

**Cardiovascular and Renal Drugs  
Advisory Committee  
December 10, 2008**

**Imagify™  
(Perflubutane Polymer Microspheres)**

**ACUSPHERE**

# AI-700: Introductory Remarks

Michael Slater  
Senior VP, Regulatory Affairs and Operations

Acusphere, Inc.  
Watertown, MA

# Agenda

## **Introductory Remarks**

**Michael R. Slater**  
Acusphere, Inc.

## **Use of Ultrasound Contrast for the Detection of Coronary Artery Disease**

**Michael H. Picard, MD, FACC, FASE**  
Director, Clinical Echocardiography  
Massachusetts General Hospital

## **AI-700 Imaging**

**Professor R. Senior MD, DM, FRCP, FESC, FACC**  
Consultant Cardiologist & Director of Cardiac Research,  
Northwick Park Hospital, London

## **AI-700 Clinical Efficacy**

**Richard C. Walovitch, PhD**  
Acusphere, Inc.

## **AI-700 Clinical Safety**

**Howard C. Dittrich, MD, FACC**  
Clinical Professor of Medicine  
University of California, San Diego

## **Innate Immune Response to AI-700**

**John D. Lambris, PhD**  
Dr. Ralph and Sallie Weaver Professor of Research Medicine  
Department of Pathology & Laboratory Medicine  
University of Pennsylvania

## **Concluding Remarks**

**Richard C. Walovitch, PhD**  
Acusphere, Inc

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# Limitations of SPECT and Stress ECHO

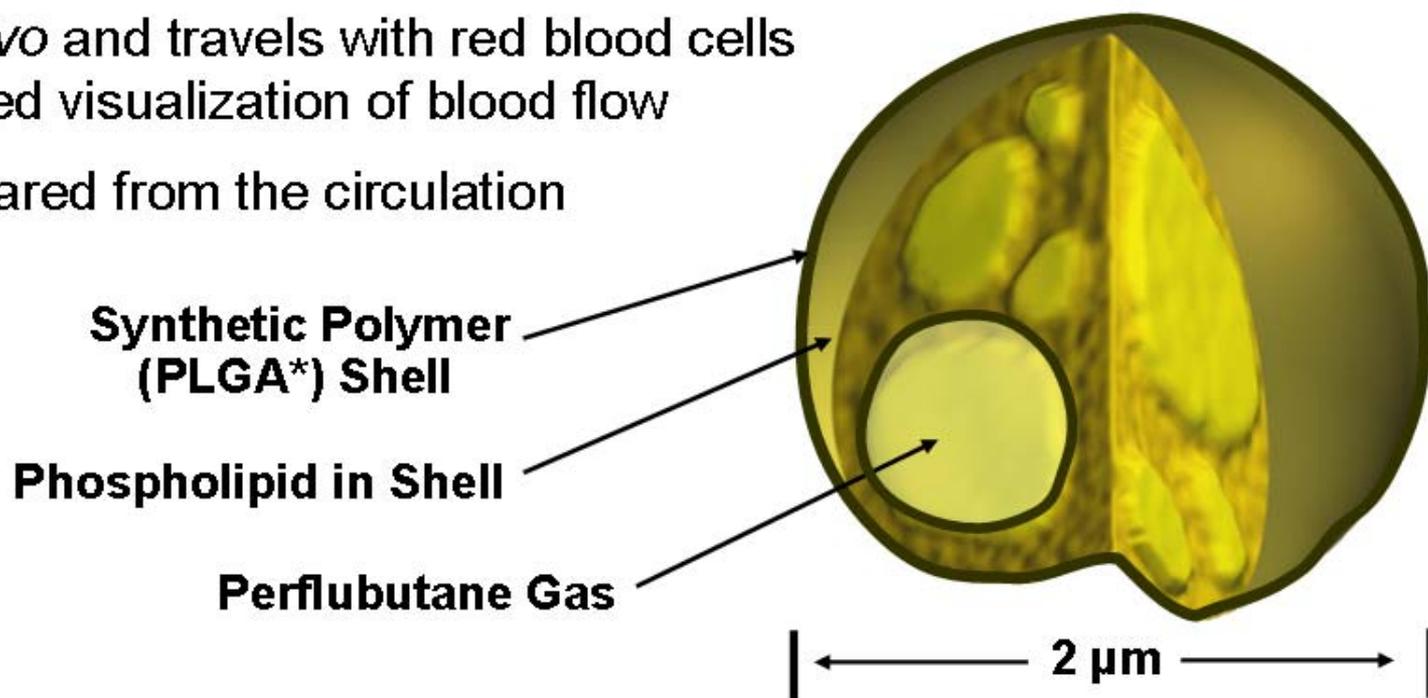
- SPECT provides perfusion information but:
  - Expensive
  - Time-consuming
  - And has ionizing radiation risk
- Stress ECHO is low cost and provides anatomical information but:
  - No perfusion information
  - Poor image quality in some patients

# Early Ultrasound Contrast Agents Were Not Designed for Perfusion Imaging

- Agitated saline
- Early ultrasound contrast agents
  - Thin, breakable natural shells
  - Filled with insoluble, fluorinated gas
- None approved for perfusion imaging

# AI-700: Engineered for Perfusion and Wall Motion Imaging

- Synthetic biodegradable polymer microspheres
- Primary shell material used in dissolvable surgical sutures
- Tight control of size distribution
  - At least 99% of microspheres  $\leq 10 \mu\text{m}$
- Stable *in vivo* and travels with red blood cells for prolonged visualization of blood flow
- Rapidly cleared from the circulation



[1] Straub et al. *J Control Release*. 2005. \*Poly-(D,L-lactide-co-glycolide)

# Proposed Use for AI-700 (Imagify™):

## Indication

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

## Patient Selection

Use will initially be limited to those patients who are indicated for pharmacologic stress testing.

## Clinical Application

Used with other clinical information to triage the patient for more invasive testing, such as angiography.

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# Agreement with FDA on SPECT as Phase 3 Comparator

- Phase 2
  - Comparisons with non-contrast ECHO
  - Dose-response
- End of Phase 2 meeting with FDA
- Phase 3 initiated
  - Disease detection study relative to ANGIO and SPECT in stable chest pain patients
- FDA requested change during Phase 3
  - To non-inferiority design vs. SPECT
  - Loss of patients to efficacy population

# AI-700 ECHO: Simultaneous Assessment of Perfusion and Wall Motion

- Potential benefits over non-contrast ECHO:
  - Perfusion and wall motion in one test
  - Increased number of evaluable images
- Potential benefits over SPECT:
  - Real-time imaging with high resolution
  - Improved wall motion information
  - Faster, widely available, cost-effective
  - No ionizing radiation

# Safety of AI-700 ECHO

- No deaths or immediate life-threatening events
- Safety signals from nonclinical studies and clinical trials
  - Cardiopulmonary effects in a small cohort of patients
  - Self-limiting or treated with standard care
- Some adverse events due to stressor agent
- Risk mitigation strategies
  - Initial use in patients indicated to receive stressor agents
  - Post-approval safety surveillance studies
  - Contraindications for at-risk patients

# Contraindications

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

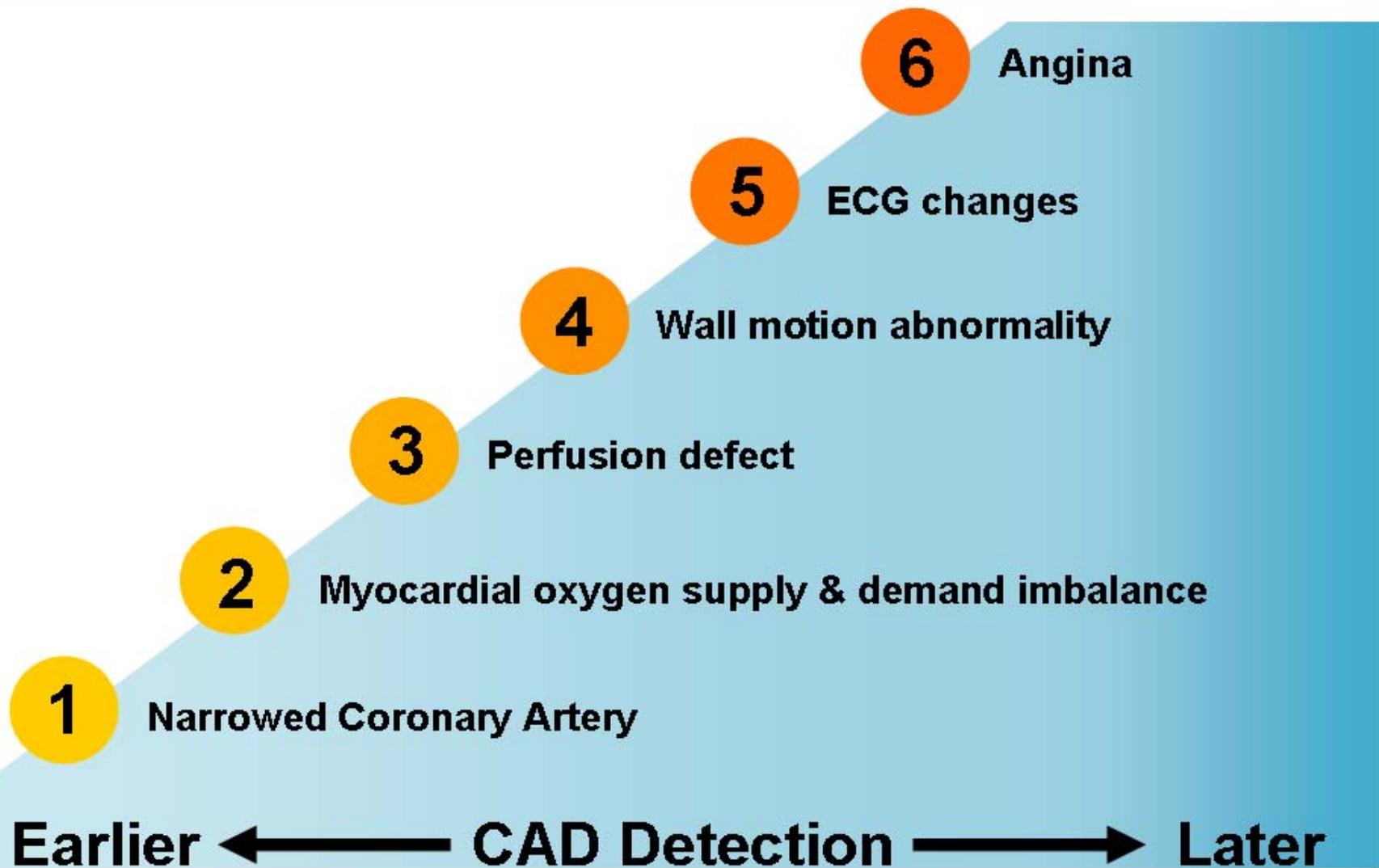
# AI-700 ECHO Benefits Outweigh the Risks

- First effective imaging agent to conveniently provide assessment of cardiac perfusion and wall motion
- Acute safety risk of AI-700 ECHO can be managed with standard care
- AI-700 has a favorable risk:benefit ratio
  - With the selected target patient population
  - With appropriate contraindications
  - With proposed post-approval safety surveillance studies

# Use of Ultrasound Contrast for the Detection of Coronary Artery Disease

Michael H Picard, MD  
Director, Clinical Echocardiography  
Massachusetts General Hospital and  
Harvard Medical School  
Boston, MA

# Testing for Coronary Artery Disease (CAD): Exploiting the Ischemic Cascade



# Clinical Need for Detecting Coronary Artery Disease

- Coronary angiography (ANGIO) gold standard for CAD diagnosis
- ANGIO limitations:
  - Invasive test with mortality risk<sup>1</sup>
  - Fatal cancer risk due to ionizing radiation exposure<sup>2</sup>
  - Contrast-induced nephropathy<sup>3</sup>
  - Up to 1/3 of cardiac catheterizations are in non-diseased patients<sup>4</sup>
- Clinical need for ANGIO “gatekeeper”
- ACC/AHA Guideline Recommendations for ANGIO triage (Class I)<sup>5</sup>
  - Rest/Stress Myocardial Perfusion Imaging (SPECT)
  - Rest/Stress Echocardiography (Stress ECHO)

[1] Main et al. *J Am Coll Cardiol*. 2007; [2] Einstein et al. *J Am Med Assoc*. 2007; [3] Jo et al. *J Am Coll Cardiol*. 2006.

[4] Shaw et al. In: Zaret and Beller. *Clinical Nuclear Cardiology State of the Art and Future Directions*. 3rd ed. 2005;

[5] Gibbons et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina. 2002.

# Exercise vs. Pharmacologic Stress Imaging

- Stress testing induces controlled state of ischemia
- Exercise is preferred method for stress testing
  - Approximately 50% of patients cannot exercise or have suboptimal exercise test (ref EAE guidelines)<sup>1</sup>
- Pharmacologic agents mimic exercise effects in patients unable to exercise<sup>2</sup>
- Patients requiring pharmacologic stress testing are sicker than patients who can exercise
  - ACC/AHA Guidelines (Class I) recommendation for patients unable to exercise<sup>1</sup>
    - ECHO – dobutamine
    - SPECT – vasodilatory

[1] Sicari et al. EAE Guidelines; *European Journal of Echocardiography* (2008).

[2] Gibbons et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina. 2002.

