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EMA/CHMP/312452/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Imagify

International non-proprietary name: perflubutane

Procedure No. EMEA/H/C/002347

Note

List of Outstanding Issues as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

µL Microlitre
ACC American College of Cardiology
AHA American Heart Association
ANGIO Coronary angiography
ASE American Society of Echocardiography
AUC Area under the curve
AWQ Acoustic window quality
BMI Body mass index
CA Coronary angiography
CABG Coronary artery bypass graft
CAD Coronary artery disease
CHF Congestive heart failure
CI Confidence interval
cm Centimetre
COPD Chronic obstructive pulmonary disease
CSR Clinical Study Report
CT Computed tomography
ECG Electrocardiogram
ECHO Echocardiogram or echocardiography
FDA Food and Drug Administration
GCP Good Clinical Practice
GJS Global Jeopardy Score
Hz Hertz
ICH International Conference on Harmonisation
ITT Intent-to-Treat
i.v. Intravenous
kg kilogram
LVG Left ventriculography
LVO Left ventricular opacification
m Metre
Max Maximum
MCE Myocardial contrast enhancement
mg Milligram
MI Myocardial infarction
min Minute
Min Minimum
mITT Modified Intent-to-Treat
MRI Magnetic resonance imaging
mL Millilitre
mROC Multi-reader receiver operating characteristic
ms Millisecond
ND Not done
NPV Negative predictive value
OR Odds ratio
PET Positron emission tomography
PFB Perflubutane

PLGA Poly-(D,L-lactide-co-glycolide)
PP Per protocol
PPV Positive predictive value
Pt Patient
ROC Receiver operating characteristic
RR Relative risk
RWMA Regional wall motion abnormality
SAP Statistical Analysis Plan
SD Standard deviation
sec Second
SmPC Summary of Product Characteristics
SPECT Single photon emission computed tomography
99mTc Technetium-99m

1. Recommendation

Based on the review of the data and the Applicant's response to the CHMP LoQ on quality, safety and efficacy, the CHMP considers that the application for Imagify, to be used as:

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

is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the list of questions (Section VI).

The major objections precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

1. Sufficient specificity of Imagify ECHO to detect CAD has not been demonstrated in the clinical program. Accuracy is not considered an adequate primary endpoint and the non-inferiority of the method in terms of sensitivity and specificity against the comparator SPECT has not been consistently demonstrated in the pivotal trials.

2. In AI-700-32, specificity of SPECT readings was in an unacceptably low range, when compared to ANGIO/LVG as a truth standard. Specificity of SPECT was subject to bias, when compared to SPECT itself as part of the alternative truth standard. In addition, in study AI-700-33 non inferiority of specificity has not been demonstrated. Therefore, the overall clinical program failed to demonstrate sufficient specificity of Imagify stress ECHO.
   The added value of Imagify stress ECHO over standard ECHO has not been demonstrated in the clinical program.

Other concerns:

1. The applicant has amend the SmPC wording to address safety concerns. It cannot be confidently said that no life-threatening cardiopulmonary or anaphylactoid reactions have occurred with Imagify? The wording proposed offers an inappropriate assurance as to the safety of Imagify. The applicant suggests that the potential risk of a life threatening reaction with Imagify has to be considered in the context of radiation associated with SPECT. Whilst minimising radiation exposure in any patient is desirable, exposure to a fatal reaction is clearly not. Partially resolved (Slight SmPC modification required).

2. The applicant is still obliged to provide evidence that non-inferiority (NI) of Imagify ECHO vs. SPECT for accuracy can be robustly concluded based on an adequately justified NI margin which rules out clinically unacceptable loss of efficacy. If this is not possible, it should be justified why a formal proof of NI is not needed.

3. Impact on therapeutic decisions and clinical outcome, which refers to a description and quantification of impact of diagnostic information on management of a patient and clinical outcome, has not been addressed.

Inspection issues

GMP inspection

At a pre-submission meeting with the applicant on 02/10/2012, the Rapporteur was informed that an inspection of the contract manufacturing site by an EU competent authority was planned.

The applicant has now provided evidence that the site has been inspected by the competent authority of the United Kingdom and complies with the relevant GMP provisions for a dosage form of this type. Therefore, there are no outstanding GMP issues.

2. Executive summary

2.1. Problem statement

Imagify (AI-700) is a new ultrasound product which is suggested to overcome limitations of the earlier generation ultrasound contrast agents (e.g., Optison®, Luminity® and Sonovue®), which are thin shelled microbubbles made of natural materials (e.g. proteins, lipids) that are fragile and easily destroyed during imaging. In comparison, AI-700 is comprised of thicker shelled and more durable synthetic polymer microspheres which do not break under haemodynamic pressure and have mechanical strength and stability, so when ultrasound energy is targeted at them they do not break. This enables them to persist and produce prolonged duration of enhancement that is required to perform a robust assessment of myocardial perfusion.

2.2. About the product

Imagify™, also referred to as AI-700, is an ultrasound imaging agent for use during echocardiography (ECHO). It is provided as a lyophilisate for dispersion in water for injection. It consists of microspheres composed of the polymer poly-(D,L-lactide-co-glycolide) (PLGA) and a phospholipid which are synthetic and biodegradable. The microspheres contain high molecular weight perflubutane (PFB) gas as the active substance and have been engineered to have a high degree of mechanical strength and to have a mean diameter of between 2.0 and 2.6 µm so they can pass through small blood vessels and allow transpulmonary passage. The microspheres reflect the ultrasound beam back to the transducer and then an ultrasound machine containing a computer with appropriate software transforms the reflected signals into images.

The primary advantage of the AI-700 ECHO technique is that, unlike most current imaging techniques, it does not result in exposure to any form of radiation. It could also allow simultaneous real time evaluation of myocardial perfusion for more than 5 minutes along with very high quality cardiac wall motion. Myocardial perfusion imaging is known to provide enhanced diagnostic value over that of wall motion alone [Elhendy, 2004] for detection of coronary artery disease (CAD).

2.3. The development programme/compliance with CHMP guidance/scientific advice

The applicant appears to have sought scientific advice from the MPA. It is unclear what package was provided for the MPA but issues discussed seemed to relate to the standard of truth to be used. The first pivotal study was complete at the time of seeking advice and recruitment was well advanced for the second pivotal study.

The issue of non inferiority was discussed and explained by the applicant by saying that non-inferiority was defined according to the usual confidence interval approach requiring the lower limit of the interval for the difference in sensitivity and specificity to be above the non-inferiority margin (-δ). The applicant explained that δ is defined as 0.15 and explained the reasons for this value which may be perceived as a little high. The main reason put forward was the post-test referral bias in favour of SPECT which results in SPECT having a too high apparent sensitivity and a correspondingly low specificity. Three other factors in favour of SPECT were: The large quantitative reference database that is available to SPECT readers, the “Minimal criteria for success”, and the uncertainty concerning where the true sensitivity and specificity for SPECT are due to the large variability to be found in the literature.

The applicant states that Study designs, particularly the Phase III studies, were conducted in accordance with Committee for Proprietary Medicinal Products (CHMP) Points to Consider on the Evaluation of Diagnostic Agents.

2.4. General comments on compliance with GMP, GLP, GCP

The applicant states that all the clinical studies in the AI-700 development programme were conducted in compliance with current Good Clinical Practice (GCP) requirements, as described in the International Conference on Harmonisation (ICH) Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guideline E6). Compliance with these regulations is also stated to constitute compliance with the ethical principles described in the Declaration of Helsinki.

Evidence of GMP inspection by an EU authority of the site of finished product manufacture and packaging has now been provided. A GMP certificate has been provided in respect of the site of EU batch release.

A declaration has been provided by the QP of the site of EU batch release to confirm GMP compliance of the site of active substance manufacture.

2.5. Type of application and other comments on the submitted dossier

- Legal basis

This is a full stand-alone centralised application under Article 3 (2)(a) New active substance of regulation (EC)No 726/2004.

3. Scientific overview and discussion

3.1. Quality aspects

Drug substance

Perfluorobutane is a saturated perfluorinated alkane; a chemically inert gas with poor solubility in water. It is not the subject of a monograph of the European Pharmacopoeia. The relevance of its physico-chemical characteristics in a drug product of this nature has been suitably discussed.

The information provided on the raw materials, synthesis, purification and quality control of the active substance provides good assurance that pharmaceutical-grade material of acceptable quality can be consistently produced. Adequate data has been provided to support the stability of the active substance and the proposed re-test period of 60 months is acceptable. No unresolved issues remain in relation to the active substance.

Drug product

The drug product is presented as a freeze-dried (lyophilised) powder for dispersion for injection in a glass vial. The powder is to be reconstituted with water for injection prior to intravenous injection.

The powder is composed of porous microspheres combined with excipients. The manufacturing process introduces the active substance to the microspheres and the vial headspace.

The manufacturing process includes aseptic compounding, microencapsulation and lyophilisation; in accordance with the European *Note for Guidance on Process Validation* (CPMP/QWP/848/96), the process is considered "non-standard" and full process validation data has been submitted and considered acceptable.

The finished product specifications are considered generally sufficient to ensure adequate and reproducible quality control over the product at release and throughout shelf-life. The proposed shelf-life of 2 years for the finished product can be accepted. No unresolved issues related to the drug product remain.

3.2. Non clinical aspects

Pharmacology

The in vitro study pharmacodynamic study suggests that while AI-700 IBP is decreased at higher frequency compared to the reference product Optison, attenuation is decreased, and echogenicity is improved with AI-700 at comparable frequencies and acoustic powers. Overall, the in vitro study indicates AI-700 has potential to decrease attenuation and increase image enhancement. The in vivo studies indicate myocardial enhancement is achieved for 1.5 minutes in dog and monkey, at AI-700 dose of 4.0 mg/kg. AI-700 image duration was greater than that of Optison in monkey, but was not compared in dog. The assessment of cardiovascular safety in the dog myocardial enhancement study showed a dose-dependent hypotensive response at 8 and 10 mg/kg AI-700. There appears to be a possible trend toward decreased BP at 4 mg/kg AI-700 but this is unclear. The applicant suggests that this is due to the presence of one of the excipients in the treatment solution, which has been shown to cause hypotension at similar doses in dog (Millard et al, 1977). The applicant's argument is plausible, but cannot be determined in this study as there is no adequate group to control for the excipient. In the myocardial perfusion defect study in dogs, there was also decreased BP with AI-700 at 4 mg/kg,

but the severity of the occlusion model makes it difficult to determine if the effect was related to AI-700. As some effects on blood pressure were seen in safety studies at higher doses, an AI-700-related hypotensive effect cannot be discounted. The applicant's justification for the lack of secondary pharmacodynamic studies is based on the fact that PFB is inert and therefore effects unrelated to the desired transient image enhancement are not expected. As PFB is not pharmacologically active, and AI-700 excipients are metabolised to known components, the occurrence of secondary pharmacological effects is considered unlikely, and the lack of studies is considered acceptable.

In the safety pharmacology studies in rhesus monkeys, cynomolgus monkeys, pigs and dogs with PH, the most significant finding was a hypotensive effect. In the GLP cardiovascular study in rhesus and cynomolgus monkeys, MAP was decreased by 15-25% with 40 mg/kg AI-700, which was greatest up to 15 minutes post-dose and returned to baseline prior to the next dose or by the end of the monitoring period. Increased HR was also seen, which was considered a compensatory effect following BP decrease. Some decreases were seen with 4 and 20 mg/kg doses but measurements were generally within normal ranges and were not accompanied by increased HR. In contrast to other studies in monkeys, no PVCs were reported. In the GLP cardiovascular study in pigs, animals were given two doses separated by 1 hour, up to the maximum clinical dose. AI-700 was associated with a significant decrease in blood pressure, which was over 50% in 5/9 animals. This decrease led to increased heart rate and a subsequent compensatory increase in blood pressure. The mechanism of hypotensive induction is unknown. PVCs occurred but were sporadic and not considered dose related. The hypotensive effect of AI-700 was not associated with any clinical pathology parameters. No NOAEL was determined for the study, however the maximum clinical dose is of 2 x 0.04 mL/kg doses was the NOAEL in animals pretreated with indomethacin. The applicant is asked to discuss the apparent thromboxane mediated hypotensive effect in pigs in terms of potential mechanism and its clinical relevance. Cardiopulmonary safety was investigated in non-GLP studies in rhesus monkeys, cynomolgus monkeys, and in dogs with induced pulmonary hypotension. In the monkey studies, no significant respiratory adverse effects were identified. In the close-chest dog model of PH, three animals died in the pilot study. Mortality appeared to be associated with mechanical ventilation, as when the main study was amended so that the animals were not mechanically ventilated, no mortality was seen. Thus, the mortality is not considered AI-700-related.In the main study all treatment groups exhibited elevations in mean pulmonary arterial pressure, which was dependent on dose and PH severity. This was accompanied by transient decreases in systolic arterial pressure and heart rate, which were greater in high dose AI-700/severe PH groups. The applicant is asked to discuss the implications of lack of a GLP study of respiratory safety, considering the effects of AI-700 on respiration and lung in the toxicology studies. The response submitted by the applicant was considered acceptable.

PVCs were observed in pharmacodynamic and cardiopulmonary safety studies in monkeys, and the cardiovascular safety study in pigs. The occurrence appeared to be dependent on the experimental protocol or imaging procedures, was also seen with the reference product Optison, and has been observed to be dependent on imaging parameters during clinical imaging. Thus the occurrence of PVCs appears not to be AI-700 related, and is not considered a safety concern.

The cerebrovascular study in rats showed some neurological effects which had uncertain toxicological significance. Animals with partly closed right eyes were seen in all AI-700 treatment groups. The clinical signs are not correlated with histopathological or microscopic signs, as necrosis and gliosis within the cerebral cortex, hippocampus, midbrain and thalamus was apparent in control groups and did not increase with AI-700 dose. Overall the cerebrovascular study does not suggest a safety concern at up to 4 mg/kg AI-700.

The lack of pharmacodynamic drug interaction studies is acceptable, as PFB is an inert compound and therefore interactions with co-administered drugs are not expected.

Pharmacokinetics

The pharmacokinetic studies are limited to two GLP-compliant studies performed in rats to determine the distribution and clearance of the components of AI-700. Absorption studies were not performed as the product is intended for IV administration. Metabolism studies were not performed; a review of the available literature is provided.

Distribution of [Pd]-AI-700 microspheres was evaluated in SD rats following a single IV tail vein injection. The method of administration in the initial Set 1 animals led to poor delivery of test article; therefore the data for set 2 animals is assessed. The distribution study indicates that the test article-related material is quickly removed from the blood, with a decline of up to 85% of Pd within 7 minutes of dosage. Measurable levels of Pd were present at 24 hours in the blood, and in the liver, lung, and spleen at necropsy. However the applicant appears to describe organ distribution data from set 1 animals, where the distribution was confounded by inadequate administration of the test article. Taking the data from Table 4 (Study 98-7089), the Pd concentration is as follows: spleen (28.3 ± 3.75 and 22.7 ± 6.74 µg/g), lung (35.9 ± 9.61 and 39.0 ± 10.8 µg/g), and liver (12.4 ± 0.3 and 13.1 ± 2.64 µg/g) for cohorts 1 and 2. The applicant should update the Pharmacokinetic summary to reflect the more relevant set 2 data. From this data it appears that total recovery of Pd from these tissues was 62-67%. Considering the low total recovery rate, and the appearance of spheroid bodies in other organs such as the kidney in repeat dose studies, the applicant is asked to further justify the lack of full organ analysis at necropsy as significant levels of Pd are unaccounted for in the study. The response submitted by the applicant was considered acceptable.

In agreement with the expected rapid respiratory elimination of perfluorocarbons, the majority of PFB was eliminated through expiration. 61.2% of the cumulative dose was recovered at 10 minutes post dose using sample tube collection. 69% of PFB was recovered within 180 minutes. Using the trapezoidal rule it was estimated recovery totalled 71.4% by 48 hours post dosing. Using the air bag collection method, 77% of PFB was expired at 180 minutes post-dose, the earliest time-point analysed. Recovery of PFB was estimated to be up to 78.7% after 48 hours post dosing, but this value is based on one animal and is not considered statistically significant. From these studies, as much as ~30% of administered PFB was not measurable in exhaled air. The applicant suggests this discrepancy is caused by inefficient collection methods, or the use of trapezoidal rule for estimation of elimination. The applicant is asked to discuss the potential for other routes of elimination of PFB, and any associated toxicological implications. The applicant is asked also to discuss the absence of analysis of Pd concentration in excreta, in light of the discrepancy between dose administered and dose recovered from spleen, lung, liver, and injection site in the distribution study. The response submitted by the applicant was considered acceptable.

No pharmacokinetic drug interaction studies were performed. As PFB is expected to be rapidly excreted through respiration without metabolism, it is unlikely that concomitantly administered medications will have clinically relevant interactions. The microsphere components are taken up by RES macrophages and their metabolites are incorporated into metabolic cycles which are not expected to cause clinically relevant drug interactions. The lack of pharmacokinetic drug interaction studies is acceptable.

Toxicology

The toxicological studies for AI-700 comprise in vivo studies in mice, rats, rabbits guinea pigs and monkeys, to characterise single- and repeat-dose toxicity, genotoxicity, reproductive and developmental toxicity, local tolerance and antigenicity. The single dose toxicity studies were performed with AI-700 in rats and Cynomolgus monkeys. Selection of dose levels and route of administration was considered appropriate. In rats, significant mortality occurred at 100 mg/kg AI-700 which was most likely due to respiratory arrest following capillary blockage by microspheres. Altered/laboured respiration was likely as a result of this effect. The lethal dose is over ten times the expected clinical dose and the margin of safety is acceptable. Refractile nonbirefringent spheroid bodies, assumed to be AI-700 microspheres were present at 14 days post dose in lung liver and spleen, and injection site. Therefore, in relation to the pharmacokinetics of AI-700, it can be expected that microspheres may persist for at least 14 days after administration.

14-day repeat dose studies were performed in rat and monkey, with a further 28 day recovery period. Although AI-700 is a single-use diagnostic agent, repeat dose studies of at 1 month are expected for any product whose intended duration of use is up to 2 weeks. The applicant should justify the absence of adequate repeat dose studies. No toxicokinetic parameters were investigated in the repeat-dose studies. In the rat study, mortality occurred in 3 animals receiving 60 mg/kg/day at day 9-13. Cause of death was due to respiratory insufficiency, similar to that of acute lethal doses in the single-dose studies. Histopathology revealed associated spheroid body-containing macrophage/synctial cell infiltrate in the alveolar septa, hemorrhage and acute inflammation of the lung. Spheroid bodies were also observed within macrophages at the site of injection, and in RES cells of the spleen, liver and renal glomeruli. These effects occurred with all AI-700 doses and severity was dose related. Reversibility was seen following the recovery period in all tissues but the injection site. In the cynomolgus monkey study, up to 65 mg/kg AI-700 did not lead to mortality in any animals. Refractile, nonbirefringent spheroid bodies were found in pulmonary septa and alveolar macrophages, and this correlated with increased lung weight in high dose animals. Spheroid bodies were also found in RES cells of the splenic red pulp and marginal zone of the periarteriolar lymphoid sheath in animals in all treatment groups. Spheroid bodies were also observed in the sinusoidal macrophages of the liver and glomeruli. Statistically significant alterations in haematological parameters were not dose dependent and/or were low in magnitude, and are not considered toxicologically significant.

The applicant considers the presence of spheroid bodies in RES cells to represent normal the response of the body to clear material from the circulation, which is not in itself an adverse effect. Thus, the NOAEL for the rat study was considered 10 mg/kg/day, as organ weights at this dose were not altered. Similarly, the NOAEL for the cynomolgus monkey study was determined to be 65 mg/kg/day - the highest dose administered. The presence of spheroid bodies was generally not correlated with macroscopic/microscopic findings indicating toxicity or alterations in clinical chemistry to indicate impaired organ function. Furthermore reversibility in all tissues other than the injection site was seen at the end of the recovery period. Taken together, the histopathological findings do not suggest a safety concern at the doses which do not cause respiratory insufficiency. As significant lung toxicity and respiratory insufficiency occurred in rat only at 60 mg/kg, this provides an acceptable margin of safety for the clinical dose of 3.3 mg/kg.

AI-700 was negative in the standard battery of genotoxicity studies. The studies are GLP compliant, use AI-700 at sufficient concentrations for the assay sensitivities. Overall, genotoxic potential of AI-700 can be discounted. The absence of carcinogenicity testing is acceptable considering the negative genotoxicity results, and that AI-700 is an infrequently administered diagnostic agent.

The study of fertility and early embryonic development did not identify any effects on maternal toxicity or male or female reproduction up to 30 mg/kg. The paternal toxicity was seen at lower doses than those in the repeat dose studies, where mortality occurred only at 60 mg/kg/day. Considering the extended dosage period, and that 4 of the 5 animals died after at least day 19, the disparity between the toxicity in the two studies is not unexpected. The 30 mg/kg/day dose is also almost ten times that of the maximum clinical dose, so taken together this is not a concern. In the main embryo-fetal development rat study, no evidence of embryotoxicity or fetotoxicity was observed up to the highest dose of 30 mg/kg/day. In the main rabbit study, AI-700 was well tolerated in adults at up to 15 mg/kg/day, which caused toxicity in the dose range-finding studies. There were isolated malformations in two foetuses at 3 mg/kg/day and one fetus at 15 mg/kg/day, however as these were low in incidence and were not related to dose, they are not considered to be toxicologically significant. The NOAEL for embryotoxicity, fetotoxicity and teratogenicity was 30 mg/kg/day in rats and 15 mg/kg/day in rabbits.

Toxicokinetic parameters were not monitored in parallel with the reproductive and developmental toxicity studies. Consequently, it was not determined if AI-700 microspheres cross the placenta. Although embryo-fetal toxicity and teratogenicity were not observed, the effect of AI-700 administration in late pregnancy on offspring survival is unknown. The applicant is asked to comment on the absence of offspring survival data following administration of AI-700 during gestation, and discuss the post-natal toxicity implications of transmission of microspheres to the offspring. No segment III studies were performed, so the potential effects transmission of microspheres through lactation offspring during lactation is unknown. It was not determined if excretion of AI-700 microspheres in milk occurs. Considering the lack of pharmacokinetic and toxicity data, the applicant is asked discuss the potential for transmission of microspheres to the offspring prior to clearance from circulation. The pharmacokinetic basis for SmPC recommendation of a 1 hour cessation of breast-feeding should be provided. The response submitted by the applicant was considered acceptable.

The positive data in the ASA phase of the antigenicity study is in contrast to Phase I clinical data described by the applicant. When nine healthy volunteers were re-challenged with AI-700, no immune mediated adverse effects were observed, and markers of hypersensitivity reactions were unchanged. Despite the limited number of subjects, the clinical data offers some reassurance. However in light of the positive ASA result and the small number of volunteers re-challenged with AI-700, the applicant is asked to discuss the potential for anaphylactoid reactions (see clinical question). The response submitted by the applicant was considered acceptable.

In the study of the effect on reconstitution of AI-700 on acute toxicity, it was found that reconstitution of AI-700 with pressure venting minimised the onset of adverse effects, compared to the reconstitution protocols used in initial clinical studies. As the method of reconstitution used in the non-clinical safety studies appeared to cause less adverse effects, the applicant's decision to change the clinical reconstitution procedure to reflect this. The data supports this approach.

Ecotoxicity / environmental risk assessment

Perflubutane PEC surfacewater value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5. Therefore perflubutane is not expected to pose a risk to the environment. The absence of a phase II Environmental Fate and Effects Analysis is acceptable. The microsphere constituents are carbohydrates or lipids and are incorporated into normal metabolic processes. They are not expected to pose a significant risk to the environment. An adjuvant is used as a solubilising agent in ecotoxicological tests at higher concentrations than are expected in the environment due to AI-700 use. Therefore it is not expected to pose an increased environmental risk.

Discussion on non-clinical aspects

Overall the non-clinical dossier is well presented and the studies are accurately represented and summarised. The pharmacodynamic studies are limited, but as AI-700 is a diagnostic agent and pharmacologically inactive, the studies provide sufficient proof of efficacy alone and in comparison to the reference product, Optison. Overall, similar or improved echogenicity was shown compared to the comparator. Safety pharmacology suggests a reasonable safety profile in rats, dogs, and non-human primates. Alterations in blood pressure were generally low in magnitude and returned to normal levels prior to subsequent dosing or the end of study.

