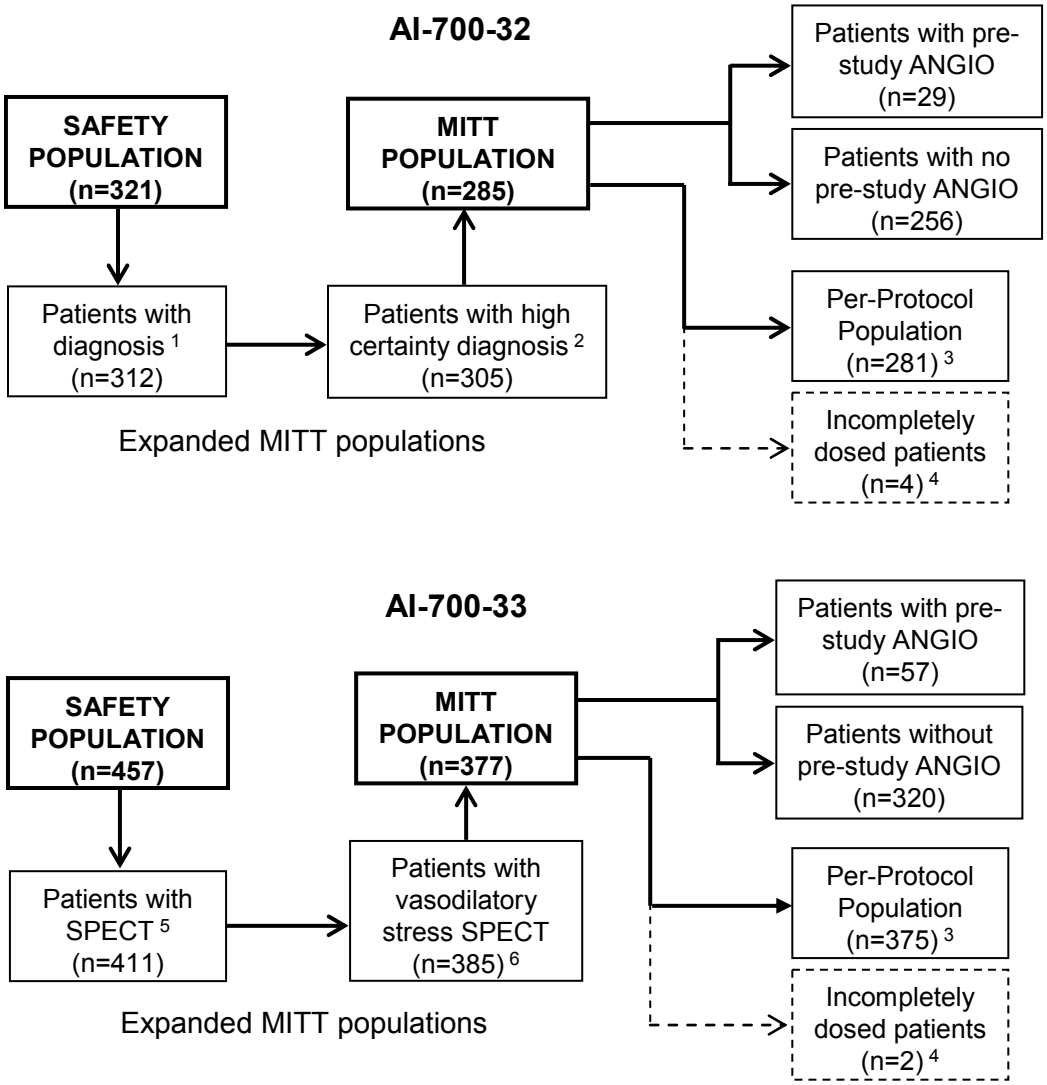
Using a ratio rather than a difference allows the magnitude of the allowable difference between the two modalities to change as the absolute values change. Thus, a non-inferiority

margin defined by a ratio translates into a larger absolute difference between the performance of 2 groups when values are higher. As sensitivity and specificity values decrease, the allowable absolute difference also decreases, thereby lowering the potential for ECHO to be declared non-inferior to SPECT. Conversely, as sensitivity and specificity values increase, the allowable absolute difference also increases, which is acceptable because the point estimates for ECHO would still be at clinically relevant values.

APPENDIX 9: SENSITIVITY ANALYSES

Analysis populations in Studies AI-700-32 and -33 are presented by study in Appendix Figure 1.

Appendix Figure 1: Populations for Analysis, by Phase 3 Efficacy Study



1. MITT plus 27 patients with Non-blinded CAD review of disease "Probably" or "Almost definitely" present with or without supporting SPECT or ECG data.
2. MITT plus 20 patients with Non-blinded CAD review of disease "Almost definitely" present with or without supporting SPECT or ECG data.
3. MITT patients having complete, intended AI-700 dosing.
4. Subpopulations not part of sensitivity analyses.
5. MITT plus all patients with SPECT reviewed by blinded readers otherwise disallowed.
6. MITT plus lost/poor quality/disallowed but vasodilatory stress SPECT.

For both pivotal studies, results similar to primary analysis results were obtained for analyses performed in MITT subpopulations and expanded MITT populations (see Appendix Table 6).

Appendix Table 6: Sensitivity of Primary Efficacy Analysis: Non-inferiority Analysis of MITT Subpopulations and Expanded MITT Populations (Studies AI-700-32 and AI-700-33)

Analysis Population	n	Accuracy	Sensitivity	Specificity
AI-700-32				
MITT	285	Non-Inferior	Not Non-Inferior	Superior
All patients with diagnosis	312	√√	√√	√√
High certainty of diagnosis	305	√√	√√	√√
Patients with pre-study ANGIO	29	√	√	√
Patients without pre-study ANGIO	256	√√	√√	√√
Per-protocol population	281	√√	√√	√√
AI-700-33				
MITT	377	Non-Inferior	Superior	Not Non-Inferior
All patients with SPECT	411	√√	√√	√√
All patients with vasodilatory stress SPECT	385	√√	√√	√√
Patients with pre-study ANGIO	57	√√	√√	√√
Patients without pre-study ANGIO	320	√√	√√	√√
Per-protocol population	375	√√	√√	√√

√ Due to small sample size, non-inferiority testing was not meaningful. No clinically relevant difference from MITT results. Inspection of estimates for relative risk ratio and associated 95% confidence intervals did not suggest heterogeneity between analysis performed with the subgroup and MITT population.

√√ Conclusion the same as MITT based on non-inferiority test results

APPENDIX 10: AI-700 SAFETY STUDIES

A tabular listing of safety studies is presented in Appendix Table 7.