# Rest/Stress Myocardial Perfusion Imaging (SPECT)

- The most common technique for triaging to ANGIO
  - Myocardial perfusion most important
    - Quantification available
  - Limited anatomical information obtained (resting wall motion and EF)
- Limitations
  - High ionizing radiation dose
    - Fatal malignancy<sup>1</sup>
    - Environmental impact and biohazard for patients and physicians
  - Major contributor to escalating cardiac imaging cost
  - Less convenient and less available than ECHO
    - Up to 6 hr procedure
    - Dependent on <sup>99m</sup>Tc (technetium-99m) supply

[1] Thompson and Cullom. *J Nucl Cardiol*. 2006

# Non-Contrast Stress Echocardiography

- Advantages over SPECT:
  - Comprehensive real-time anatomic and functional evaluation of all cardiac structures
    - Stress LVEF, valve diseases, pericardial disease, right heart pressures
  - No ionizing radiation
  - Fast (<60 minutes)
  - Wide availability<sup>1</sup>
  - Higher spatial and temporal resolution
- Less common technique due to limitations:
  - No information on perfusion
  - Non-diagnostic quality images in 10-25% of patients due to poor Acoustic Window Quality (AWQ)<sup>2</sup>

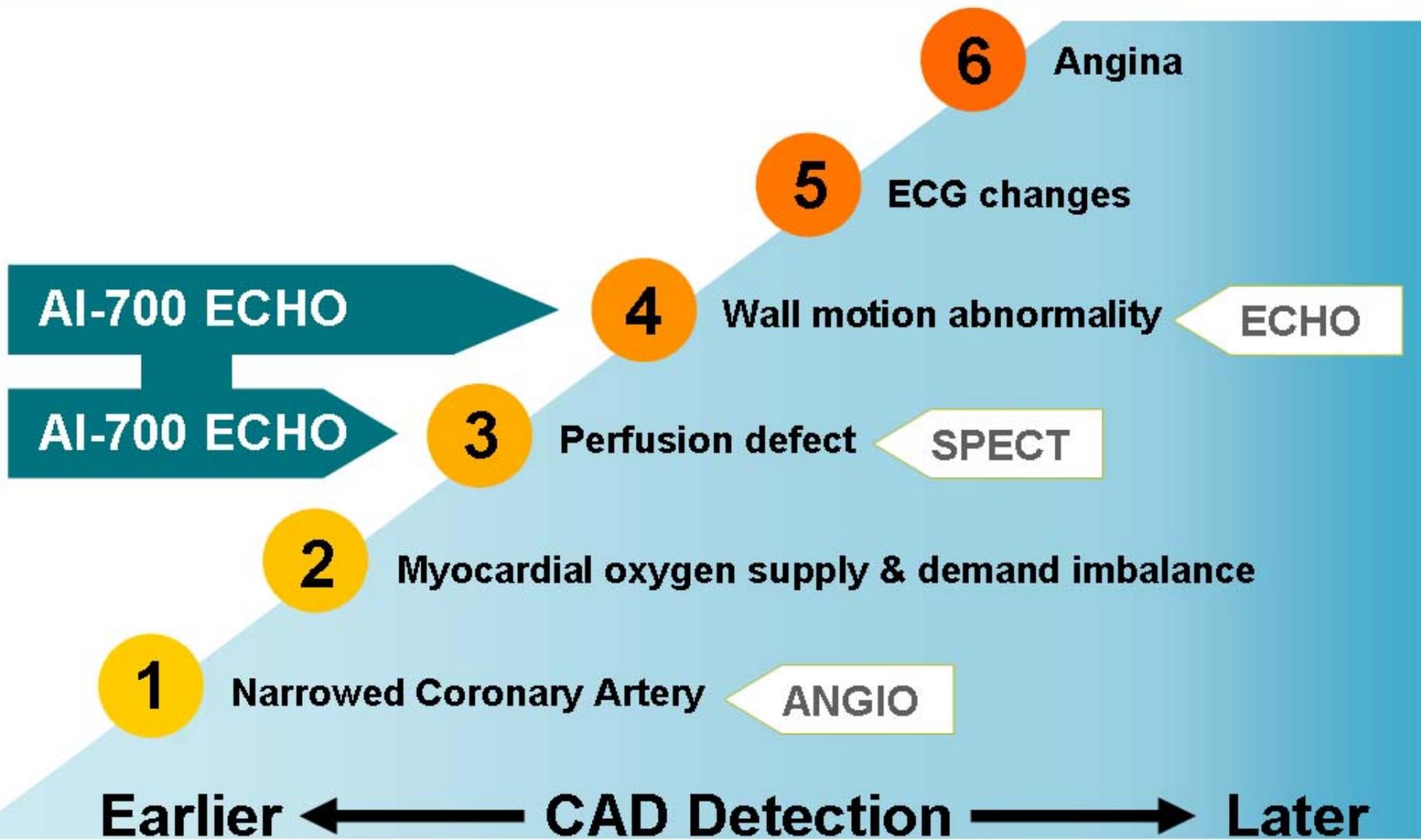
[1] Klein Biomedical Consultants I. The Ultrasound Contrast Agent Imaging Market in the USA On the Threshold of Explosive Growth. New York (NY); Sep 2003; [2] Cukon-Buttignoni et al. *J Am Soc Echocardiogr.* 2008.

## Currently Approved Contrast Agents Indicated to Enhance Image Quality – Not Perfusion

Classification	Perflutren-containing microspheres
Indication	Optison™ <sup>1</sup> and Definity® <sup>2</sup> are indicated for use in patients with suboptimal echocardiograms to opacify the left ventricle and to improve the delineation of the left ventricular endocardial border
Indication Limitation	'The safety and efficacy of (Optison™/Definity®) with exercise stress or pharmacologic stress testing have not been established'

[1] Optison (package insert), GE Healthcare; 2008; [2] Definity (package insert), Bristol-Myers Squibb; 2008.

# CAD Diagnosis: Ischemic Cascade



# AI-700 Addresses Important Clinical Need

- Advantage over SPECT
  - No ionizing radiation
  - Faster
  - Wide availability and lower cost
  - Real time imaging and assessment with higher spatial and temporal resolution
  - Rest and stress wall motion
- Advantages over non-contrast ECHO
  - Perfusion
  - Safer pharmacologic stressor
  - 99% evaluable images
- Advantages over both procedures
  - Synergy of simultaneous wall motion and perfusion imaging

# AI-700 Imaging

Roxy Senior, MD, DM, FRCP, FESC, FACC

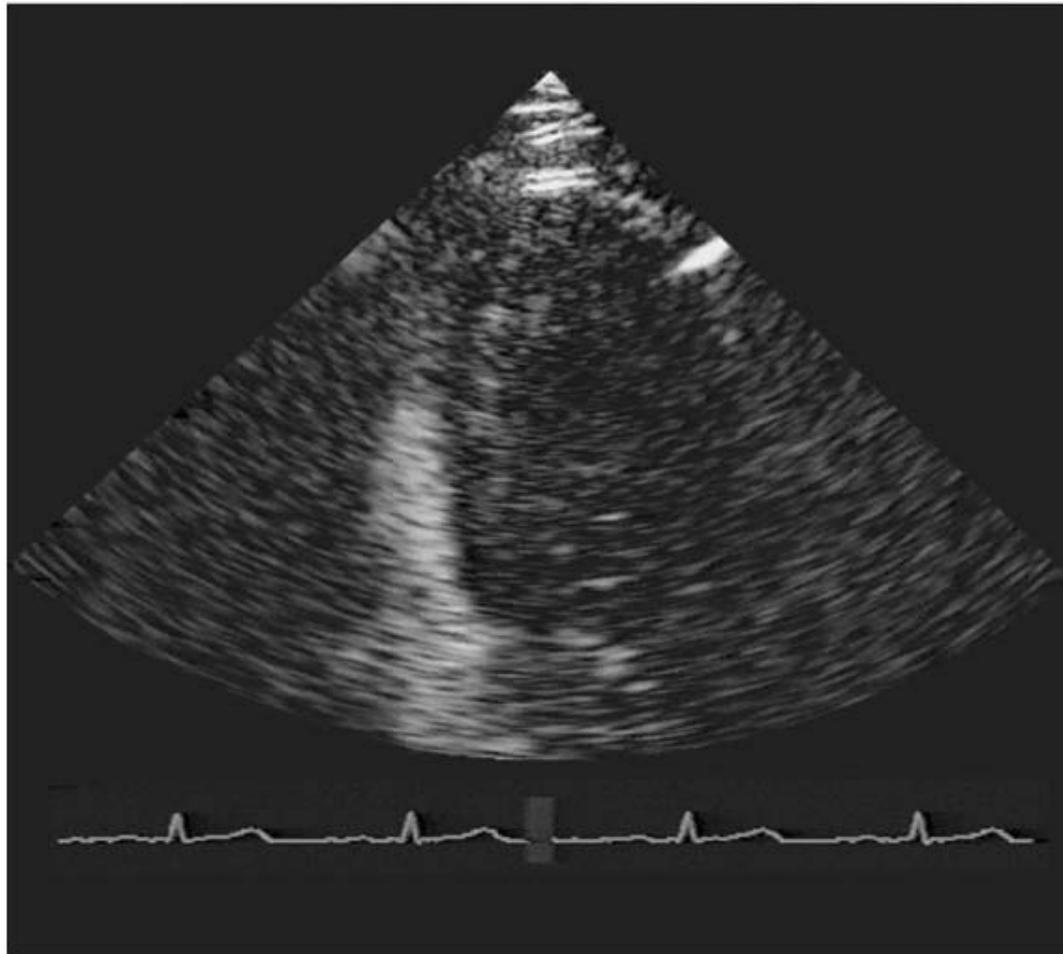
Consultant Cardiologist and  
Director of Cardiac Research,  
Northwick Park Hospital, Harrow, UK

# Interpreting AI-700 Images

- AI-700 advantages over other contrast agents
  - Robust performance (easy-to-use, simple reconstitution) with prolonged contrast enhancement
  - Provides simultaneous evaluation of wall motion and perfusion
  - Wall motion and perfusion are synergistic
- Overview of case studies
  - Myocardial enhancement anatomy and scan planes
  - Detection of normal myocardial perfusion with AI-700
  - Ability to detect subendocardial defect with AI-700
  - Non-diagnostic ECHO converted to diagnostic study with AI-700
  - Perfusion defect without wall motion abnormality

# AI-700 Myocardial Enhancement Anatomy

## Apical Four Chamber View



### Real-time Imaging Phases

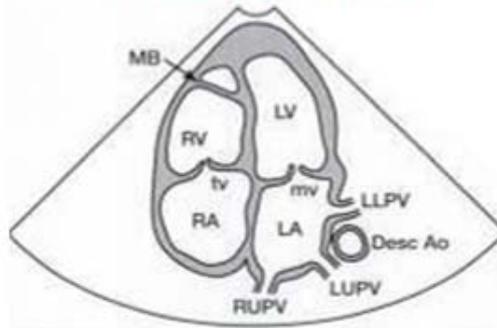
1. Left Ventricular Opacification
2. Myocardial Perfusion
3. Flash
4. Replenishment

### Real-time Ultrasound Settings

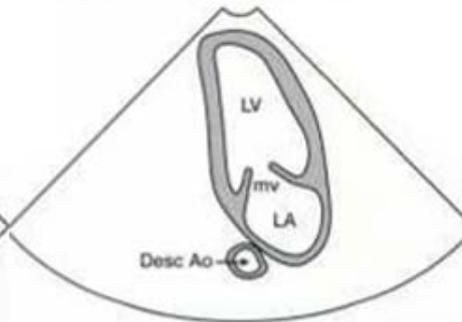
- Mechanical Index (MI)-0.3
- Imaging Frame rate- ~ 30 Hz
- Flash ~1 second-MI 1.0

# Echocardiographic Apical Scan Planes

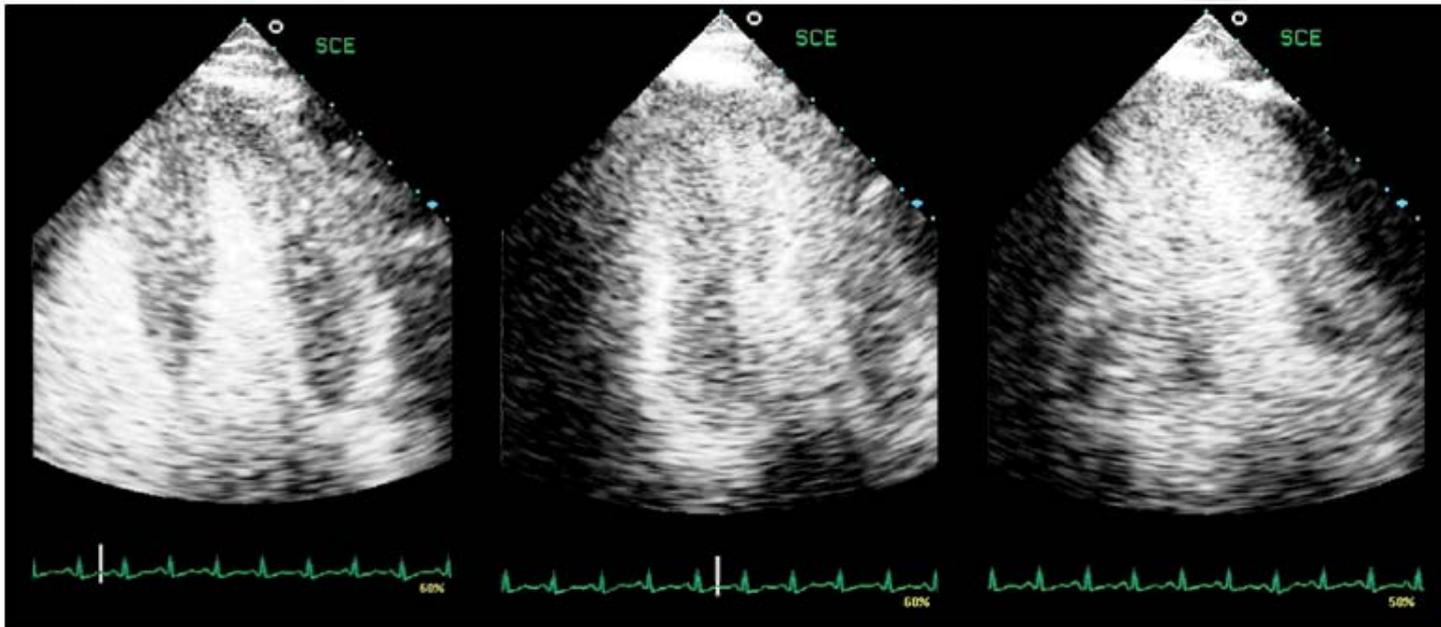
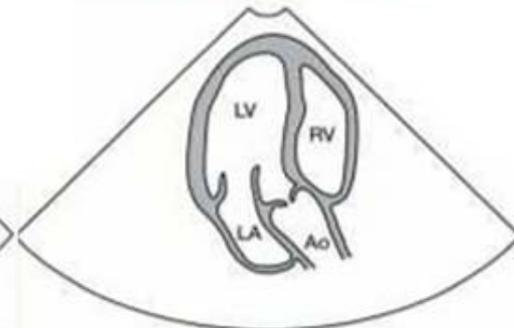
4-Chamber View



2-Chamber View



3-Chamber View



Normal study: bright, homogenous AI-700 enhancement

## Patient 32-18-009: Medical History

- 64 year old white male
- Atypical exertional chest pain
- Diabetes
- Peripheral vascular disease
- Hyperlipidemia
- Obesity
- Former smoker
- Mild COPD