The toxicity studies did not highlight overt adverse effects within the expected clinical dose range. Mortality in single dose studies due to respiratory insufficiency occurred at substantially supratherapeutic doses, and clinical microsphere concentration is not expected to reach levels sufficient to interfere with the lung vasculature. Effects seen in repeat-dose studies were largely due to the presence of microspheres in cells of RES, which is considered part of the mechanism of clearance from the circulation. The toxicity profile of AI-700 was generally favourable; however repeat dose studies of sufficient length were not conducted. Determining accurate margins of safety was also confounded by inadequate or absent pharmacokinetic and toxicokinetic studies. However based on the characteristics of PFB elimination and the absence of toxicity correlates in tissues where microspheres were detected, these limitations are not expected to pose significant clinical safety concerns.

Conclusion on non-clinical aspects

The overall the non-clinical data could be considered appropriate to support the use of AI-700 as an ultrasound imaging agent provided the clinical data justifies its use and a satisfactory response is made to the list of questions.

3.3. Clinical aspects

Table 1: Overview of AI-700 Clinical Studies

Study Number	Study Phase	Design	Study Population N = total (1241) (AI-700/placebo) (1194/47)	Location Number of Sites (n)
AI-700-01	I	Single-blind, placebo-controlled, parallel-group safety study of rising single i.v. doses	n = 48; Healthy (33/15)	US Single centre
AI-700-02	I	Single-blind, placebo-controlled, parallel-group safety & myocardial imaging study of rising single i.v.doses	n = 28; Healthy (20/8)	US Single centre
AI-700-04	I	Single-blind, placebo-controlled, PK & safety study with rechallenge	n = 12; Healthy (10/2)	US Single centre
AI-700-05	I	Double blind, placebo-controlled, crossover safety & PK study	n = 19; CHF (8) Moderate COPD (11) (18/19)	US Multicentre (3)
AI-700-06	I	Randomized, single-blind, active-control safety study	n = 10; Healthy (10/0)	US Single centre
AI-700-20	II	2-stage paired comparison, safety & ECHO imaging study of a single i.v. dose	n = 53; Healthy (18) CAD patients (35) (53/0)	US Multicentre (4)
AI-700-21	II	Placebo-controlled, 3-stage, safety & ECHO imaging study	n = 122; Healthy (25) CAD patients (75) (100/22)	US Multicentre (9)
AI-700-23	III	Pilot, open-label, dual-injection, safety & ECHO imaging study	n = 133; CAD patients (133/0)	International Multicentre (35)
AI-700-32	III	Pivotal, open-label, dual-injection, safety & ECHO imaging study	n = 321; CAD patients (321/0)	International Multicentre (17)
AI-700-33	III	Pivotal, open-label, dual-injection, safety & ECHO imaging study	n = 457; CAD patients (457/0)	International Multicentre (11)
AI-700-34	III	Exploratory, open-label, dual-injection, ECHO imaging settings & safety study	n = 39; Healthy (14) Stable Cardiac patients (25) (39/0)	US & UK Multicentre (4)

Healthy = healthy volunteers, CHF = congestive heart failure, COPD = chronic obstructive pulmonary disease, PK = pharmacokinetics, CAD = coronary artery disease, i.v. = intravenous, ECHO = echocardiography
Placebo patients received normal saline.
* 19 subjects from Study AI-700-05 received both AI-700 and placebo

Pharmacokinetics

Pharmacokinetic parameters were delineated in healthy volunteers in Study AI-700-04. Additional supportive information was derived from a single study in patients with CHF and/or COPD (Study AI-700-05). Data supporting the choice of AI-700 dose for the Phase III studies were acquired from pharmacodynamic evaluation of imaging characteristics of AI-700 in early studies (AI-700-02 and AI-700-21).

The PK of PFB in blood and expired air were simultaneously fitted and well described using a 2-compartment PK model. The results of this study indicate that the active component of AI-700, PFB gas, is rapidly cleared from blood and excreted from the lungs following dosing. AI-700 does not appear to be immunogenic. There was however evidence of C3a complement activation. Mean CRP levels in all A1-700 subjects were increased above the upper limits of normal at 24 hours post dose during phase 1. WBCs and neutrophils decreased transiently immediately post dose but by 8 hours, transient increased in WBC and neutrophil counts were seen.

Data from pre-clinical metabolism studies suggests that the microsphere component of AI-700 is removed from the blood via phagocytosis by cells of the RES, and undergoes chemical degradation within the digestive vacuoles of the macrophages until the polymer is hydrolysed into water-soluble fragments and eventually eliminated. This is similar to the disposition found for other types of microparticles.

Pre-clinical studies of AI-700 in rats indicated that the polymeric microsphere component of AI-700 was cleared from the blood in this manner. In AI-700 pre-clinical studies, spheroid bodies presumed to be test material were observed in histologic preparations in the reticuloendothelial cells of the lung, liver, kidney, and spleen after dosing. These inclusions were cleared within 14-28 days.

PRB is an inactive gas that is considered chemically non-reactive and is not metabolized *in vivo*. No metabolism studies for PFB were performed.

In a specific study in patients with COPD and CCF, transient mean decreases in baseline in FEV1 were observed in moderate COPD patients following both active and placebo although decreases at all-time points were 7-14% greater following AI-700 than with placebo. The difference was most notable at 15 to 20 minutes following second injection of AI-700 (decrease of 21.9% for AI-700 versus 8.1% for placebo).

At this time point, all patients who received AI-700 had decreased FEV1 as compared to Baseline. At 15 to 20 minutes following the second injection of AI-700, the mean decrease in the FEV1 (measured volume) was 0.35 L (range: decrease of 0.08 to 0.80 L) as compared to mean decrease of 0.09 L for placebo (range: decrease of 0.18 L to increase of 0.17 L).

For FVC, there was little difference between the 2 treatments in the mean percent change from Baseline, with the exception of 15 to 20 minutes following the second dose of test article (-9.0% for placebo *versus* -15.0% for AI-700).

Small decreases from Baseline were observed in mean FEV1/FVC during AI-700 dosing.

At 15 to 20 minutes following the second dose, the mean percent change from Baseline was an increase of 0.8% for placebo (range: decrease of 7.3% to increase of 12.8%) versus a decrease of 8.0% for AI-700 (range: decrease of 21.6% to no change).

For FEF25-75, the largest mean percent change from Baseline also occurred at 15 to 20 minutes following the second dose of test article (-5.8% for placebo versus -25.5% for AI-700).

Two of 8 moderate COPD patients (25%) had FEV1 decreases of ≥15%. These 2 patients (01-202 and 02-205) had maximum decreases of FEV1 (% predicted) of 23% and 19%, respectively. Both patients demonstrated asymptomatic declines in FEV1 at the initial post-dose spirometry time point; these declines persisted throughout the first hour post-dose. The fluctuations in FEV1 values did not change appreciably following the second dose of AI-700 as compared to the first dose. For Patient 01-202, pulse oximetry values dropped to 89% at 20 minutes following the first dose of AI-700 and to 88% at 13 minutes and 15 minutes after the second dose of AI-700.

Pharmacodynamics

AI-700 was designed to optimise MCE by maximising the reflected ultrasound energy, rather than simply maximising the scattering cross section, as this also tends to maximise the attenuation coefficient. High attenuation leads to low imaging depth and loss of tissue images further from the ultrasound probe. Effective MCE in a clinical sense is measured by subtracting the duration of attenuation from the duration of MCE. For effective MCE to be of clinical value, it must be maintained for a sufficient period of time so that all areas of the myocardium can be imaged.

The studies sought to evaluate the relationship between MCE and AI-700 ultrasound imaging. Because MCE is needed to visualise myocardial perfusion, and perfusion is needed for diagnostic evaluation in CAD, MCE is the parameter that best represents the pharmacodynamics of AI-700. MCE occurs when blood containing the contrast agent fills the small blood vessels supplying the myocardium. Areas of the myocardium that are hypoperfused (e.g. from stenosed or occluded arteries) do not give a strong ultrasound reflection since the contrast agent is depleted in these areas.

Another important acoustic parameter which relates to the clinical utility of AI-700 is opacification of the left ventricle (LVO), which occurs when the chamber fills with blood containing the contrast agent. This can highlight the endocardial border and enable easier visualisation of the regional ventricular wall motion abnormalities (i.e., resulting from prolonged or severe hypoperfusion or infarcted myocardium) and can provide an indication of the extent of CAD. Imaging of wall motion requires sufficient temporal resolution (measured as frame rate [Hz]) to observe the ventricular wall moving in real time (a minimum of approximately 20 Hz). Optimal imaging therefore requires simultaneous imaging of both prolonged perfusion and real-time wall motion. When using AI-700, the ultrasound signal is concentration-dependent and so, when the ventricle fills with blood containing AI-700 the resulting duration of LVO will always be greater than the duration of MCE (the amount of blood in the myocardial circulation is only 5-10% of that present in the ventricle) [Kaul,1997]. Evaluation of perfusion and wall motion requires both MCE and LVO, and the limiting parameter is duration of MCE [Yao, 2002].

The clinical pharmacology studies were designed to assess the degree and duration of LVO and MCE, and to determine optimal equipment settings for AI-700 imaging.

In clinical practice, approximately 1.0-1.5 minutes of LVO and MCE, without significant attenuation, is considered to be required for a diagnostically useful exercise stress ECHO. This 1.0-1.5 minute time requirement corresponds to the duration of time in which valid changes in cardiac function can still be observed after cessation of exercise stress.

Longer duration of enhancement can provide additional clinical utility, particularly under pharmacologic stress conditions where peak stress can be maintained for several minutes.

In 3 of the 4 clinical studies that enrolled CAD patients (AI-700-21, AI-700-32, and AI-700-33), the duration of MCE was not characterised beyond 5 minutes. This was because in the majority of patients, full examination of the myocardium was completed within 5 minutes, and additional imaging had the potential to cause additional minor discomfort to patients without offering any clinical benefit.

In Studies **AI-700-02 and AI-700-21**, individual doses of 0.014-0.108 mL/kg AI-700 were evaluated for duration of MCE using various low-power (non-linear, real-time perfusion) imaging techniques and moderate to high power (harmonic greyscale, power Doppler) imaging techniques with frame rates varying from 1-30 Hz. The level of effective MCE was shown to be dependent upon the AI-700 dose in these 2 studies and upon the imaging mode employed and AI-700 dose (0.054 or 0.081 mL/kg) in Study AI-700-20. These studies showed strong dependence of the duration of MCE on injection rate, imaging mode, acoustic power, and frame rate. Longer MCE duration was associated with lower acoustic power and lower frame rates.

In Studies **AI-700-02 and AI-700-20**, the duration of injection was determined by the specifics of the protocol, with no flexibility to adjust dosing rate and this resulted in excessive attenuation in some patients.

Study **AI-700-20** was a phase 2 study of safety and myocardial imaging in healthy volunteers and in patients with coronary artery disease**.** This was designed as an open label study with paired comparison in which each subject was to serve as his own control for safety and efficacy. There were two stages with a pilot in healthy volunteers and an open stage with clinically stable patient with CAD who had a perfusion defect localised by SPECT. The pilot was conducted to define optimum ultrasound instrumentation for the open stage.47 subjects including 35 with CAD received AI-700. The study suggested that doses of 2.0mg/kg and 3.0mg./kg (higher than the proposed marketing dose) were tolerated by patients and volunteers with flushing in 25%, headache and cough 9%, and nausea and dyspnoea in 6%.This study was an exploratory study using doses which were greater than those being proposed for use with the marketed product

In Study **AI-700-02**, the optimal dose for use with harmonic greyscale or harmonic power Doppler imaging techniques was in the range of 0.054 to 0.108 mL/kg. Below this dose range, the duration and/or extent of enhancement was too low to permit successful imaging. Above this dose range, the increase in attenuation could obscure the observation of critical cardiac structures for an unacceptably long fraction of the imaging session.

Low-power, real-time perfusion imaging techniques first became available on many ultrasound imaging systems just prior to Study AI-700-20. These imaging techniques were designed to allow for simultaneous imaging of myocardial perfusion and wall motion.

Imaging could be performed at lower mechanical indices (0.2-0.3) than greyscale harmonic techniques (0.6-1.3), resulting in less destruction of the contrast agent. This was hypothesised to have a net effect of increasing the duration of MCE. This benefit had a trade-off, in that low-power imaging techniques do not allow penetration of the myocardium as well as techniques that use higher mechanical indices. In addition, the lower frame rates, compared with those used with higher power techniques, result in a reduction of the ability to image wall motion and to acquire satisfactory images in patients with poor acoustic windows.

The results of Study **AI-700-20** demonstrated that contrast-enhanced images were evaluable with minimal attenuation at a lower AI-700 dose (0.054 mL/kg), using low-power imaging techniques. High-power imaging techniques (harmonic greyscale imaging at mechanical indices of 0.8-1.3) required more contrast, resulting in better images at a dose of 0.081 mL/kg.

The poor performance of the low-power imaging techniques at the higher AI-700 dose was attributed to excess attenuation, secondary to the mandated injection rate (which was too rapid for the more sensitive low-power imaging technique). This study demonstrated the need to allow the rate and therefore the duration of AI-700 injection to be modified based upon the observed image enhancement characteristic (real-time feedback from ultrasound images to the person administering the contrast agent). Different doses of AI-700 would be needed, depending on what imaging mode, frame rate, and mechanical index were to be used.

Study **AI-700-21** was a Phase 2, Placebo-Controlled, Safety and Myocardial Imaging Study of AI-700 in Healthy Volunteers and in Patients with Suspected Ischaemic Heart Disease. The objectives of this study were to evaluate the safety of AI-700 administered intravenously to healthy volunteers at rest and patients with suspected ischaemic heart disease at rest and under pharmacologic stress; and to evaluate the sensitivity and specificity of AI-700 for detecting myocardial perfusion defects in patients with suspected ischaemic heart disease.

The study was to be conducted in 3 stages: the Pilot, Bolus, and Ancillary Stages. Each stage was to include 2 imaging sessions: Imaging Session I and Imaging Session II. In the Pilot Stage, echocardiographic (ECHO) contrast imaging of healthy volunteers was to be performed at rest during Imaging Sessions I and II. In the Bolus and Ancillary Stages, ECHO contrast imaging and single-photon emission computed tomography (SPECT) imaging were to be performed in patients with suspected ischaemic heart disease at rest in Imaging Session I, and under pharmacologic stress (induced with dipyridamole) during Imaging Session II. SPECT was to be performed for all patients enrolled in the Bolus and Ancillary Stages to provide a reference standard against which evaluation of myocardial defect detection from ECHO was to be compared. ECHO and SPECT images were to be evaluated for myocardial defects by modality-specific panels of 3 independent readers who were blinded to subject dose group, subject medical history, and institutional reader results.

Efficacy: To meet the primary efficacy endpoint, 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.

Enrolled subjects were to receive 2 doses of AI-700 or placebo, 1 dose per imaging session

The study enrolled a total of 122 subjects: 25 healthy volunteers in the Pilot Stage and 97 patients in the Bolus and Intermediate Dose Stages (22 patients received placebo and 75 patients received AI-700). Because ultrasound machines from several different manufacturers were used for image acquisition in this study, each clinical site was to determine its own optimum imaging parameters (with approval from the applicant) for visualisation of the myocardium. These imaging parameters were to remain consistent for the duration of the study.

The primary efficacy analysis was based on the majority assessment from the results of the 3 independent blinded readers for detecting myocardial defects when comparing SCE to stress SPECT on a subject-by subject basis for each of the 4 patient dose groups (ITT Population). Specificity ≥80% was obtained for all 4 patient dose groups (range: 80% to 93%), but only the lowest (0.028 mL/kg) and highest (0.108 mL/kg) AI-700 dose groups had values for sensitivity ≥60%. Both the 0.028 mL/kg and 0.108 mL/kg AI-700 dose groups met the primary endpoint for sensitivity and specificity, with at least 1 of these 2 statistics being greater than 10% above placebo levels.

Table 29: SCE vs. Stress SPECT, Subject-by-Subject Comparison, Majority Blinded Read Assessment (ITT Population)

Dose Group (Total, mL/kg)	N	Sensitivity (%)	Specificity (%)	Agreement (%)	PPV (%)	NPV (%)
CAD Patients						
AI-700 (0.108)	28	62	93	79	89	74
AI-700 (0.028)	25	63	82	76	63	82
Placebo	22	43	80	68	50	75
AI-700 (0.081)	22	33	88	73	50	78
Healthy Volunteers						
AI-700 (≤0.108)	17	*	94	*	*	*

Source: Section 14.2, Table E-1.1
*Healthy volunteers did not have SPECT imaging as a reference standard and were presumed to be CAD-free; therefore, sensitivity, agreement, and predictive positive and negative values could not be derived.

The results of Study AI-700-21 suggested that total AI-700 doses of 0.028 mL/kg and 0.108 mL/kg administered during dual injection rest-stress echocardiographic imaging were equivalent for the detection of myocardial defects in patients with suspected ischaemic heart disease, obtaining sensitivity results ≥62% and specificity results ≥82%. The 0.081 mL/kg AI-700 dose did not meet the primary endpoints for sensitivity and specificity.

Although numbers of patients are relatively small the sensitivity and specificity for placebo and the intermediate dose which is closest to the marketed dose, are not very different. Sensitivity of placebo is 43% compared to 33% with AI-700.

The study showed not surprisingly that all 4 single doses tested (0.014, 0.04, and 0.054 mL/kg AI-700 in patients; ≤0.054 mL/kg AI-700 in healthy volunteers) produced different durations of LVO and MCE. Some titration of the dosing rate was allowed in Study AI-700-21 (dosing was to be completed within 1 to 5 minutes), which mitigated attenuation at all but the highest AI-700 dose. At the highest dose, sufficient attenuation occurred to decrease the duration of effective MCE as well as LVO, such that the intermediate dose (0.04 mL/kg) produced longer mean effective enhancement and had the highest percentage of patients having MCE greater than 4 minutes.

More importantly, all patients at this dose had at least the 2 minutes of MCE needed to perform a stress study.

Although the highest duration of effective MCE occurred at a cumulative dose of 0.081 mL/kg (0.046 mL/kg [rest imaging] followed by 0.035 mL/kg [stress imaging]), adequate MCE still occurred in a substantial fraction of patients at the low cumulative AI-700 dose of 0.028 mL/kg (0.014 mL/kg during rest and stress imaging), suggesting that the minimally efficacious dose is patient-specific.

Therefore, the conclusion was that all patients should receive at least the low cumulative dose (0.028 mL/kg), but that it may be necessary to use up to the cumulative AI-700 dose of 0.081 mL/kg to achieve diagnostic results in some patients during dual-injection rest-stress imaging. Results from Study AI-700-21 also suggested that subject body habitus may have an effect on the duration of MCE. More healthy volunteers (≤0.054 mL/kg dose group) had MCE greater than 5 minutes, compared with MCE obtained using the same dose in patients with a history of chest pain. This was attributed to the perceived better ECHO acoustic window quality in healthy volunteers.

The results of Studies AI-700-02, AI-700-20, and AI-700-21 illustrate the limitations of fixed dosing, namely that because fixed doses were assigned without regard to body habitus or imaging mode, too little or too brief MCE was obtained in some patients and too much enhancement (with concomitant visual attenuation) was observed in others. In addition, attenuation was exacerbated by the less flexible injection duration of these pre-Phase III trials.

These data therefore support the use of dosing-to-effect, whereby:

- contrast would be administered at a rate dictated by a real-time review of the contrast-enhanced images

- contrast dosing would conclude after all imaging necessary to make a diagnostic assessment was completed (not based on when a dose was administered in its entirety)

This dose-to-effect method should result in all patients obtaining the minimally efficacious dose.

Since the cumulative AI-700 dose of 0.081 mL/kg administered in Study AI-700-21 resulted in the best combination of MCE and low attenuation with all patients having at least 2 minutes of MCE, this dose (nominally 0.080 mL/kg) was chosen as the maximum dose for Phase III studies.

Discussion on clinical pharmacology

The pharmacokinetic properties of PFB gas were evaluated in preclinical and clinical studies, with microsphere metabolism being investigated in preclinical studies only.

The PK of AI-700 depends in part upon disposition of the microspheres, which have a mean diameter between 2.0 and 2.6 µm and their size allows for their transpulmonary passage. Preclinical studies demonstrated that AI-700 microspheres are cleared from the vascular space and taken up by cells of the reticulo-endothelial system (RES). Microspheres remain within RES macrophages until the polymer is hydrolysed and eventually eliminated.

The insoluble PFB rapidly leaks out of the microspheres and is eliminated via the lungs. After i.v. administration of AI-700 in rats, 70% of the PFB was eliminated in expired air within 3 hours after dosing. In addition, 50-85% of the palladium-labelled microspheres were cleared from the circulation within 5 to 7 minutes after completion of dosing. By 24 hours post-dose, the majority of the recovered palladium-labelled microspheres were localised in organs within the RES system.

Human PK studies also demonstrated rapid clearance of PFB. A 2-compartment simultaneous fit of data for blood and expired air showed that, following i.v. injection, PFB 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 within 10 minutes after completion of dosing, and almost 75% was cleared within 1 hour in healthy volunteers.

In patients with COPD and/or CHF, clearance of PFB was so rapid that very few samples showed measurable amounts of gas in the blood after completion of dosing. In this study there were mean changes in FEV1 in the moderate COPD cohort. Although these changes were subclinical and not accompanied by changes in SaO2, the Sponsor elected not to enrol a cohort of severe COPD patients into this study, because severe COPD patients might be less able to adjust to any changes in functional lung volume. The mean differences in spirometry were most notable at 15-20 minutes following the second dose of AI-700. At this time point, all patients receiving AI-700 had some decrease in FEV1 (range: decrease of 0.08 L to 0.80 L).

Pharmacokinetic studies have not been assessed in children, in renal or hepatic impairment.

There were no specific studies in older people although some older patients were included in the studies. The Applicant also carried out an analysis relating efficacy to age (and an increased prevalence of CAD in older patients).

The pharmacodynamics of AI-700 have been well characterised and relate to the duration of effective MCE, with an MCE sufficient to allow a comprehensive examination of the myocardium being required.

The duration of MCE is dependent upon multiple factors such as dose and injection rate of AI-700, but also acoustic characteristics for each patient (which includes the position of the heart, the space between ribs, and the degree of attenuation secondary to variations in the anatomy of chest cavities, presence of underlying disease (e.g. lung) which interferes with the transmission of the ultrasound beam), duration of time the contrast agent remains in the ventricle (which is related to ejection fraction and heart rate) and the ultrasound equipment and settings used.

Conclusions on clinical pharmacology

Pharmacokinetic studies relate only to the PFB part of Imagify and this has been adequately characterised. Only PFB concentrations could be assessed in order to estimate PK parameters. Performing studies of the clearance of microspheres of AI-700 following i.v. administration was not possible, as the microspheres are not water soluble and cannot be separated from the blood.

The pharmacodynamics of AI-700 have been well characterised and relate to the duration of effective MCE, with an MCE sufficient to allow a comprehensive examination of the myocardium being required.

Clinical efficacy

Dose-response studies and main clinical studies

The two Phase III AI-700 efficacy trials (Studies AI-700-32 and AI-700-33) enrolled patients from 28 sites representing 9 countries and 3 continents, with approximately 45% of patients coming from the EU. Sites represented a variety of clinical practice settings and included private, public, and academic, with institutions situated in urban, suburban, and rural settings. Enrolled patients ranged from 33 to 83 years of age, and represented a heterogeneous demographic profile, with 25% female, 29% non-White, and 11% Hispanic.

1,241 subjects received either AI-700 or placebo in 11 clinical studies of AI-700, including 1,194 subjects who received any amount of AI-700 and 66 subjects who received placebo. A total of 911 (76.3%) of these 1,194 subjects were patients with suspected CAD and recent history of chest pain who were to receive the intended maximum dose of 0.08 mL/kg AI-700 administered as 2 intravenous (i.v) injections (each of 0.04 mL/kg) during same-day rest and pharmacologic (dipyridamole) stress ECHO imaging sessions.

The Phase III studies included the 2 pivotal safety and efficacy studies mentioned above as well as 2 method development studies (AI-700-23 and AI-700-34). Study designs, particularly the

Phase III studies, were conducted in accordance with CHMP Points to Consider on the Evaluation of Diagnostic Agents.

The applicant points out that for each Phase III study, 3 independent, monitored, blinded readers were used for the assessment of the AI-700 ECHO images, and independent core laboratories and blinded readers were used to assess the comparative standard (SPECT) and the truth standard (ANGIO). These blinded readers were selected from institutions that were not participating in patient enrolment.