Appendix Table 7: AI-700 Clinical Safety Studies

Study/ Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/ Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-01, Phase 1 (USA)	**AI-700** CB98002A, CB98002B **PBO** 47-687-FW 47-368-DK	Safety assessment of rising single IV doses of AI-700 in HV	Single-blind, placebo-controlled, parallel-group design.	48/48 (34/14)	48 HV (mean age was not calculated for this study; 18-46 yrs)	**IV AI-700 doses** **0.014 mL/kg** (n = 6) **0.054 mL/kg** (n = 6) **0.081 mL/kg** (n = 5) **0.108 mL/kg** (n = 16) **PBO** N = 15	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: 26/33 (79%) Number of subjects with ≥1 AE post **PBO**: 7/15 (47%) Most frequent post **AI-700** AEs: flushing 13/33 (39%), headache 8/33 (24%), and dyspnea 7/33 (21%) Most frequent post **PBO** AEs: injection site edema 3/15 (20%) and headache 2/15 (13%)

HV = healthy volunteers; P = patients; PBO = placebo; AE = adverse event; SAE = serious AEHV = healthy volunteers; P = patients; PBO = placebo; AE = adverse event; SAE = serious AE

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/ Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-02, Phase 1 (USA)	**AI-700** CB98002A, CB98002B **PBO** 47-687-FW 47-368-DK	Safety and myocardial imaging characteristics of rising single IV doses of AI-700 in HV	Single-blind, placebo-controlled, parallel-group design	28/28 (17/11)	28 HV (mean age was not calculated for this study; 18-49 yrs)	**IV AI-700 doses**: **0.014 mL/kg** (n = 5) **0.054 mL/kg** (n = 10) **0.108 mL/kg** (n = 5) **PBO** (n = 8)	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: 9/20 (45%) Number of subjects with ≥1 AE post **PBO**: 5/8 (63%) Most frequent post **AI-700** AEs: dizziness, palpitations, cough, pallor, and injection site edema (2/20 each, 10%) Most frequent post **PBO** AEs: dizziness (3/8, 38%) and erythema (2/8, 25%)

HV = healthy volunteers; P = patients; PBO = placebo; AE = adverse event; SAE = serious AE; IV = intravenous

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-04, Phase 1 (USA)	**AI-700** CB98005B, 6258T **PBO** 67-163-DK 65-206	Safety and PK of DFB in blood and expired air after IV AI-700 in HV; Assessment of potential immunogenicity of AI-700, with rechallenge	Single-blind, placebo-controlled, 2-stage study: Stage 1: safety Stage 2: immune rechallenge at ~2 yrs	12/9 (8/4) 10/10 **AI-700** and 2/2 **PBO** subjects completed initial dosing phase; 7/10 **AI-700** and 2/2 **PBO** completed rechallenge phase	12 HV 28.3 yrs (19-42 yrs)	**IV AI-700 doses 0.081 mL/kg** 2 doses (n = 10) **PBO** 2 doses (n = 2)	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: 4/10 (40%) Number of subjects with ≥1 AE post **PBO**: 1/2 (50%) Most frequent post-**AI-700** AEs: headache, dizziness – no AE seen in >1 subject. Most frequent post-**PBO** AEs: headache – no AE seen in >1 subject. No evidence of immunogenicity was observed with AI-700, initially or with rechallenge at ~2 yr.

HV = healthy volunteers; P = patients; PBO = placebo; AE = adverse event; SAE = serious AE; IV = intravenous

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-05, Phase 1 (USA)	**AI-700** 6550TR, 6604TR **PBO** 0.9% normal saline, multiple Lot Numbers	Safety of IV AI-700 and PK study of DFB in patients with D_LCO and moderate COPD and/or CHF	Double-blind, PBO-controlled crossover design	19/18 (CHF: 6/2) (COPD: 4/7)	8 CHF P: 63.5 yrs (45-77 yrs) 11 COPD P: 68.1 yrs (46-78 yrs)	**IV AI-700 doses** 2 doses of **0.040 mL/kg** or 2 doses of an equivalent volume of **PBO**	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: 1 CHF P; 1/19 (5%) Number of subjects with ≥1 AE post **PBO**: 0/19 (0%) Most frequent post **AI-700** AEs: cough, chest pain and discomfort, dizziness, and nausea in 1 CHF P (5%) Most frequent post **PBO** AEs: no AEs.

HV = healthy volunteers; P = patients; AE = adverse event; SAE = serious AE; IV = intravenous; PK = pharmacokinetics; COPD = chronic obstructive pulmonary disease; CHF = congestive heart failure; DFB = decafluorobutane gas; D_LCO = diminished pulmonary diffusing capacity

AI-700 Clinical Safety Studies

Study/ Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/ Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-06, Phase 1 (USA)	**AI-700** *Control lot:* 6258T *Test Lot:* 6454T	Safety evaluation of 2 separate lots of IV AI-700: 1 lot (6454T: Cohort 1) having 1% of microspheres with a slightly larger mean diameter than usual (ref. active control: lot 6258T: Cohort 2)	Randomized, single-blind, active control study	10/10 (4/6) (Cohort 1: 3/2) (Cohort 2: 1/4)	10 HV 40 yrs (18-66 yrs) (Cohort 1: 47 yrs, 33-66) (Cohort 2: 33 yrs, 18-61)	**IV AI-700 doses** 2 doses of **0.040 mL/kg** in each of 2 treatment sessions Total dose of **0.160 mL/kg** for each HV	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: Cohort 1: 1/5 (20%); Cohort 2: 3/5 (60%) Most frequent post **AI-700** AEs: headache was the only AE reported by >1 subject (2/10, 20%) in either imaging session. Overall safety profiles for both **AI-700** lots were similar.

HV = healthy volunteers; P = patients; AE = adverse event; SAE = serious AE; IV = intravenous

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-20, Phase 2 (USA)	**AI-700** CB98002A, CB98003B	Safety evaluation of a single IV dose of AI-700 and the utility of AI-700 as an ECHO contrast agent	2-stage, paired comparison study in HV and CAD P Stage 1: pilot, HV Stage 2: open, P	53/47 HV: (6/12) P: (31/4)	18 HV: 33 yrs (26-45 yrs) 35 CAD P: 63 yrs (52-77 yrs)	**IV AI-700 doses** **0.054 mL/kg** or **0.081 mL/kg**	Deaths: none SAEs: none Number of subjects with ≥1 AE post **AI-700**: Pilot stage: 12/18 (67%); Open stage: 15/35 (43%). Most frequent post **AI-700** AEs: flushing (13/53, 25%), cough and headache (each 5/53, 9%), and nausea and dyspnea (each 3/53, 6%).

HV = healthy volunteers; P = patients; AE = AE; SAE = serious AE; IV = intravenous; CAD = coronary artery disease

AI-700 Clinical Safety Studies

Study/ Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Subjects Entered/ Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-21, Phase 2 (USA)	**AI-700** CB98003A, CB98003B, CB98005A, CB98005B, CB98007A, CB98007B **PBO** Lot numbers not available	Evaluation of safety and the sensitivity and specificity of IV AI-700 in HV at rest and in P under DIPY stress. ECHO contrast imaging compared with SPECT imaging at rest and under DIPY stress in bolus and ancillary stages	**PBO**-controlled, 3-stage study: Stage 1: Pilot; 2 ECHO contrast imaging of HV with **AI-700** Stage 2: bolus; ECHO imaging of P at rest and under stress with IV **AI-700** or **PBO**, Results with **AI-700** compared with those for SPECT Stage 3: ancillary (P); ECHO imaging at rest and under stress with IV **AI-700**, results compared with those for SPECT	122 HV: 25/24 P: 97/97 (80/42)	25 HV: 33 yrs (23-52 yrs) 97 P: 61 yrs (39-79 yrs)	**IV AI-700** mL/kg in each of 2 image sessions Pilot: **≤0.054** Bolus: **0.014** or **0.054** or **PBO** normal saline Ancillary: **0.046** (session I) and **0.035** (session II) DIPY & Aminophylline administered in Bolus & Ancillary (Image Session II)	Deaths: none SAEs: none **Number of subjects…** discontinued after **AI-700** dosing: 1 with **AI-700** dose interruptions: none with ≥1 AE: *Pilot* (HV): 16/25 (64%) *Bolus* (P): 19/22 (86%) **PBO**; 44/53 (83%) **AI-700** *Ancillary* (P): 17/22 (77%) Most frequent post-**AI-700** AEs: headache (39/100, 39%) flushing (19/100, 19%) chest pain (11/100, 11%) nausea, chest tightness, and dizziness (each 10/100, 10%). Most frequent post-**PBO** AEs: headache (11/22, 50%) flushing (5/22, 23%) chest pain (4/22, 18%)

HV = healthy volunteers; P = patients; ECHO = echocardiographic; AE = adverse event; SAE = serious AE; IV = intravenous; PBO = placebo;