# Ability to Detect Subendocardial Defect

## Apical Four Chamber View



**Non-Contrast**

**Rest AI-700**

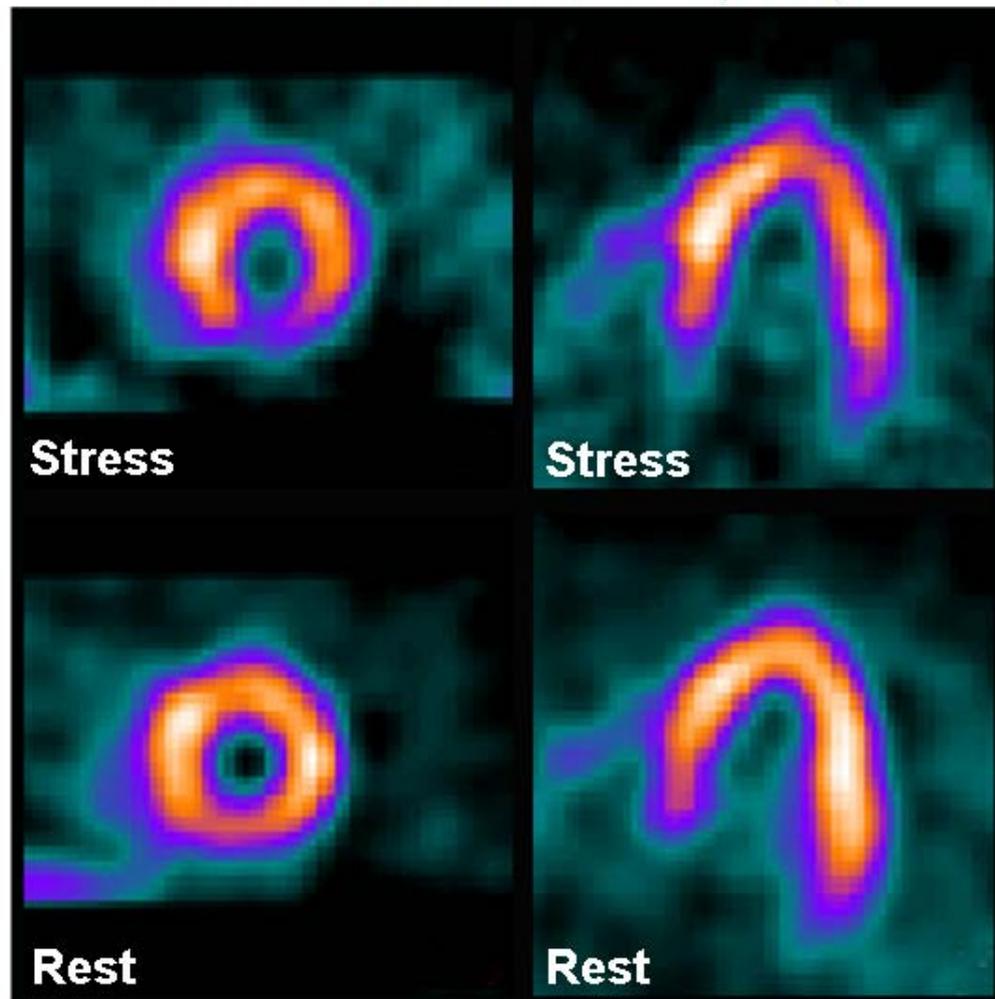
**Stress AI-700**  
(subendocardial defect)

# Angiography Result: Flow-limiting Circumflex Stenosis (79% and 84%)



# Concordant SPECT Results: Circumflex and Right Circulation Disease

SPECT Results: Circumflex (CX-R)



# Patient 32-18-009: Study Interpretations

- AI-700 diagnostic features
  - Hypoechoic apical lateral perfusion defect with hypokinetic apical wall motion abnormality
- SPECT results: disease
- Angiography results: flow-limiting LCX stenosis

## Patient 33-35-037: Medical History

- 71 year old white male
- Typical exertional chest pain
- Hypertension
- Hyperlipidemia
- Controlled atrial fibrillation

# Non-Diagnostic ECHO Converted to Diagnostic Study with AI-700

## Apical Four Chamber View

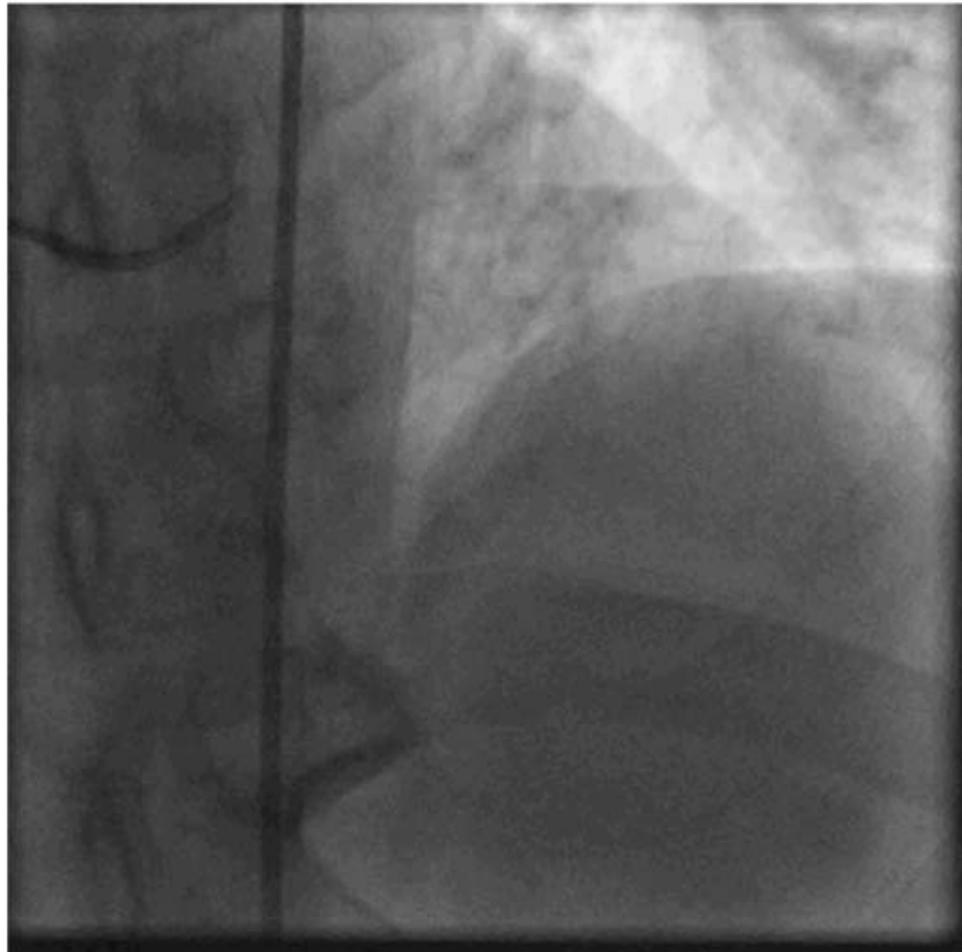


**Non-Contrast**  
(Poor AWQ)

**Rest AI-700**

**Stress AI-700**  
(subendocardial defect)

# Angiography Result: Flow-limiting Mid-LAD Stenosis (84%)



# Patient 33-35-037: Study Interpretations

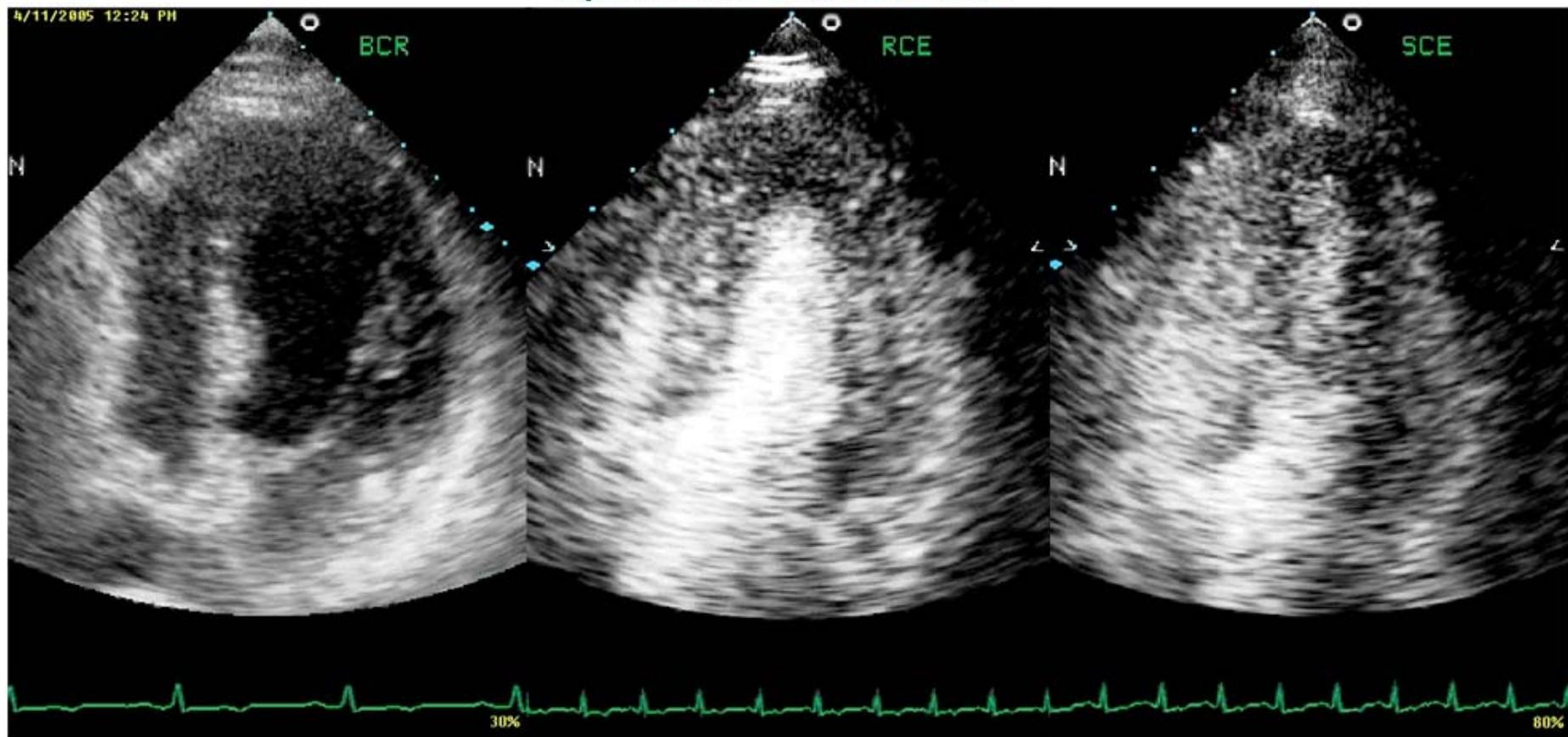
- AI-700 diagnostic features
  - Converted non-diagnostic imaging window
  - Hypoechoic apical perfusion defect
  - Severely hypokinetic apical wall motion
- SPECT results – disease
- Angiography results – flow-limiting LAD stenosis

## Case 35-045-33: Medical History

- 77 year old white female
- Myocardial infarction - 2004
- Typical/exertional chest pain
- Hypertension
- Hyperlipidemia

# Perfusion Defect Without Wall Motion Abnormality

## Apical Four Chamber View



**Non-Contrast**  
(good AWQ)

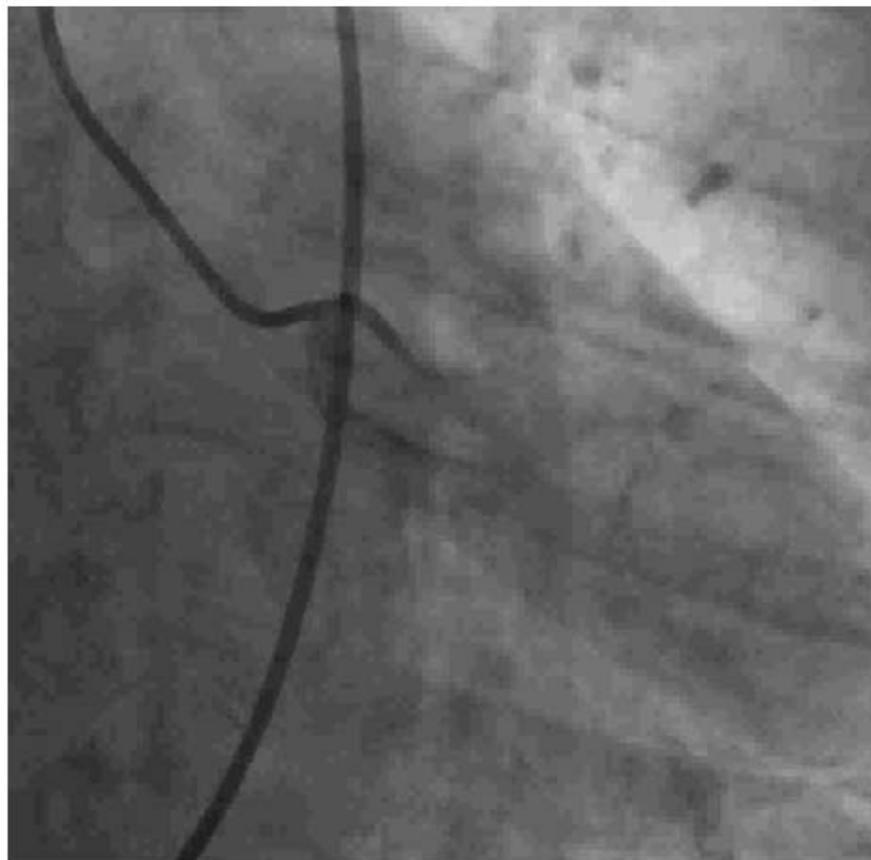
**Rest AI-700**

**Stress AI-700**  
(subendocardial defect-  
normal wall motion)

From the lab of Dr Mark Monaghan, Kings College Hospital, London, England

# Angiography Result: Flow-limiting Stenoses (LAD 77%, LCx 74%, RCA 77%)

## Flow-limiting stenoses, triple-vessel disease



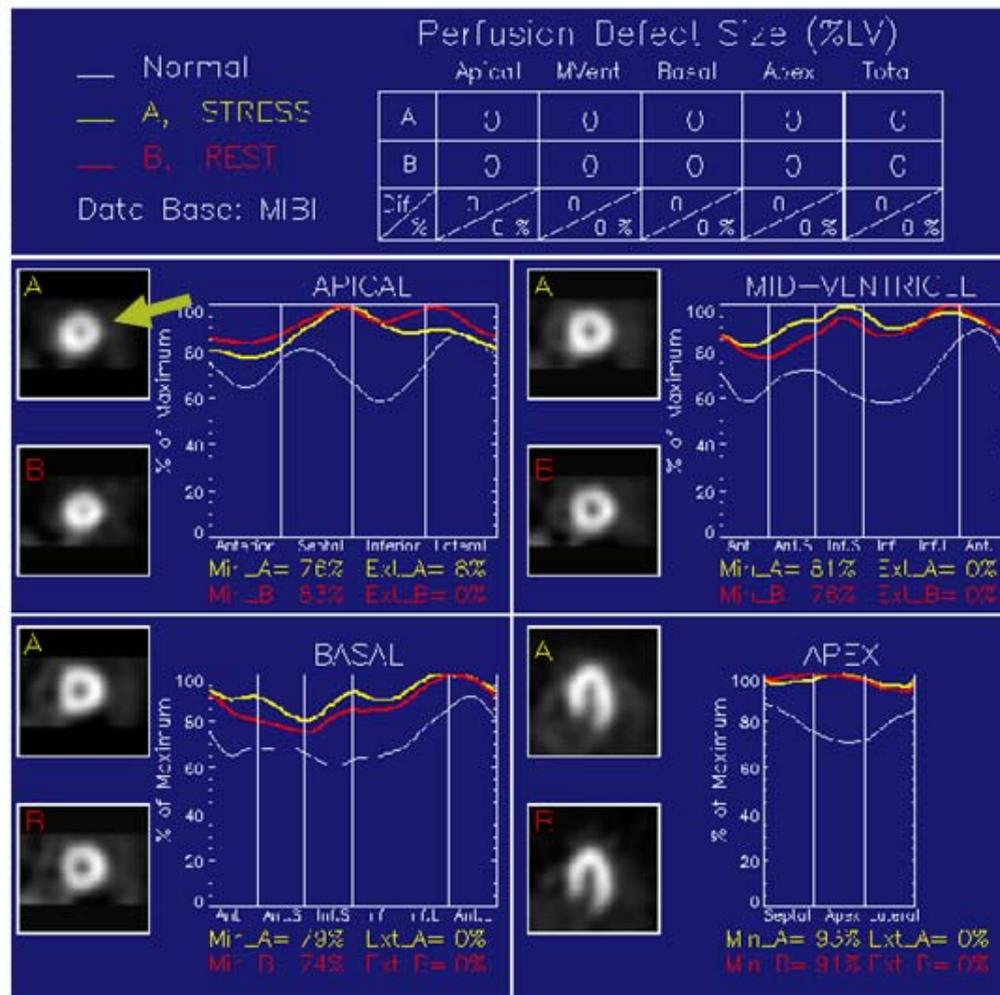
**Circumflex (LCX)**



**Left Anterior Descending (LAD)**

# SPECT Result: False Negative

SPECT Results: Negative (small area of artifact noted in apex?)