The blinded readers were also trained extensively (using images acquired from prior AI-700 clinical studies) and standard reading stations were provided.

Study AI-700-23: A Pilot Phase III International, Multicentre, Open-Label, Dual Injection Myocardial Imaging and Safety Study of AI 700 in Patients with Suspected Ischaemic Heart Disease.

Objectives

The objectives of this study were to familiarise investigators and clinical sites in the use of AI-700 imaging techniques, to assess the appropriateness and feasibility of planned safety measures for the Pivotal Phase III studies, and to assess the Phase III methods for collecting AI-700 ECHO accuracy, sensitivity and specificity data for detecting CAD when compared with available ANGIO and SPECT imaging data.

Investigators/clinical sites participating in this Pilot Phase 3 study were to be considered sufficiently familiar with AI-700 imaging techniques and study procedures if they were able to:

- perform all required imaging and safety evaluations in accordance with the study protocol,
- obtain acceptable AI-700 contrast images using ultrasound machine settings designated in the Investigator's AI-700 ECHO and SPECT Imaging Manual,
- collect accurate safety and imaging data as specified in the protocol, in a timely manner.

There was a Blinded Reader Training Subgroup of 18 patients (13.5% of patients) whose AI-700-23 ECHO images were reviewed by the applicant and were selected to evaluate the ability of 6 independent ECHO Blinded Readers to accurately interpret similar images to be collected in the planned Phase 3 efficacy studies of AI-700.

Study Design

This was a pilot Phase III, open-label, dual-injection study. 133 patients were enrolled at 35 sites in the United States, Canada and in the EU. Patients with suspected or previously diagnosed CAD underwent an exclusion ECHO (to exclude patients having clinically significant intra-cardiac shunts or any significant cardiovascular structural and/or functional abnormality), a baseline reference ECHO (to assess patient imaging quality and to optimise equipment settings for subsequent contrast images), a rest contrast ECHO (to evaluate myocardial defects following the first injection of AI-700), and a stress contrast ECHO (to evaluate myocardial defects following induction of pharmacological stress by administration of dipyridamole followed by a second AI-700 injection). Some patients also underwent optional SPECT imaging performed during rest and stress. ANGIO and any available left ventriculography (LVG) data were also collected from patients who had cardiac catheterisation performed within 30 days prior to or following AI-700 administration. All patients received two bolus injections of 0.04 mL/kg AI-700 (over a maximum duration of 5 minutes each), giving a total dose of 0.08 mL/kg. The first injection was administered during rest ECHO imaging and then at least 1 hour later (to allow a full battery of longitudinal safety evaluations to be performed), the second injection was given for stress ECHO imaging (following the infusion of 0.56 mg/kg dipyridamole). At the conclusion of stress imaging, aminophylline was administered as an antagonist to the vasodilatory stressor to rapidly reduce symptoms associated with stress testing.

Efficacy Results

All contrast AI-700 ECHO and SPECT images were qualitatively evaluated for myocardial defect location by expert readers. Available ANGIO and LVG data were also evaluated by the investigator at each site and by an independent ANGIO core laboratory. Significant CAD was defined as the presence of significant coronary artery stenosis on ANGIO or the presence of regional wall motion abnormalities on LVG. Individual site difficulties in obtaining ECHO and SPECT imaging and ANGIO data were identified during the study and appropriately addressed to ensure that an efficient data collection process was established for the subsequent Phase III efficacy studies.

Following a site-by-site review of the study procedures, 28 of the 35 investigators/clinical sites were considered proficient in all study procedures i.e. were competent at ECHO, SPECT, and ANGIO imaging and had the logistical infrastructure to follow the protocol requirements.

Each of these 28 clinical sites subsequently went on to participate in the Phase III efficacy studies (AI-700-32 or AI-700-33). Six echocardiologists were chosen based on their review of a subset of very clear ECHO cases from this study and then trained to serve as blinded readers in the Phase III efficacy studies. All six blinded readers demonstrated good diagnostic performance for all parameters: accuracy (75% to 94%), sensitivity (75% to 100%), and specificity (75% to 100%).

Safety

There was a minimal but consistent trend for oxygen saturation to decrease after both injections of AI-700. This decrease was not clinically relevant, with the vast majority of patients maintaining levels in excess of 95%. Maximum absolute mean decreases were (CSR AI-700-23)<1% during both imaging sessions. High frequency pulse oximeter monitoring captured approximately 3800 values, as SaO2 measurements were performed on average every 90 seconds during the first 15 minutes post dosing. Sixteen (12.0%) of 133 patients had decreases from Baseline in SaO2 that were to less than 90%, and/or had consecutive decreases of at least 5% from Baseline). Two of these patients experienced mild clinical symptoms, each reported as an AE (dyspnoea, respiratory rate increased).

Summary

The majority (80%) of the investigators/clinical sites that participated in this pilot Phase III study were able to demonstrate proficiency in the use of AI-700 imaging techniques and other protocol requirements. The study design, including AI-700 dosing methods, imaging parameters, and reference data collection methods was assessed as being appropriate for use in the Phase III efficacy studies. Six ECHO blinded readers were able to adequately interpret a subset of AI-700 ECHO images from this study with good results for accuracy, sensitivity, and specificity.

Study AI-700-32: A Phase III, International, Multicentre, Open-Label, Dual-Injection, Echocardiographic Imaging and Safety Study of AI-700 in Patients with Suspected Ischaemic Heart Disease Undergoing Single-Photon Emission Computed Tomography.

Objectives

The objectives of this study were to determine the accuracy, sensitivity, and specificity of AI-700-enhanced ECHO imaging for assessing CAD in patients being evaluated for inducible ischaemia, to determine if the diagnostic accuracy of AI-700 ECHO was non-inferior to SPECT in the same patients,

and to demonstrate the safety of AI-700 administered intravenously to patients with suspected ischemic heart disease.

Study Design

This was an open-label, safety and blinded efficacy study of the ability of AI-700 to detect CAD based on assessment of myocardial perfusion and wall motion in patients with known or suspected ischaemic heart disease. All patients underwent two AI-700 ECHO imaging sessions: a rest contrast ECHO in Session I, followed by a stress contrast ECHO in Session II. The stress testing used pharmacologic stress induced by dipyridamole (0.56 mg/kg, infused over 4 minutes), with aminophylline or theophylline used after the stress test as the stress antagonist.

SPECT myocardial perfusion imaging using gating and quantification was the comparative standard, with patients having SPECT carried out on the same day, using Technetium-99m (99mTc)-labelled sestamibi imaging before and after the same dipyridamole administration as used for ECHO. Where available, ANGIO or LVG results were used as the truth standard for CAD. An alternative truth standard, including patient history and unblnded SPECT review, was used when ANGIO was not available. Three independent ECHO blinded readers evaluated the ECHO images for myocardial defect detection and localisation (via an integrated assessment of perfusion and wall motion). SPECT images with quantification were evaluated in a similar manner by an independent SPECT blinded reader. An independent core laboratory evaluated the ANGIO/LVG data.

Patients were dosed by body weight, with each patient receiving two separate series of small bolus injections for a total of 0.04 mL/kg AI-700 (over a maximum 10-minute duration) at the resting and stress imaging evaluations, giving an overall total AI-700 dose of 0.08 mL/kg.

Imaging sessions occurred on the same day and were separated by a period of at least 1 hour to allow for longitudinal safety evaluations.

Conduct of the study

Study enrolment began in December 2003 following discussion with the US FDA Division Medical Imaging and Radiopharmaceutical Drug Products. Further discussions with FDA in March 2005 revealed that the FDA preferred null hypothesis testing of the primary efficacy endpoints, where success criteria are based on pre-specified sensitivity and specificity thresholds. After discussion with FDA regarding the use of individual blinded reader results instead of a majority blinded read score, the proposed sensitivity and specificity criteria were revised based on the weighted mean average of the individual blinded read results.

Subsequently (June 2005), FDA recommended that the study design be changed to a non-inferiority analysis of ECHO vs. SPECT imaging, with ANGIO/LVG as the preferred truth standard:

- Historical gating criteria were to be prospectively defined to ensure adequate performance of the SPECT comparator, and

- Primary efficacy was to be based on independent achievement of the non-inferiority margin by at least 2 of the 3 ECHO blinded readers.

One important consequence of adopting a non-inferiority approach, at FDA's request, well into the conduct of the study, was that many patients were enrolled without the preferred truth standard of ANGIO/LVG data. This required the design of an alternative truth standard (which included review of CAD status at 90 days post dose, and/or review of unblinded SPECT data by a cardiologist certified to interpret SPECT, the non- blinded CAD reviewer).

Accordingly, the protocol and the statistical plan were amended to include a non-inferiority primary efficacy analysis based on FDA recommendations, with an alternative truth standard for patients who did not have ANGIO data, and modified sample size estimates.

The statistical methods and plan were revised and data lock occurred in May 2006.

The selection of margins for the non-inferiority analyses was based primarily on clinical considerations. As no data were available the applicant chose a margin that is described as "conservative, yet achievable". The selected margin used the relative risk ratio approach of 0.83 for accuracy, sensitivity and specificity.

Inclusion/Exclusion

Inclusion: Eligible patients were clinically stable men and non-pregnant/non-lactating women 18 to 80 years, who were being evaluated for the presence of inducible ischemia, and were indicated for SPECT perfusion imaging. Patients were to have a low to intermediate probability of CAD based upon recent history of angina, with or without prior history of MI.

Exclusion: Candidates with any of the following were to be excluded: any clinically unstable conditions within 7 days prior to AI-700 dosing; an acute MI, cerebrovascular accident, or transient ischaemic attack within 30 days of dosing; severe congestive heart failure; significant left main CAD; oxygen saturation (SaO2) <90% at rest; or moderate to severe chronic obstructive pulmonary disease. Patients with a history of MI of non-CAD aetiology or who exhibited new or changing electrocardiogram (ECG) abnormalities between Screening and AI-700 dosing were also excluded, as were patients with prior coronary artery bypass graft (CABG) within 6 months.

Patient Characteristics at Study Entry: The study enrolled 216 males (67.3%) and 105 females (32.7%). Most patients were White (195, 60.7%); there were 62 (19.3%) Asian, 51 (15.9%) Black, and 13 (4.0%) patients identified as "other" race. Twelve patients (3.7%) were of Hispanic ethnicity. Mean age was 61 years (range: 33 to 81 years). At Screening, 309 patients (96.3%) had a history of or current angina pectoris, 241 (75.1%) had a history of hypertension, and 235 (73.2%) had hyperlipidaemia.

As measured by the truth standard, CAD prevalence was 44% in this study population.

Primary efficacy: The study 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. Primary analysis (MITT Population) involved sequential non-inferiority testing of the ratios (≥ 0.83; alpha=0.025) of AI-700 ECHO to SPECT diagnostic statistics, first for accuracy followed by the accuracy components of sensitivity and specificity. Each of the 3 ECHO readers was compared to SPECT Reader 1. 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. For the primary efficacy analysis, any AI-700 SCE images that were unavailable or unevaluable for any reason were considered discordant to the truth standard.

Efficacy Results

A total of 321 patients were enrolled at 17 clinical sites in North America, Europe and Australia, of which 119 patients had ANGIO or LVG results. After the blind was broken, 81 patients were found to be positive for CAD based on ANGIO or LVG results, and a further 44 patients without ANGIO or LVG were considered positive for CAD based on documented MI.

Overall therefore, in the MITT Population, 125 patients (43.9%) had disease; 160 patients (56.1%) had no disease. Disease prevalence was similar in the Per-Protocol Population.

The primary analysis (based on the modified Intent-to-Treat [mITT] population, 285 patients) involved hierarchical non-inferiority testing of the ratios (≥0.83; alpha=0.025) of AI-700 ECHO to SPECT diagnostic statistics versus the truth standard.

Null hypothesis (H0):

- ECHO is inferior to SPECT for accuracy of CAD detection (for at least 2 of 3 ECHO readers)

Alternative hypothesis (HA):

- 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 were calculated. If the lower bound of the 2-sided 95% CI for relative risk ratio was >0.83 for at least 2 of the 3 AI-700 ECHO blinded readers, then the primary efficacy endpoint was met. The ratio of the accuracy of each AI-700 ECHO reader to the accuracy of SPECT reader 1 (MITT Population) was evaluated with a margin of 0.83.

Accuracy was to be tested first and if accuracy met the non-inferiority criterion, then the components of accuracy - sensitivity and specificity - were tested. Each of the three ECHO readers was compared to the SPECT reader. The criteria for success were that two of the three ECHO blinded readers had to demonstrate non-inferiority. To ensure the adequate performance of the SPECT comparative standard, the SPECT reader was required to achieve pre-determined levels of sensitivity (set at 76%) and specificity (set at 59%) (determined from a literature review and on agreement with the FDA) compared to the truth standard. The SPECT reader had a sensitivity and specificity of 77.6% and 63.8%, respectively and thus met these predefined criteria.

All 3 ECHO readers had accuracy estimates that were statistically non-inferior to that of SPECT 66.3%, 67.0%, 71.2% and 69.8% for ECHO Readers 1-3 and SPECT Reader 1, respectively; p ≤ 0.004). Greater variation was observed in ECHO reader sensitivity (76.8%, 56.8%, and 49.6% for ECHO Readers 1-3, respectively) and specificity (58.1%, 75.0%, and 88.1%, for ECHO Readers 1-3, respectively). The sensitivity for ECHO Reader 1 was non-inferior to SPECT (p = 0.002). The specificity for ECHO Readers 2 and 3 was superior to SPECT (p ≤ 0.006).Therefore only one reader (ECHO 1) achieved non-inferior sensitivity, while the other two readers (ECHO 2 and 3) showed superior specificity to SPECT. Sensitivity and specificity are inversely related, and therefore these results are expected given the tendency of individual readers to be slightly more or less aggressive for identification of smaller defects. The MAH suggests that the results from this study suggest that there was one “aggressive” SPECT reader compared to one “aggressive” ECHO reader and two conservative ECHO readers.

Table 28: Non-Inferiority Analysis: ECHO Concordance vs. SPECT Concordance with the Truth Standard for Defect Detection (MITT Population)

Truth	SPECT Reader 1			ECHO Reader 1			ECHO Reader 2			ECHO Reader 3			ECHO Majority		
	Disease	No Disease	Total	Disease	No Disease	Total	Disease	No Disease	Total	Disease	No Disease	Total	Disease	No Disease	Total
Disease	97	28	125	96	29	125	71	54	125	62	63	125	75	50	125
No disease	58	102	160	67	93	160	40	120	160	19	141	160	41	119	160
Total	155	130	285	163	122	285	111	174	285	81	204	285	116	169	285
Accuracy		**69.8%**			**66.3%**			**67.0%**			**71.2%**			68.1%	
RR					0.950			0.960			1.020			0.975	
95% CI					0.862, 1.045			0.872, 1.056			0.928, 1.122			0.888, 1.069	
p-value					0.004			0.002			<0.001			<0.001	
Sensitivity		**77.6%**			**76.8%**			**56.8%**			**49.6%**			60.0%	
RR					0.990			0.732			0.639			0.773	
95% CI					0.882, 1.110			0.626, 0.836			0.535, 0.741			0.669, 0.874	
p-value					0.002			0.968			>0.999			0.869	
Specificity		**63.8%**			**58.1%**			**75.0%**			**88.1%**			74.4%	
RR					0.912			1.176			1.382			1.167	
95% CI					0.777, 1.065			1.037, 1.346			1.240, 1.570			1.028, 1.334	
p-value					0.120			<0.001			<0.001			<0.001	
p-value for superiority					0.879			0.006			<0.001			0.009	

Source: Section 14.2.1, Table 11.4.1.

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

To evaluate the consistency of the primary efficacy analysis, the analysis was repeated in sub-populations and expanded versions of the mITT population. Results of these analyses were similar to those of the primary efficacy analyses. Logistic regression analyses were also performed to determine the odds ratio (OR) of CAD associated with AI-700 defect detection.

These analyses demonstrated that the OR of CAD increased with the number of abnormal myocardial regions detected by AI-700 ECHO and that the ORs were similar to those for SPECT. Secondary and tertiary efficacy analyses evaluated ECHO and SPECT readers’ sensitivity and specificity for disease detection under a variety of different situations. These results also confirmed the primary efficacy conclusions. Among the 91 patients in the Per Protocol (PP) population with ANGIO but no prior coronary artery bypass graft (CABG) or confirmed MI, the ECHO majority sensitivity estimates demonstrated that as disease severity increased (as measured by GJS), AI-700 ECHO sensitivity increased. This was observed in both patients with disease defined as ≥ 50% diameter stenosis (used

in some CAD efficacy trials in the literature) and disease defined as ≥ 70% diameter stenosis (as used in this trial). When disease severity was analysed as a function of the number of vessels involved, all readers had lower sensitivity estimates in early stage disease (single-vessel) and higher sensitivity estimates in late stage disease (multi-vessel). Analysis of regional disease detection (anterior, lateral or infero-posterior: correlating with the regions served by the 3 major coronary arteries) showed that ECHO had numerically higher accuracy and specificity compared to SPECT. Paired agreement for regional disease detection between the SPECT reader and each of the three ECHO readers demonstrated statistically significant results in all cases ($p<0.001$). Inter-reader agreement was also statistically significant ($p<0.001$) for all possible combinations of ECHO readers, and intra-reader agreement was similar for the 3 ECHO readers and the SPECT reader. Subgroup analyses suggested no appreciable differences in disease detection by gender or body mass index (BMI).

Accuracy remained fairly consistent among the 4 readers (range of 66.3-71.2% for the ECHO readers; 69.8% SPECT Reader 1), with no statistically significant differences between any pair of readers.

Sensitivity ranged from 49.6-76.8% for the ECHO readers; sensitivity was 77.6% for SPECT Reader 1. Specificity ranged from 58.1-88.1% for the ECHO readers; specificity was 63.8% SPECT Reader 1.

Conclusion

This study met the requirement regarding accuracy and suggested that AI-700 is as accurate as quantitative 99mTc-labelled sestamibi SPECT imaging in detecting CAD in patients with chest pain who are being evaluated for inducible ischaemia, based on an assessment of perfusion and wall motion.

It did not meet the endpoints regarding sensitivity. Intra- and inter-methodology differences between ECHO and SPECT readers in sensitivity and specificity were attributed to differences in each reader's disease detection bias and are not considered by the applicant as indicative of superior or inferior performance for either method regarding sensitivity or specificity.

To minimise AI-700 and SPECT reader bias issues in the subsequent trial (which was already running), the 3 new AI-700 readers were retrained to more aggressively read smaller observed defects as disease, and 3 SPECT readers were selected instead of just the one used in this study.

Study AI-700-33: A Phase III, International, Multicentre, Open-Label, Dual-Injection, Echocardiographic Imaging and Safety Study of AI-700 in Patients with Suspected Ischaemic Heart Disease Undergoing Diagnostic Coronary Angiography.

Objectives

The primary objectives of this study were to estimate the accuracy, sensitivity, and specificity of AI-700-enhanced ECHO imaging for assessing CAD in patients being evaluated for inducible ischemia, to determine if the diagnostic accuracy of AI-700 ECHO was non-inferior to SPECT in the same patients, and to demonstrate the safety of AI-700 administered i.v. to patients with suspected ischemic heart disease.

Study Design

This was an open-label, safety and blinded efficacy study in 11 sites in the US and Europe of the ability of AI-700 to detect CAD based on assessment of myocardial perfusion and wall motion in patients with known or suspected ischemic heart disease. All patients underwent two AI-700 ECHO imaging sessions: a rest contrast ECHO in Session I, followed by a stress contrast ECHO in Session II. The stress testing used pharmacologic stress induced by dipyridamole (0.56 mg/kg, infused over 4 minutes), with aminophylline or theophylline used after the stress test as the stress antagonist. SPECT myocardial perfusion imaging using gating and quantification was the comparative standard, with about half of the patients having SPECT carried out on the same day, using two injections of 99mTc radionuclide, one at rest and the other under the same vasodilatory stress conditions as the ECHO images. Coronary ANGIO and, if applicable, LVG were used to provide a truth standard to calculate the accuracy, sensitivity, and specificity of ECHO and SPECT in evaluating the presence or absence of CAD. An alternative truth standard, including patient history and unblinded SPECT review, was used in the few patients when ANGIO was not available. Three independent ECHO blinded readers evaluated the ECHO images for myocardial defect detection and localisation (via an integrated assessment of perfusion and wall motion).

After the results of Study AI-700-32 had been evaluated and showed that 2 of the 3 readers had exhibited conservative bias (undercalled disease), the 3 different readers in this study, while still blinded to all data, were retrained to minimise this type of bias. Following this retraining, blinded reading was re-initiated for all the images in the study. Three independent SPECT-blinded readers evaluated SPECT images quantitatively for myocardial defect detection and localisation, similarly to the AI-700 readers.

An independent laboratory evaluated the ANGIO/LVG data.

Patients were dosed by body weight, with each patient receiving two separate series of small bolus injections for a total of 0.04 mL/kg AI-700 (over a maximum 10-minute duration) at the resting and stress imaging evaluations, giving an overall total AI-700 dose of 0.08 mL/kg.

AI-700 imaging sessions were to occur on the same day and were separated by a period of at least 1 hour to allow for longitudinal safety evaluation.

Inclusion/Exclusion Criteria

Eligible patients were 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.

Exclusion**:** Candidates with any of the following were to be excluded: any clinically unstable conditions within 7 days prior to AI-700 dosing; an acute MI, cerebrovascular accident, or transient ischaemic attack within 30 days of dosing; severe congestive heart failure; significant left main CAD; oxygen saturation (SaO2) <90% at rest; or moderate to severe chronic obstructive pulmonary disease. Patients who had a history of MI of non-CAD aetiology or who exhibited new or changing electrocardiogram (ECG) abnormalities between Screening and AI-700 dosing were also excluded. Patients with prior history of CABG were not eligible for enrolment.

Patient Characteristics

The study enrolled 366 males (80.1%) and 91 females (19.9%). Most patients were White (359, 78.6%); there were 33 (7.2%) Black, 6 (1.3%) Asian, and 59 (12.9%) patients identified as “other” race. There were 72 patients (15.8%) of Hispanic ethnicity.

Mean age was 62.4 years (range: 34-83 years). In accordance with the eligibility criteria, all 457 patients (100%) had a history of cardiovascular conditions or were suspected of having CAD. At Screening, 444 patients (97.2%) had history of or current angina pectoris, 351 (76.8%) had hyperlipidaemia, and 348 (76.1%) had hypertension.

As measured by the truth standard, CAD prevalence was 58% in this study population.

Conduct of the Study

Investigators participating in Study AI-700-33 were required to first complete the Pilot Phase 3 Study AI-700-23 and to be trained in the clinical use and evaluation of data acquired from AI-700 contrast imaging. Following completion of Pilot Study AI-700-23, clinical sites that had demonstrated competence conducting AI-700 rest and stress imaging were invited to participate in study AI-700-33 (or in the concurrent study AI-700-32). All patients in Study AI-700-33 were to have a recent prior angiogram (ANGIO) or were to be scheduled for ANGIO at the time of dosing. All patients in AI-700-32 were to have on-study SPECT perfusion imaging.

Study enrolment began in December 2003 with this design, following discussion with the US FDA Division of Medical Imaging and Radiopharmaceutical Drug Products. Further discussions with FDA in March 2005 revealed that FDA preferred null hypothesis testing of the primary efficacy endpoints, where success criteria are based on pre-specified sensitivity and specificity thresholds. After discussion with FDA regarding the use of individual blinded reader results instead of a majority blinded read score, the proposed sensitivity and specificity criteria were revised based on the weighted mean average of the individual blinded read results.

Subsequently (June 2005), FDA recommended that the study design be changed to a non-inferiority analysis of ECHO vs. SPECT imaging, with ANGIO/LVG as the preferred truth standard. FDA also recommended prospectively defined historical gating criteria for SPECT.

Primary efficacy was recommended to be based on independent achievement of the non-inferiority margin by at least 2 of the 3 ECHO blinded readers.

One important consequence of adopting a non-inferiority approach was that patients previously enrolled without evaluable SPECT results would be considered non-evaluable and excluded from the primary analyses. The statistical plan was revised in February 2006 (Revision 1) to include a non-inferiority primary efficacy analysis based on FDA recommendations, with an alternative truth standard for patients without ANGIO data, and modified sample size estimates.