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-23, Phase 3 (North America, Europe, Australia)	**AI-700** 6258T, 6530T, 6530TR, 6550TR	To familiarize Investigators with study procedures and to assess safety, study methodology, and ECHO parameters used with IV AI-700 used for the diagnosis of P with suspected ischemic heart disease. Comparison of results with those for ANGIO and SPECT.	Open-label design with both rest and stress ECHO contrast imaging	133/129 (95/38)	133 P: 61 yrs (37-80 yrs)	**IV AI-700 doses** **0.040 mL/kg** in each of 2 imaging sessions (rest and stress) Stress imaging included DIPY and Aminophylline Dosing	Deaths: none SAEs: 1 P reported 2 SAEs (hypertension and vertigo) **Number of patients…** discontinued after **AI-700** dosing: 2 with **AI-700** dose interruptions: 1 with ≥1 AE post **AI-700**: 107/133 (81%) Most frequent post **AI-700** AEs: headache (57/133, 43%) chest pain (18/133, 14%) nausea (18/133, 14%) feeling hot (13/133, 10%)

HV = healthy volunteers; P = patients; ECHO = echocardiographic; ANGIO = coronary angiography; SPECT = single photon emission computed tomography; AE = adverse event; SAE = serious AE; IV = intravenous

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/ Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-32 [RAMP-1], Phase 3 (North America, Europe, Australia)	**AI-700** 6258T, 6530TR, 6550TR, 6604TR, 6538T, 6530T	To determine the safety, accuracy, sensitivity, and specificity obtained with AI-700 contrast ECHO imaging, compared with SPECT, for assessing CAD in P being evaluated for inducible ischemia	Open-label dosing with blinded efficacy evaluation	321/314 (216/105)	321 P: 61 yrs (33-81 yrs)	**IV AI-700 doses** **0.040 mL/kg** in each of 2 ECHO imaging sessions (rest and stress) Stress imaging included DIPY and Aminophylline Dosing	Deaths: none SAEs: 4 P reported 6 SAEs (adverse drug reaction, vasovagal reaction, mental status change, vision blurred, eye pain, visual disturbance). **Number of patients…** discontinued after **AI-700** dosing: 6 with **AI-700** dose interruptions: 2 with ≥1 AE post-**AI-700**: 251/321 (78%) Most frequent post-**AI-700** AEs: headache (135/321, 42%) chest pain (45/321, 14%) chest discomfort (36/321, 11%) nausea (35/321, 11%) flushing (29/321, 9%).

HV = healthy volunteers; P = patients; ECHO = echocardiographic; CAD = coronary artery disease; SPECT = single photon emission computed tomography; RAMP = **R**eal-time **A**ssessment of **M**yocardial **P**erfusion; IV = intravenous; AE = adverse event; SAE = serious AE

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-33 [RAMP-2], Phase 3 (USA, Europe)	**AI-700** 6258T, 6530T, 6530TR, 6550TR, 6550T, 6604TR, 6530TR	To determine the safety, accuracy, sensitivity, and specificity obtained with AI-700 contrast ECHO imaging, compared with SPECT imaging, for assessing CAD in P being evaluated for inducible ischemia	Open-label dosing with blinded efficacy evaluation	457/449 (366/91)	457 P: 62 yrs (34-83 yrs)	**IV AI-700 doses** **0.040 mL/kg** in each of 2 ECHO imaging sessions (rest and stress) Stress imaging included DIPY and Aminophylline Dosing	Deaths: none SAEs: 6 P had 6 SAEs: (myocardial infarction [2 P], chest pain [1 P]), hypersensitivity [1 P], syncope vasovagal [2 P]. **Number of patients…** discontinued after **AI-700** dosing: 7 with **AI-700** dose interruptions: 4 with ≥1 AE post **AI-700**: 294/457 (64%) Most frequent post-**AI-700** AEs: headache (125/457, 27%) leukocytosis (59/457, 13%) flushing (45/457, 10%) nausea (42/457, 9%) chest pain (38/457, 8%) ANC increased (37/457, 8%)

HV = healthy volunteers; P = patients; ECHO = echocardiographic; CAD = coronary artery disease; SPECT = single photon emission computed tomography; IV = intravenous; RAMP = **R**eal-time **A**ssessment of **M**yocardial **P**erfusion; AE = adverse event; SAE = serious AE; ANC = absolute neutrophil count

AI-700 Clinical Safety Studies

Study/Protocol Number, Phase of Development (Country)	Product ID/Batch Number (NME)	Study Objectives	Study Design	Number Subjects Entered/Completed (M/F)	HV/P (Age: Mean, range)	Treatments	Safety Results
AI-700-34 (ACCESS), Phase 3 [USA and UK]	**AI-700** 6667TR	To evaluate and optimize settings on multiple imaging platforms for AI-700 contrast ECHO and to evaluate safety and efficacy of a fixed IV dose of AI-700 in HV and stable cardiac P	Open-label design using multiple ECHO imaging platforms. Each subject had 2 rest ECHOs using AI-700	39/38 (30/9)	14 HV 25 P (49 yrs, 23-70 yrs)	**IV AI-700 doses** **0.040 mL/kg** in each of 2 ECHO imaging sessions	Deaths: none SAEs: none No. subjects with ≥1 AE post-**AI-700**: 11/39 (28%) Most frequent post-**AI-700** AE: rigors (3/39, 8%) after Discharge from the study was the only AE reported by >1 subject.

HV = healthy volunteers; P = patients; ACCESS = **A**cquiring **C**onsensus on **C**ontrast **E**chocardiographic **S**ystem **S**ettings; ECHO = echocardiographic; AE = adverse event; SAE = serious AE; IV = intravenous

APPENDIX 11: PROPOSED LABELING

PROPOSED LABELING

1 INDICATIONS AND USAGE

Imagify™ is an ultrasound imaging agent indicated for patients with stable chest pain being evaluated for inducible ischemia for the detection of coronary artery disease (CAD) based on assessment of myocardial perfusion and wall motion. Imagify™ echocardiography is accomplished with rest and stress techniques.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

Imagify™ is intended for intravenous administration after reconstitution with 5 mL sterile Water for Injection. See *Reconstitution, Preparation, and Administration* (2.3) for more details concerning reconstitution.

Imagify™ is administered by an intravenous injection of 1–10 minutes duration followed by a saline flush. See *Image Evaluation* (2.2) for more details concerning rate of administration.

The total dose should not exceed 0.08 mL/kg administered as two injections each of up to 0.04 mL/kg (1.6 mg/kg). The first injection is administered for the rest echocardiogram and the second injection is administered approximately 1 hour later for the stress echocardiogram.

Utilize an intravenous catheter, which should be flushed with saline prior to and immediately following administration of the injection into a peripheral vein.

2.2 Image Evaluation

The Imagify™ dose should be administered at a rate sufficient to produce myocardial enhancement, commencing with approximately 33% of the total volume per minute for approximately 30 seconds. Once myocardial contrast enhancement (MCE) is observed, the health care provider will evaluate the image enhancement and any attenuation in real time and adjust the rate of administration accordingly. The rate of administration should be adjusted as necessary according to the image appearance in the individual patient and administration should be discontinued as soon as all images have been obtained.

2.3 Reconstitution, Preparation, and Administration

Upon reconstitution with 5 mL of sterile Water for Injection, the Imagify™ vial yields a 41 mg/mL suspension containing 280 µg perflubutane/mL.
Vials of unreconstituted Imagify™ should be allowed to warm to room temperature prior to reconstitution. Vials of unreconstituted Imagify™ should not remain at room temperature longer than 8 hours. Vials held at room temperature longer than 8 hours should be discarded. Do not re-refrigerate product that has been equilibrated to room temperature.