## Case 35-045-33: Study Interpretations

- AI-700 diagnostic features
  - hypoechoic perfusion defect with normal wall motion
- SPECT results: no disease
- Angiography results: multiple flow-limiting stenoses

# AI-700 ECHO Imaging

- Robust performance (easy-to-use, simple reconstitution) with prolonged contrast enhancement
- Both wall motion and perfusion in real time
- Clear depiction of subendocardial perfusion defects
- Converting poor quality images to diagnostic quality images

# AI-700 Clinical Efficacy Overview

Richard Walovitch, PhD  
Senior VP, Clinical Research

Acusphere, Inc.  
Watertown, MA

# Overview of Pivotal Studies

	AI-700-32	AI-700-33
<b>Design</b>	Phase 3, international, multicenter, open-label, safety and blinded efficacy	
<b>Investigator Sites</b>	11 (North America, EU, Australia)	17 (North America, EU)
<b>Objectives</b>	Demonstrate efficacy and safety of AI-700 stress ECHO in stable chest pain patients being evaluated for inducible ischemia for the detection of coronary artery disease (CAD)	
<b>Primary Efficacy Analysis</b>	Non-inferiority of AI-700 ECHO vs SPECT nuclear perfusion imaging	

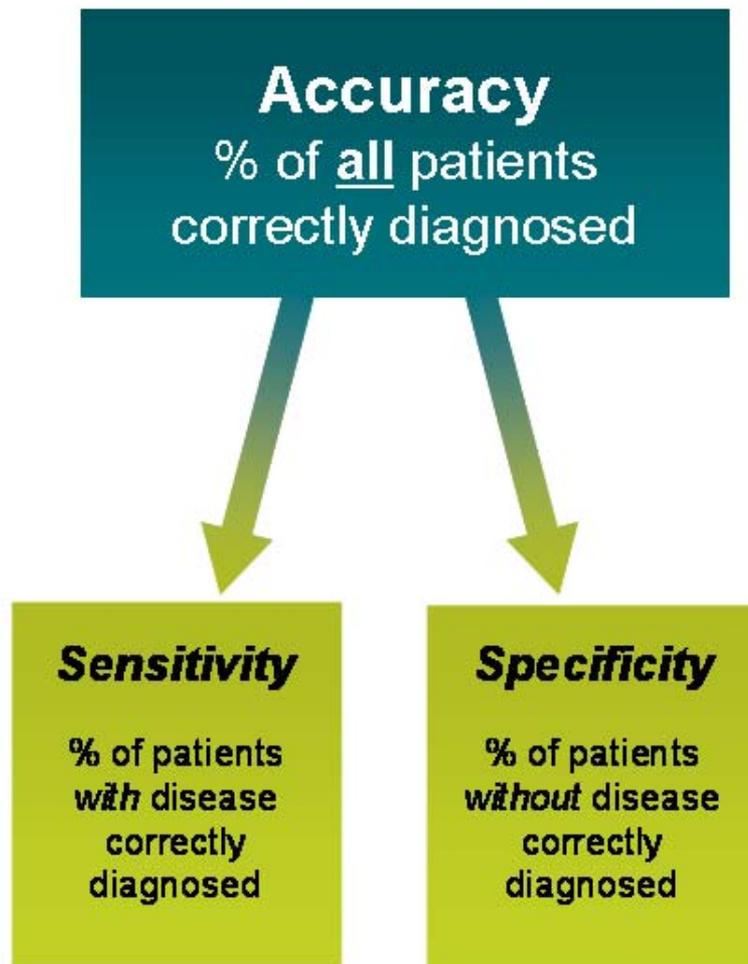
# Overview of Pivotal Studies

	AI-700-32	AI-700-33
<b>Comparative Standard</b>	Tc99m SPECT imaging with gating and quantification	
<b>Dose</b>	Two injections of 0.04 mL/kg AI-700, one during rest and one during dipyridamole (0.56 mg/kg, IV) stress	
<b>Key Inclusion/Exclusion Criteria</b>	<p><b>Inclusion:</b> 18-80 years of age, stable chest pain, indicated for pharmacologic stress testing</p> <p><b>Exclusion:</b> clinically unstable condition within 7 days, change in status between protocol imaging evaluations</p>	
<b>Difference in Inclusion/Exclusion Criteria</b>	<p><b>Inclusion:</b> Same day stress SPECT</p> <p><b>Exclusion:</b> CABG within 6 months</p>	<p><b>Inclusion:</b> Recent Coronary ANGIO</p> <p><b>Exclusion:</b> CABG</p>

# Components of Efficacy Evaluation in Pivotal Studies

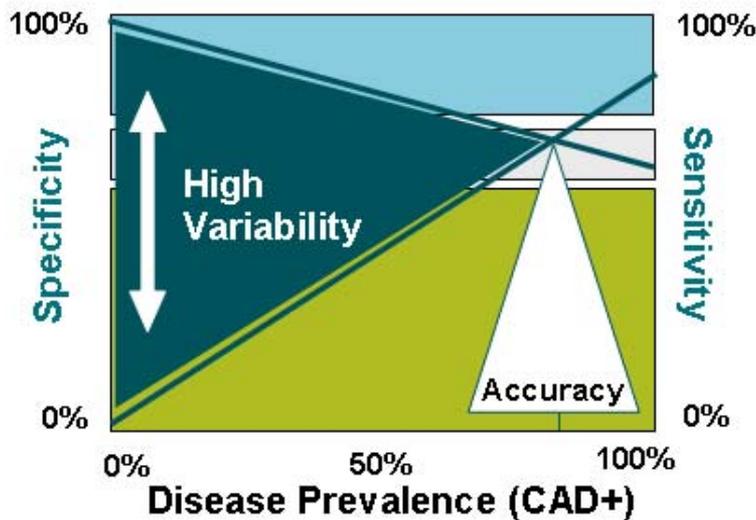
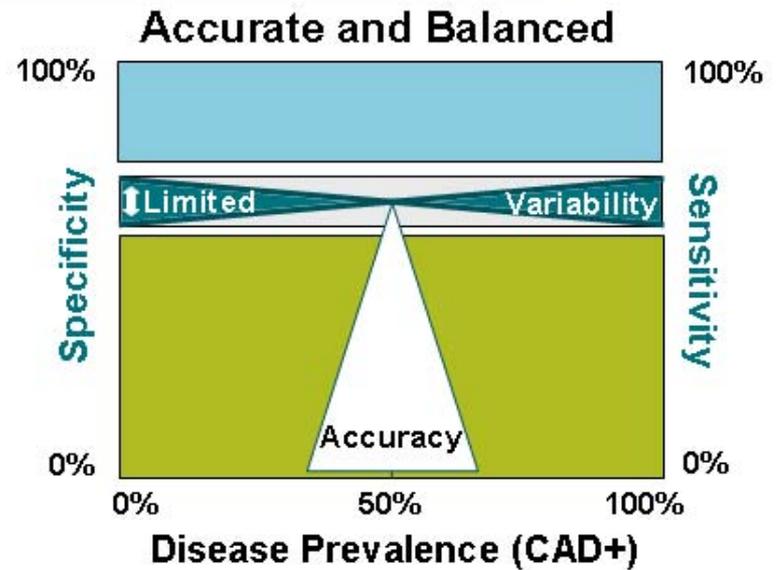
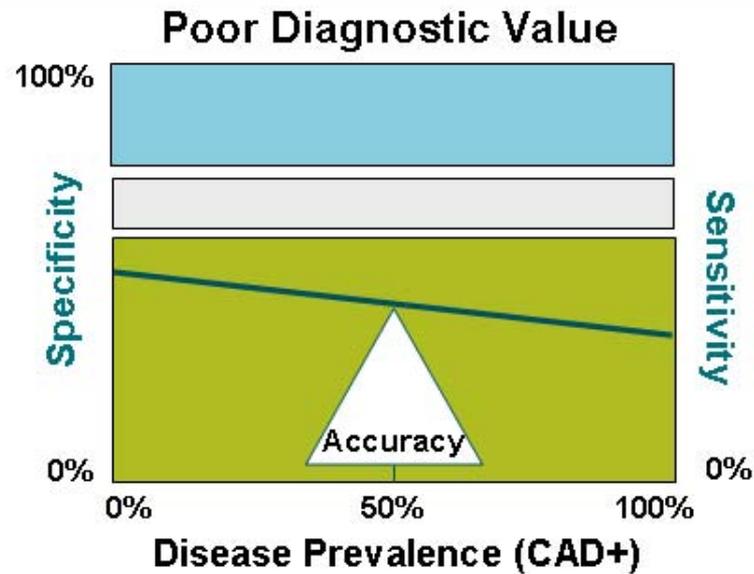
	AI-700-32	AI-700-33
<b>ANGIO/LVG</b>	Independent Core Lab assessed: <ul style="list-style-type: none"> <li>– Stenosis by quantitative coronary angiography</li> <li>– Wall motion by LVG</li> </ul>	
<b>Non-Blinded CAD Review in absence of ANGIO/LVG</b>	One independent reviewer of SPECT, ECG and clinical data	
<b>AI-700 ECHO</b>	Three independent blinded readers for each study	
<b>SPECT</b>	One independent blinded reader	Three independent blinded readers

# Primary Efficacy Analysis



- Evaluated ratio of AI-700 ECHO performance to SPECT (margin of 0.83 alpha=0.05)
- Preserves the Type I error rate (alpha=0.05) for each step
- “Success”
  - Non-inferiority for  $\geq 2$  of 3 ECHO readers

# High Accuracy Required for Diagnostic Value



- Superiority Zone
- Non-inferiority Zone
- Inferiority Zone

# Lowest Allowable ECHO Point Estimates With 0.83 Margin

SPECT Value (%)	Sensitivity (Margin=0.83)		Specificity (Margin=0.83)	
	Minimum ECHO Value (%)	RR	Minimum ECHO Value (%)	RR
90	80	0.89	83	0.92
85	77	0.91	79	0.93
80	73	0.91	76	0.95
75	70	0.93	72	0.96
70	66	0.94	68	0.97
65	62	0.95	64	0.98
60	59	0.98	60	1.00

Simulated relative risk ratio non-inferiority analysis assuming a population similar to AI-700-33 study (220 CAD+ and 157 CAD-) with a one-sided p-value of <0.025.

# Definition of Truth for Coronary Artery Disease

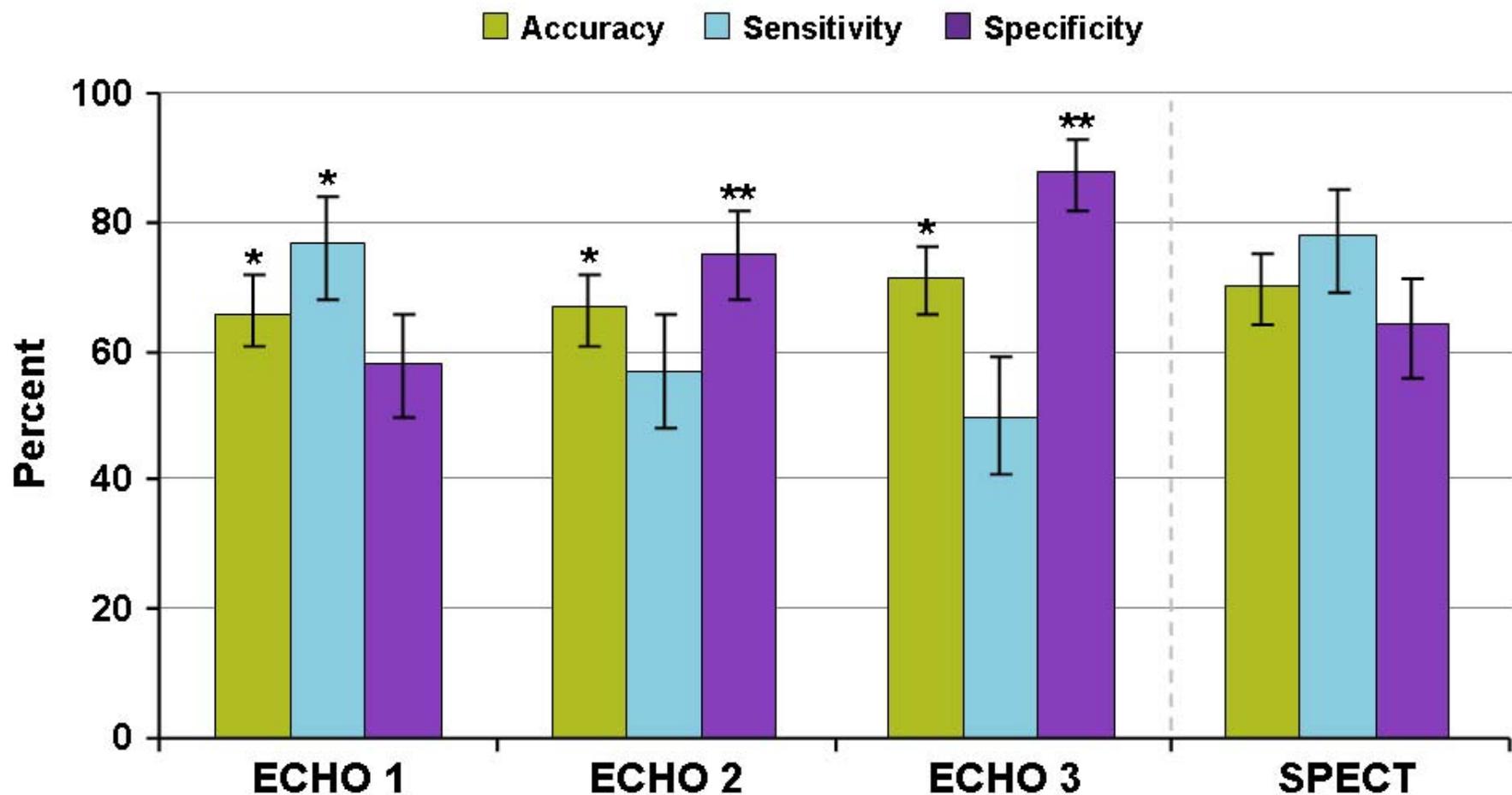
- **ANGIO/LVG, if available (74%)**
  - 70% diameter stenosis or regional wall motion abnormality on LVG --► CAD+
- **ANGIO/LVG unavailable, CAD history (7%)**
- **ANGIO/LVG unavailable and no history of CAD (19%)**
  - Non-blinded nuclear cardiologist (CAD reviewer) performed overall assessment using:
    - SPECT
    - Clinical history
    - ECG
    - Not AI-700 imaging