Following further discussions with FDA regarding analysis of imaging data from the previous study, AI-700-32, the Sponsor modified the statistical plan for Study AI-700-33 in April, 2007 (Revision 2) to

eliminate historical gating criteria for SPECT and to increase the number of SPECT blinded readers from 1 to 3.

Efficacy Results

A total of 457 patients were enrolled at 11 clinical sites. After the blind was broken, 219 patients were found to have CAD based on ANGIO or LVG results, and 1 further patient without ANGIO or LVG was considered positive for CAD based on documented MI. The primary analysis (based on the mITT population) involved hierarchical non-inferiority testing of the ratios (≥0.83; alpha=0.025) of AI-700 ECHO to SPECT diagnostic statistics. Accuracy was to be tested first and if accuracy met the non-inferiority criterion, the components of accuracy - sensitivity and specificity - were tested. Accuracy, sensitivity, and specificity were also estimated for each of the three SPECT readers, and the statistic-specific median SPECT reader was identified. Each of the three ECHO readers was then compared with the median SPECT reader. The criteria for success were that two of the three ECHO blinded readers had to demonstrate non-inferiority.

Table 28: Non-Inferiority Analysis: ECHO Concordance vs. SPECT Concordance with the Truth Standard for Defect Detection (MITT Population)

Truth	Median SPECT Reader[1]			ECHO Reader 1			ECHO Reader 2			ECHO Reader 3		
	Disease	No Disease	Total	Disease	No Disease	Total	Disease	No Disease	Total	Disease	No Disease	Total
Disease	134	86	220	160	60	220	149	71	220	161	59	220
No disease	38	119	157	70	87	157	44	113	157	54	103	157
Total	172	205	377	230	147	377	193	184	377	215	162	377
Accuracy	**67.1%**			**65.5%**			**69.5%**			**70.0%**		
RR				0.976			1.036			1.043		
95% CI				0.892, 1.068			0.960, 1.118			0.964, 1.130		
p-value				<0.001			<0.001			<0.001		
Sensitivity	**60.9%**			**72.7%**			**67.7%**			**73.2%**		
RR				1.194			1.112			1.201		
95% CI				1.075, 1.337			1.005, 1.237			1.089, 1.338		
p-value				<0.001			<0.001			<0.001		
p-value for superiority				<0.001			0.020			<0.001		
Specificity	**75.8%**			**55.4%**			**72.0%**			**65.6%**		
RR				0.731			0.950			0.866		
95% CI				0.622, 0.850			0.844, 1.064			0.758, 0.980		
p-value				0.951			0.013			0.259		

Source: Section 14.2.1.1, Tables 11.4.1, 11.4.1.1, and 11.4.1.2.
RR = relative risk (for each diagnostic statistic, RR was defined as the ratio of ECHO to SPECT); CI = confidence interval
[1] The Median SPECT Reader for accuracy, sensitivity, and specificity, was SPECT Reader 1.

The results showed that the accuracy of all 3 ECHO readers was statistically non-inferior to the median SPECT reader. Accuracy was similar for the Median SPECT Reader (67.1%) and

ECHO Readers 1-3: 65.5%, 69.5%, and 70.0%, respectively. The accuracy of each ECHO readers was statistically non-inferior to the Median SPECT Reader ($p < 0.001$).

Sensitivity and specificity showed greater variations between readers. The Median SPECT Reader had a sensitivity of 60.9%. Sensitivity was 72.7%, 67.7%, and 73.2% for ECHO Readers 1-3, respectively. Sensitivity values for all 3 ECHO blinded readers were statistically superior to those of the Median SPECT Reader ($p \leq 0.020$). The specificity for the Median SPECT Reader was 75.8%; ECHO Readers 1, 2 and 3 had specificities of 55.4%, 72.0%, and 65.6%, respectively. The specificity of ECHO Reader 2 was statistically non-inferior to that of the Median SPECT Reader ($p = 0.013$).

Sensitivity values for all three ECHO blinded readers were statistically superior to those of the median SPECT reader and the specificity of ECHO reader 2 was statistically non-inferior to that of the median SPECT reader. As noted for study AI-700-32, this pattern of missing one endpoint and over-achieving on the other was considered by the applicant to reflect the different reader biases towards either aggressive or conservative reading. The 3 SPECT readers showed similar values for accuracy but with a much wider range (one high, one low) for sensitivity and specificity, just like the 3 AI-700 readers in this trial (and the 32 trial).

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Final Analysis

Table 11.4.14
Individual AI-700 ECHO Blinded Readers Diagnostic Performance Compared with Median SPECT Reader For CAD Detection
(ECHO Data Collected Prior to the Retraining of the ECHO Blinded Readers)

	Median Spect Reader	ECHO Reader 1	ECHO Reader 2	ECHO Reader 3
Accuracy	66.8%	60.9%	66.8%	65.8%
Rel. Risk		0.911	1.000	0.985
95% CI		(0.826, 1.003)	(0.915, 1.093)	(0.896, 1.083)
p-value		0.032	<0.001	<0.001
Sensitivity	58.8%	52.5%	63.3%	65.5%
Rel. Risk		0.894	1.077	1.115
95% CI		(0.771, 1.032)	(0.959, 1.216)	(0.985, 1.270)
p-value		0.157	<0.001	<0.001
Specificity	78.0%	72.4%	71.7%	66.1%
Rel. Risk		0.929	0.919	0.848
95% CI		(0.812, 1.059)	(0.799, 1.053)	(0.728, 0.979)
p-value		0.049	0.074	0.383

Acusphere, Inc.
Protocol AI-700-33
Final Analysis

Table 11.4.22
Non-Inferiority Analysis: Individual AI-700 ECHO Blinded Readers Diagnostic Performance Compared with Median SPECT Reader for CAD Detection
(including all patients with any stress SPECT results)

	Median Spect Reader	ECHO Reader 1	ECHO Reader 2	ECHO Reader 3
Accuracy	67.6%	65.2%	69.3%	70.1%
Rel. Risk		0.964	1.025	1.036
95% CI		(0.884, 1.050)	(0.954, 1.103)	(0.960, 1.119)
p-value		<0.001	<0.001	<0.001
Sensitivity	62.6%	72.4%	67.5%	72.4%
Rel. Risk		1.158	1.079	1.158
95% CI		(1.049, 1.286)	(0.981, 1.191)	(1.053, 1.281)
p-value		<0.001	<0.001	<0.001
Specificity	75.0%	54.8%	72.0%	66.7%
Rel. Risk		0.730	0.960	0.889
95% CI		(0.622, 0.847)	(0.857, 1.073)	(0.781, 1.006)
p-value		0.954	0.007	0.143

Retraining of readers

Conduct of the blinded reads for Study AI-700-33 had commenced prior to the unblinding of Study AI-700-32. Following unblinding of the -32 study, it became evident that the -32 study blinded readers

had read images in what was considered by the applicant to be an overly conservative manner (i.e., reading with high specificity). As a result, the decision was made to stop the blinded reads in the -33 study and to re-train the -33 study readers to read with increased sensitivity while maintaining high accuracy.

At no time prior to the unblinding of the -33 study did the Sponsor or the readers have access to the results of the initially read cases from the -33 study.

Following database lock, the primary efficacy analyses were repeated using the 304 patients whose data were read prior to the retraining of the ECHO readers. For these patients, 177 (58.2%) had disease and 127 (41.8%) did not, according to the truth standard.

Following retraining, accuracy values increased for each of the 3 ECHO readers. The increases were small (<5%): 65.5% vs 60.9% (ECHO Reader 1), 69.5% vs 66.8% (ECHO Reader 2), and 70.0% vs 65.8% (ECHO Reader 3). Accuracy for the Median SPECT Reader was 66.8%.

Accuracy values prior to retraining were statistically non-inferior to the Median SPECT Reader for 2 ECHO readers ($p<0.001$ for ECHO Readers 2 and 3). The RRs ranged from 0.911 to 1.000.

Following retraining, sensitivity values increased for each of the 3 ECHO readers. The increases ranged from 4.4% to 20.2%: 72.7% vs 52.5% (ECHO Reader 1), 67.7% vs 63.3% (ECHO Reader 2), and 73.2% vs 65.5% (ECHO Reader 3). Sensitivity for the Median SPECT Reader was 58.8%. Sensitivity values prior to retraining were statistically non-inferior to the Median SPECT Reader for 2 ECHO readers ($p<0.001$ for ECHO Readers 2 and 3). The RRs ranged from 0.894 to 1.115.

Following retraining, specificity values showed little change for 2 of the ECHO readers: 72.0% vs 71.7% (ECHO Reader 2) and 65.6% vs 66.1% (ECHO Reader 3). Specificity following retraining was lower for ECHO Reader 1 (55.4% vs 72.4%). Specificity for the Median SPECT Reader was 78.0%. Prior to retraining, none of the ECHO readers were statistically non-inferior to the Median SPECT Reader. The RRs ranged from 0.848 to 0.929.

The applicant states that the objective of retraining was met (increased sensitivity while maintaining accuracy). In this smaller data set (304 vs. 377 MITT patients in the primary efficacy analysis), reader retraining resulted in a slight improvement in accuracy for all 3 ECHO readers. Retraining readers to more aggressively call disease resulted in higher sensitivity for all 3 ECHO readers.

Specificity values were essentially unchanged for 2 ECHO readers. Thus, training increased the readers' ability to correctly classify diseased patients.

To evaluate the consistency of the primary efficacy analysis, the analysis was repeated in sub-populations and expanded versions of the mITT population. Results of these analyses were similar to those of the primary efficacy analyses. Relative risk (RR) ratios were also determined for all primary efficacy parameters for all ECHO and SPECT pairs. These pair wise comparisons demonstrated some variation among individual pairs of readers; however, taken collectively, the performance of ECHO and SPECT did not differ appreciably.

Logistic regression analyses were also performed to determine the odds ratio of CAD associated with AI-700 defect detection. These analyses demonstrated that the odds of CAD increased with the number of defective myocardial regions detected by AI-700 ECHO.

Secondary and tertiary efficacy analyses evaluated ECHO and SPECT readers' sensitivity and specificity for disease detection under a variety of different situations. These results also confirmed the primary efficacy conclusions. ECHO reader sensitivity estimates demonstrated that as disease severity increased (as measured by GJS), AI-700 ECHO sensitivity increased.

This was observed both in patients with disease defined as ≥50% diameter stenosis (used in some CAD efficacy trials in the literature) and disease defined as ≥70% diameter stenosis (as used in this trial). For the SPECT readers, sensitivity tended to increase as disease severity increased. When disease severity was analysed as a function of the number of vessels involved, all readers had lower sensitivity estimates in early stage disease (single-vessel) and higher sensitivity estimates in late stage disease (multi-vessel). Analysis of regional disease detection (anterior, lateral or inferoposterior: correlating with the regions served by the 3 major coronary arteries) demonstrated that accuracy for all six readers was similar for each of the three myocardial regions, however, sensitivity and specificity varied both by reader and by region. Inter-reader agreement was statistically significant for all possible combinations of ECHO readers ($p<0.001$) and for all possible combinations of SPECT readers ($p<0.001$).

Intra-reader agreement was statistically significant for all three ECHO readers ($p\leq0.002$) and for all three SPECT readers ($p<0.001$). Paired agreement analyses across the two imaging methods showed the percent agreement between individual ECHO and SPECT readers ranged from 63.9% to 74.3%. Paired agreement between ECHO and SPECT majority readings was 72.4%. Subgroup analyses suggest no clinically significant differences in disease detection by gender or BMI.

Conclusion

AI-700 enhanced echocardiography is as accurate as quantitative 99mTc sestamibi SPECT perfusion imaging in detecting CAD in patients with chest pain who had recently undergone or were scheduled to undergo ANGIO, based on an assessment of perfusion and wall motion. Inter- and intra-method differences between ECHO and SPECT readers in sensitivity and specificity were attributed to differences in individual reader's disease detection bias and are not indicative of superior or inferior performance for either method regarding sensitivity or specificity.

Summary of main efficacy results

The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 10: Primary Efficacy Results from AI-700 Phase III Efficacy Studies

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		0.950	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
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		0.990	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
p-value[2]		0.002*	0.968	>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		0.912	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
p-value[2]		0.120	<0.001*	<0.001*		0.951	0.013*	0.259
p-value for superiority		0.879	0.006	<0.001				

Source: Module 5, AI-700-32 and AI-700-33 CSRs

RR=relative risk 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

Clinical studies in special populations

Small PK studies were performed in patients with CCF and COPD but there were no other studies in special populations.

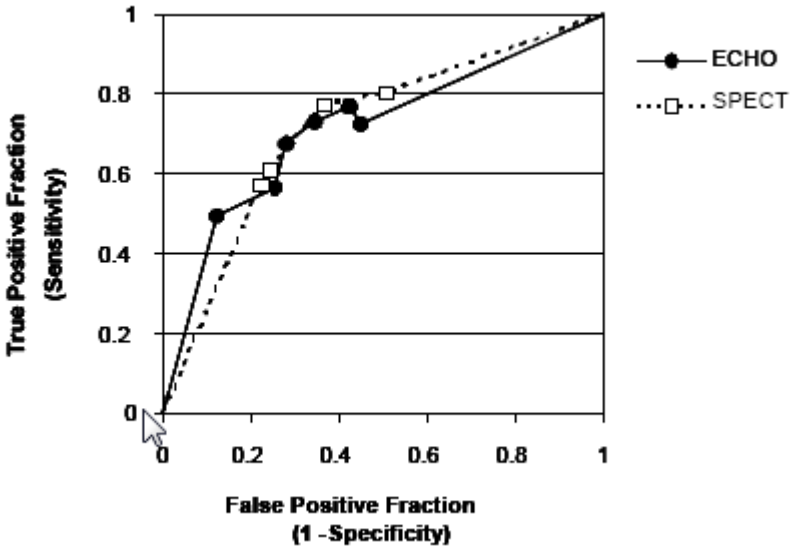
Analysis performed across trials (pooled analyses AND meta-analysis)

Pooled Analysis of AI-700 Compared with SPECT

When the Pivotal Phase III trials are considered collectively or when the data are pooled, several analyses support the proposal that AI-700 ECHO and SPECT have similar ability to diagnose CAD. Firstly, sensitivity and specificity estimates of more than 70% (weighted mean) were obtained when combining the results from studies AI-700-32 and AI-700-33 for both AI-700 ECHO and SPECT. All ECHO and SPECT readers' true positive fraction (sensitivity) versus the false positive fraction (1-specificity) were plotted by modality (Figure 5). The resulting multi-reader receiver operating characteristic curves (mROC) for AI-700-enhanced ECHO and SPECT perfusion imaging were very similar, with an equal area under the curve value of 0.72, indicating that, when both Phase III studies are considered collectively, the diagnostic performance of the 2 modalities is similar. The mROC curves

also illustrate that the variations in reader bias (the position along the mROC curve) are similar for both SPECT and ECHO, thereby supporting the idea that both ECHO and SPECT readers make the same degree of trade-offs between sensitivity and specificity. Similar results were seen when the ROC analysis was performed excluding patients with history of prior MI (i.e. evaluating the ability of ECHO and SPECT to detect only inducible ischaemia).

Figure 5: Multi-Reader ROC Curve for ECHO and SPECT Readers



The ROC curves can provide information about the relation between sensitivity and specificity for individual readers but do not allow statistical comparison of sensitivity and specificity.

Supportive study(ies)

Clinical Utility of AI-700 ECHO

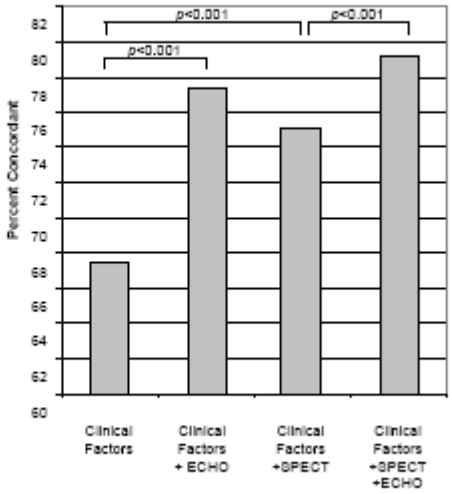
The intended clinical use of AI-700 ECHO is to integrate imaging information along with other clinical variables to screen for those patients at elevated risk of CAD who would benefit most from further, more invasive evaluation with ANGIO. The clinical utility of AI-700 was therefore examined by analysing the value of a positive AI-700 result versus clinical risk factors such as those typically used to stratify patients for CAD risk e.g. age (10-year ordinal categories), gender, and angina symptoms (atypical, typical and unstable), as well as other well-characterised risk factors such as hypertension, smoking, and diabetes.

In pooled multivariate analysis of Studies AI-700-32 and AI-700-33, positive AI-700 ECHO (majority assessment of 3 readers) was a clear, significant marker of CAD even after adjustment for clinical factors. In fact, this analysis showed that a positive AI-700 ECHO patient had a 5.3 fold higher risk of CAD than a negative AI-700 ECHO patient (odds ratio [OR][95% CI] = 5.3 [3.7, 7.5]; p<0.001). Similar results were observed with SPECT (OR [95% CI] = 4.2 [3.0, 5.9]; p<0.001). In addition, 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).

AI-700 ECHO provides significant incremental diagnostic value over standard clinical factors, similar to SPECT (Figure 8). It is reasonable to assume that ECHO performance under non-blinded conditions (i.e., in clinical practice, when the clinician has access to clinical data such as patient demographics,

medical history, ECG findings, etc.) will provide even better results than the ECHO blinded readings obtained in the clinical trials setting.

Figure 8: Incremental Value of AI-700 and SPECT Perfusion Imaging Over Clinical Factors



Note: p-values were derived from Wald test associated with addition of covariate.

The CHMP guidelines of diagnostic agents require adequate diagnostic performance and reader/technique dependent precision in relation to a comparator. Applications are required to address technical and diagnostic performance. The studies performed do not provide a clear indication of the expected sensitivity and specificity associated with Imagify. Impact on diagnostic thinking refers to quantifying the impact of a test on diagnostic thinking and the analysis above does suggest that AI-ECHO is potentially a significant marker of CAD.

However, impact on therapeutic decisions and clinical outcome, which refers to a description and quantification of impact of diagnostic information on management of a patient and clinical outcome, has not been addressed.

Discussion on clinical efficacy

Design and conduct of clinical studies

All 5 efficacy studies (AI-700-20, AI-700-21, AI-700-23, AI-700-32, and AI-700-33) were performed in stable adults with confirmed or suspected CAD. CAD patients in study AI-700-20 were stable post-myocardial infarction patients, while patients in studies AI-700-21, AI-700-23, AI-700-32, and AI-700-33 were clinically stable, with a recent history of chest pain.

The two pivotal studies AI-700 32 and AI-700 33 which were single arm, multicentre studies with each centre qualifying for participation on the basis of adequate performance in Pilot study 23. Both studies involved rest and dipyridamole ECHO and SPECT. The same dose was administered in each study (0.04mL/kg in two doses). The primary endpoint was accuracy in comparison of ECHO and SPECT.

In study AI-700-32 all patients were recruited from patients scheduled for SPECT and so had a relatively lower CAD risk. In study AI-700-33 all patients were scheduled for or had recently undergone ANGIO and so had a relatively higher CAD risk.

Based on the evaluation of the true prevalence of CAD, both studies AI-700-32 and AI-700-33 were found to have enrolled 44% and 58% of patients with CAD, respectively.

Truth standard in AI-700 32 was coronary angiography/LVG (42% patient) or clinical history and SPECT; Truth standard in AI-700 33 was coronary angiography/LVG (94% patients) or clinical history and SPECT.

There are major issues that question the reliability of the analyses of the whole study. First, in a considerable number of patients an alternative truth standard was used that included SPECT instead of ANGIO/LVG as a truth standard, biasing specificity in favour of SPECT. For those subjects, where ANGIO, LVG or LVG+ANGIO was used as a truth standard, specificity was below 40% for SPECT. This is not acceptable for an appropriate comparator. It is concluded that it is not possible to get reliable information for specificity of Imagify stress ECHO based on the pivotal study AI-700-32. This is at least partly related to the choice of the alternative truths standard.

It can be considered as a drawback that there is no information on the screening and selection of patients. A possible selection bias cannot be assessed. It is not likely however that selection bias was a key factor that influenced the comparison between SPECT and Imagify ECHO.

Primary efficacy analyses involved the comparison of AI-700 ECHO imaging to an independent truth standard (usually ANGIO or LVG), and in the pivotal studies, also to a comparative standard (SPECT).

All the tests were conducted at the clinical site and then the images were sent via independent core laboratories to “blinded” readers who had access only to the images and no clinical information. AI-700 ECHO images were read by three blinded ECHO readers in each trial using standardised criteria based upon objective features of disease in each image, and pre-specified rules for artefact interpretation. Readers reviewed perfusion and wall motion simultaneously.

The SPECT images were read by one blinded reader in the AI-700-32 trial and three blinded readers in the AI-700-33 trial.

The pivotal Phase III studies were non-inferiority analyses designed to compare the diagnostic performance of AI-700 stress ECHO with that of SPECT for detection of CAD versus primarily

ANGIO/LVG as truth. The criterion for success in each study was that two of the three ECHO blinded readers were non-inferior to the SPECT blinded reader(s) and the that the lower bound of the 2 sided 95% CI for relative risk ratio was greater than 0.83 for at least 2 of the 3 AI-700 readers.

The endpoint was originally to be an estimate of sensitivity and specificity of ECHO in comparison to the truth standard but this was modified on the basis of discussions with the FDA who recommended a sensitivity/ specificity comparison of ECHO to SPECT and a non-inferiority design. The applicant finally proposed a sequential non inferiority analysis for ECHO versus SPECT, first for accuracy and then for sensitivity/specificity. If the accuracy endpoint was met, similar analyses would be performed in relation to sensitivity and specificity.

It is unclear why this approach regarding accuracy as distinct from sensitivity or specificity was taken. The applicant clarified how FDA recommendations influenced the changes of the analyses of the ongoing studies. Neither the choice of accuracy as the primary endpoint nor the choice of the NI margin as RRR=0.83 was based on a recommendation of the FDA. In the minutes of the last meeting with the FDA that were provided (May 2006), the FDA recommended sensitivity and specificity as primary endpoints and a NI margin of 0.87.The FDA did suggest changing to non-inferiority analyses of ECHO versus SPECT using a relative risk ratio non inferiority margin. The selected non-inferiority margin (>0.83 relative ratio of the AI-700 ECHO result to the SPECT result for each diagnostic

parameter) was selected" with the primary focus on a value that would ensure clinically similar performance in detecting CAD". The justification of the NI margin that was given by the applicant is not acceptable. The applicant should further discuss whether differences between Imagify ECHO and SPECT at the magnitude of the NI margin 0.83 can be considered as clinically irrelevant.

For each patient in the trial, the truth standard was used to determine whether the patient was positive (i.e. with disease) or negative (i.e. without disease). Then the efficacy of AI-700

ECHO and SPECT was measured by determining whether the blinded readers for each method got the patient diagnosis correct or not, based on the truth standard.

As noted above readers had been selected on the basis of successful participation in study AI-700-23. The results of study AI-700-32 became available after study AI-700-33 had commenced and before it was completed and the applicant decided to retrain readers in study AI-700-33 and reread those parts of the study that had already been performed.

Albeit it would have been preferable if the training and reading process would not have been repeated during an ongoing study, the approach over all is accepted. It approaches a clinical situation of adapting the reading process to the baseline risk of the patients.

Demographic information from all five efficacy studies in patients with suspected or previously diagnosed CAD showed that the majority of patients were male (range 65% to 89% across the studies), White (range 61% to 83%), with a mean age ranging from 61 to 63 years, which was considered representative of the target patient population in clinical practice. Baseline characteristics in the two pivotal studies were similar with 67% male and 61% Caucasian subjects in study 32 and 80% male and 79% Caucasian in study 33.

Efficacy data and additional analyses

Phase II Results

Results from the Phase II studies indicated that the appropriate dose of AI-700 was dependent on the imaging method employed and the imaging characteristics of each individual patient.

In study AI-700-21, a cumulative AI-700 dose of 0.081 mL/kg (administered as individual doses at rest and at stress) produced the best duration of effective MCE, with mean durations of 3.8 and 3.9 minutes during rest and stress imaging, respectively. Therefore, a cumulative dose of 0.08 mL/kg AI-700 (administered as individual doses of 0.04 mL/kg during rest and stress) was selected for the Phase III studies. It remains an issue of concern that in this study sensitivity and specificity of an intermediate dose, that was not identical but in the range of the (to be) marketed dose, was not different from the results of placebo. There are no data comparing Stress ECHO with and without Imagify. The added value of Imagify stress Echo over stress ECHO alone remains to be addressed.