Imagify™ Preparation and Handling Instructions:

1. Withdraw 5 mL of sterile Water for Injection into a sterile 5 mL syringe fitted with a sterile 20-23 gauge needle. Use only sterile Water for Injection.
2. Remove the flip off button cover on the Imagify™ vial, and insert a sterile 20 gauge filtered venting needle through the septum and into the vial of Imagify™.
3. Inject the 5 mL of sterile Water for Injection into the septum of the Imagify™ vial.
4. Remove the syringe and venting needle, and shake the vial vigorously for a total of 10 oscillations (1 oscillation is a single inversion and righting movement of the vial with a sharp snap of the wrist). After reconstitution, Imagify™ should appear as a white to off-white suspension with no visible particulate matter. If Imagify™ does not appear as a white to off-white suspension or if visible particulate matter is observed, DO NOT USE.
5. Withdraw reconstituted Imagify™ into a sterile 5 mL syringe fitted with a sterile, 20-23 gauge needle. DO NOT INJECT AIR into the vial prior to withdrawal. DO NOT USE A VENTING NEEDLE DURING WITHDRAWAL. If Imagify™ is not withdrawn from the vial immediately after reconstitution, it should be resuspended by shaking the vial sharply 2-3 oscillations prior to removal. Vials of reconstituted Imagify™ held longer than 60 minutes should be discarded.
6. Verify that bubbles are removed from the syringe prior to dosing. If separation in the microsphere suspension occurs before dosing, resuspend Imagify™ in the syringe by holding the syringe horizontally and gently rolling the syringe between the fingers until Imagify™ again appears as a white to off-white suspension with no visible particulate matter.
7. Once the total dose has been withdrawn into the syringe, the Imagify™ administration should be completed within 15 minutes or the material should be discarded.

VIALS ARE FOR SINGLE USE ONLY.
Imagify™ does not contain preservatives. Bacterial contamination may occur if the container has been damaged or following puncture of the rubber septum.

3 DOSAGE FORMS AND STRENGTHS

A single-use 20-mL glass vial containing a white to off-white lyophilized powder for reconstitution containing 205 mg of microspheres. Upon reconstitution, the vial yields a 41 mg/mL white to off-white suspension containing 280 µg perflubutane/mL.

4 CONTRAINDICATIONS

Imagify™ should not be administered to patients with known hypersensitivity to perflubutane or to any of the other product ingredients.

Do not administer Imagify™ to patients with known or suspected:

- Unstable acute myocardial infarction or acute coronary syndrome
- Respiratory failure, as manifested by signs or symptoms of carbon dioxide retention or hypoxia
- Severe obstructive lung disease such as severe Chronic Obstructive Pulmonary Disease (COPD), severe emphysema, pulmonary emboli
- Second or third degree AV block without a pacemaker

5 WARNINGS AND PRECAUTIONS

5.1 General Precautions

The safety of Imagify™ in subjects on mechanical ventilation or after direct intra-arterial injection has not been studied.
Diagnostic procedures involving the use of Imagify™ should be conducted under supervision of a physician with the prerequisite training and a thorough knowledge of the procedure to be performed. Appropriate facilities should be immediately available to treat any complication of the procedure, and to treat severe reactions.

5.2 Cardiac Shunts or Congenital Heart Defects

The safety of Imagify™ has not been studied in patients with congenital heart defects, right-to-left, bi-directional, or transient right-to-left cardiac shunts.

5.3 Cardiopulmonary Compromise

The safety of Imagify™ and pharmacological stress has not been studied in patients with worsening or clinically unstable congestive heart failure.
Caution should be used when performing Imagify™ echocardiography in patients with mild to moderate COPD or with resting oxyhemoglobin (SaO_2) levels below 90%.
In two pivotal Phase 3 rest and vasodilatory stress studies, 23 (3.0%) of 778 patients had transient decreases in oxyhemoglobin (SaO_2 below 90% with at least 5% decrease from baseline); 6 patients (0.89%) experienced concurrent respiratory AEs. Treatment of symptoms should consist of non-invasive supplemental oxygen therapy.
In a Phase 1 study, transient mean decreases from baseline in forced expiratory volume in 1 second (FEV_1) were observed in moderate COPD patients following both Imagify™ and placebo dosing. Mean decreases at all post-dose timepoints were 7-14% greater following Imagify™ administration compared with the placebo. The greatest difference was observed at 15-20 minutes following the second test article dose (mean FEV_1 decrease of 21.9% for Imagify™ versus 8.1% for placebo). All spirometry measurements returned to baseline values by the 24-hour follow-up.

5.4 Electrocardiographic (ECG) Changes

Premature Ventricular Contractions (PVCs) may occur during high mechanical index (MI) imaging (>1.0) but they terminate when the MI is decreased or the probe is

removed. Imaging should always be performed at an MI of 1.0 or below to minimize the occurrence of PVCs.

5.5 Clinical Labs

Fluctuations in white blood cell counts, driven primarily by changes in neutrophil counts, were observed following Imagify™ dosing. These fluctuations were transient and asymptomatic; values for both parameters returned to baseline by 24 hours post dose, were not associated with active infection, and were likely the result of the normal clearance mechanism of particulate matter from the blood.

5.6 Hypotension

Imagify™ has the potential to induce hypotension and should be used with caution in patients with autonomic dysfunction, stenotic valvular heart disease, pericarditis or pericardial effusions, carotid artery disease with cerebrovascular insufficiency, or uncorrected hypovolemia.

5.7 Chest Pain

Chest pain has been reported coincidently in patients undergoing stress echocardiography. The potential for fatal cardiac arrest or myocardial infarction exists for patients with suspected or known coronary disease who are undergoing stress testing.

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.
A total of 911 patients with suspected CAD and stable chest pain were scheduled for rest and dipyridamole (DIPY) stress echocardiography and were administered Imagify™. In this group, 677 patients were male, and 234 were female, 660 were White, 96 were Black, 76 were Asian, 76 were classified as "Other" racial group (3 did not indicate race); 91 patients reported their ethnicity as Hispanic. The mean age of patients was 61.7 years (range: 33-83). Of these 911 patients, 652 (71.6%) experienced at least one adverse event (see Table 1). Most AEs occurred following dipyridamole administration and the second dose of Imagify™. Eleven patients experienced 14 non-life-threatening serious adverse events (SAEs). No deaths were reported. Fourteen patients had AEs that resulted in permanent discontinuation of Imagify™.
<u>Serious Adverse Events (SAEs):</u>

- Six patients had SAEs within 1 hour of Imagify™ dosing, including 3 who experienced isolated *syncope vasovagal* (without loss of consciousness), and one patient who had *hypertension* and *vertigo* following the first Imagify™ dose; individual isolated cases of *mental status change* and *chest pain* were reported following administration of the vasodilator dipyridamole and the second Imagify™ dose.
- Three patients had delayed onset events (onset between 1 and 18 hours post dose), which included *eye pain*, *blurred vision*, and *vision disturbance* reported in 1 patient,

and events of *hypersensitivity* and *adverse drug event* (right-sided chest pain, headache, fever, chills, and dizziness) reported in one patient each.

- Two patients reported serious adverse events at least 24 hours after dosing including 1 episode of *myocardial infarction* and one episode of *NSTEMI*.

Discontinuations:
There were 14 patients with an AE resulting in permanent discontinuation of Imagify™. These events included headache, hypertension, vertigo, hypotension, rigors, flushing, lacrimation, wheezing, cough, sweating, tachypnea, hypersensitivity, syncope vasovagal, allergic reaction, angina, ST-segment depression, cyanosis, decreased SaO_2, weakness, diarrhea, dizziness, feeling hot, 2:1 A-V block, bradycardia, hyperhidrosis, nausea, and QT prolongation.