# Study Patient Populations and CAD Prevalence

	AI-700-32	AI-700-33
Enrolled / Safety	321	457
MITT / Efficacy	285	377
Prevalence of CAD	44%	58%

- Differences between safety and efficacy populations attributable to changes in trial design requested by FDA during in-life phase
  - Requiring SPECT comparator for all patients in Study 33
  - Change in acceptable SPECT comparator
  - Change in truth standard for Study 32

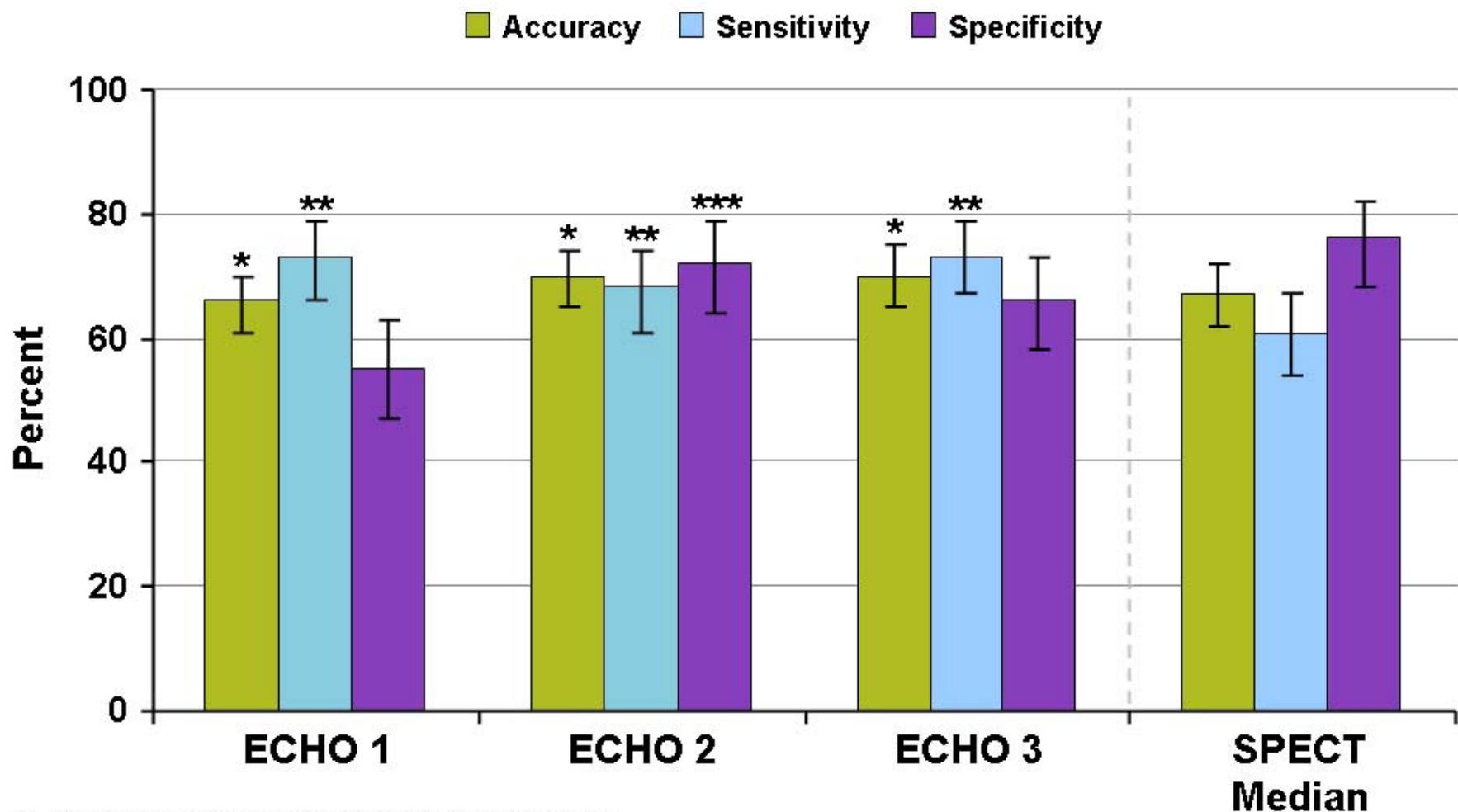
# AI-700-32: AI-700 ECHO Showed Non-Inferior Accuracy with Superior Specificity