Results also suggested that AI-700 might offer advantages over placebo ECHO imaging by providing perfusion information and high quality diagnostic images in almost all patients. The diagnostic performance of perfusion plus wall motion information obtained with AI-700 (when dose and imaging settings were optimised) was also superior to placebo (i.e. ECHO imaging without the use of contrast media) or when AI-700 was used to assess wall motion alone.

As a result of this information, the imaging in the Phase III studies was always carried out in the early post-contrast injection time period to ensure that perfusion and wall motion imaging were both evaluated. This was considered to reflect clinical practice where both components would be evaluated together to give the most accurate diagnostic result for the patient.

Phase III Results

Primary Efficacy Results

The results showed that in both studies, all 3 ECHO readers in each trial met the standard for non-inferior accuracy to SPECT, exceeding the criteria for success for the primary endpoint (only 2 of 3 readers were required to meet the non-inferiority standard). Values for accuracy were similar for all 6 ECHO readers (range 66% to 71%) and for the 4 SPECT readers (range 66% to 70%).

Table 10: Primary Efficacy Results from AI-700 Phase III Efficacy Studies

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		0.950	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
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		0.990	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
p-value[2]		0.002*	0.968	>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		0.912	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
p-value[2]		0.120	<0.001*	<0.001*		0.951	0.013*	0.259
p-value for superiority		0.879	0.006	<0.001				

Source: Module 5, AI-700-32 and AI-700-33 CSRs

RR=relative risk 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

Sensitivity and specificity, however, varied by reader, with ECHO reader sensitivity ranging from 50% to 77%, and specificity ranging from 55% to 88%. Similarly, sensitivity varied for the SPECT readers, ranging from 57% to 80%, and specificity ranging from 50% to 78%.

Without re-training in 33, the sensitivity for the three ECHO readers were 52.5, 63.3 and 65.5 versus 58.8 for SPECT.

In study AI-700-32 non-inferior sensitivity was only achieved by 1 of 3 readers, but superior specificity was seen in 2 of 3 readers; whereas in study AI-700-33 superior sensitivity was seen in 3 of 3 readers but non-inferior specificity was only seen in 1 of 3 readers. It has been noted that following retraining of readers, sensitivity values increased for each of the 3 ECHO readers.

Overall performance of the Imagify stress ECHO appeared to be similar in patients with different CV risk factors. Sensitivity and specificity were numerically higher in patients with hypertension. This should be interpreted with caution.

As discussed above, it was not possible to get reliable information for specificity of Imagify stress ECHO based on study AI-700-32 due to bias in subjects with the alternative truths standard and an unacceptably low specificity of those patents assessed based on ANGIO/LVG as a truth standard. Since in study AI-700-33 NI of specificity of Imagify-Stress ECHO as compared to SPECT could not be demonstrated, the clinical program failed overall to demonstrate sufficient specificity of Imagify stress ECHO to detect CAD. Further explanation is required for the finding that in study AI-700-32 local specificity was much higher than overall specificity to detect CAD.

Unresolved issues relevant for both studies are related to the inclusion of patients with and without a history of myocardial infarction and the lack of differentiation between detection of scar and ischaemia. In some instances assignment of patients with MI to disease positive or negative was based on the availability of data but not based on results. For patients with a scar that may be detectable with a standardised resting ECHO in many instances, the added value of Imagify stress ECHO is not clear since the evaluation did not differentiate between scar and ischaemia. The applicant has provided ROC curves to support that the results were independent of whether patients with MI were included or not, but the sensitivity of ROC curves to detect differences is unclear.

Overall, the specificity of both methods in patients with MI was low, being 13% and 29% for SPECT and ECHO in individual readers. It remains open, whether these results are due to an inappropriate assignment of patients with MI to disease positive or negative in the standard of truth. It is concluded that both pivotal studies failed to demonstrate that Imagify ECHO is a reliable diagnostic tool to diagnose CAD in patients with previous MI with sufficient specificity.

The applicant is asked, whether data from baseline ECHO are available for comparison prior to administration of Imagify and prior to pharmacological stress in order to evaluate, how many patients would have been diagnosed appropriately for scar at baseline and how many additional patients can be diagnosed after administration of Imagify and after induction of stress.

Clinical Utility

The clinical utility of AI-700 was examined by analysing the value of a positive AI-700 result versus clinical risk factors such as those typically used to stratify patients for CAD risk e.g. age (10-year ordinal categories), gender, and angina symptoms (atypical, typical and unstable), as well as other well-characterised risk factors such as hypertension, smoking, and diabetes.

In pooled multivariate analysis of Studies AI-700-32 and AI-700-33, positive AI-700

ECHO (majority assessment of 3 readers) was a clear, significant marker of CAD even after adjustment for clinical factors.

The CHMP guidelines of diagnostic agents require adequate diagnostic performance and reader/technique dependent precision in relation to a comparator. Applications are required to address technical and diagnostic performance. The studies performed do not provide a clear indication of the expected sensitivity and specificity associated with Imagify. Impact on diagnostic thinking refers to quantifying the impact of a test on diagnostic thinking and the analysis above does suggest that AI-ECHO is potentially a significant marker of CAD.

However, impact on therapeutic decisions and clinical outcome, which refers to a description and quantification of impact of diagnostic information on management of a patient and clinical outcome, has not been addressed. In particular, the following issues are not clear: The results from the clinical program do not allow differentiating between scar and ischaemia. The added value in patients with known scar, identified by conventional ECHO, is unclear, It would be desirable to know in these

patients, whether they have ischaemia or not. This was not addressed in the clinical program, however. Efficacy of Imagify in conjunction with other stress agents than dipyridamole has not been investigated either. A study including a rereading based on readers partially unblinded would have reflected the clinical situation more closely, but this was not considered feasible in such clinical trials. The applicant is asked to discuss, whether based on the data available from the studies a reliable assessment of sensitivity and specificity addressing a differentiation between fixed effects and induced effects is feasible. In this context in those patients with negative ANGIO but positive LVG an additional analysis differentiating between fixed effects and inducible effects would be of value.

Conclusions on clinical efficacy

Overall, considering the issues regarding design and conduct of the pivotal studies, it is difficult to draw firm conclusions regarding the efficacy of Imagify and efficacy or benefit has not been well characterised.

It is unclear why the approach regarding accuracy as distinct from sensitivity or specificity was taken. Sensitivity seems of greatest importance for a screening test in patients with intermediate and high risk as long as specificity is acceptable.

Further information on the appropriateness of the chosen inferiority margin is requested.

In the pivotal studies all 3 ECHO readers in each trial met the standard for non-inferior accuracy to SPECT. However in study AI-700-32 non inferior specificity was seen in 2 of 3 readers but not sensitivity and in study AI-700-33 non inferior sensitivity was seen in 3 of 3 readers but not specificity. Since evaluation of specificity in study AI-700-32 is not considered reliable, the overall study program failed to demonstrate sufficient specificity of the method, results for sensitivity were not consistent.

Furthermore, for patients with previous MI, specificity was low. This may partially be due the choice of the truth standard. The added value over ECHO alone and over stress Echo without Imagify remains to be determined especially in these patients.

As Imagify is intended as a screening test, the results for sensitivity are particularly important and success for sensitivity was seen in study 33.

In the pilot study designed to recruit and train appropriate readers, accuracy is noted as being between 75 and 94%, sensitivity between 75 and 100% and specificity between 75 and 100%.

These figures are in all cases substantially higher than those actually seen with the same readers in the pivotal studies.

SPECT sensitivity is noted as 77.6% in AI-700-32 and 60.9% in AI-700-33 and specificity was 63.8 and 75.8% respectively. When only SPECT reads standardised by ANGIO and/or LVG were considered, specificity in study AI-700-32 was below 40 %. For comparison, this is by far lower than indicated e.g. by ACC/AHA/ASNC Guidelines for the Clinical Use of Cardiac Radionuclide Imaging—Executive Summary, Circulation 2003; 108:1404-1418.

1. Sensitivity and Specificity

Tables 5 and 6⇓ in the full-text guideline summarize studies reporting sensitivities and specificities of exercise and vasodilator stress perfusion SPECT for the detection of angiographically significant (more than 50% stenosis) CAD. Sensitivities (generally uncorrected for referral bias) average 87% and 89%, respectively; specificities (also uncorrected) average 73% and 75%.

Information on the lack of renal or hepatic studies should be added to section 4.2 of the SmPC. As excretion of the gas is through the lungs, additional comments about COPD could also be included in section 4.2.

Clinical safety

The clinical safety of AI-700 was evaluated in 11 completed clinical studies that enrolled a total of 1,241 subjects. Of these subjects, 1,194 received at least one dose of AI-700, of which 130 were healthy volunteers, 18 were CHF/COPD patients, and 1,046 were CAD patients.

Of the 1,046 CAD patients who received AI-700, 911 patients (87%) were enrolled based upon a recent history of stable chest pain (Intended Dose with Stressor Population). The remaining patients were enrolled based upon moderate to high pre-test probability of CAD. All efficacy patients had single or dual injection exposure to AI-700. In clinical practice, patients are anticipated to be exposed to AI-700 no more than once a year, with the potential to be re-evaluated several times during their lives.

Patients were excluded from participation in AI-700 studies if they were deemed to have unstable CAD or other acute or unstable clinical conditions. These conditions included, but were not limited to unstable angina, acute MI, history of moderate or severe COPD, severe CHF, significant cardiovascular structural or functional abnormality, or serious arrhythmias. Patients screened for the pharmacological stress studies (studies AI-700-21, AI-700-23, AI-700-32 and AI-700-33) were also excluded if they were contraindicated for dipyridamole or the

aminophylline/theophylline stress antagonist agents. Vasodilatory stressors of the adenosine agonist class such as dipyridamole are considered contraindicated, and warnings exist for their use in unstable or compromised patients (such as those with second- or third-degree atrioventricular [AV] block, bronchoconstrictive or bronchospastic lung disease, or acute MI).

Therefore, the clinical utilisation of AI-700 (which is intended for stress imaging) is limited by the contraindications for vasodilatory stress testing, which are similar to the exclusion criteria in the Phase III studies.

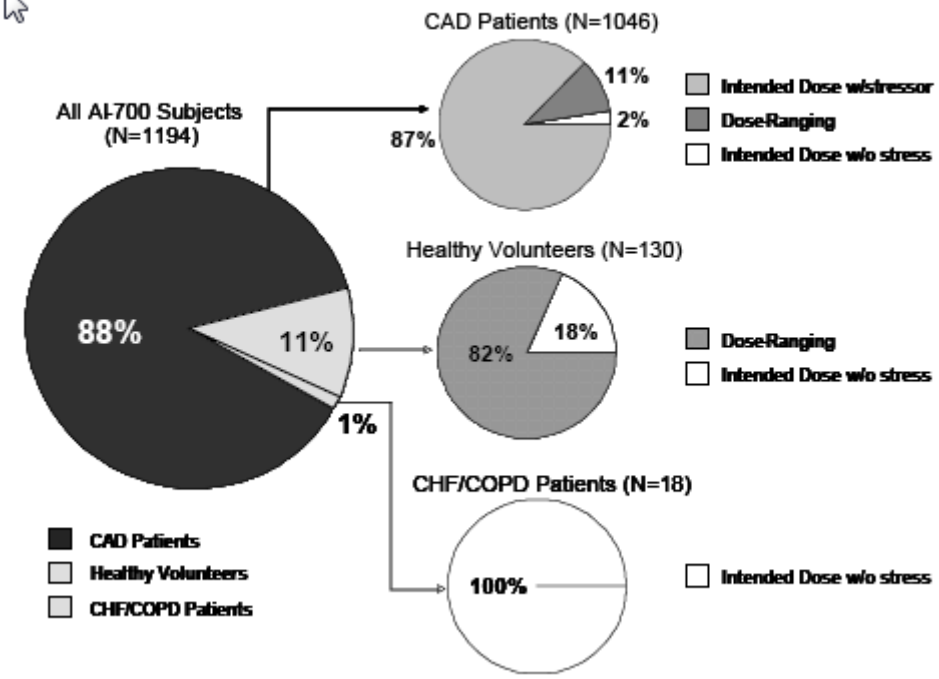
The patient population in the Phase III studies is considered to represent a broad cross-section of chronic stable chest pain patients from a variety of clinical practice settings. Patients had heterogeneous demographic profiles, with medical histories that were indicative of a population with intermediate risk of CAD. Thus, the applicant considers that the data presented in this dossier are a valid estimation of the safety profile of AI-700 in clinical practice.

Patient exposure

The clinical safety of AI-700 was evaluated in 11 clinical studies that enrolled a total of 1,241 subjects, 1,194 of whom received at least one dose of AI-700, and which include 130 healthy volunteers, 18 CHF/COPD patients. 1,046 were CAD patients.

Of the 1,046 CAD patients who received AI-700, 911 patients (87%) were enrolled based upon a recent history of stable chest pain (Intended Dose with Stressor Population). The remaining patients were enrolled based upon moderate to high pre-test probability of CAD. All efficacy patients had single or dual injection exposure to AI-700.

Figure 1: Distribution of Subjects who Received AI-700



There were 1194 patients and healthy volunteers, and 911 patients in the phase 3 database. These numbers are adequate and the patients are representative of the type of patient intended to receive this diagnostic agent.

Adverse events

Of the 911 CAD patients treated with AI-700, the most frequently reported AEs were headache (35%), chest pain (12%), nausea (10%), flushing (9%), chest discomfort (8%), dizziness (6%), feeling hot (5%), dyspnoea (4%), rigors (4%), ECG ST segment depression (4%), hypotension (3%), abdominal pain (2%), pain in extremity (2%), pyrexia (2%), and neck pain (2%).

A review of AE data for patients who received AI-700 with and without dipyridamole showed a clear correlation between the incidence of these AEs and administration of dipyridamole.

These AEs were reported in a small number of patients (≤3%) following the first dose of AI-700 and then the incidence increased 5-fold following dipyridamole administration and peak pharmacological stress (consistent with the dipyridamole package insert). Approximately 47% of patients given intravenous dipyridamole experienced an AE. The following AEs are typically associated with dipyridamole use: chest pain/angina pectoris, ECG changes (most commonly ST-T changes), arrhythmias (e.g., sinus node arrest, heart block, tachycardia, bradycardia, fibrillation), syncope and cerebrovascular events (e.g. stroke, TIA, seizures), hypotension and hot flushes. Hypersensitivity reactions such as rash, urticaria, angio-oedema, laryngospasm, severe bronchospasm, and rarely anaphylactoid reactions, have also been reported. Other adverse reactions reported include: abdominal pain, vomiting, diarrhoea, nausea, dizziness, headache, paraesthesia, myalgia, hypertension, blood pressure lability, fatigue and dyspepsia.

Some differences in common AE rates were observed in the AI-700 studies in various subpopulations (age, gender, BMI, race, and history of smoking), but the differences were modest and with uncertain clinical significance.

Adverse events in Healthy Volunteers - Dose-Ranging Studies (AI-700-01, AI-700-02, AI-700-04, AI-700-20, and AI-700-21)

Table 9: Number of Healthy Volunteers with AEs Reported by ≥4% of Healthy Volunteers Following a Single Injection, by Dose Group (Dose-Ranging Studies: AI-700-01, AI-700-02, AI-700-04, AI-700-20, AI-700-21)

		AI-700 (mL/kg) Dose				
	Placebo (N=25) n (%)	0.014 (N=11) n (%)	0.054[1] (N=43) n (%)	0.08 (N=31) n (%)	0.11 (N=21) n (%)	Total Reported (N=106) n (%)
At least one adverse event	**13 (52)**	**3 (27)**	**17 (40)**	**21 (68)**	**18 (86)**	**59 (56)**
Flushing[2]	0	0	3 (7)	10 (32)	9 (43)	22 (21)
Headache NOS	4 (16)	1 (9)	3 (7)	4 (13)	5 (24)	13 (12)
Cough[2]	0	0	2 (5)	4 (13)	5 (24)	11 (10)
Dizziness (excluding Vertigo)	3 (12)	1 (9)	0	3 (10)	5 (24)	9 (9)
Dyspnoea NOS[2]	1 (4)	0	0	2 (7)	6 (29)	8 (8)
Nausea[2]	0	0	1 (2)	2 (7)	2 (10)	5 (5)
Chest tightness[2]	1 (4)	0	1 (2)	1 (3)	2 (10)	4 (4)
Injection site pain	0	0	2 (5)	2 (7)	0	4 (4)
Ventricular extrasystoles	0	1 (9)	3 (7)	0	0	4 (4)

Source: Integrated Summary of Safety, Table S-1.1
n (%) = Number and percentage of subjects with at least one incidence of the specified AE.
NOS = Not otherwise specified.
[1] Healthy volunteers who participated in Study AI-700-21 and received a cumulative AI-700 dose of 0.11 mL/kg as 2 separate injections of 0.054 mL/kg are included in the 0.054 mL/kg dose
[2] The AEs of flushing, cough, dyspnoea, nausea, and chest tightness each showed a dose-dependent increase in incidence.

Volunteer Studies

AI-700 was discontinued because of AEs in one healthy volunteer treated with AI-700 in study AI-700-21 in a subject who experienced an AE classified as chest pain (Investigator term “warm feeling in chest”) with PVCs observed during intermittent triggered destruction pulse imaging (mechanical index 1.1 to 1.9). The induction of PVCs at mechanical indices above 1.0 is possibly associated with some ultrasound contrast agents, including AI-700 during intermittent triggered ultrasound imaging, and it is now a requirement that all contrast agent imaging be performed below a mechanical index of 1.0, and so all subsequent AI-700 trials (all Phase III studies) complied with this regulation.

A second AE of tachycardia was recorded 1 minute after the start of the second dose, so the subject was withdrawn from the study. Both AEs were considered by the Investigator to be moderate in intensity and definitely related to AI-700 administration. Both AEs resolved without needing treatment and with no residual effects. (One further subject discontinued from this study because lack of i.v. access prevented administration of the second dose).

Overall, 59 (56%) of 106 healthy volunteers treated with AI-700 in the dose-ranging studies experienced at least one AE. The most frequently reported AEs among AI-700 healthy volunteers were flushing 22 (21%) of 106 patients, headache 13 (12%), cough 11 (10%), dizziness 9 (9%), and dyspnoea 8 (8%). A dose-dependent increase in the incidence of overall AEs was observed following a single injection of AI-700 (Table 9 above). Dose-dependent increases for individual AEs were noted for flushing (the most frequently reported AE), cough, dyspnoea, and nausea. Respiratory, thoracic, and mediastinal disorders, primarily reports of coughing and dyspnoea, occurred in 9%, 7%, 26% and 48% of healthy volunteers in the 0.014 mL/kg, 0.054 mL/kg, 0.081 mL/kg and 0.108 mL/kg AI-700 dose groups, respectively.

Overall 13 (52%) of 25 healthy volunteers who received placebo experienced at least one AE.

The most frequently reported AEs among the 25 placebo-treated healthy volunteers were headache (4 subjects; 16%), dizziness and injection site induration (3 subjects each; 12%), and erythema (2 subjects; 8%). All other AEs reported in the placebo group were experienced by one subject only. No incidences of flushing, the most commonly reported AE among AI-700 healthy volunteers, were reported for placebo-treated healthy volunteers.

Of the 22 (21%) out of 106 AI-700 healthy volunteers who experienced flushing, all occurred within 1 hour post-dose. Among healthy volunteers who experienced AEs in the placebo group, the majority experienced AEs between 4 hours post-dose and the end of the study.

Fifty-four of the 106 healthy volunteers (51%) experienced AEs considered by the Investigator to be mild or moderate in intensity, and 5 healthy volunteers (5%) experienced at least one event of severe intensity.

In the phase 1 safety study AI-700-01**,** AI-700 was administered as an IV injection with rising single doses at 0.05mg/kg to 8.0mg/kg in 33 healthy male and female subjects.

The study assessed tolerability and included brief neurological assessments and mini mental status assessments pre and post dose

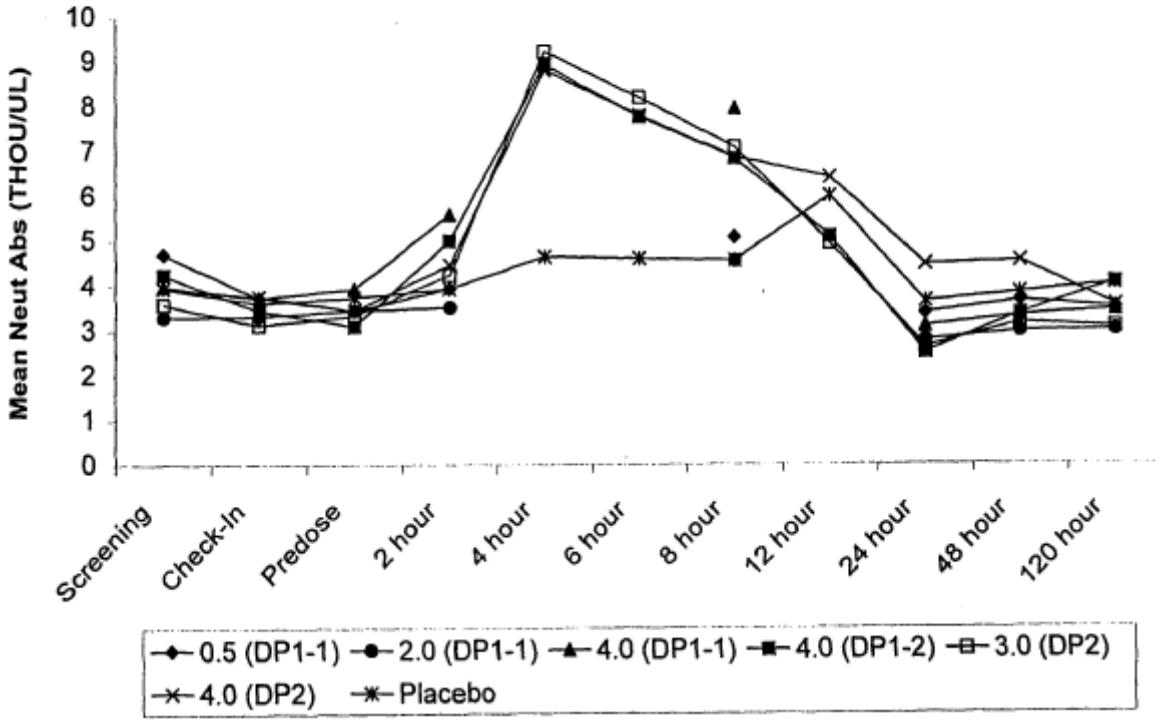
A total of 88 AEs were reported: 14 reported by 7/15 placebo subjects and the remaining 74 were reported in 26/33 subjects treated with active. All AEs were mild except for subject 407 treated with 4.0mg/kg who had 3 moderate and 4 severe AEs. The most frequent AEs were vasodilatation 39% active), headache 24% vs 13%) and dyspnoea 21% vs 0% and AEs attributed as possibly or probably related to AI-700 were more prevalent at 4.0mg/kg than at any other lower doses.

In AI-700 treated cohorts, mean WBC and neutrophil values showed a trend to increased values with correspondingly decreased lymphocytes. They were not deemed clinically significant and were not associated with any clinical findings. In subjects who received more than 2.0mg/kg mean neutrophil showed a trend to increase between 2 and 8 hours with peaks at 4hours (see graph below)

Subject 407, (see table below) a black male of 30 years (had a WBC of 4.9 pre-dose, increasing to 8.7 at 2 hours and 10.8 at 4 hours. This subject who received the 4.0mg/kg dose experienced a “constellation of neurological and respiratory AEs within minutes of administration of A1-700. AEs of difficulty communicating, shortness of breath, cough, pain were severe on onset but abated within 20 minutes and resolved within 40 minutes” At the time of the event” difficulty communicating” the subject had a decrease in MMSE score to 22 (5 minutes post dose) from a pre-dose of 27 without a corresponding change to BNA. This subject also had an increase in blood pressure which returned to normal in 60 minutes. Although this subject recovered there is no specific discussion of this case (likely cause or explanation) in the study report. Subject 207 had a skin rash which arose after dosing but which was not considered as drug related (!).