Most Common Adverse Events:
The most common AEs observed in the Phase 3 studies are summarized in Table 1, which presents AEs by onset and MedDRA System Organ Class (SOC). In the Phase 3 studies, patients received an injection (1-10 minutes) of Imagify™ of 0.04 mL/kg during rest. Approximately 1 hour later, following an IV dose (0.56 mg/kg) of DIPY to induce pharmacological stress, a second Imagify™ injection of the same dose was administered. Onset of AEs was reported for 4 timepoints:

- **1st Dose to DIPY** = after the first dose of Imagify™, but before DIPY stressor administration (rest imaging session - duration of approximately 1 hour),
- **DIPY to 2nd Dose** = from the administration of DIPY stressor but before the second dose of Imagify™ (duration of approximately 7 minutes),
- **2nd Dose to Discharge** = from the administration of the second Imagify™ dose during pharmacologic stress until patient discharge (stress imaging session - duration of 2-3 hours),
- **Discharge to Follow-up** = from patient discharge through the follow-up assessment (duration of 72 hours ± 48 hours).

Table 1: Common Adverse Events (Reported by ≥4% of Patients)

	Time of Onset				
Adverse Event or Category	**1st Dose to DIPY N = 911 n (%)**	**DIPY to 2nd Dose N = 894 n (%)**	**2nd Dose to Discharge N = 894 n (%)**	**Discharge to Follow-up N = 911 n (%)**	**Total N = 911 n (%)**
Number of Patients with ≥1 AE	105 (11.5)	391 (43.7)	402 (45.0)	151 (16.6)	652 (71.6)
Total Number of AEs	179	672	768	260	1879
Blood and lymphatic system disorders	**1 (0.1)**	**0**	**61 (6.8)**	**2 (0.2)**	**64 (7.0)**
Leukocytosis*	1 (0.1)	0	57 (6.4)	1 (0.1)	59 (6.5)
Gastrointestinal disorders	**8 (0.9)**	**58 (6.5)**	**66 (7.4)**	**27 (3.0)**	**148 (16.2)**
Nausea	5 (0.5)	34 (3.8)	45 (5.0)	13 (1.4)	95 (10.4)
General disorders and administration site conditions	**32 (3.5)**	**151 (16.9)**	**110 (12.3)**	**60 (6.6)**	**280 (30.7)**
Chest pain	2 (0.2)	60 (6.7)	41 (4.6)	10 (1.1)	101 (11.1)
Chest discomfort	4 (0.4)	45 (5.0)	22 (2.5)	4 (0.4)	68 (7.5)
Feeling hot	6 (0.7)	29 (3.2)	6 (0.7)	4 (0.4)	45 (4.9)
Rigors	10 (1.1)	2 (0.2)	16 (1.8)	13 (1.4)	39 (4.3)
Investigations	**12 (1.3)**	**39 (4.4)**	**108 (12.1)**	**22 (2.4)**	**162 (17.8)**
Neutrophil count increased*	2 (0.2)	0	46 (5.1)	3 (0.3)	51 (5.6)
Nervous system disorders	**43 (4.7)**	**200 (22.4)**	**155 (17.3)**	**36 (4.0)**	**370 (40.6)**
Headache	27 (3.0)	167 (18.7)	128 (14.3)	28 (3.1)	317 (34.8)
Dizziness	7 (0.8)	27 (3.0)	16 (1.8)	5 (0.5)	52 (5.7)
Respiratory, thoracic, and mediastinal disorders	**12 (1.3)**	**37 (4.1)**	**27 (3.0)**	**12 (1.3)**	**79 (8.7)**
Dyspnea	4 (0.4)	20 (2.2)	16 (1.8)	3 (0.3)	40 (4.4)
Vascular disorders	**27 (3.0)**	**64 (7.2)**	**43 (4.8)**	**9 (1.0)**	**129 (14.2)**
Flushing	17 (1.9)	52 (5.8)	18 (2.0)	3 (0.3)	85 (9.3)

* Post-dose clinical laboratory samples were collected only at Discharge and Follow-up.

The incidence of most common AEs was similar for various subpopulations, including age, gender, body mass index, race, history of smoking, and diabetes.
Other treatment-emergent adverse events that occurred in >1% of these patients by MedDRA SOC included:
Cardiac disorders: Angina pectoris
Gastrointestinal disorders: Abdominal pain, abdominal pain upper and vomiting
General disorders and administration site conditions: Pyrexia, fatigue and injection site burning
Investigations: Electrocardiogram ST segment depression, lymphocyte count decreased, monocyte count decreased and white blood cell count increased
Musculoskeletal and connective tissue disorders: Pain in extremity, neck pain, pain in jaw and myalgia
Nervous system disorders: Dysgeusia, tremor, burning sensation and paraesthesia

Respiratory, thoracic and mediastinal disorders: Throat tightness
Skin and subcutaneous tissue disorders: Hyperhidrosis
Vascular disorders: Hypotension and hot flush

Dose-ranging studies in 110 patients have demonstrated that there was a dose-dependent increase in the overall AE rate following administration of Imagify™. The most frequent AEs reported in the absence of the pharmacological stressor and which demonstrated a dose-dependent increase were flushing and headache.

7 DRUG INTERACTIONS

Drug-drug interactions for Imagify™ have not been studied.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B. Reproduction studies have been performed in rats at doses up to 15 mg/kg/day and rabbits at 30 mg/kg/day (up to 9.1 times the human dose on a body weight basis and 1.5 times the human dose on a body surface area basis). The studies have revealed no evidence of impaired fertility or harm to the fetus due to Imagify™. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

8.2 Labor and Delivery

There is no known information on the effect of Imagify™ on a mother or fetus during delivery or labor.

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Imagify™ is administered to a nursing woman.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been studied.

8.5 Geriatric Use

Of the total number of subjects in clinical studies of Imagify™, 44% were 65 or over. No overall differences in safety or efficacy were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients but greater sensitivity in some older individuals cannot be ruled out.

10 OVERDOSAGE

The consequences of an overdose with Imagify™ are not known. Treatment of an overdose should be directed toward support of vital functions and prompt institution of symptomatic therapy.

11 DESCRIPTION

Imagify™ is a diagnostic drug intended for use as a perfusion stress echocardiographic imaging agent.

Imagify™ (Perflubutane Polymer Microspheres) for Injectable Suspension is a sterile, non-pyrogenic, white to off-white lyophilized powder that, upon reconstitution is used for contrast myocardial enhancement during ultrasound imaging of the heart.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Imagify™ contains gas that increases the reflected acoustic signal during ultrasound imaging.

12.2 Pharmacodynamics

In two Phase 3 clinical studies including patients with suspected or previously diagnosed CAD, Imagify™ administered intravenously over 1 to 10 minutes provided >5 minutes of left ventricular opacification (LVO) and >5 minutes of myocardial contrast enhancement (MCE) in >80% of patients. Imaging was performed using triggered/intermittent imaging mode (mechanical index = 0.8-1.0) and real-time/low-power imaging mode (mechanical index = 0.2-0.3).

12.3 Pharmacokinetics

Imagify™ is a lyophilized preparation of synthetic, biodegradable polymer microspheres composed of poly-(D,L-lactide-co-glycolide) [PLGA] and a phospholipid (1,2 -diarachidoyl-sn -glycero-3 -phosphocholine [DAPC]) that contains perflubutane gas. Perflubutane concentrations in blood and expired air were measured using gas chromatography-mass spectroscopy. Performing studies of the clearance of Imagify™ microspheres following IV administration was not possible. Pharmacokinetic parameters were studied in healthy volunteers after intravenous administration of a single dose of 0.08 mL/kg.