\*p<0.001 as compared to SPECT for non-inferiority

\*\*p<0.001 as compared to SPECT for superiority

# AI-700-33: AI-700 ECHO Showed Non-Inferior Accuracy with Superior Sensitivity

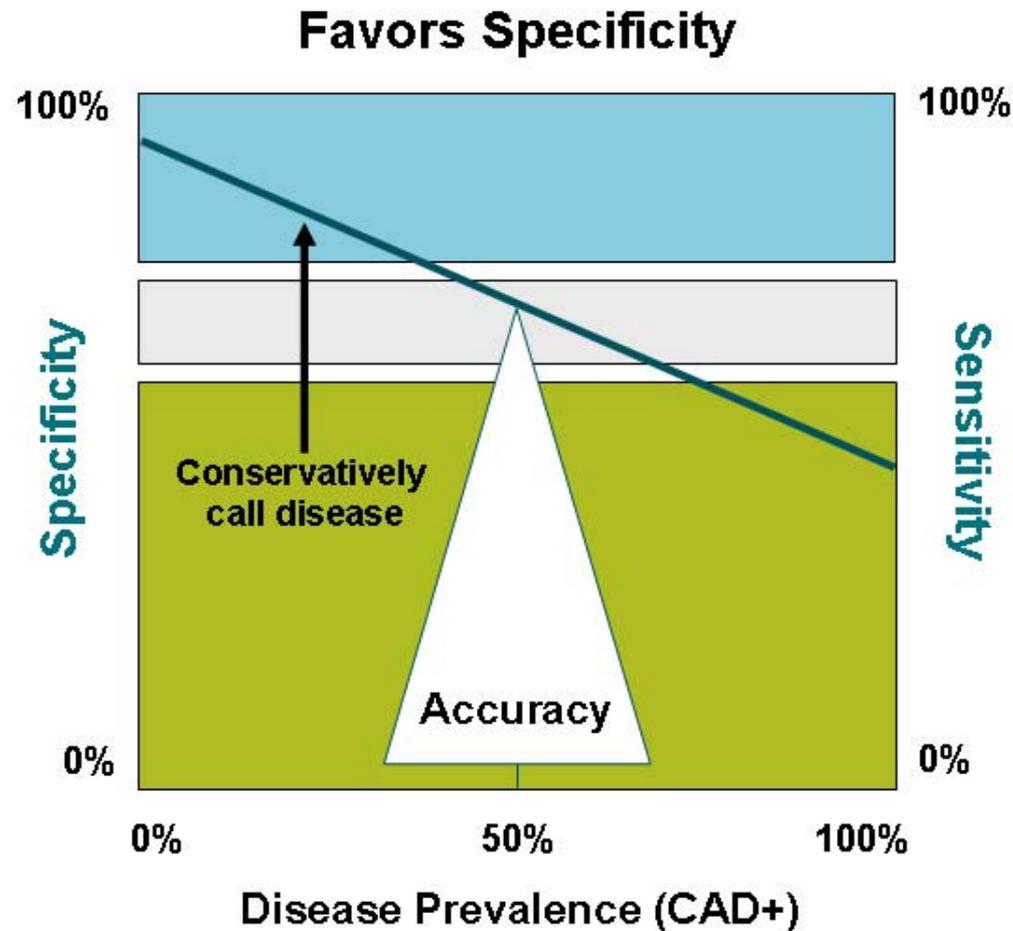


\*p<0.001 as compared to SPECT for non-inferiority

\*\*p<0.001 as compared to SPECT for superiority

\*\*\*p=0.013 as compared to SPECT for non-inferiority

# Disease Detection Threshold Impacts Sensitivity and Specificity When Accuracy is Non-Inferior



Superiority Zone

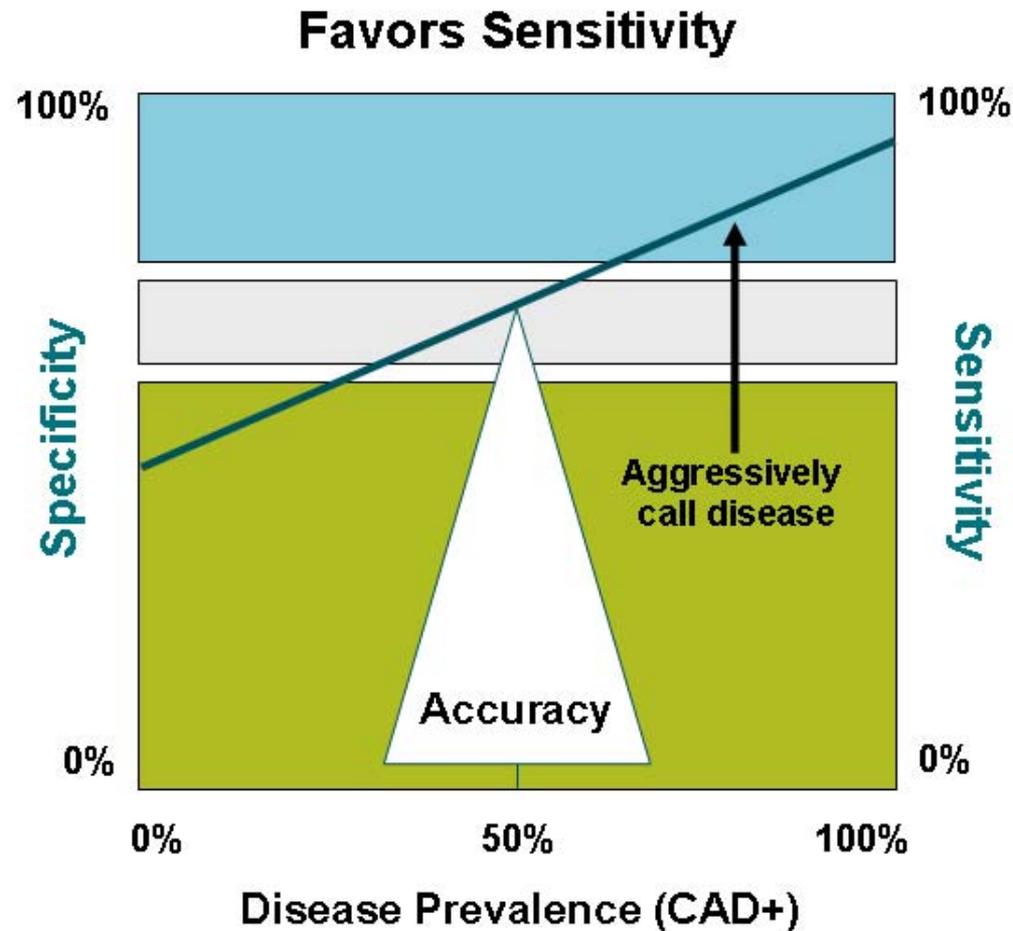


Non-Inferiority Zone



Inferiority Zone

# Disease Detection Threshold Impacts Sensitivity and Specificity When Accuracy is Non-Inferior



Superiority Zone

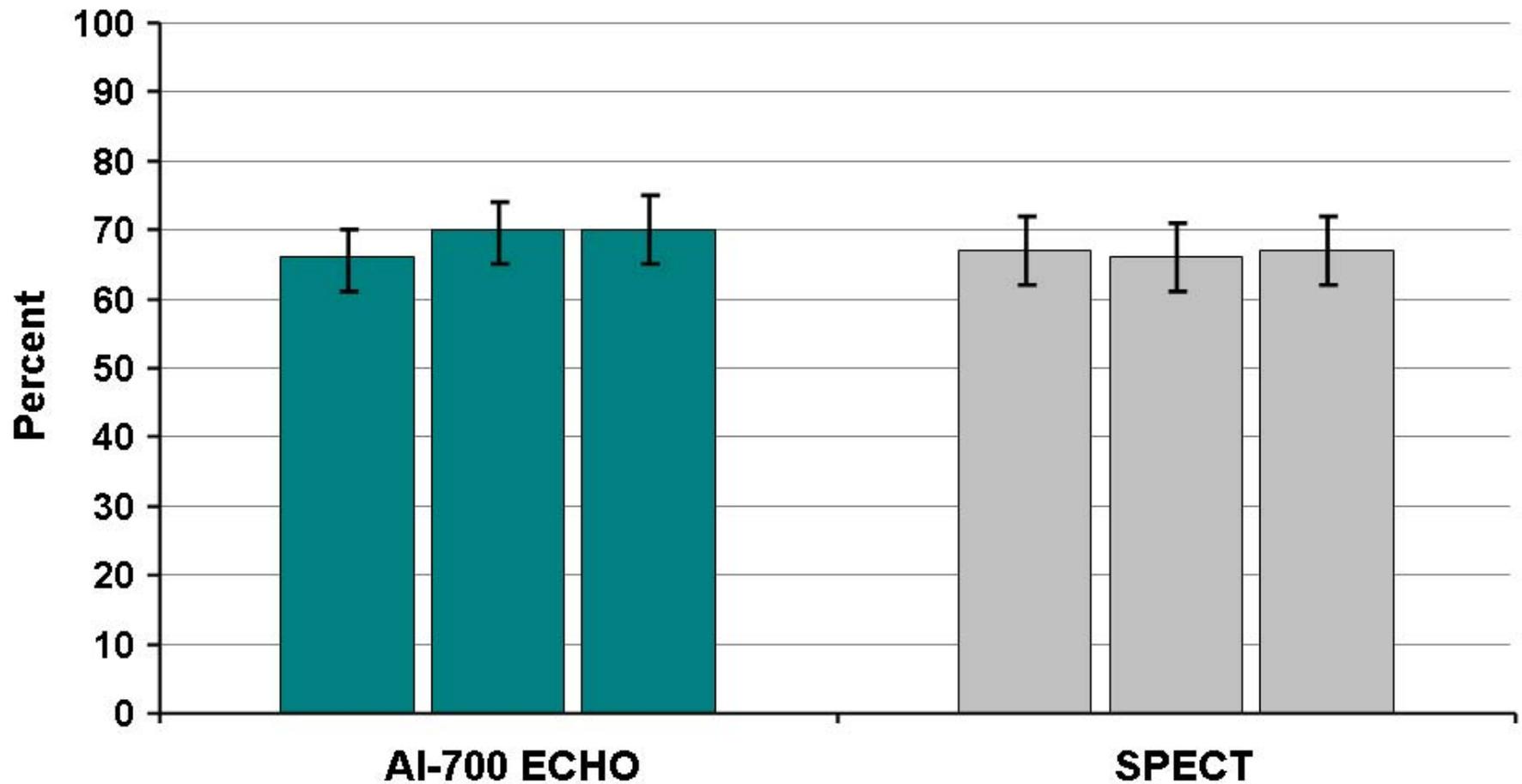


Non-Inferiority Zone

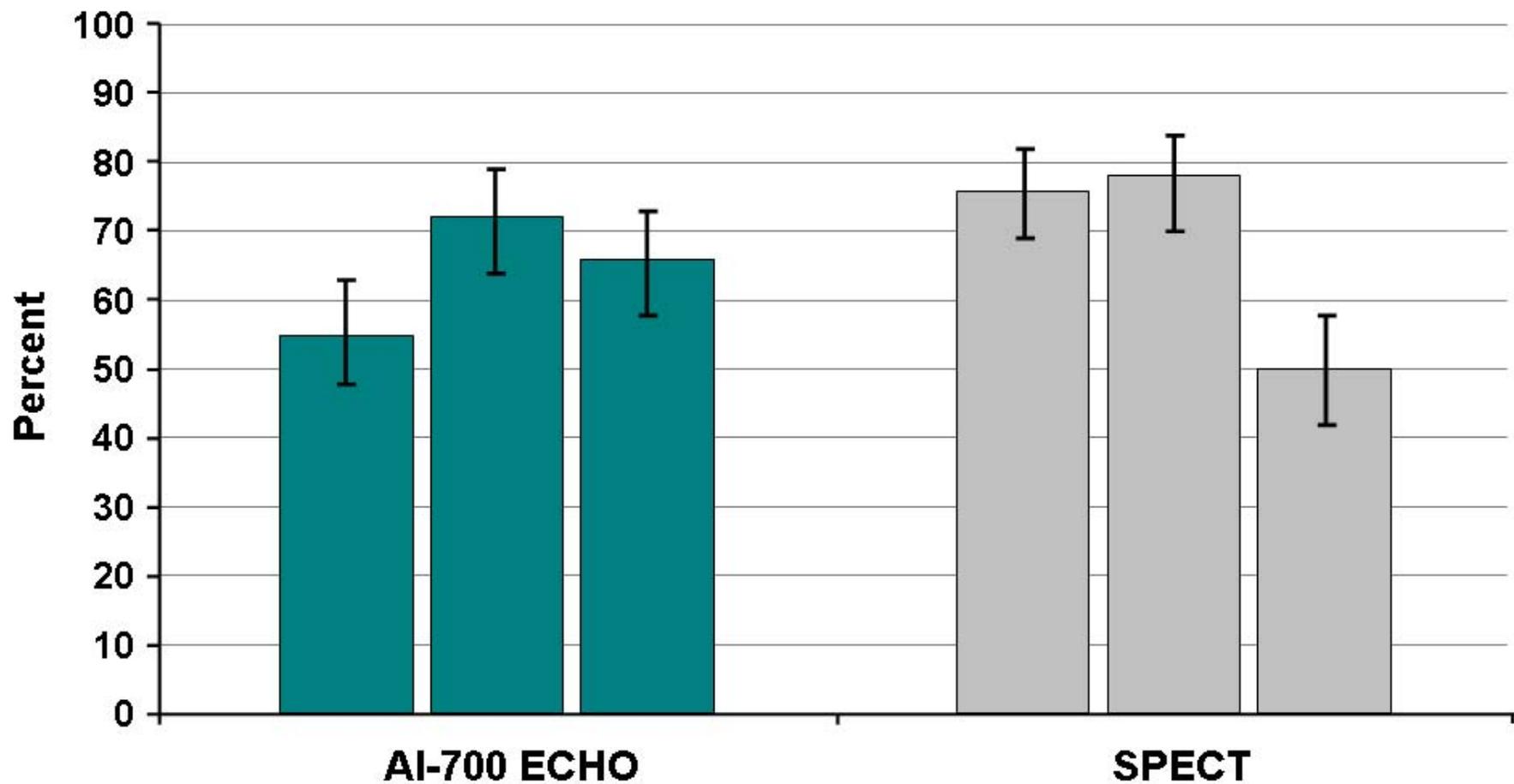


Inferiority Zone

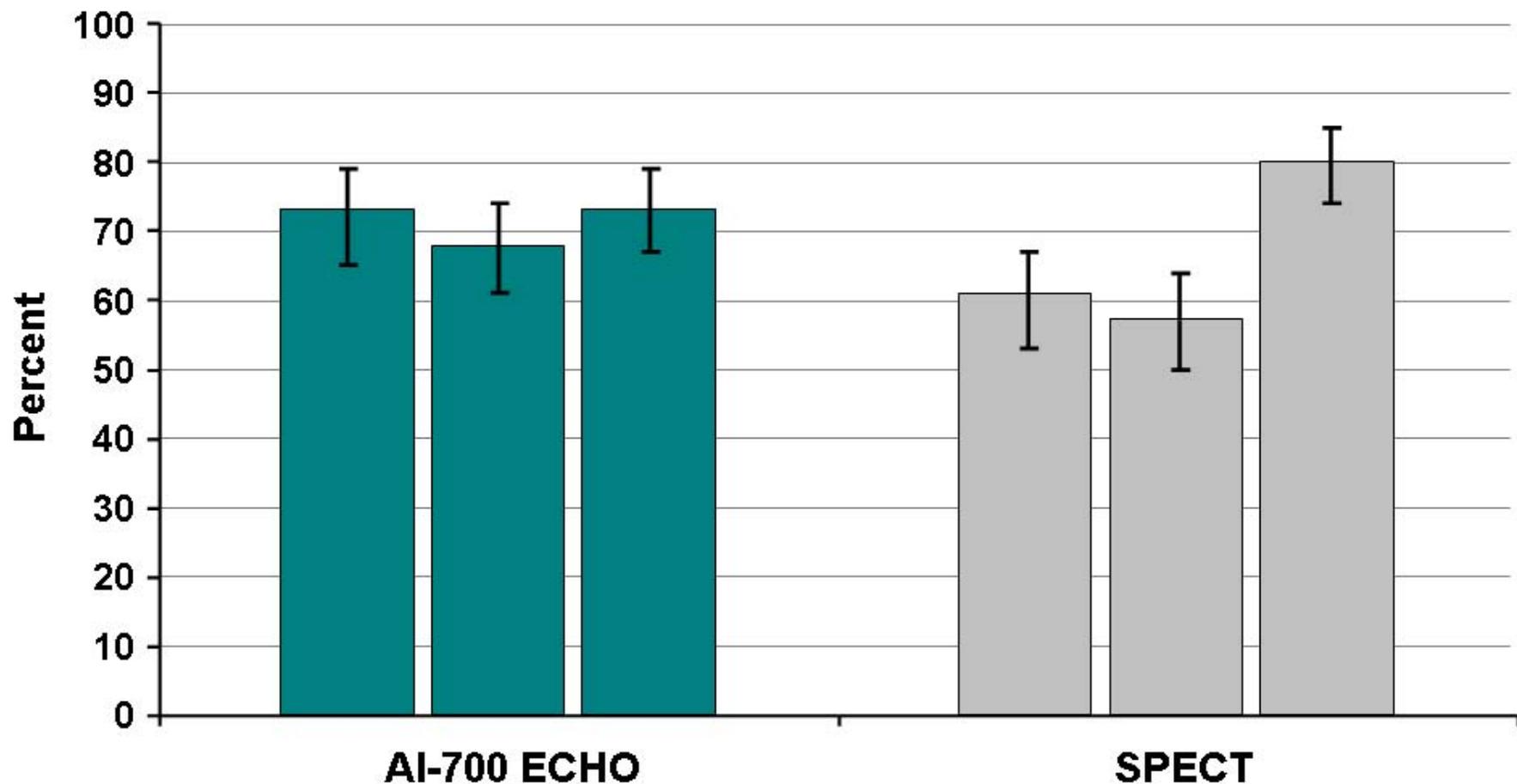
# Accuracy for ECHO and SPECT Readers (Study AI-700-33)



# Specificity for ECHO and SPECT Readers (Study AI-700-33)



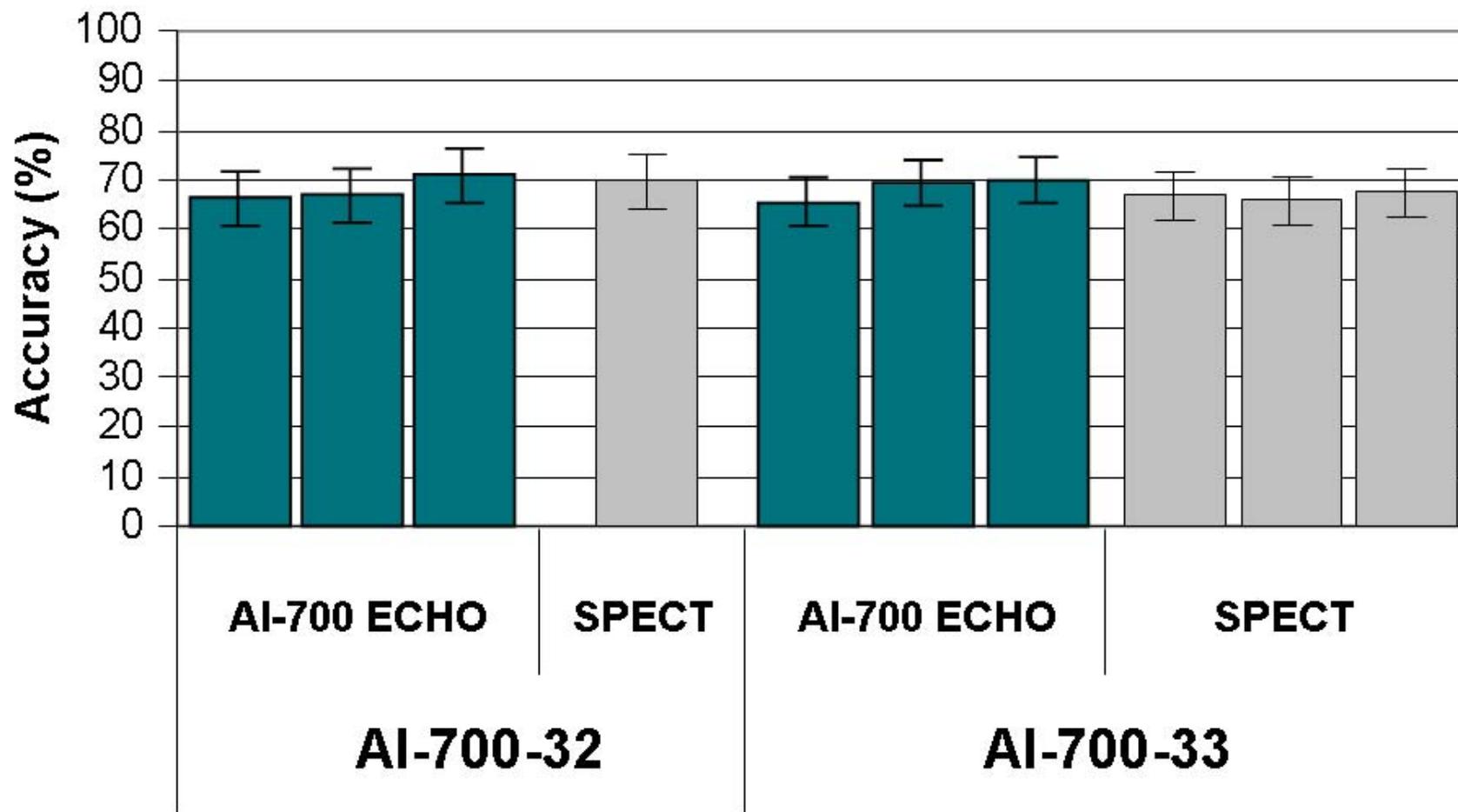
# Sensitivity for ECHO and SPECT Readers (Study AI-700-33)



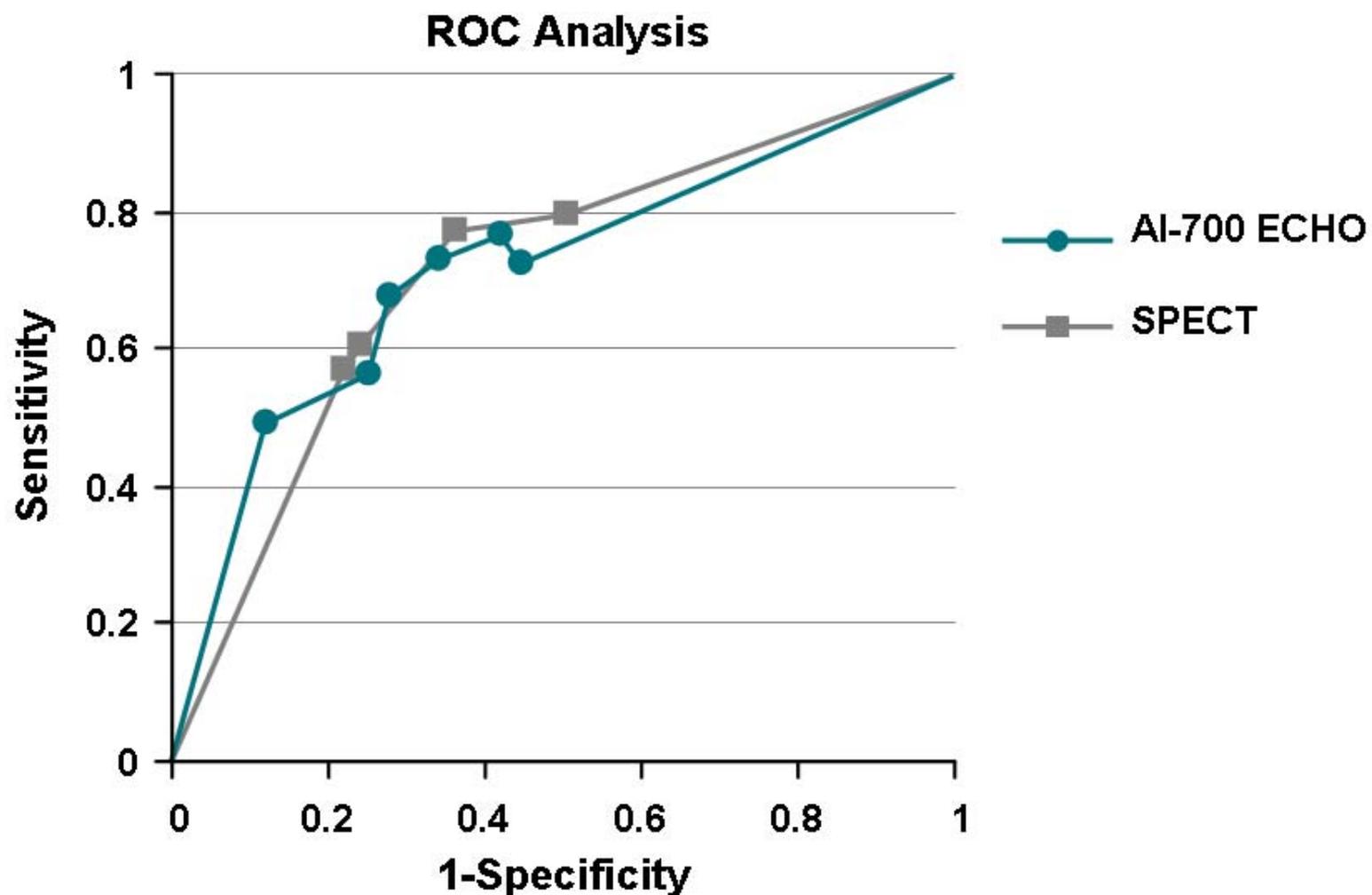
# Consistent Results Support Diagnostic Efficacy