Dose escalation beyond 4.0mg/kg was deemed unnecessary based on safety data from the 4.0mg/kg dose and a companion study A1-700-02 indicated feasibility of myocardial perfusion at single bolus doses of 2.0-4.0.

Figure 11.1-1
PLOT FOR MEAN NEUT ABS vs. TIMEPOINTS BY COHORT



Subject Number	Cohort	PREFERRED TERM Verbatim Term	Onset Date	Onset Time 24hr clock	Time to Onset HH:MM	Duration of AE HH:MM
403	PLACEBO	INJECTION SITE EDEMA Indurated area right forearm at injection site	02/10/1999	08:03	48:33	6:57
404	4.0 mg/kg (DP1-2)	VASODILATATION Flushing	02/08/1999	07:49	0:04	0:08
405	4.0 mg/kg (DP1-2)	CHILLS Feeling cold	02/08/1999	08:08	0:08	0:07
407	4.0 mg/kg (DP1-2)	THINKING ABNORMAL Intermittent difficulty communicating	02/08/1999	08:32	0:02	0:33
		VASODILATATION Hot sensation	02/08/1999	08:33	0:03	0:27
		DYSPNEA Intermittent shortness of breath	02/08/1999	08:37	0:07	0:35
		SWEATING Clammy	02/08/1999	08:37	0:07	0:25
		DIZZINESS Dizzy	02/08/1999	08:37	0:07	0:11
		COUGH INCREASED Intermittent cough	02/08/1999	08:38	0:08	2:07
		CHEST PAIN Intermittent chest pain	02/08/1999	08:40	0:10	0:15
		HEADACHE Intermittent headache	02/08/1999	08:50	0:20	13:38
		ABDOMINAL PAIN Abdominal pain	02/08/1999	08:55	0:25	0:20
		PAIN Left arm pain	02/08/1999	09:10	0:40	0:07

Study AI-700-32: A Phase III, International, Multicentre, Open-Label, Dual-Injection, Echocardiographic Imaging and Safety Study of AI-700 in Patients with Suspected Ischaemic Heart Disease Undergoing Single-Photon Emission Computed Tomography

A total of 321 patients received an injection of AI-700 in Imaging Session I, and 316 received a second AI-700 injection at stress during Imaging Session II. Five patients did not receive a second dose of AI-700. No deaths were reported during this study.

Overall, 251 (78.2%) of 321 patients reported at least one AE, with a total of 765 AEs reported.

Four patients experienced a total of 6 non-life-threatening SAEs. Three of these patients experienced single SAEs: preferred terms of adverse drug reaction (with symptoms including dizziness, hot flushes, headache, and right-sided chest pain, syncope, vasovagal, and mental status change. One patient experienced 3 SAEs: preferred terms vision blurred, eye pain, and visual disturbance. All SAEs resolved without residual effects. Five patients had AEs that resulted in permanent discontinuation of AI-700 (3 patients during Imaging Session I and 2 patients during Imaging Session II). One patient had an AE that resulted in a temporary interruption of AI-700 dosing. One patient had an AE that resulted in an adjustment of the AI-700 dosing regimen.

Overall, the most frequently reported AEs among the 321 patients were headache (135 patients; 42.1%), chest pain (45 patients; 14.0%), chest discomfort (36 patients, 11.2%), nausea (35 patients; 10.9%), flushing (29 patients; 9.0%), dizziness (23 patients, 7.2%), hypotension (20 patients, 6.2%), feeling hot (20 patients, 6.2%), and dyspnoea (18 patients, 5.6%).

Most of the reported AEs (744 of 765 AEs; 97.3%) were considered by the Investigator to be of mild or moderate intensity. Twenty-one (2.7%) AEs were rated severe. Severe AEs were reported by 14 (4.4%) of the 321 patients, and included headache (3 patients, 0.9%), chest pain, chest discomfort, rigors, hyperhidrosis, hypotension (2 patients each, 0.6%), bradycardia, lethargy, limb discomfort, tremor, mental status changes (an SAE), night sweats, hypertension, and pallor (1 patient each, 0.3%).

Therefore, 321 patients experienced a total of 6 SAEs. Patient 07.060 experienced syncope vasovagal after the first dose of AI-700. Patient 02.030 had mental status changes (diagnosed as a probable conversion disorder) after the second dose of AI-700. During the Follow-up period,

Patient 09.015 was hospitalised for eye pain, blurred vision, and visual disturbance; Patient 07.018 was hospitalised for an adverse drug reaction (symptoms included dizziness, hot flushes, headache, right-sided chest pain, fever, and chills).

Five patients were permanently discontinued from AI-700 due to AEs. Three of these patients were discontinued after receiving 1 injection of AI-700: Patient 02.002 (mild headache and moderate rigors), Patient 16.010 (mild flushing, mild increased lacrimation, mild wheezing, and moderate coughing), and Patient 16.021 (severe hyperhidrosis, severe hypotension, moderate tachypnoea, and mild vertigo). Two patients had AEs during their second dose of AI-700 which resulted in AI-700 being

permanently discontinued before the full dose was given: Patient 07-032 (mild dizziness and moderate hypotension) and Patient 07.091 (mild hypotension).

Two patients withdrew consent (severe rigors and vasovagal attack).

Mean WBC count increased from 6.66×103/µL at Baseline to 8.57×103/µL at Discharge, returning to 6.60×103/µL at Follow-up. The increase appears to be primarily related to an increase in absolute neutrophil count (ANC). ANC increased from 4.17×103/µL at Baseline to 6.87×103/µL at Discharge, returning to 4.05×103/µL at Follow-up. A concurrent mean reduction in absolute lymphocyte count (ALC) was observed: 1.88×103/µL at Baseline, 1.25×103/µL at Discharge, and 1.91×103/µL at Follow-up.

Vital signs reflected procedures and reports of hypotension during second session with dypyrimadole.

Small, transient fluctuations (<1%) from Baseline in mean SaO2 values were observed after both injections of AI-700. Though there appears to be a temporal relationship to the administration of AI-700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation). These decreases were not specifically related to respiratory AEs, resolved quickly, and did not appear to be of clinical significance. None of the changes occurred immediately following AI-700 dosing, thus reducing the possibility of a microvasculature obstruction following dosing.

Mental Status Change

A 54 year old black female 2 minutes into the second AI-700 injection and approximately 4 minutes after completion of the dipyridamole infusion reported that her legs were feeling “tight” and she repositioned herself. AI-700 dosing was completed at 12:19:40.

Immediately upon completion of the AI-700 dosing and prior to the aminophylline injection at 12:20 hours, she reported that her legs were feeling “heavy and numb” and she was feeling “dizzy.” Her ECG was unchanged and her blood pressure was 172/93 mmHg; 50 mg of aminophylline was administered. Blood pressure increased to 194/103 mmHg. At 12:23 hours, she seemed lethargic and was non-verbal. Her response to commands was sluggish. Pupils were equal in size, round, reactive to light and accommodation. After administration of the Solu-Cortef, Benadryl, and IV fluid, she became more responsive neurologically and was able to move all extremities spontaneously, but followed commands inconsistently. She appeared confused and disoriented and spoke only single-syllable words with great difficulty. She was admitted to intensive care for a brief period of observation and then was transferred to the medical ward for the duration of her hospital stay. Follow-up head CT scans (on 2 occasions), MRI, and MRA were all negative for acute infarct or other CNS lesion and showed no change from the previous scans on 15 Nov 2005. No neurologic deficit was identified.

Study AI-700-33: A Phase III, International, Multicentre, Open-Label, Dual-Injection, Echocardiographic Imaging and Safety Study of AI-700 in Patients with Suspected Ischaemic Heart Disease Undergoing Diagnostic Coronary Angiography.

A total of 457 patients received an injection of AI-700 in Imaging Session I (rest), and 449 patients received a second AI-700 injection during Imaging Session II (stress). Eight patients did not receive a second dose of AI-700: 7 patients had AEs leading to permanent discontinuation of AI-700

Overall, 294 (64.3%) of 457 patients reported at least 1 AE, with a total of 786 AEs reported. Six non-life-threatening, SAEs were reported by 6 patients (1.3%) during the study: 2 patients experienced syncope vasovagal following the first AI-700 dose (within approximately 5 and 30 minutes post dose, respectively), 2 patients had MIs (both ≥24 hours after Discharge), and 1 patient each reported chest pain (post dipyridamole dosing) and a hypersensitivity reaction (approximately 1 hour following the SCE). Five of the 6 SAEs resolved without residual effects; one MI resulted in post MI bradycardia requiring a pacemaker.

Overall, the most frequently reported AEs among the 457 patients were headache (125 patients; 27.4%), leucocytosis (59 patients, 12.9%), flushing (45 patients, 9.8%), nausea (42 patients, 9.2%), chest pain (38 patients, 8.3%), neutrophil count increased (37 patients, 8.1%), chest discomfort (27 patients, 5.9%), and ST-segment depression (25 patients, 5.5%).

Thirty-seven (8.1%) of the 457 patients experienced at least 1 AE after the first dose of AI-700, before receiving dipyridamole. No AEs reported in this time frame occurred in >5% of patients. The most frequently reported AEs during this time period were flushing (10 patients, 2.2%), headache (6 patients, 1.3%), and nausea (5 patients, 1.1%).

There were 449 patients who underwent stress imaging. After dipyridamole administration and prior to the second dose of AI-700, 173 of the 449 patients (38.5%) experienced at least 1 AE. The most frequently reported AEs during this period were headache (68 patients, 15.1%), flushing (30 patients, 6.7%), chest pain (24 patients, 5.3%), chest discomfort (21 patients, 4.7%), dizziness (14 patients, 3.1%), nausea (12 patients, 2.7%), and ST-segment depression (11 patients, 2.4%).

Following the second dose of AI-700 and through the Discharge assessments, 187 patients (40.9%) experienced at least 1 AE. The most frequently reported AEs during this interval were leucocytosis (57 patients, 12.7%), headache (48 patients, 10.7%), neutrophil count increased (34 patients, 7.6%), nausea (21 patients, 4.7%), chest pain (15 patients, 3.3%), ST-segment depression (14 patients, 3.1%), and flushing (9 patients, 2.0%).

Most of the reported AEs (767 of 786 AEs; 97.6%) were assessed by the Investigator as mild or moderate. A total of 19 severe AEs (including 4 SAEs rated severe) were reported by 11 (2.4%) of 457 patients. The 4 severe SAEs included syncope vasovagal, hypersensitivity, myocardial infarction, and chest pain. The remaining severe AEs were chest pain (4 events), asthenia, A-V block, bradycardia, diarrhoea, dizziness, feeling hot, hypersensitivity, hyperhidrosis, hypotension, nausea, ST-segment depression, and ventricular fibrillation.

All severe AEs resolved without residual effects. Patients 35.025 (hypersensitivity) and 48.047 (multiple AEs) had AI-700 permanently discontinued as a result of severe AEs; Patient 35.030 had AI-700 permanently discontinued as a result of a severe SAE (syncope vasovagal, see narrative in Section 12.3.2.1). One patient (Patient 49.082) required temporary interruption of AI-700 dosing as a result of a severe AE (chest pain).

Mean WBC count increased from 6.9×103/µL at Baseline to 9.4×103/µL at Discharge, returning to 7.1×103/µL at Follow-up. The increase appears to be primarily related to an increase in absolute neutrophil count (ANC). ANC increased from 4.4×103/µL at Baseline to 7.7×103/µL at Discharge, returning to 4.6×103/µL at Follow-up. The most common haematologic abnormality reported as an AE was leucocytosis or WBC count increased (61 of 457 patients, 13.3%).A concurrent mean reduction in absolute lymphocyte count (ALC) was observed: 1.9×103/µL at Baseline, 1.3×103/µL at Discharge, and 1.8×103/µL at Follow-up.

Serious adverse events and deaths

No deaths were reported in any studies of AI-700.

No SAEs were reported in any of the Phase I or II clinical studies of AI-700.

In the Phase III studies, a total of 11 (<1%) out of 1,194 subjects who received any dose of AI-700 experienced 14 non-life threatening SAEs: 6 subjects in study AI-700-33, 4 subjects in study AI-700-32, and 1 subject in study AI-700-23.

Six patients experienced 7 acute SAEs (onset within 30 minutes following AI-700 dosing).

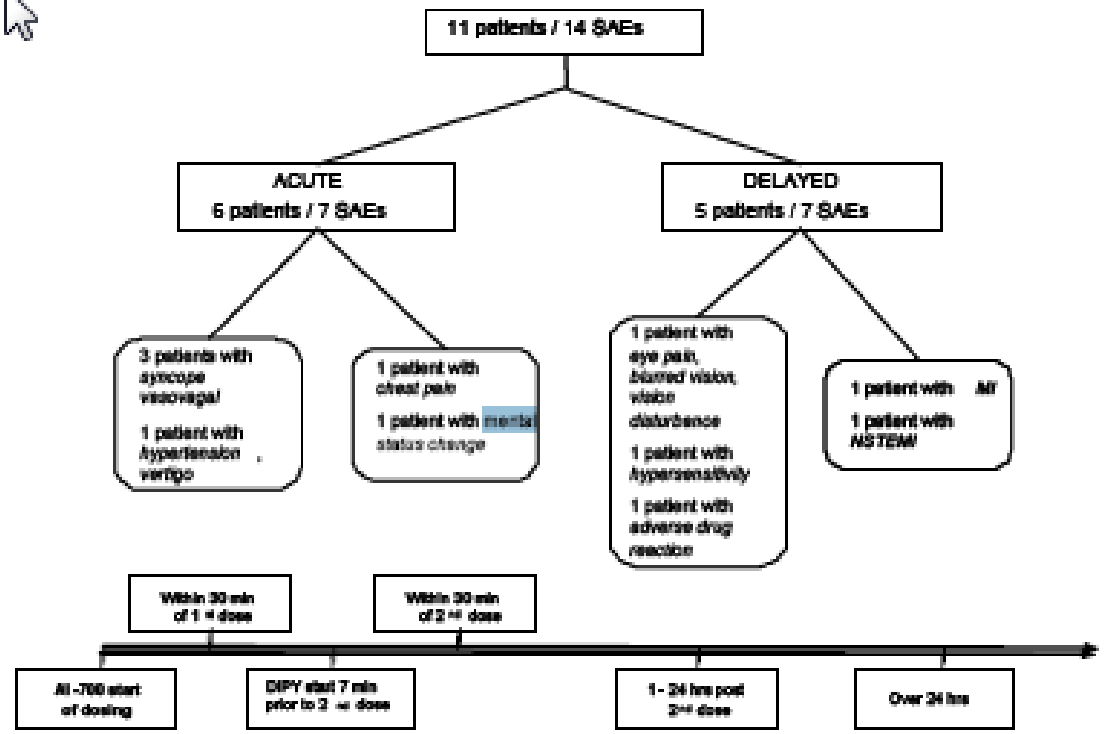
Following the first dose of AI-700, 3 patients experienced individual episodes of vasovagal syncope without loss of consciousness, and 1 patient experienced hypertension and vertigo.

Following the second AI-700 dose, 2 additional patients experienced acute SAEs. One patient experienced mental status changes (considered a possible conversion disorder by the Investigator possibly related to dipyridamole and aminophylline and probably related to AI-700), and one experienced an SAE of chest pain.

Five additional patients reported 7 delayed onset SAEs (onset ≥1 hour post dosing). Onset of 5 of these SAEs was reported within 1 to 18 hours following completion of the study. These events comprised eye pain, blurred vision and vision disturbance in 1 patient (subsequent brain scan and ophthalmological examination did not reveal any cerebral damage), hypersensitivity, adverse drug reaction, MI, and non-ST segment elevation MI (NSTEMI).

Both cardiac events occurred at least 24 hours following AI-700 dosing and were considered unrelated by the Investigator to AI-700, dipyridamole or aminophylline. All but one of the SAEs resolved without residual effects; the NSTEMI resulted in post-MI bradycardia requiring a pacemaker.

Figure 7: Display of Acute and Delayed Serious Adverse Events by Time of Onset



Source: Integrated Summary of Safety, Listing 1.0.

Table 25: Serious Adverse Events

Patient No./Age/Race	SAE(s) and Intensity	Timing	Investigator's Assessment of Relationship	Resolution and Treatment
33.16.003 (76-year-old White female)	mild vertigo and moderate hypertension	after the first dose of AI-700	Possibly related to AI-700, unrelated to dipyridamole, unrelated to aminophylline	Vertigo resolved without treatment or residual effects; hypertension resolved with treatment (i.v. metoprolol)
32.07.060 (79-year-old White male)	moderate syncope vasovagal	after the first dose of AI-700	Possibly related to AI-700, unrelated to dipyridamole	Treated with atropine and the plasma expander 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	Probably related to AI-700, possibly related to dipyridamole, possibly related to aminophylline.	Treated with i.v. Solu-Cortef, Benadryl, and a normal saline i.v 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	Unrelated to AI-700, unrelated to dipyridamole, unrelated to aminophylline.	Resolved without treatment or residual effects
32.07.018 (63-year-old Arab male)	hospitalized for a moderate general disorders and administration site condition adverse drug reaction (symptoms included dizziness, hot flushes, headache, right-sided chest pain, fever, and chills)	during the Follow-up period	Probably related to AI-700, unrelated to dipyridamole unrelated to aminophylline	Resolved with treatment (paracetamol) without residual effects
33.35.008 (69-year-old White male)	severe hypersensitivity reaction to dipyridamole or AI-700	after the second dose of AI-700	Possibly related to AI-700, possibly related to dipyridamole unrelated to aminophylline	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	Probably related to AI-700, unrelated to dipyridamole.	Resolved with treatment (i.v. saline, atropine) without residual effects
33.37.008 (59-year-old White female)	moderate syncope vasovagal	after the first dose of AI-700	Probably related to AI-700, unrelated to dipyridamole.	Resolved with treatment (atropine) without residual effects
33.36.011 (76-year-old White male)	severe myocardial infarction	during the Follow-up period	Unrelated to AI-700, unrelated to dipyridamole.	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	Unrelated to AI-700, unrelated to dipyridamole, unrelated to aminophylline	Resolved with treatment (heparin, nitroglycerin, ramipril, aspirin, pravastatin, clopidogrel) Residual effects treated with a pacemaker
33.42.031 (64-year-old White male)	severe chest pain	after the second dose of AI-700	Possibly related to AI-700, probably related to dipyridamole, probably related to aminophylline	Resolved with treatment (nitroglycerin, tirofiban) without residual effects

Source: Integrated Summary of Safety, Listing 1.

Laboratory findings

In all 11 clinical studies of AI-700, blood and urine samples were collected for clinical laboratory evaluations (including haematology, serum chemistry, and coagulation parameters).

Urinalysis was also performed in all studies with the exception of studies AI-700-21 and AI-700-34.

There were no noteworthy findings for laboratory parameters (other than WBC and ANC) observed in studies other than the pivotal studies AI-700-32 and AI-700-33.

Transitory elevations in white blood cells (WBC) and absolute neutrophil count (ANC) were observed at 2 to 8 hours post-dose, returning to baseline levels within 24 hours.

In the pivotal studies mean WBC counts increased from 6.8×103/μL at Baseline to 9.1×103/μL at Discharge, returning to 6.9×103/μL at Follow-up (WBC normal range: 3.8 to 10.7×103/μL). The increase appeared to be primarily related to a transient increase in ANC. Neutrophils increased from

4.3×103/µL at Baseline to 7.4×103/µL at Discharge, above the normal range (1.96 to 7.23×103/µL), before returning to 4.4×103/µL at Follow-up. A concurrent transient mean reduction in ALC was observed: 1.9×103/µL at Baseline, 1.3×103/µL at Discharge, then returning to 1.9×103/µL at Follow-up.

Vital Signs

No clinically significant trends in any vital signs were observed in moderate COPD or CHF patients. In addition, AI-700 had little to no effect on respiratory rate or body temperature in healthy volunteers or stable chest pain patients. Changes in heart rate were occasionally observed in healthy volunteers, particularly at the 0.108 mL/kg AI-700 dose, which is above the maximum intended clinical dose (0.080 mL/kg). Changes in heart rate were seen in all CAD patients after dipyridamole administration. Blood pressure changes were seldom observed in healthy volunteers with the exception of a small increase in blood pressure at the 0.108 mL/kg dose.

However, changes in blood pressure were observed in the Intended Dose with Stressor Population (stable chest pain patients). Approximately 75% of these patients had a history of hypertension and were being managed with antihypertensive therapy. In this patient population, a small reduction (6% to 7%) from Baseline was observed for both mean systolic and mean diastolic blood pressures between 5 and 10 minutes after AI-700 dosing. The reductions in mean systolic and diastolic blood pressures returned towards baseline values by 30 minutes following AI-700 dosing in both sessions. The majority of blood pressure decreases reported as AEs occurred following dipyridamole and during stress imaging.

Hypotension was the most common vital sign abnormality reported as an AE (22 episodes in 21 [7%] patients). Most hypotensive AEs occurred following pharmacologic stress with dipyridamole (17 of 22 AEs). None of the hypotensive patients experienced a loss of consciousness. All hypotensive AEs resolved without residual effects.

The applicant suggests that the decrease in blood pressure is related to stimulation of the alternate pathway of complement activation. Complement activation is the body's normal response mechanism for clearance of microparticles from the bloodstream and AI-700 has been demonstrated to induce a moderate increase in the complement marker C3a and may also have caused transient changes in neutrophil counts that are also believed to be related to complement activation. However dipyridamole and other vasodilators also cause decreases in blood pressure and individual patient characteristics, such as baseline clinical conditions (e.g. ECG abnormalities, diarrhoea, dehydration) or other study procedures (e.g. i.v. injection, breath holding) may all play a role in triggering some of the changes in blood pressure (particularly during rest and in the absence of the dipyridamole stressor).

Adverse events of pyrexia and increased body temperature were reported in 24 (3%) of the 911 patients. However, these events were not associated with other signs of infection. The majority of these events were mild in intensity, required only symptomatic treatment and resolved with no residual effects.

ECGs

No noticeable clinically significant differences in ECG parameters (PR, QRS, QT/QTc) were observed that indicated an effect of AI-700 on ECG intervals. When changes were observed, they were isolated and were either attributable to the underlying disease, induction of ischaemia during stress, or the related to the methodology of reading ECG intervals.

PVCs, common in CAD patients, have been demonstrated to increase during stress imaging procedures. High mechanical index cardiac imaging with ultrasound contrast agents are known to

induce PVCs. Induction of PVCs is dependent on acoustic energy and frequency (Hz) of ultrasound imaging.

Recently, regulatory oversight restricting high mechanical index imaging (>1.0) during contrast ultrasound imaging has been enacted. Prior to the Phase III studies and in animal studies with AI-700 and 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. In humans during the Phase II AI-700 studies, 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.

The most frequently reported ECG AEs in patients in the Phase I and II studies were related to an increase in PVC rates that only occurred during high mechanical index imaging following administration of AI-700. In the Intended Dose with Stressor Population, imaging was limited to a mechanical index of <1.0 and no association between PVC and any imaging mode was observed. In addition, the AEs reported in relation to ECG changes were almost exclusively attributed to dipyridamole.

Safety in special populations

Cardiovascular Events

Due to the nature of the use of AI-700 in patients with suspected CAD, cardiovascular events were of particular interest. Three individual SAEs of MI, NSTEMI, and chest pain were reported in 3 patients (representing 0.3% of all subjects treated with AI-700).

An in-depth review of ischaemic changes in all patients (including a review of ischaemic changes on ECG and AEs of chest pain at rest and at other post AI-700 time points) did not reveal an additional cardiac risk from the administration of AI-700 to stable chest pain. In fact, the incidence of chest pain or chest discomfort with AI-700 dipyridamole stress ECHO was similar to that observed with dipyridamole alone. Nonetheless, the potential for fatal cardiac arrest or MI exists for patients with suspected or known CAD who are undergoing stress testing of any type so appropriate precautions should be taken.

The safety of AI-700 has not been evaluated in unstable patients with acute MI or acute coronary syndromes.

COPD and CCF

Two doses of 0.040 mL/kg AI-700 were administered to 18 patients with CHF or mild/moderate COPD in study AI-700-05. One patient in the CHF cohort reported 5 AEs, including chest discomfort, chest pain, nausea, dizziness, and cough. The AEs of cough and chest discomfort were reported following the first dose of AI-700, and the AEs of chest pain, dizziness and nausea occurred following the second dose. All 5 AEs resolved following treatment with supplemental oxygen and i.v. Decadron. All 5 AEs were considered possibly related to AI-700 by the Investigator. No other AEs were reported in either CHF or COPD patients.