Absorption
In healthy volunteers maximum mean perflubutane concentration in blood was 1.450 (± 1.024) nL/mL. Time of peak perflubutane blood concentration was 3.2 (± 1.4) minutes following the start of the injection.

Distribution
Observed blood concentrations in healthy volunteers and levels of perflubutane in expired air were simultaneously fitted using a 2-compartment model. Perflubutane has a terminal elimination half-life of approximately 4 minutes and a longer rate-limiting distribution half-life of approximately 84 minutes. However, this longer distribution half-life is not clinically significant because the majority of the dose is eliminated within minutes, as described below.

Metabolism
Data from pre-clinical studies suggests that the microsphere components of Imagify™ are removed from the blood via phagocytosis by cells of the reticulo-endothelial system (RES) and undergo degradation within the digestive vacuoles of the macrophages. In pre-clinical studies, spheroid bodies presumed to be test material were observed in histologic preparations in the RES cells of the lung, liver, kidney, and spleen after dosing. These inclusions were cleared within 14-28 days.
Perflubutane is considered chemically non-reactive and is not metabolized *in vivo*. No metabolism studies for perflubutane were performed.

Elimination
Results of human PK studies indicated that the perflubutane gas component of Imagify™ was rapidly cleared from the blood following IV injections of 0.08 mL/kg to healthy volunteers. Measurable blood concentration data demonstrated that perflubutane concentrations decreased steadily following administration of Imagify™ at a dose of 0.08 mL/kg to healthy volunteers.

Approximately 70% of the perflubutane gas component of the Imagify™ dose administered to healthy volunteers was eliminated in expired air within 1 hour of dosing. A 2-compartment simultaneous fit for blood and expired air revealed that a large percentage of the perflubutane dose was eliminated within minutes of cessation of IV administration: 91.6% was expired in the first 4 hours and virtually 100% was eliminated by 24 hours. These results indicate that the perflubutane gas component of Imagify™ was rapidly cleared from blood and expired by the lungs following IV dosing.
As perflubutane is excreted via the pulmonary route, no studies in patients with impaired renal or hepatic function were conducted.

Specific Populations

The pharmacokinetics of Imagify™ has not been studied in patients with hepatic diseases. The effects of gender, age, and/or race on the pharmacokinetics of Imagify™ have not been studied. The pharmacokinetics of Imagify™ in pediatric subjects has not been studied.

12.4 Immunogenicity

The Phase 1, placebo-controlled, single-blind study of the pharmacokinetics and safety of Imagify™ administered intravenously to healthy subjects incorporated a re-challenge approximately 2 years after the first IV administration. Following initial dosing with 0.08 mL/kg to healthy adult volunteers in Stage 1 of the study, levels of tryptase (a marker of histamine release in Type I hypersensitivity reactions [allergic reactions]), did not increase. Increases from baseline in C3a and CRP concentrations following Imagify™ dosing – and transient decreases from baseline in WBC counts, including the differential counts – were seen at 6 minutes post dosing, followed by a transient increase in WBC and neutrophil counts within 8 hours post dose. Values returned to baseline levels by 24 hours post dose.

Immediately prior to Stage 2, there was no evidence of hypersensitivity to Imagify™, as indicated by pre-dose skin prick test results.

As seen during Stage 1, evidence of asymptomatic complement activation was observed as part of the normal innate immune response to particulate material in the blood. During Stage 2, a similar response to injection of Imagify™ microspheres occurred, as there was a transient increase of C3a levels immediately following dosing, but post-dose C3 levels were unchanged from baseline and tryptase concentrations were not elevated following dosing, indicating no mast cell degranulation or histamine release and fluctuations in WBC and neutrophil counts were smaller than those seen during the first dosing session. No subject showed any indication of an allergic reaction to Imagify™, either initially or upon re-challenge.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies with Imagify™ have not been performed to evaluate carcinogenic potential. No evidence of genotoxicity was found in the following studies with Imagify™: 1) bacterial reverse mutation assay, 2) *in vitro* mammalian chromosome aberration test with Chinese Hamster Ovary (CHO) cells, and 3) *in vivo* mouse micronucleus assay.
Imagify™ was found to have no effect on fertility of male and female rats at intravenous dose levels up to 30 mg/kg/day (180 mg/m^2). For a 65 kg person (1.71 m^2 body surface area) this dose represents 9.1 times the maximum clinical dose on a body weight basis and 1.4 times the maximum clinical dose on a body surface area basis.

14 CLINICAL STUDIES

Two pivotal Phase 3, international, multicenter, open-label safety and blinded efficacy studies were conducted, RAMP1 and RAMP2. Patients with stable, suspected anginal chest pain underwent perfusion stress echocardiography (PSE), which includes a complete Imagify™ rest and stress procedure. Patients also underwent gated, quantitative ^{99m}Tc (technetium-99m) single-photon-emission-computed tomography (SPECT) perfusion imaging at rest and during dipyridamole pharmacologic stress. Independent readers, completely blinded to all patient information, history, and results of

other tests, interpreted the images. For both PSE and SPECT, readers were instructed to consider both perfusion and wall motion in their evaluation. The coronary artery disease truth standard was defined by quantitative coronary angiography [ANGIO (≥70% stenosis)] plus left ventriculogram (LVG) if available, or 90-day outcome with clinical review. Accuracy, sensitivity, and specificity were evaluated using non-inferiority analysis compared to SPECT. For each diagnostic statistic, the relative ratio (RR), which equals the PSE value divided by the SPECT value, was calculated with non-inferiority being established if ≥ 0.83 (one sided alpha=0.025) and superiority if greater than 1.00. Three ultrasound blinded readers evaluated the RAMP1 trial images and three other readers evaluated the RAMP2 images. The diagnostic results were compared to a single SPECT reader in the RAMP1 trial and the median of three SPECT readers in the RAMP2 trial. The endpoint was considered achieved if at least 2 of the 3 ultrasound readers' evaluations demonstrated non-inferiority to the SPECT comparator.
The primary efficacy analysis compared the RRs in a hierarchical manner [accuracy was evaluated first; then if the non-inferiority endpoint was achieved, sensitivity and specificity were then evaluated similarly versus SPECT.] Additional analyses were performed to support the primary analysis [receiver-operating characteristic curve (ROC) and logistical regression models]. Sensitivity analyses were also conducted on multiple populations.

Subjects received two injections (1-10 minutes) of Imagify™, each of 0.04 mL/kg, one during rest and one following standard IV infusion of dipyridamole to induce pharmacologic stress. Total dose of Imagify™ was 0.08 mL/kg.
Efficacy Analyses
Comparison of the subjects included in the RAMP1 and RAMP2 trials are shown in Table 2.

Table 2: Comparison of Studies Subjects

			RAMP1	RAMP2
Safety Population (N)			321	457
Male [n (%)]			216 (67)	366 (80)
Age [yr. mean (range)]			61 (33-81)	62 (34-83)
CAD Risk Factors [n (%)]				
	- Chest pain		309 (96)	444 (97)
	- Hyperlipidemia		235 (73)	351 (77)
	- Hypertension		241 (75)	348 (76)
	- Diabetes		86 (27)	157 (34)
	- Obesity (BMI ≥30 kg/m^2)		104 (32)	160 (35)
	- Smoking History			
		- Never	128 (40)	145 (32)
		- Current	51 (16)	96 (21)
		- Former	142 (44)	216 (47)
Efficacy Population (N)			285	377
CAD by Truth Std. [n (%)]			125 (44)	220 (58)
Truth Standard				
	- ANGIO/LVG		119	369
	- Cardiovascular History or Non-Blinded CAD Review		166	8

The diagnostic statistics for PSE and SPECT in the RAMP1 and RAMP2 trials are shown in Table 3. The accuracy of all six ultrasound blinded readers was similar and was demonstrated to be statistically non-inferior to SPECT (p<0.02).
Greater variation (attributed to reader subjectivity) was observed in sensitivity and specificity evaluations in both trials for both PSE and SPECT. In the RAMP1 trial, 2 of 3 ultrasound blinded readers demonstrated superiority to SPECT for specificity, but missed the sensitivity endpoint since only one of the ultrasound blinded readers achieved non-inferiority. In the RAMP2 trial, all three ultrasound blinded readers were shown to be superior to SPECT for sensitivity but missed the specificity endpoint because only one of the ultrasound blinded readers demonstrated non-inferiority. With all readers, PSE sensitivity increased as disease severity increased, based on the myocardial volume subtended by the stenotic vessels. No clinically significant differences in disease detection were noted in patients <65 years old as compared with those ≥ 65.