- Efficacy analysis was robust; conclusions not affected by:
  - Sensitivity analyses in multiple populations
  - Definition of disease as determined by 50% diameter stenosis or different truth standards
- Sensitivity was positively correlated with disease severity
  - All 6 ECHO readers had at least 70% sensitivity in multi-vessel disease
- ECHO defect size positively correlated with probability of CAD
- In patients with ANGIO AI-700 ECHO was able to correctly localize disease more often than SPECT
  - 84-97% for AI-700 ECHO vs 69-74% for SPECT

# Consistent Accuracy for All Readers



# Diagnostic Performance of AI-700 ECHO is Comparable to SPECT

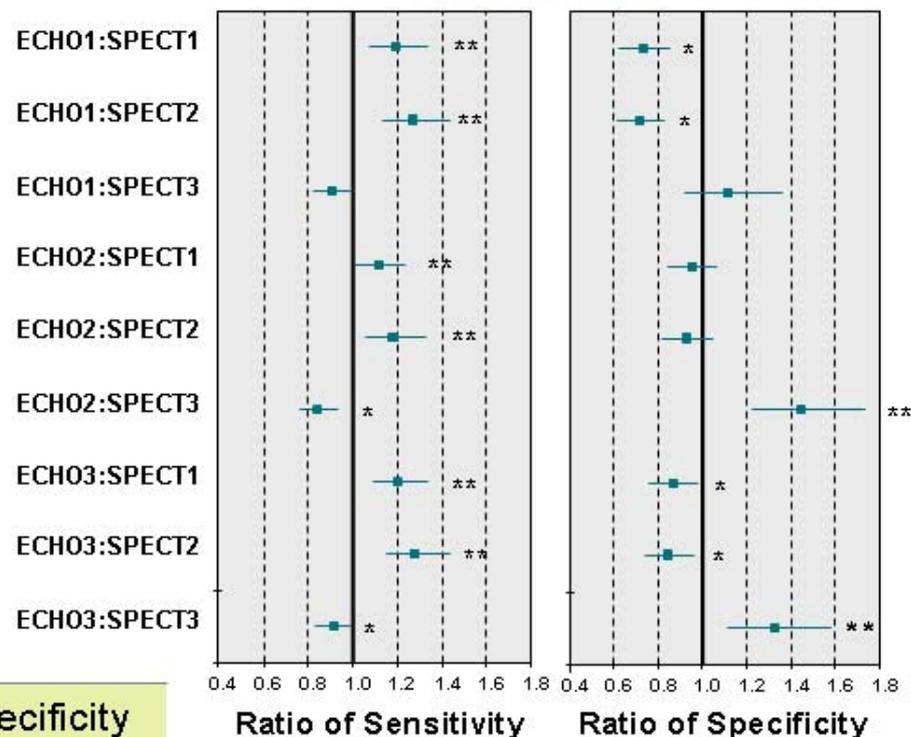
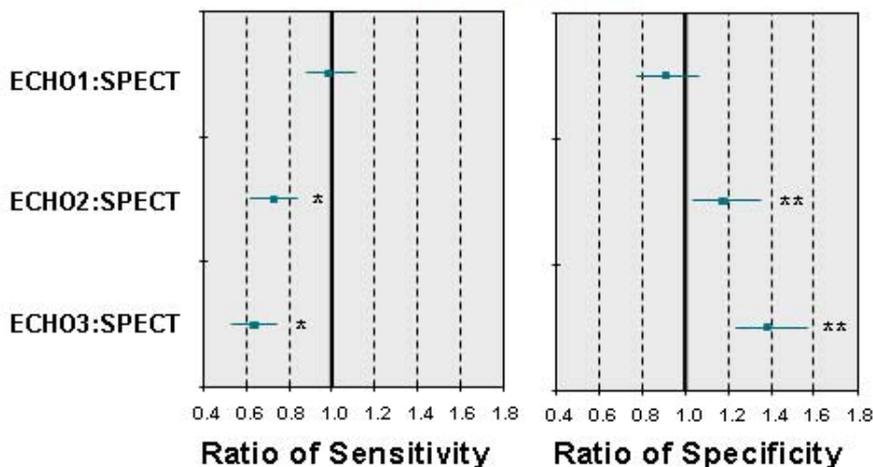


Area Under the Curve: ECHO=0.72; SPECT=0.72

# Pairwise Comparison: Both AI-700 ECHO and SPECT Readers Trade-off Sensitivity and Specificity<sup>1</sup>

## AI-700-32

## AI-700-33



	Superior Sensitivity		Superior Specificity	
	ECHO	SPECT	ECHO	SPECT
AI-700-32	0	2	2	0
AI-700-33	6	2	2	4
<b>Combined</b>	<b>6</b>	<b>4</b>	<b>4</b>	<b>4</b>

<sup>1</sup>Relative risk ratios with 95% confidence intervals

\* SPECT superior to AI-700 ECHO

\*\* AI-700 ECHO superior to SPECT

# AI-700 Provides Diagnostic Information Even When Non-Contrast Images Are Poor

- Acoustic Window Quality (AWQ) was evaluated independent of CAD assessment
- 27% of ECHO patients had poor AWQ
- Diagnostic information was the same regardless of AWQ
  - >99% images were evaluable
- AWQ diagnostic results attributed to:
  - Prolonged myocardial enhancement
  - Acoustic properties of AI-700

# Integrated Analyses Conclusions: AI-700 ECHO and SPECT Are Clinically Comparable

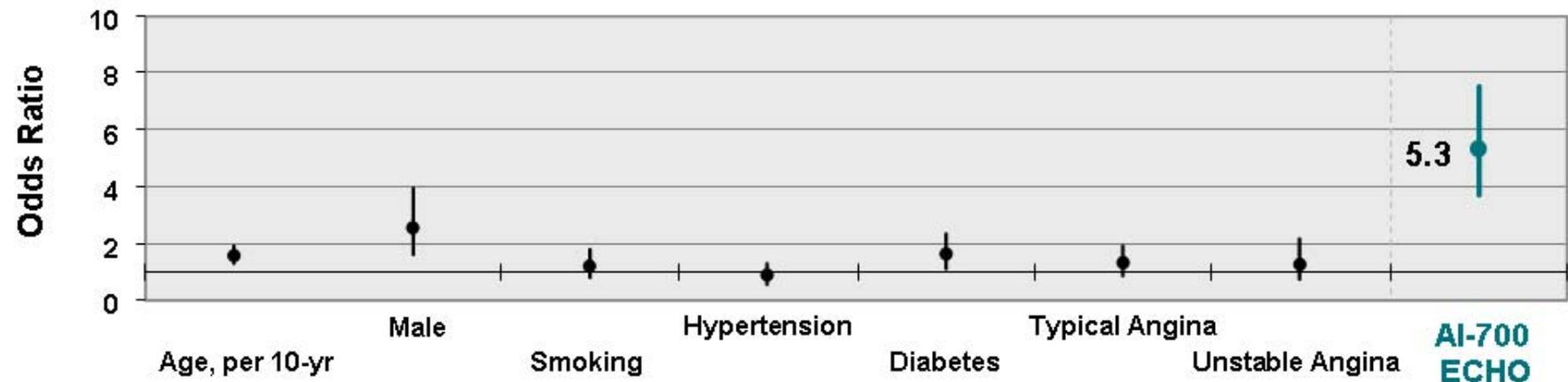
- Similar trade-offs in sensitivity and specificity
  - ROC analysis
  - Pairwise comparison
- Difference within and between trials in sensitivity and specificity are primarily attributed to reader bias and are not modality-specific

# AI-700 Increases Diagnostic Value Over Clinical Risk Factors

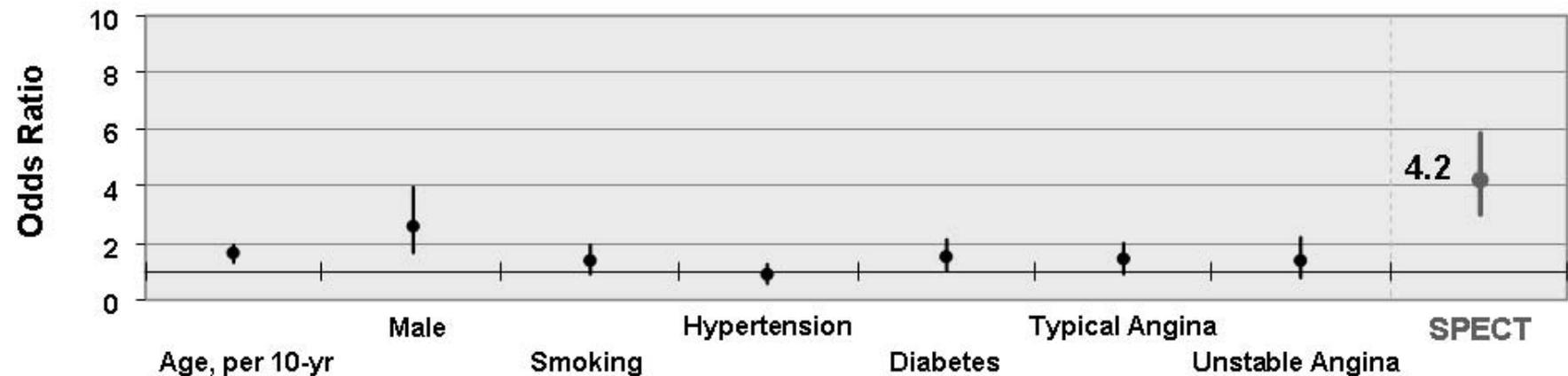
- **Objective:** Evaluate the ability of AI-700 ECHO to predict CAD in the context of standard clinical risk factors
- **Population:** Pooled mITT populations from AI-700-32 and AI-700-33
- **Clinical Risk Factors:** Age, male, history of typical or unstable angina (compared to atypical), diabetes, hypertension, smoking (current/former compared to never)
- **Logistic Regression Analysis:** Odds ratio (odds of CAD among patients with risk factor compared to those without)

# Multivariate Analysis: AI-700 ECHO is a Strong Predictor of CAD

**Model: Clinical factors + AI-700 ECHO**



**Model: Clinical factors + SPECT**



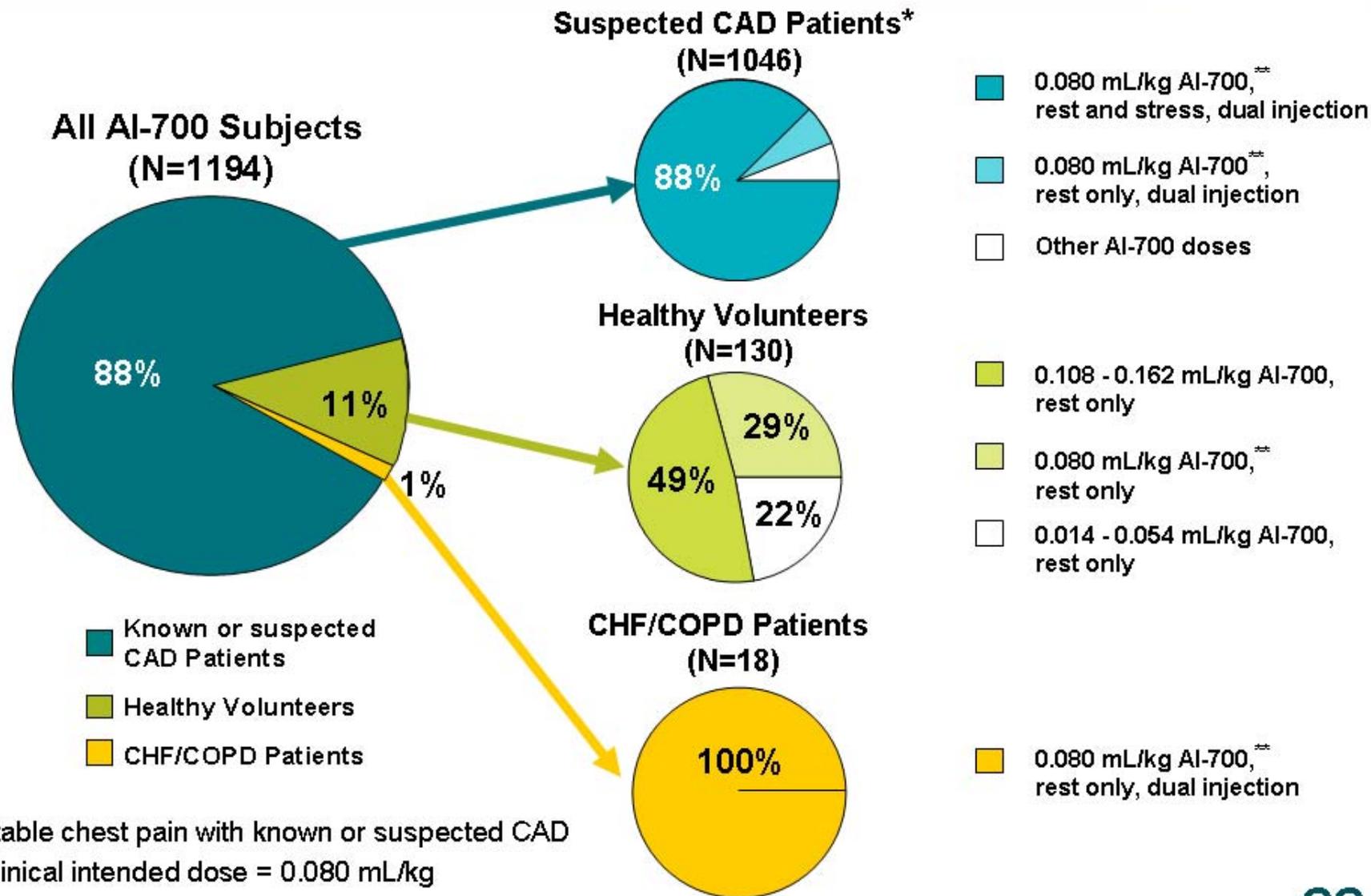
# Consistent Evidence that AI-700 ECHO is Comparable to SPECT