8 patients completed the CHF cohort. 11 patients were recruited into the moderate COPD cohort and 8 completed the protocol.

There were mean changes in FEV1 in the moderate COPD cohort. Although these changes were subclinical and not accompanied by changes in SaO2, the applicant elected not to enrol a cohort of

severe COPD patients into this study, because severe COPD patients might be less able to adjust to any changes in functional lung volume.

The mean differences in spirometry were most notable at 15-20 minutes following the second dose of AI-700. At this time point, all patients receiving AI-700 had some decrease in FEV1 (range: decrease of 0.08 L to 0.80 L). Return to Baseline levels was documented for all patients.

A modest cumulative effect of multiple doses of AI-700 was seen in moderate COPD.

Table 35: FEV1 (% predicted) in Moderate COPD Patients (Study AI-700-05)

Moderate COPD Patient	AI-700 (N=8)			Placebo (N=8)		
	FEV_1 at First Baseline	Minimum FEV_1 during Treatment*	FEV_1 at 24-Hour Follow up	FEV_1 at First Baseline	Minimum FEV_1 during Treatment*	FEV_1 at 24-Hour Follow Up
05.01.202	69	46	68	64	57	63
05.02.201	46	38	44	43	33	48
05.02.202	48	34	49	44	35	48
05.02.203	55	47	59	56	51	52
05.02.204	47	33	48	49	44	61
05.02.205	59	40	66	56	51	60
05.03.204	43	37	49	54	43	55
05.03.205	56	48	59	51	46	57

Source: Integrated Summary of Safety, CSR AI-700-05, Table 13
*Lowest FEV_1 measured at any post-dose timepoint during Dosing Periods 1 & 2.

Oxyhaemoglobin Saturation (SaO2) by Pulse Oximetry

Measurement of SaO2 by pulse oximetry was performed in all clinical studies of AI-700.

Frequency of SaO2 measurement ranged from 5 collection time points following single AI-700 doses in Phase I Studies AI-700-01 and AI-700-02, to 13 time points following each injection in the dose-ranging Study AI-700-21 and in all subsequent intended dose studies (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).

Volunteers

During Treatment Session I in Study AI-700-06, mean SaO2 at Baseline was 98.6% in Cohort 1 (AI-700 Test Lot) and 97.6% in Cohort 2 (AI-700 Control Lot). In Cohort 1, slight mean decreases in SaO2 were seen at all post-dose time points, with the maximum mean decrease from Baseline being 4% that was seen 30 minutes following the first dose of AI-700. In Cohort 2, the maximum mean decrease from Baseline was also 4%, seen 7 minutes following the second AI-700 dose. SaO2 at the remaining time points was generally unchanged from Baseline. Two healthy volunteers experienced 3 decreases in SaO2 that were below 90% during Treatment Session 1, one in each cohort. In both subjects SaO2 decreases occurred within 30 minutes following administration of AI-700 and were greater than 5% from Baseline. SaO2 decreased to 87% in Subject 06.01.003 (Control Lot) and was preceded by AEs of “awareness of taking deep breaths” and “tightness in chest when taking deep breaths”. The lowest SaO2 for Subject 06.01.004 was 89% and it was asymptomatic. SaO2 recovered in approximately 30

minutes following the decreases to ≤90% for both patients w/o treatment. In Treatment Session 2, mean SaO2 at Baseline was 97.5% in Cohort 1 and 98.7% in Cohort 2. In Cohort 1, mean SaO2 remained unchanged or was slightly decreased from Baseline at all post-dose time points assessed; a maximum mean decrease of 2% was seen 11 minutes following the first AI-700 dose. In Cohort 2, mean SaO2 was generally unchanged from Baseline at all post-dose time points assessed. All subjects had SaO2 values ≥ 90% at all timepoints evaluated during Treatment Session 2, with decreased SaO2 not being reported as an AE for any subject.

CHF/COPD Patients - Intended Dose Study (AI-700-05)

Mean SaO2 following AI-700 dosing in both the CHF and COPD cohorts was greater than 95% and changed less than 1% from Baseline (less than 2% for placebo) at all-time points. No notable differences between treatments were seen with regard to mean SaO2 levels over time in CHF patients following AI-700 dosing. No CHF patient had a decrease in SaO2 to <90% at any measured time point.

Small changes from Baseline in mean SaO2 were observed in the moderate COPD cohort during AI-700 and placebo dosing.

Among patients with CAD, mean and individual SaO2 values were ≥90% at Baseline and at all post-dose time points evaluated in all dose groups, with a few listed exceptions. No trend was seen with regard to mean change from Baseline in SaO2 among patients treated with AI-700 or placebo.

Ten patients with CAD experienced a decrease from Baseline in SaO2 to <90% following administration of AI-700.

Overall, respiratory AEs or notable decreases in SaO2 were occasionally recorded shortly after dosing in healthy volunteers and patients who received AI-700 in the absence of dipyridamole. These decreases in SaO2 were usually asymptomatic. In addition, all respiratory AEs were transient and resolved without residual effects. A possible mechanism for these SaO2 decreases is via complement activation.

Immunological events

During stage 1 C3a levels increased immediately following AI-700 dosing in all subjects. Mean C3a increased from 30.6 ng/mL at baseline to 441.9 at 6 minute post dose. For 2 subjects levels exceeded 940ng/mL, the upper limit of normal. Levels returned to around baseline 2 hours post dose. C3a findings in Stage 2 were similar to those seen in Stage 1, although the magnitude of the increase from Baseline to 6 minutes post dose in mean C3a for the AI-700 subjects was notably smaller than that seen in Stage 1. During Stage 2, C3a levels were higher at 6 minutes post dose than at Baseline for all 7 subjects who were rechallenged with AI-700, but returned to near Baseline levels by 2 hours post dose. The mean C3a level at Baseline was 68.6 ng/mL. Mean post-dose values for the AI-700 group were 342.4 ng/mL, 153.7 ng/mL, and 75.9 ng/mL at 6 minutes, 30 minutes, and 2 hours post dose, respectively.

During both Stage 1 and Stage 2, C3 was measured at Baseline and at 2 hours post dose.

Slight decreases from Baseline to 2 hours post dose were seen in mean C3 levels among AI-700- and placebo-treated subjects during Stage 1. There was no notable change in mean C3 level for the AI-700 group during Stage 2. As a further measure of the activation of complement following administration of AI-700, the observed peak instantaneous rate of complement activation (6 minutes postdose) was calculated using C3 and C3a values. The average rate of consumption of C3 at 6 minutes post dose was determined to be <1%, assuming that no appreciable synthesis of new C3 occurred between Baseline and 6 minutes post dose.

Levels of tryptase did not rise (indicating that rising C3a levels immediately after dosing did not precipitate any anaphylactic response). CRP levels from AI-700-04 are shown in the table below 24 hours after administration in stage 1 and 120 minutes after administration in stage 2.

Table 22: Individual C-Reactive Protein Results (mg/dL) by Treatment Group, Stage, and Timepoint

	AI-700 Subjects										Placebo Subjects	
	901	902	002	003	005	007	008	009	012	014	001	006
Stage 1												
-10 min	0.10	0.05	0.02	0.06	0.13	0.08	0.06	0.49	0.02	0.34	0.02	0.76
24 hours	0.40	0.33	0.30	0.69	1.10	0.62	0.42	0.86	0.11	0.92	0.02	0.47
Stage 2												
-10 min	0.36	0.08	0.05	0.21	NA	0.05	0.16	NA	NA	0.08	<0.02	0.38
120 min	0.33	0.09	0.05	0.19	NA	0.05	0.16	NA	NA	0.08	<0.02	0.35

Source: Section 16.2.8, Data Listing 8.5
NA = not assessed (Subject Nos. 005, 009, and 012 did not return for Stage 2 dosing.)

Safety related to drug-drug interactions and other interactions

AI-700 is intended for administration with dipyridamole and this is considered in assessment of adverse reactions. It is of note that in many studies adverse events were substantially increased following dipyridamole and this has been considered in reviewing safety.

Discontinuation due to AES

There were 16 subjects with an AE that resulted in permanent discontinuation of AI-700, including 1 healthy volunteer in Phase II study AI-700-21, 2 CAD patients from the pilot study AI-700-23, and 13 CAD patients from studies AI-700-32 and AI-700-33. The most common AEs in these patients were hypotension and vasovagal syncope (Table 26).

Table 26: Adverse Events Resulting in Permanent Discontinuation of AI-700

Patient Number/Age/Race	Adverse Event(s) and Intensity	Timing	Resolution and Treatment
21.07.904 (36-year-old White female HV)	moderate chest pain and tachycardia	during the second dose of AI-700	both AEs resolved w/o treatment
23.16.003 (76-year-old White female)*	moderate headache, mild vertigo, moderate hypertension	after the first AI-700 dose	resolved with treatment (metoprolol i.v.)
23.34.003 (68-year-old White male)	moderate hypotension	after the first AI-700 dose	resolved w/o treatment
32.02.002 (56-year-old Black female)	mild headache and moderate rigors	after the first dose of AI-700	resolved w/o 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 w/o treatment; coughing resolved with treatment
32.16.021 (68-year-old White male)	severe sweating, severe hypotension, moderate tachypnoea, and mild vertigo	after the first dose of AI-700	resolved with treatment (promethazine hydrochloride)
32.07.032 (66-year-old Asian female)	mild dizziness and moderate hypotension	during the second dose of AI-700	dizziness resolved w/o 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
32.07.091 (65-year-old Asian female)	mild hypotension	during the second 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 w/o 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, diarrhoea, dizziness, feeling hot, 2:1 A-V block, bradycardia, hyperhydrosis, hypotension, and nausea (all severe), mild QT prolongation	after the first dose of AI-700	resolved w/o treatment mild QT prolongation resolved within 4 hours

Temporary Interruption

There were 6 patients with an AE resulting in temporary interruption of AI-700, and 1 patient with an AE (moderate hypotension) resulting in an adjustment of the AI-700 dosing regimen

(Table 27). Of these 7 patients, 4 were enrolled in study AI-700-33, 2 were enrolled in study AI-700-32, and 1 was enrolled in study AI-700-23.

Table 27: Adverse Events Resulting in Temporary Interruption or Dose Adjustment of AI-700

Patient Number/Age/Race	Adverse Event(s) and Intensity	Timing	Resolution and Treatment
23.08.001 (64-year-old White male)	severe chest pain	temporary interruption of second dose of AI-700	resolved with treatment (aminophylline)
32.31.020 (50 year-old White male)	mild cold sweat	temporary interruption of second dose of AI-700	resolved w/o treatment
32.02.015 (78 year-old White male)	moderate hypotension	adjustment of the AI-700 dosing regimen during Imaging Session II *	resolved with treatment (250cc normal saline bolus)
33.35.073 (64-year-old White female)	bradycardia, nausea, and vomiting (all mild)	temporary interruption of second dose of AI-700	resolved with treatment (aminophylline)
33.49.047 (64-year-old White male)	moderate nausea, mild headache, moderate decreased blood pressure, and moderate dizziness	temporary interruption of second dose of AI-700	resolved w/o treatment
33.49.082 (58-year-old Hispanic male)	severe chest pain, mild headache, mild ear pain, mild neck pain, and mild throat pain	temporary interruption of second dose of AI-700	resolved w/o treatment
33.49.093 (52-year-old Hispanic male)	moderate chest pain	temporary interruption of second dose of AI-700	resolved with treatment (aminophylline, glyceryl trinitrate

Therefore, 16 patients were withdrawn, most (13) during or after the first dose. There were different symptoms, such as hypotension (at least 5), syncope, rigors, weakness and flushing. Seven subjects required temporary interruption with various symptoms including chest pain and hypotension.

Discussion on clinical safety

The majority of AEs in the AI-700 studies were mild in intensity, of short duration, and resolved without treatment or residual effects. However, a small proportion of subjects experienced severe AEs and drug discontinuation that were considered related to AI-700 administration, but all were self-limiting or resolved with medical intervention.

It is accepted that the type and temporal relationship of AEs to the administration of dipyridamole and its peak pharmacological effect may suggest that many of the most common AEs observed in the rest/stress AI-700 trials were likely to be due in part if not completely to dipyridamole administration. The issue regarding increase in AEs following administration of dipyridamole prompts careful examination of AEs seen before such administration. However, the increase in AEs following administration is significant and it cannot be excluded that this increase may represent an additive effect (rather than solely an effect of the dipyridamole).

The clinical safety of AI-700 was evaluated in 11 completed clinical studies that enrolled a total of 1,241 subjects. Of these subjects, 1,194 received at least one dose of AI-700, of which 130 were healthy volunteers, 18 were CHF/COPD patients, and 1,046 were CAD patients. Of the

1,046 CAD patients who received AI-700, 911 patients (87%) were enrolled based upon a recent history of stable chest pain (Intended Dose with Stressor Population). The remaining patients were enrolled based upon moderate to high pre-test probability of CAD. All efficacy patients had single or dual injection exposure to AI-700. In clinical practice, patients are anticipated to be exposed to AI-700 no more than once a year, with the potential to be re-evaluated several times during their lives.

During the Phase I and Phase II studies, 66 subjects received placebo. The common AEs in subjects who received placebo were similar to those seen in subjects who received AI-700, i.e. headache, chest tightness, dizziness, and flushing. In the dose-ranging studies, a dose-dependent increase in the number of AEs was observed following a single injection of AI 700 at rest in healthy volunteers. This dose-dependent increase was driven primarily by the most common AEs of flushing, cough, and nausea.

Of the 911 CAD patients treated with AI-700, the most frequently reported AEs were headache (35%), chest pain (12%), nausea (10%), flushing (9%), chest discomfort (8%), dizziness (6%), feeling hot (5%), dyspnoea (4%), rigors (4%), ECG ST segment depression (4%), hypotension

(3%), abdominal pain (2%), pain in extremity (2%), pyrexia (2%), and neck pain (2%). A review of AE data for patients who received AI-700 with and without dipyridamole showed a clear correlation between the incidence of these AEs and administration of dipyridamole.

These AEs were reported in a small number of patients (≤3%) following the first dose of AI-700 and then the incidence increased 5-fold following dipyridamole administration and peak pharmacological stress (consistent with the dipyridamole package insert).

A number of issues support the idea of complement mediated reactions. C3a levels were increased with no associated elevation of tryptase. CRP levels were increased.

Small, transient fluctuations (<1%) from Baseline in mean SaO2 values were observed after both injections of AI-700. Though there appears to be a temporal relationship to the administration of AI-

700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation). Further discussion of these changes and their significance (and mechanism) is requested.

A clear dose-dependent trend was observed in the WBC spectrum. A consistent neutrophil mediated effect on WBC was observed. In Study AI-700-04, a transient decrease in circulating neutrophils was observed at 6 minutes post AI-700 dosing and this was followed by a more gradual recovery phase in which the WBC count increased to reach pre-dosing levels after 30-60 minutes. Similar elevations in both parameters were observed consistently across AI-700 studies at approximately 2 to 3 hours post dosing. Correlative changes in monocytes were also observed, while lymphocyte count tended to be inversely related to neutrophils. All values returned to Baseline levels within 24 hours post dosing.

It is of note that Mini Mental State Examinations were performed in a number of studies (01, 02, 20). One subject (a volunteer) in the first study had transient changes. Did changes occur in any other volunteers and patients? In study 02, subject 2005 had a drop from 30 to 26, 5 minutes after drug administration; subject 3002 from 30 to 28 and 1001 and 1006 had drops of 1 at 5 minutes. In the -20 study only mean results are presented. What if anything was the significance of these changes? The Applicant should provide a listing of all changes and discuss the changes and their significance. Further discussion on the case of mental status change in study AI-700-32 would be helpful in particular considering possible causes. This issue had been resolved at the time of withdrawal.

Small, transient fluctuations (<1%) from Baseline in mean SaO2 values were observed after both injections of AI-700. Though there appears to be a temporal relationship to the administration of AI-700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation). Further discussion of these changes and their significance (and mechanism) is requested. This issue had been resolved at the time of withdrawal.

Transient decreases of <1% in mean SaO2 were observed following of AI-700 in CAD patients, with a return toward Baseline by the 60-minute post-dose time point. The greatest decreases in SaO2 occurred 11 to 13 minute post-dosing. Again the applicant suggests that a potential mechanism is that this is another manifestation related to complement activation.

Changes in blood pressure were observed in the Intended Dose with Stressor Population (stable chest pain patients). Approximately 75% of these patients had a history of hypertension and were being managed with antihypertensive therapy. In this patient population**,** a small reduction (6% to 7%) from Baseline was observed for both mean systolic and mean diastolic blood pressures between 5 and 10 minutes after AI-700 dosing. The reductions in mean systolic and diastolic blood pressures returned towards baseline values by 30 minutes following AI-700 dosing in both sessions.

Adverse events of pyrexia and increased body temperature were reported in 24 (3%) of the 911 patients and these events were not associated with other signs of infection.

Complement activation is presumably occurring on the basis of the presence of the polymeric microsphere component of AI-700 which is presumed to be cleared by the reticuloendothelial cells of the lung, liver, kidney, and spleen after dosing over a period of days or weeks.

Conclusions on clinical safety

The majority of AEs in the AI-700 studies were mild in intensity, of short duration, and resolved without treatment or residual effects. However, a small proportion of subjects experienced severe AEs and drug discontinuation that were considered related to AI-700 administration. These severe

reactions, manifesting during the drug development phase, are not dissimilar to severe reactions that have been seen with some similar marketed products.

Of the 911 CAD patients treated with AI-700, the most frequently reported AEs were headache (35%), chest pain (12%), nausea (10%), flushing (9%), chest discomfort (8%), dizziness (6%), feeling hot (5%), dyspnoea (4%), rigors (4%), ECG ST segment depression (4%), hypotension (3%), abdominal pain (2%), pain in extremity (2%), pyrexia (2%), and neck pain (2%). A review of AE data for patients who received AI-700 with and without dipyridamole showed a clear correlation between the incidence of these AEs and administration of dipyridamole.

These AEs were reported in a small number of patients (≤3%) following the first dose of AI-700 and then the incidence increased 5-fold following dipyridamole administration and peak pharmacological stress (consistent with the dipyridamole package insert). However, it cannot be excluded that there is an additive effect when dipyridamole is used.

Also of concern, especially in the context of the infrequent but severe adverse reactions, is the evidence of complement mediated reactions. C3a levels were increased with no associated elevation of tryptase. CRP levels were increased.

Small, transient fluctuations (<1%) from Baseline in mean SaO2 values were observed after both injections of AI-700. Transient decreases of <1% in mean SaO2 were observed following of AI-700 in CAD patients, with a return toward Baseline by the 60-minute post-dose time point. The greatest decreases in SaO2 occurred 11 to 13 minute post-dosing. Though there appears to be a temporal relationship to the administration of AI-700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation). Again the applicant suggests that a potential mechanism is that this is another manifestation related to complement activation and the applicant makes acceptable proposals for changes to the SmPC to address use in patients at risk of respiratory symptoms.

A clear dose-dependent trend was observed in the WBC spectrum. A consistent neutrophil mediated effect on WBC was observed. In Study AI-700-04, a transient decrease in circulating neutrophils was observed at 6 minutes post AI-700 dosing and this was followed by a more gradual recovery phase in which the WBC count increased to reach pre-dosing levels after 30-60 minutes. Similar elevations in both parameters were observed consistently across AI-700 studies at approximately 2 to 3 hours post dosing. Correlative changes in monocytes were also observed, while lymphocyte count tended to be inversely related to neutrophils. All values returned to Baseline levels within 24 hours post dosing.

Changes in blood pressure were observed in the Intended Dose with Stressor Population (stable chest pain patients). Approximately 75% of these patients had a history of hypertension and were being managed with antihypertensive therapy. In this patient population**,** a small reduction (6% to 7%) from Baseline was observed for both mean systolic and mean diastolic blood pressures between 5 and 10 minutes after AI-700 dosing. The reductions in mean systolic and diastolic blood pressures returned towards baseline values by 30 minutes following AI-700 dosing in both sessions.

Adverse events of pyrexia and increased body temperature were reported in 24 (3%) of the 911 patients and these events were not associated with other signs of infection.

Complement activation is presumably occurring on the basis of the presence of the polymeric microsphere component of AI-700 which is presumed to be cleared by the reticuloendothelial cells of the lung, liver, kidney, and spleen after dosing over a period of days or weeks.

It is of note that Mini Mental State Examinations were performed in a number of studies (01, 02, 20). One subject 407 (a volunteer) in the first study had transient changes. Did changes occur in any other

subjects? In study 02, subject 2005 had a drop from 30 to 26, 5 minutes after drug administration; subject 3002 from 30 to 28 and 1001 and 1006 had drops of 1 at 5 minutes. In the -20 study only mean results are presented. What if anything was the significance of these changes? The applicant suggests that some of these changes may be occurring as manifestations of complement activation. This issue had been resolved at the time of withdrawal.

There were unexplained effects in Subject 407, a black male of 30 years had a WBC of 4.9 pre-dose, increasing to 8.7 at 2 hours and 10.8 at 4 hours. This subject who received the 4.0mg/kg dose experienced a "constellation of neurological and respiratory AEs within minutes of administration of AI-700. AEs of difficulty communicating, shortness of breath, cough, pain were severe on onset but abated within 20 minutes and resolved within 40 minutes". At the time of the event "difficulty communicating" the subject had a decrease in MMSE score to 22 (5 minutes post dose) from a pre-dose of 27 without a corresponding change to BNA. This subject also had an increase in blood pressure which returned to normal in 60 minutes. This subject recovered fully. There is limited discussion of this case (likely cause or explanation) in the study report some of this subject's symptoms may be due to complement activation but difficulty communicating is less likely to be explained in this way. This issue had been resolved at the time of withdrawal.

Thus the safety of Imagify has not yet been adequately characterised both in terms of cardiovascular events and in terms of other infrequent, but severe adverse reactions, including complement activation (CARPA), with signs of inflammation, changes in blood pressure and decrease in SaO2. Comparison with SPECT should be made, based on the findings of studies AI-700-32 and 33. Identification of High risk groups for side effects has been considered but not extensively discussed, taking into account findings with other agents for CV indications using microspheres.

Discussion on clinical safety

The majority of AEs in the AI-700 studies were mild in intensity, of short duration, and resolved without treatment or residual effects. However, a small proportion of subjects experienced severe AEs and drug discontinuation that were considered related to AI-700 administration, but all were self-limiting or resolved with medical intervention.

It is accepted that the type and temporal relationship of AEs to the administration of dipyridamole and its peak pharmacological effect may suggest that many of the most common AEs observed in the rest/stress AI-700 trials were likely to be due in part if not completely to dipyridamole administration. The issue regarding increase in AEs following administration of dipyridamole prompts careful examination of AEs seen before such administration. However, the increase in AEs following administration is significant and it cannot be excluded that this increase may represent an additive effect (rather than solely an effect of the dipyridamole).

Of the 911 CAD patients treated with AI-700, the most frequently reported AEs were headache (35%), chest pain (12%), nausea (10%), flushing (9%), chest discomfort (8%), dizziness (6%), feeling hot (5%), dyspnoea (4%), rigors (4%), ECG ST segment depression (4%), hypotension (3%), abdominal pain (2%), pain in extremity (2%), pyrexia (2%), and neck pain (2%).

It is of note that Mini Mental State Examinations were performed in a number of studies (01, 02, 20). One subject (a volunteer) in the first study had transient changes. In study 02, subject 2005 had a drop from 30 to 26, 5 minutes after drug administration; subject 3002 from 30 to 28 and 1001 and 1006 had drops of 1 at 5 minutes.

Small, transient fluctuations (<1%) from Baseline in mean SaO2 values were observed after both injections of AI-700. Though there appears to be a temporal relationship to the administration of AI-700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation). Further discussion of these changes and their significance (and mechanism) is requested. The applicant considers that the changes could be due to Imagify and because of complement activation. They make acceptable proposals to contraindicate in patients with severe COPD and advise caution when performing Imagify echocardiography in patients with moderate COPD or with resting oxyhaemoglobin (SaO2) levels below 90 %. It is noted that Imagify has the potential to cause transient drops in FEV1 and SaO2 and Caution should be exercised in at risk patients. The applicant should further elaborate on examples or specify what is meant by " at risk" patients. This issue had been resolved at the time of withdrawal.