Table 3: Non-Inferiority Analysis: PSE vs. SPECT (Efficacy Populations)

Diagnostic Statistic	RAMP1				RAMP2			
	SPECT Reader	UltSnd Reader 1	UltSnd Reader 2	UltSnd Reader 3	Median SPECT Reader	UltSnd Reader 1	UltSnd Reader 2	UltSnd Reader 3
Accuracy	**69.8%**	**66.3%***	**67.0%***	**71.2%***	**67.1%**	**65.5%***	**69.5%***	**70.0%***
RR		0.950	0.960	1.020		0.976	1.036	1.043
95% CI		0.862, 1.045	0.872, 1.056	0.928, 1.122		0.892, 1.068	0.960, 1.118	0.964, 1.130
Sensitivity	**77.6%**	**76.8%***	**56.8%**	**49.6%**	**60.9%**	**72.7%†**	**67.7%†**	**73.2%†**
RR		0.990	0.732	0.639		1.194	1.112	1.201
95% CI		0.882, 1.110	0.626, 0.836	0.535, 0.741		1.075, 1.337	1.005, 1.237	1.089, 1.338
Specificity	**63.8%**	**58.1%**	**75.0%†**	**88.1%†**	**75.8%**	**55.4%**	**72.0%***	**65.6%**
RR		0.912	1.176	1.382		0.731	0.950	0.866
95% CI		0.777, 1.065	1.037, 1.346	1.240, 1.570		0.622, 0.850	0.844, 1.064	0.758, 0.980

RR=relative ratio (the ratio of PSE to SPECT); CI = confidence interval; UltSnd = Ultrasound.

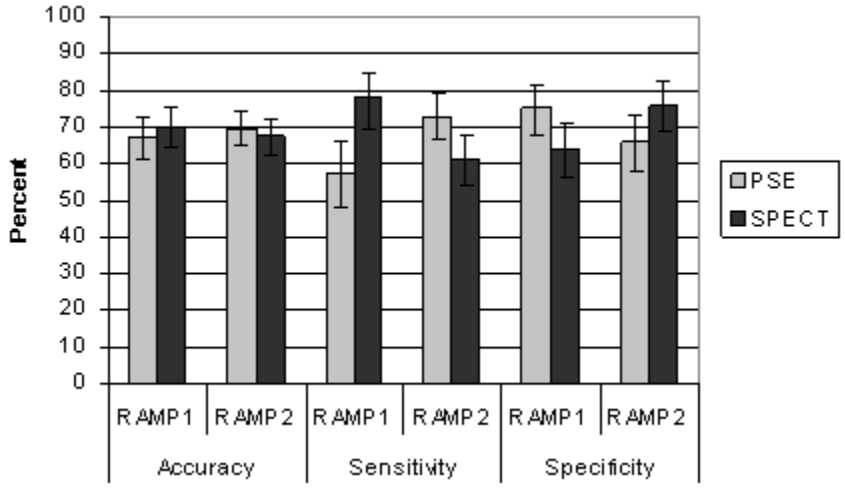
* non-inferior, p-value <0.02

† superior, p-value ≤0.02

These data support the conclusion that Imagify™ PSE is non-inferior to gated, quantitative ^{99m}Tc SPECT perfusion imaging for the assessment of CAD.

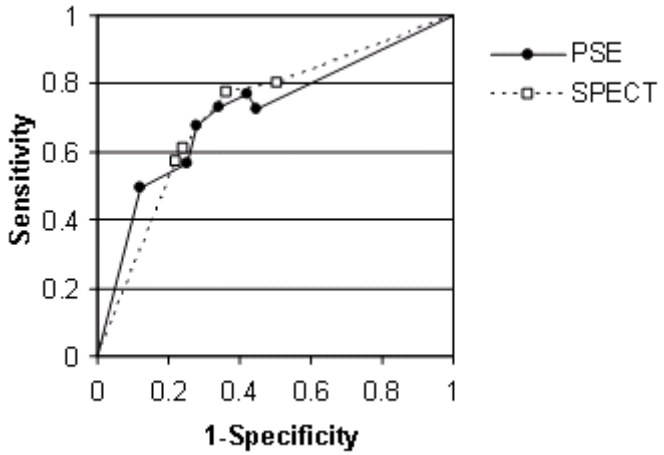
Analyses Supportive of the Primary Efficacy Analyses:
Median values for ultrasound and SPECT blinded readers in both trials were determined and are shown in Figure 1.

Figure 1: Median Values Chart (95% CI)



The magnitude and direction of reader bias (subjectivity) for all 6 ultrasound and all 4 SPECT blinded readers was quantitatively assessed by plotting the true positive fraction (sensitivity) versus false positive fraction (1-specificity). The resulting multi-reader receiver operating characteristics curves (mROC) shown in Figure 2 demonstrate almost identical shapes for PSE and SPECT. Area-under-the-curve (AUC) calculated using the trapezoidal rule was also equal.

Figure 2: mROC Curve – Trial RAMP1 and RAMP2 Combined Analysis

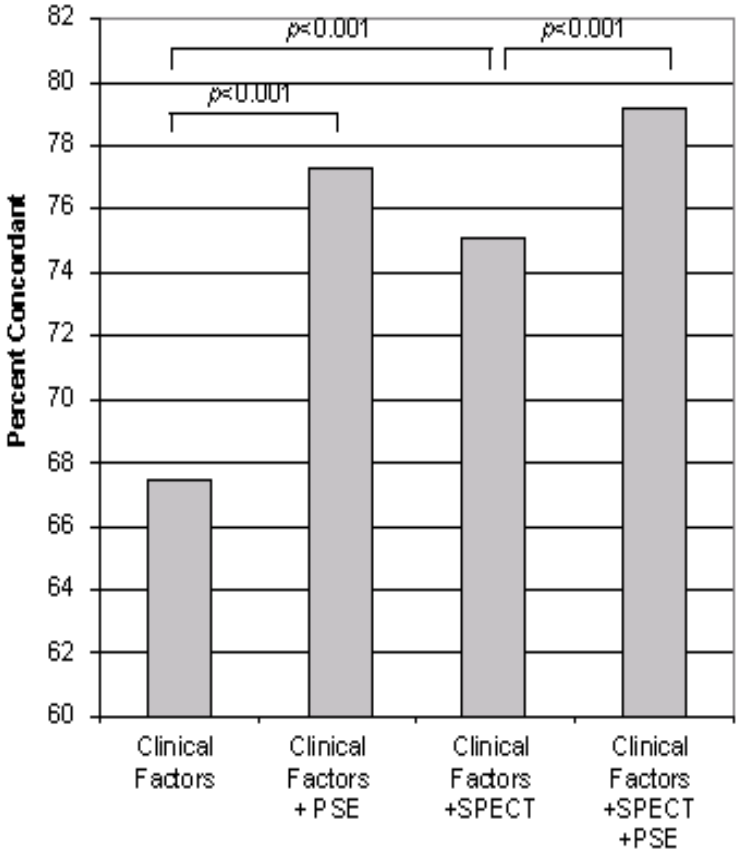


AUC PSE = 0.72; AUC SPECT = 0.72

The relative value of four models for predicting CAD was evaluated based on the RAMP1 and RAMP2 data: clinical factors alone (age; gender; smoking; hypertension; diabetes; angina type); clinical factors + Imagify™ PSE; clinical factors + SPECT; clinical factors + Imagify™ PSE + SPECT. Comparison of the percent concordance statistic (derived from the probability predicted for each observation) revealed that percent concordance was higher for models with clinical factors plus either Imagify™ PSE or SPECT than clinical factors alone. (See Figure 3.) Percent concordance was highest for the multivariate model by including both PSE and SPECT. The additional predictive value associated with adding either PSE or SPECT to a model with clinical

factors alone was significant (p<0.001). Moreover, PSE provided additional predictive value to a model already containing clinical factors and SPECT.