Although serious AEs were not frequent, they were nevertheless of potential concern, particularly in the context of this application relating to a diagnostic rather than therapeutic product. Serious adverse reactions in the context of a diagnostic product must have a strong influence of assessment of benefit risk ratio.

In the Phase III studies, a total of 11 (<1%) out of 1,194 subjects who received any dose of AI-700 experienced 14 non-life threatening SAEs: 6 subjects in study AI-700-33, 4 subjects in study AI-700-32, and 1 subject in study AI-700-23.

Six patients experienced 7 acute SAEs (onset within 30 minutes following AI-700 dosing).

Following the first dose of AI-700, 3 patients experienced individual episodes of vasovagal syncope without loss of consciousness, and 1 patient experienced hypertension and vertigo.

Following the second AI-700 dose, 2 additional patients experienced acute SAEs. One patient experienced mental status changes (considered a possible conversion disorder by the Investigator possibly related to dipyridamole and aminophylline and probably related to AI-700), and one experienced an SAE of chest pain. There was in addition a severe adverse reaction in the first volunteer study involving an inability to communicate with associated changes in the mini mental scale.

These adverse reactions, taken in the context of the changes in white cells and elevations in complement raise the possibility of a risk of significant complement activation.

It is noted that Mini mental state and brief neurological assessments were included in early studies. The applicant has provided accounts of clinically relevant drops in MMSE in three subjects administered Imagify and suggests that dose and rate of drug delivery may have been higher with these patients. Additional discussion of the role of complement activation is expected.

A clear dose-dependent trend was observed in the WBC spectrum. A consistent neutrophil mediated effect on WBC was observed. In Study AI-700-04, a transient decrease in circulating neutrophils was observed at 6 minutes post AI-700 dosing and this was followed by a more gradual recovery phase in which the WBC count increased to reach pre-dosing levels after 30-60 minutes. Similar elevations in both parameters were observed consistently across AI-700 studies at approximately 2 to 3 hours post dosing. Correlative changes in monocytes were also observed, while lymphocyte count tended to be inversely related to neutrophils. All values returned to Baseline levels within 24 hours post dosing. The applicant suggests that a possible underlying mechanism for changes in the WBC spectrum is a concurrent increase in activated complement through the alternative pathway, as demonstrated by increased C3a levels in Study AI-700-04. Based on their review of literature and AI-700 human and animal data, the applicant suggests that moderate levels of complement activation may be part of the

clearance mechanism of particles from the systemic circulation. The pattern of severe (though not common) adverse reactions seems similar to other similar contrast agents and these severe reactions may represent more severe complement mediated reactions.

There remain issues to be discussed for the relative high number of leucocytosis / neutrophils. A table on adverse events with regard to currently ongoing trials is missing.

Changes in blood pressure were observed in the Intended Dose with Stressor Population (stable chest pain patients). Approximately 75% of these patients had a history of hypertension and were being managed with antihypertensive therapy. In this patient population**,** a small reduction (6% to 7%) from Baseline was observed for both mean systolic and mean diastolic blood pressures between 5 and 10 minutes after AI-700 dosing. The reductions in mean systolic and diastolic blood pressures returned towards baseline values by 30 minutes following AI-700 dosing in both sessions.

Adverse events of pyrexia and increased body temperature were reported in 24 (3%) of the 911 patients and these events were not associated with other signs of infection.

Changes in spirometry in patients with moderate COPD were of a degree for the applicant to decide not to proceed to assess patients with severe COPD.

Serial pulse oximetry was used to assess SaO2 in all subjects. In healthy volunteers, SaO2 values remained within the normal range for all subjects throughout the study. However, the frequency of dyspnoea, shortness of breath, and cough was greater in healthy subjects treated with AI-700 compared with placebo and these AEs tended to occur at the higher AI-700 doses. In some cases, SaO2 was seen to decrease but it usually remained above 90%.

In the CHF and moderate COPD cohorts of Study AI-700-05, mean SaO2 following AI-700 was >95% and changed <1% from Baseline (<2% for placebo) at all-time points. No patients had a decrease from Baseline of >5%. FEV1 changes were observed following AI-700 dosing in the COPD patients. Mean FEV1 decreases at all post-dose time points were 7% to 14% greater following AI-700 administration than following placebo. The greatest difference was observed at 15 to 20 minutes following the dose (mean FEV1 decrease of 21.9% for AI-700 versus 8.1% for placebo). All spirometry measurements returned to Baseline values by the 24-hour Follow-up.

A total of 40 (4%) of 911 patients in the Intended Dose with Stressor Population reported dyspnoea as an AE, with the majority (36 patients) experiencing dyspnoea after dipyridamole administration.

Transient decreases of <1% in mean SaO2 were observed following of AI-700 in CAD patients, with a return toward Baseline by the 60-minute post-dose time point. The greatest decreases in SaO2 occurred 11 to 13 minute post-dosing. Again the applicant suggests that a potential mechanism is that this is another manifestation related to complement activation.

In summary, use of Imagify seems to be associated with significant symptomatic complement activation in up to 50% of patients. The applicant has proposed SmPC changes to minimise use in at risk respiratory patients. Serious adverse reactions, possibly associated with complement activation did occur in small numbers of patients but it is unclear how such at risk patients should be identified.

Although adverse event reporting associated with dipyridamole was expected, serious adverse reactions did occur before dipyridamiole use and an additive effect cannot be excluded in association with dipyridamole.

Pharmacovigilance system

The applicant has provided a summary of their pharmacovigilance system in-line with Directive 2010/84/EU.

Risk management plan

See specific assessment report according to the PRAC timetable.

4. Orphan medicinal products

N/A

5. Benefit risk assessment

Beneficial effects

Intermediate risk patients need an accurate assessment of CAD so that if they have disease, it can be adequately and appropriately treated, and if they have no disease, they can avoid unnecessary additional invasive procedures.

The AI-700 ECHO test has been shown to provide satisfying pictures of the myocardium at rest and during exercise. There is a potential of Imagify ECHO to detect CAD in stable, intermediate risk patients with chest pain and to differentiate between scar and ischemia. If approved this test could be performed without use of cancer-inducing ionising radiation exposure.

The test could provide the cardiologist with real-time diagnostic information to quickly assess the presence or absence of significant CAD. The AI-700 ECHO test could provide simultaneous perfusion and wall motion information for improved diagnostic performance compared to placebo (non-contrast) ECHO. There is a suggestion that diagnostic accuracy is unaffected by poor non-contrast window quality.

By gating decisions the use of Imagify could avoid the risks associated with use of alternative procedures. ANGIO is the definitive test for detection of CAD but is not only costly and highly invasive, but it is also associated with significant acute morbidity (nephrotoxicity can occur in up to 50% of patients) and mortality of about 1 in 1,000. It also exposes patients to significant radiation, with its associated fatal cancer risk estimated to be 0.4 in 1,000. Due to these risks, ANGIO is only appropriate as a first line test in patients with high pre-test probability of disease.

SPECT relies primarily on myocardial perfusion information and so is considered a more sensitive procedure because its direct measurement of perfusion, as predicted by the ischaemic cascade, can reveal CAD at an earlier stage. However, SPECT is expensive, time consuming, and exposes the patient to the risk of fatal malignancy from the radiation dose of between 8 and 23mSv depending on the radiopharmaceuticals used.

Imagify can be used in patients who cannot exercise, although pharmacological stress carries significant increased risk, e.g., the risk of life-threatening events associated with dipyridamole stress testing is estimated to be 0.7 patients per thousand, in addition to a high rate of non-serious AEs. However, pharmacologic stress also provides significant benefits, allowing non-invasive detection of inducible ischaemia in patients who would otherwise have to proceed directly to ANGIO.

Stress ECHO is the other non-invasive modality recommended for additional risk stratification of intermediate pre-test probability CAD patients. ECHO, in addition to being radiation free, has

significant positive clinical, logistical, and economic attributes. However, stress ECHO also has significant risks associated with its use. Stress ECHO only detects changes in wall motion and cannot directly assess perfusion. Thus stress ECHO, as predicted by the ischaemic cascade, may miss earlier stage CAD, which may only cause small perfusion defects, but not wall motion abnormalities.

Uncertainty in the knowledge about the beneficial effects

The efficacy has not been well documented and it is unclear how AI-700 stress ECHO and SPECT compare. Non-inferiority of specificity as compared to SPECT has not been demonstrated since in one study specificity of the comparator is not reliable and in the other study non-inferiority was not achieved. Results for sensitivity were not consistent over the two pivotal trials.

In addition, the diagnostic value in patients with myocardial infarction is unclear. The studies showed a low specificity in these patients, the data are difficult to interpret and the diagnostic value of detection of ischemia in patients with a known scar has not been investigated. The added value over ECHO alone and stress ECHO alone without Imagify is unclear. Overall, considering the issues regarding design and conduct of the pivotal studies, it is difficult to draw firm conclusions regarding the efficacy of Imagify and the diagnostic value.

Unfavourable effects

The majority of AEs in the AI-700 studies were mild in intensity, of short duration, and resolved without treatment or residual effects. However, a small proportion of subjects experienced severe AEs and drug discontinuation that were considered related to AI-700 administration, but all were self-limiting or resolved with medical intervention.

Of the 911 CAD patients treated with AI-700, the most frequently reported AEs were headache

(35%), chest pain (12%), nausea (10%), flushing (9%), chest discomfort (8%), dizziness (6%), feeling hot (5%), dyspnoea (4%), rigors (4%), ECG ST segment depression (4%), hypotension

(3%), abdominal pain (2%), pain in extremity (2%), pyrexia (2%), and neck pain (2%).

Also of concern, especially in the context of the infrequent but severe adverse reactions, is the evidence of complement mediated reactions. C3a levels were increased with no associated elevation of tryptase. CRP levels were increased.

Transient decreases of <1% in mean SaO2 were observed following of AI-700 in CAD patients, with a return toward Baseline by the 60-minute post-dose time point. The greatest decreases in SaO2 occurred 11 to 13 minute post-dosing. Though there appears to be a temporal relationship to the administration of AI-700, these changes occurred in a small subset of patients (5 patients experienced 11 instances in which SaO2 values were below 90% saturation).

A clear dose-dependent trend was observed in the WBC spectrum. A consistent neutrophil mediated effect on WBC was observed. In Study AI-700-04, a transient decrease in circulating neutrophils was observed at 6 minutes post AI-700 dosing and this was followed by a more gradual recovery phase in which the WBC count increased to reach pre-dosing levels after 30-60 minutes. Similar elevations in both parameters were observed consistently across AI-700 studies at approximately 2 to 3 hours post dosing. Correlative changes in monocytes were also observed, while lymphocyte count tended to be inversely related to neutrophils. All values returned to Baseline levels within 24 hours post dosing.

Changes in blood pressure were observed in the Intended Dose with Stressor Population (stable chest pain patients). Approximately 75% of these patients had a history of hypertension and were being

managed with antihypertensive therapy. In this patient population, a small reduction (6% to 7%) from Baseline was observed for both mean systolic and mean diastolic blood pressures between 5 and 10 minutes after AI-700 dosing. The reductions in mean systolic and diastolic blood pressures returned towards baseline values by 30 minutes following AI-700 dosing in both sessions.

Adverse events of pyrexia and increased body temperature were reported in 24 (3%) of the 911 patients and these events were not associated with other signs of infection.

It is of note that Mini Mental State Examinations were performed in a number of studies (01, 02, 20). In these studies, 3 healthy volunteers and patients exhibited transient changes. These all occurred at higher than currently recommended doses and at faster than currently recommended injection rates. The applicant suggests that some of these changes may be occurring as manifestations of complement activation.

One of these subjects (#407), a black male of 30 years had a WBC of 4.9 pre-dose, increasing to 8.7 at 2 hours and 10.8 at 4 hours. This subject who received the 4.0mg/kg dose (2.7 times the clinical maximum single dose and injected >5 times faster than the recommended clinical dosing regime) experienced a “constellation of neurological and respiratory AEs within minutes of administration of AI-700. AEs of difficulty communicating, shortness of breath, cough, pain were severe on onset but abated within 20 minutes and resolved within 40 minutes”. At the time of the event ”difficulty communicating” the subject had a decrease in MMSE score to 22 (5 minutes post dose) from a pre-dose of 27 without a corresponding change to BNA. This subject also had an increase in blood pressure which returned to normal in 60 minutes. This subject recovered fully.

Uncertainty in the knowledge about the unfavourable effects

Further information is required on the safety of Imagify. The evidence above suggests complement activation in many if not all patients receiving it. Some of the serious adverse reactions, involving for instance vasovagal attack and hypotension may represent more extreme examples of complement activation and it is of note that these cases are occurring in the context of clinical trials rather than in marketed use. It is unknown if the severity of reactions seen with other similar agents will be found to occur with Imagify.

The safety of Imagify has not been adequately characterised both in terms of cardiovascular events and in terms of other infrequent, but severe adverse reactions, including complement activation (CARPA), signs of inflammation, changes in blood pressure and decrease in SaO2. Pulmonary hypertension due to trapping of microspheres should be ruled out. Comparison with SPECT should be made, based on the findings of studies AI-700-32 and 33. Identification of High risk groups for side effects should be considered and discussed, taking into account findings with other agents for CV indications using microspheres.

Importance of favourable and unfavourable effects

Imagify could be potentially useful as a screening test in considering patients for further investigation of CAD. The adverse reactions are for the most part mild but the underlying evidence of complement activation is of concern.

Benefit-risk balance

Benefit has not been clearly shown. There are a number of significant statistical issues and concerns regarding the conduct of one of the pivotal studies. Sensitivity and specificity of Imagify in the

proposed indication are not shown and the diagnostic value above ECHO and stress ECHO remains to be determined.

From a safety point of view the suggestion of complement activation and the occurrence of severe though infrequent adverse reactions is of concern.

Discussion on the benefit-risk assessment

Benefit has not been clearly shown and there is complement activation which may in some cases cause severe adverse reactions. In considering benefit risk of a diagnostic agent, greater emphasis must be given safety issues. In this case, benefit has not been shown in a satisfactory manner and there are definite safety issues which need to be examined further.

Conclusions

The overall B/R of Imagify is considered, negative.

6. CHMP list of questions

6.1. Quality aspects

Major objections

Drug Product

1. Justification in accordance with the European Note for Guidance on Process Validation (CPMP/QWP/848/96) is necessary to show that the process validation results presented in respect of the microencapsulation and lyophilisation processes are acceptable. This Note for Guidance requires successful completion of three consecutive production batches, which the applicant has failed to achieve.

   This issue had been resolved at the time of withdrawal

Other concerns

Drug Product

2. Validated sterile hold times and maximum filling time should be incorporated in Module 3.

   This issue had been resolved at the time of withdrawal

3. Apparent discrepancies in the some parameters of the lyophilisation cycle between MA dossier and validation reports should be clarified with appropriate updates to the CTD documents.

   This issue had been resolved at the time of withdrawal

4. A corrected version of the application form (with revised section 2.2.1 and 2.6.1) should be submitted. The composition and the strength (expressed by the mass of the active substance perflubutane per vial and per ml) should be given separately for the lyophilized drug product as well as for the reconstituted form.

   This issue had been resolved at the time of withdrawal

6.2. Non clinical aspects

Major objections

Pharmacology

None

Pharmacokinetics

None

Toxicology

None

Other concerns

Pharmacology

None

Pharmacokinetics

None

Toxicology

None

6.3. Clinical aspects

Major objections

Pharmacokinetics

None

Pharmacodynamics

None

Efficacy

The applicant is asked to address the following issues in an oral explanation (taking account of the more extensive major objections to address in writing) and to discuss the clinical relevance.

- Sufficient specificity of Imagify ECHO to detect CAD has not been demonstrated in the clinical program.
- The added value of Imagify ECHO stress echocardiography over standard stress ECHO is unclear
- The diagnostic value of Imagify ECHO in patients with previous myocardial infarction has not been established.

The applicant is requested to address the following in writing:

1) Sufficient specificity of Imagify ECHO to detect CAD has not been demonstrated in the clinical program.

   Accuracy is not considered an adequate primary endpoint and the non-inferiority of the method in terms of sensitivity and specificity against the comparator SPECT has not been consistently demonstrated in the pivotal trials.

   In AI-700-32, specificity of SPECT readings was in an unacceptably low range, when compared to ANGIO/LVG as a truth standard. Specificity of SPECT was subject to bias, when compared to SPECT itself as part of the alternative truth standard. In addition, in study AI-700-33 non

inferiority of specificity has not been demonstrated. Therefore, the overall clinical program failed to demonstrate sufficient specificity of Imagify stress ECHO.

2) The added value of Imagify stress ECHO over standard ECHO has not been demonstrated in the clinical program.

The pivotal trials aimed at detection of CAD based on Imagify ECHO findings. However, the trials included patients with known CAD (e.g. patients with a history of myocardial infarction) and an unknown number of patients with wall motion abnormalities that was already seen in a standard ECHO. For these patients there is no diagnostic benefit when using Imagify ECHO since ischaemia cannot be differentiated from scar.

Furthermore, it is unclear, whether sensitivity and specificity to detect CAD in this large subgroup of patients in the pivotal trials is transferable to the remaining patients. Sensitivity and specificity may be different between both groups.

3) The diagnostic value of Imagify ECHO in patients with previous myocardial infarction has not been established.

In patients with a history of a previous MI specificity to detect CAD was in a low range. This may in part be due to the definition of the standard of truth in these patients. In addition, the clinical benefit of a diagnostic tool confirming CAD in patients with known previous MI is not obvious. The relevant question in these patients is, whether they have ischemia or not. However, no data were provided demonstrating that presence or absence of stress induced ischaemia can be differentiated in these patients.

This issue had been resolved at the time of withdrawal

Safety

4) Imagify causes complement activation manifesting as pyrexia, drop in blood pressure, change on complement levels, changes in CRP, changes in SaO2 (and FEV1 in COPD patients) in approximately 50% of patients and these reactions are significant in at least 3% of patients. The difference between this product and others similar agents is that complement activation seems to take place in such a large proportion of patients. Reactions with other products were noted when the products were marketed and these reactions though rare were serious.

Acusphere acknowledges that in routine clinical practice after approval, a higher frequency of adverse reactions may occur because of less strict patient selection and suggests that in any case, serious reactions occur where trained personnel are prepared for acute emergency. Severe reactions and death occurred with other agents in similar situations. What is intended by “extreme caution” (unstable cardiopulmonary status) versus caution (pulmonary hypertension)?

Safety is still a concern in the context of the benefit and specifically the benefit risk.

This issue had been resolved at the time of withdrawal

Other Concerns

5) The applicant should provide evidence that NI of Imagify ECHO vs. SPECT for accuracy can be robustly concluded based on an adequately justified NI margin which rules out clinically unacceptable loss of efficacy. If this is not possible, it should be justified why clinically unacceptable loss of efficacy can nevertheless be reasonably excluded.

6) SmPC wording for renal and hepatic impairment has been removed from section 4.2. A section on Older people also should be added.

   This issue had been resolved at the time of withdrawal

7) Impact on therapeutic decisions and clinical outcome, which refers to a description and quantification of impact of diagnostic information on management of a patient and clinical outcome, has not been addressed.

8) Section 4.8 should be amended: paragraphs on Complement activation and Use with a pharmacologic stressor should be moved to section 4.4. The paragraph on complement activation should specify which undesirable effects are consistent..... In the paragraph on use with a pharmacologic stressor, the use of the sentence “In Phase II placebo trial.... “is questioned. It is not clearly stated that adverse reactions occurred more commonly with the Imagify/dipyridamole combination than is reported in the literature for dipyridamole alone.

   This issue had been resolved at the time of withdrawal

9) Study AI-700-21 is not a reliable study to assess the additional benefit of Imagify over placebo. The dose closest to the dose used in the pivotal trials (2 x 0.04 mL/kg) was the 0.081 ml/kg dose that was applied in a non-randomised fashion. For this dose Sensitivity and specificity were not clearly different from placebo. The applicant states that a differentiation between fixed and reversible defects was not an aim of the program. In that case, Imagify ECHO has no diagnostic role in a patient, where a scar or a wall motion abnormality can already be detected by a standard ECHO without a contrast agent. In addition to the unclear results in patients after MI discussed above the application could be restricted to patients without wall motion abnormalities at rest in a standard ECHO and without a previous myocardial infarction. However, this is not the patient population that has been investigated in the pivotal trials.

10) In the AI-700-32 trial 6 patients classified as disease positive by left ventriculography (LVG) had negative coronary angiography (ANGIO). It is concluded that these patients had scar but no relevant ischaemia. The data showed that in patients with fixed defects in the LVG without ischaemia the sensitivity of Imagify ECHO to detect defects was high. A differentiation between scar and ischaemia could not be reliably achieved. It can be concluded that in those patients, where a wall motion abnormality can be detected by standard ECHO, Imagify ECHO has no added benefit.Taken together, in case wall motion abnormalities can be seen at rest by standard ECHO with sufficient confidence, there is no diagnostic benefit when using Imagify stress ECHO in such a patiant. The clinical program has not demonstrated a higher specificity of Imagify ECHO over standard ECHO in these cases. The added value of Imagify ECHO over standard ECHO has not been shown.The applicant is asked to comment.

11) The applicant has not addressed the multiplicity that arises when, after the three readers have been NI compared with the SPECT standard, success is based on any two of them being significantly non-inferior. The applicant would need to justify, with references if possible, why they consider that multiplicity is not an issue when choosing any two out of three results. In addition, the applicant should comment on whether, in its statistical analyses of non-inferiority

or superiority, it took account of the inherent pairing involved when comparing any two readers (SPECT and ECHO). The pairing arises from the fact that the ECHO and SPECT were taken from the same patient.

This issue had been resolved at the time of withdrawal.

12) In Subject 407, the applicant considers that complement activation is a plausible explanation. It is also suggested that the dose was 2.7 times the maximum single dose and there is a suggestion but no confirmation that the rate of administration was faster than normal. Signs and symptoms in this case should be included in section 4.9.

This issue had been resolved at the time of withdrawal

13) The description of the clinical development which has now been added is not appropriate in section 4.8

This issue had been resolved at the time of withdrawal

Pharmacovigilance system

None

Risk management plan

Imagify (AI-700) RMP endorsed per PRAC Rapporteur's Assessment Report, 8 May, 2014.

Pharmacokinetics

None

Pharmacodynamics

None

Additional information provided by the applicant

During the Oral Explanation on 21 May 2014, the applicant provided additional clarification on the major objections, however the CHMP feel that there are still outstanding issues to be resolved. The CHMP agreed to convene an expert meeting in order to search clarification on some aspects of the product. A list of questions for the expert meeting was agreed

In addition the CHMP agree to adopt the 3rd List of Outstanding issues to the Applicant. The Applicant will have to submit written responses to the question as well as in an Oral explanation. The List of Oustanding issues is written below.

Major Objections

1. Sufficient specificity of Imagify ECHO to detect CAD has not been demonstrated in the clinical program. Accuracy is not considered an adequate primary endpoint and the non-inferiority of the method in terms of sensitivity and specificity against the comparator SPECT has not been consistently demonstrated in the pivotal trials.

2. In AI-700-32, specificity of SPECT readings was in an unacceptably low range, when compared to ANGIO/LVG as a truth standard. Specificity of SPECT was subject to bias, when compared to

SPECT itself as part of the alternative truth standard. In addition, in study AI-700-33 non inferiority of specificity has not been demonstrated. Therefore, the overall clinical program failed to demonstrate sufficient specificity of Imagify stress ECHO.

3. The added value of Imagify stress ECHO over standard ECHO has not been demonstrated in the clinical program.

Other concerns

4. The applicant has amended the SmPC wording to address safety concerns. It cannot be confidently said that no life-threatening cardiopulmonary or anaphylactoid reactions have occurred with Imagify? The wording proposed offers an inappropriate assurance as to the safety of Imagify. The applicant suggests that the potential risk of a life threatening reaction with Imagify has to be considered in the context of radiation associated with SPECT. Whilst minimising radiation exposure in any patient is desireable, exposure to a fatal reaction is clearly not. Partially resolved (Slight SmPC modification required).

5. The applicant is still obliged to provide evidence that non-inferiority (NI) of Imagify ECHO vs. SPECT for accuracy can be robustly concluded based on an adequately justified NI margin which rules out clinically unacceptable loss of efficacy. If this is not possible, it should be justified why a formal proof of NI is not needed.

6. Impact on therapeutic decisions and clinical outcome, which refers to a description and quantification of impact of diagnostic information on management of a patient and clinical outcome, has not been addressed.