Figure 3: The Effect of Imagify™ Stress PSE on Predicting CAD



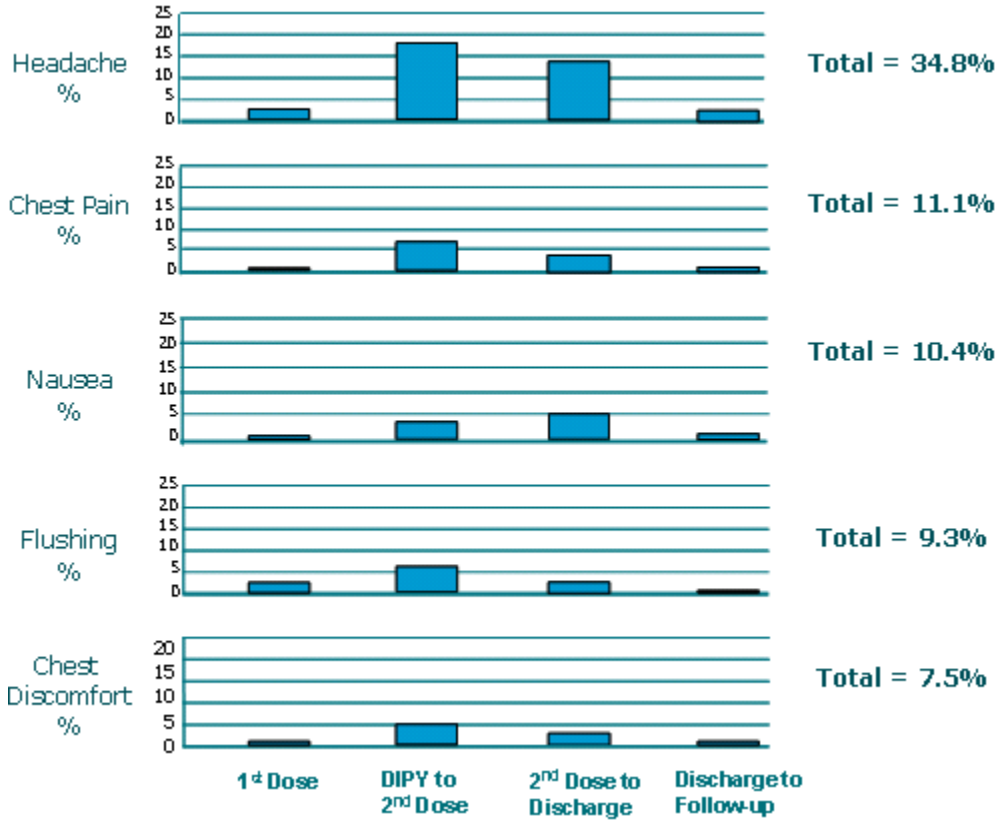
Phase 2 trials (AI700-20 {efficacy population 47 subjects with known CAD or healthy volunteers} and AI700-21 {efficacy population 97 patients with known or suspected CAD}) were also conducted to compare Imagify™ PSE to other commonly used imaging modalities. These trials confirmed that Imagify™ showed similar diagnostic performance to SPECT and that Imagify™ PSE demonstrated better diagnostic performance compared to non-contrast echocardiography.
Overall, the data from the clinical studies confirm that Imagify™ was able to identify patients with and without coronary artery disease just as well as SPECT.

Image Quality
Blinded evaluation of patients' imaging windows prior to injection of Imagify™ in Phase 3 studies, RAMP1 and RAMP2 combined, indicated that 26.6%, 55.0%, and 18.4% of patients had imaging windows of "poor", "fair", and "good" acoustic window quality. After Imagify™ injection, 99% of the PSE images were rated to be of sufficient quality to be fully interpretable. Prolonged enhancement of the myocardium (MCE) for at least 5 minutes was observed in 80% of patients and 90% of patients showed no appreciable attenuation artifact.

Safety Considerations

Analysis of the timing of onset of adverse events indicates that the majority occurred following administration of the pharmacologic stress agent, dipyridamole, and were generally consistent with its known adverse event profile. The timing relationship is shown for the five most common adverse events in Figure 4.

Figure 4: Frequent AEs by Onset Time Charts (Phase 3 Studies)



16 HOW SUPPLIED/STORAGE AND HANDLING

Imagify™ is supplied in a single use 20 mL glass vial containing white to off-white lyophilized powder for reconstitution. Each carton of Imagify™ contains 4 vials.

Storage

Store between 2°-8°C (36°-46°F) in a refrigerator.
Vials of unreconstituted Imagify™ should be allowed to warm to room temperature prior to reconstitution. Vials of unreconstituted Imagify™ should not remain at room temperature longer than 8 hours. Vials held at room temperature longer than 8 hours should be discarded. Do not re-refrigerate product that has been equilibrated to room temperature.

VIALS ARE FOR SINGLE USE ONLY.

Imagify™ does not contain preservatives. Bacterial contamination may occur if the container has been damaged or following puncture of the rubber septum.

17 PATIENT COUNSELING INFORMATION

Patients who receive Imagify™ should be instructed to:

1. Inform her physician or healthcare provider if she may be pregnant, is trying to become pregnant, or is nursing
2. Inform his/her physician or healthcare provider if he/she has a congenital heart defect or cardiac shunt
3. Inform his/her physician or healthcare provider if he/she has severe COPD

U.S. Patent Nos: xx
NDC xxx-xxx-xx
Rx Only

Manufactured by: Acusphere, Inc.
Tewksbury, MA USA
Printed in the USA
For Customer Service call 1-800-xxxx

APPENDIX 12: ADVERSE EVENTS IN AI-700 PATIENTS

In Table 34, data from multiple MedDRA (Version 6.1) preferred terms were compiled from Summary of Safety Section 2.7.4, Table 12.2.2 for patients who received AI-700 and dipyridamole and who experienced chest pain, ventricular extryasystoles, ECG abnormalities/ST-T changes, arrhythmia, and A/V block.

- Chest pain category includes those patients who experienced chest pain, chest discomfort, myocardial ischemia, myocardial infarction, and angina pectoris.
- Ventricular extrasystoles category includes patients who experienced ventricular bigeminy, ventricular trigeminy, and ventricular extrasystoles.
- ECG abnormalities/ST-T changes category includes patients who experienced ECG ST-T changes and ECG ST-T segment depression.
- Arrhythmia category includes patients who experienced sinus tachycardia, tachycardia, sinus bradycardia, bradycardia, atrial fibrillation, and ventricular fibrillation.
- A/V block category includes patients who experienced atrioventricular block, atrioventricular block first degree, and bundle branch block right.