- Both trials met primary endpoint of accuracy
  - AI-700 as accurate as SPECT for all blinded readers
- AI-700 ECHO was comparable to SPECT for sensitivity and specificity, although each trial missed on one of these second tier endpoints
  - AI-700 ECHO and SPECT blinded readers showed a similar trade-off between sensitivity and specificity
- AI-700 ECHO provided added clinical value over patient risk factors
- AI-700 ECHO can detect CAD in stable chest pain patients being evaluated for inducible ischemia

# AI-700 Clinical Safety Overview

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# Overview of Clinical Exposure to AI-700



\* Stable chest pain with known or suspected CAD

\*\* Clinical intended dose = 0.080 mL/kg

# AI-700 Safety Population

Study Number	Design	Safety Population
<b>Phase 3 (Pilot Qualification Study)</b>		
<b>AI-700-23*</b>	Pilot, open-label, dual-injection (rest/stress)	n=133
<b>Phase 3 (Pivotal Studies)</b>		
<b>AI-700-32*</b>	Open-label, dual-injection (rest/stress), safety & ECHO imaging study	n=321
<b>AI-700-33*</b>	Open-label, dual-injection (rest/stress), safety & ECHO imaging study	n=457
Pivotal Study Population		n=778
<b>Total Safety Population</b>		<b>n=911</b>

\* Stable chest pain with known or suspected CAD; no placebo arm

# Cardiovascular History of Phase 3 Patients

	Safety Population (n = 911)
Chest pain history	874 (95.9%)
Rest	134 (14.7%)
Exertion	531 (58.3%)
Atypical	283 (31.1%)
Unstable	19 (2.1%)
Hypertension	683 (75.0%)
Hyperlipidemia	645 (70.8%)
Cardiac catheterization	487 (53.5%)
BMI $\geq 30$ kg/m	317 (34.8%)
Diabetes	285 (31.3%)
Peripheral vascular disease	95 (10.4%)
Arrhythmia	82 (9.0%)
CABG	70 (7.7%)
Carotid artery disease	53 (5.8%)
Stroke/TIA	50 (5.5%)
CHF	41 (4.5%)
COPD	30 (3.3%)
Thromboembolic disease	20 (2.2%)
Cardiac surgeries other than CABG	16 (1.8%)

# Timing of Safety Assessments Based On Rapid Clearance of AI-700

- AI-700 Kinetics
  - Biologically inert gas (perflubutane) cleared from body via lungs within hours ( $t_{1/2} = 4$  minutes)
  - Microspheres are removed rapidly from bloodstream into RES and cleared from body over ensuing days
  - Microspheres mainly composed of biodegradable, biocompatible polymer used in absorbable sutures and depot drugs (e.g. Lupron Depot<sup>®</sup>)
- AEs, vital signs, ECGs, clinical laboratories, and other safety parameters collected from baseline through follow-up (72 hrs.)

# Key Safety Findings

- Transient cardiopulmonary safety signals
  - Decrease in blood pressure
  - Decrease in oxygenation
- Potential mechanism for these findings
  - Transient activation of complement as a part of innate immune response
- Risk mitigation strategy

# Overview of Adverse Events

- AI-700 Clinical Program (n=1194 received AI-700)
  - No deaths
  - 11 (1%) subjects (all from Phase 3 studies) experienced 14 SAEs
- Phase 3 Safety Population (n=911)
  - 72% of patients experienced  $\geq 1$  AE
  - 97% of AEs were mild or moderate intensity
  - Majority of AEs occurred during stress
  - Majority of AEs resolved without treatment and without residual effects

# Acute and Delayed Serious Adverse Events According to Time of Onset

11 Patients / 14 SAEs

**ACUTE**  
n=6 / 7 SAEs

**DELAYED**  
n=5 / 7 SAEs

- Patients with syncope vasovagal (n=3)
- Hypertension, vertigo (n=1)

- Chest pain (n=1)
- Mental status change (n=1)

- Eye pain, blurred vision, vision disturbance [18 hrs]
- Hypersensitivity [1 hr]
- Adverse drug reaction [3 hr]

- MI [2 days]
- NSTEMI [1 Day]

Within 30 min  
1<sup>st</sup> dose

Within 30 min  
2<sup>nd</sup> dose

Over 24 hours

time<sub>0</sub>

Start of 1<sup>st</sup>  
AI-700 dose

DIPY start 7 min  
prior to 2<sup>nd</sup> dose

1 hr post 2<sup>nd</sup>  
AI-700 dose

24 hrs post 2<sup>nd</sup>  
AI-700 dose

Aminophylline  
(end of stress imaging)

# Adverse Events Leading to Discontinuation of AI-700 Dosing

- 16 of 911 patients (14 at rest, 2 at stress)
- Most common AE resulting in discontinuation was hypotension (n=5)
  - 3 AEs at rest, 1 required treatment (lowest SBP value  $\geq 100$  mmHg, decreased from 120 mmHg at BL)
  - 2 AEs at stress, both required treatment (lowest SBP value 52 mmHg, decreased from 127 mmHg at BL)
- SAEs that resulted in discontinuation:
  - Vasovagal syncope (n=3) without loss of consciousness
  - Hypertension and vertigo (n=1)
- All AEs resolved without residual effect

# Vasovagal Syncope

- Very common in stressful situations
  - Blood donation, phlebotomy, parenteral injections
  - Open-label study of investigational drug
  - Multiple observers (4 to 7), counting down to time of injection (3,2,1.....inject)
- Clear-cut diagnosis in these studies
  - Trained observer
  - Real-time telemetry and ready access to BP reading
  - Atropine

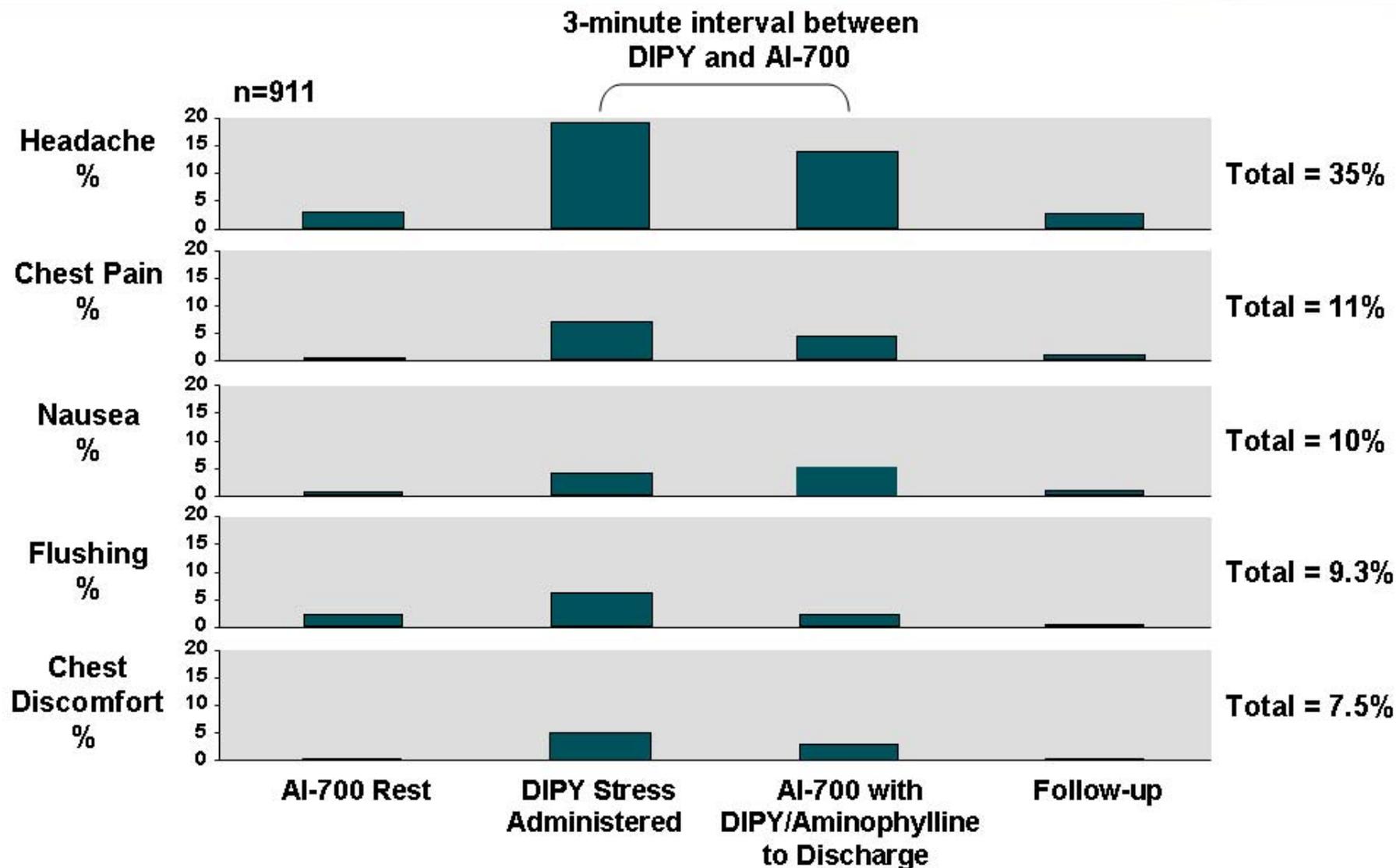
# Vasovagal Syncope Events

Patient ID (Age/Gender)	Imaging Session	Intensity	Event	Treatment (indication)	Resolution
33.35.030 (72 y/o female)	Rest	Severe	SAE, D/C	Atropine (bradycardia) Normal Saline (hypotension)	Yes, NRE
33.37.008 (59 y/o female)	Rest	Moderate	SAE, D/C	Atropine (bradycardia)	Yes, NRE
32.07.060 (79 y/o male)	Rest	Moderate	SAE, D/C	Atropine (bradycardia); Polygeline (hypotension)	Yes, NRE
33.35.009 (68 y/o female)	Stress	Mild	AE	Normal Saline (vasovagal)	Yes, NRE
23.43.001 (58 y/o male)	Stress	Mild	AE	None	Yes, NRE
32.12.001 (66 y/o male)	FU	Moderate	AE	None	Yes, NRE
33.46.001 (54 y/o female)	FU	Moderate	AE	Normal Saline (vasovagal)	Yes, NRE

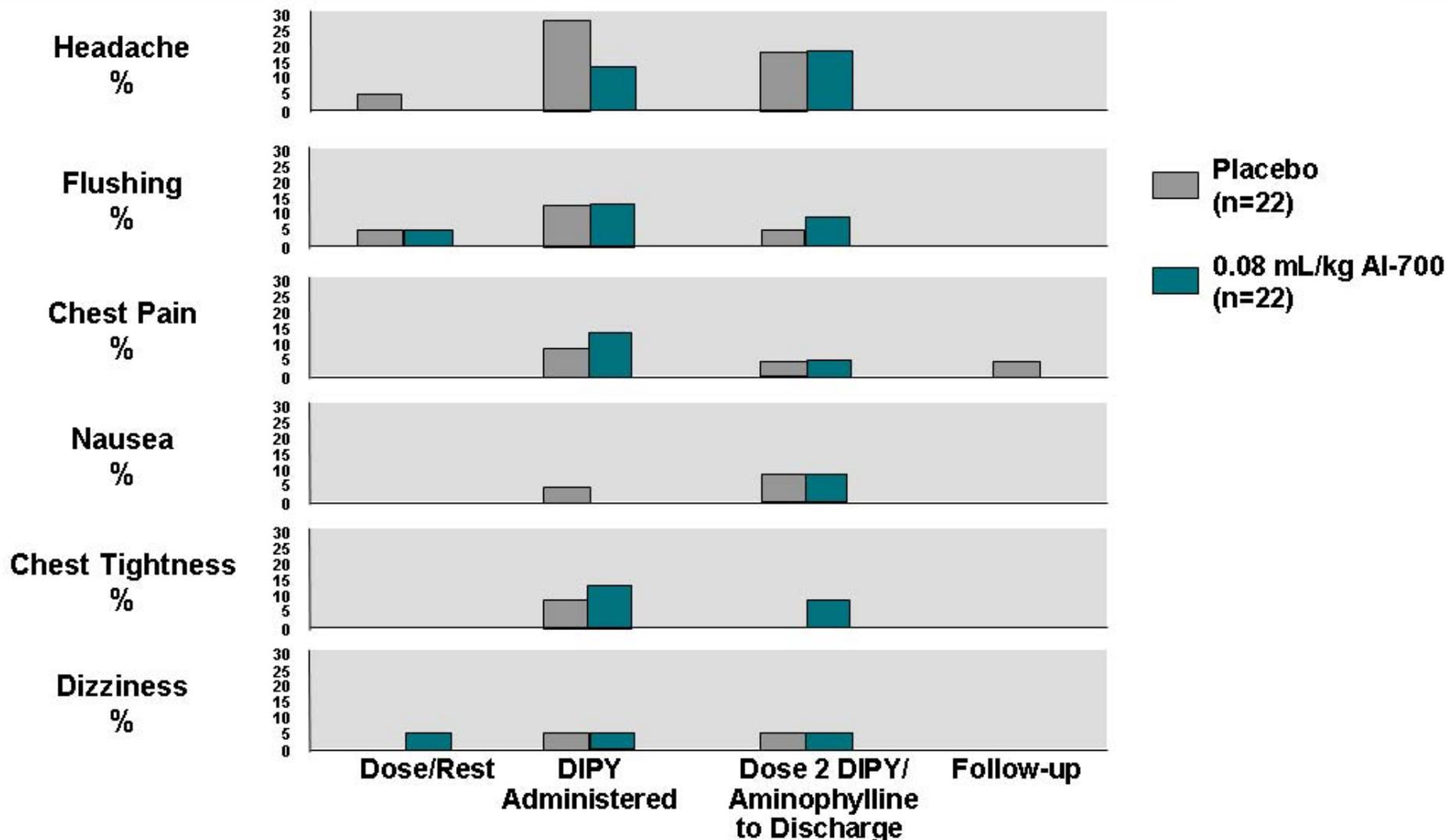
NRE = no residual effect

D/C = discontinuation

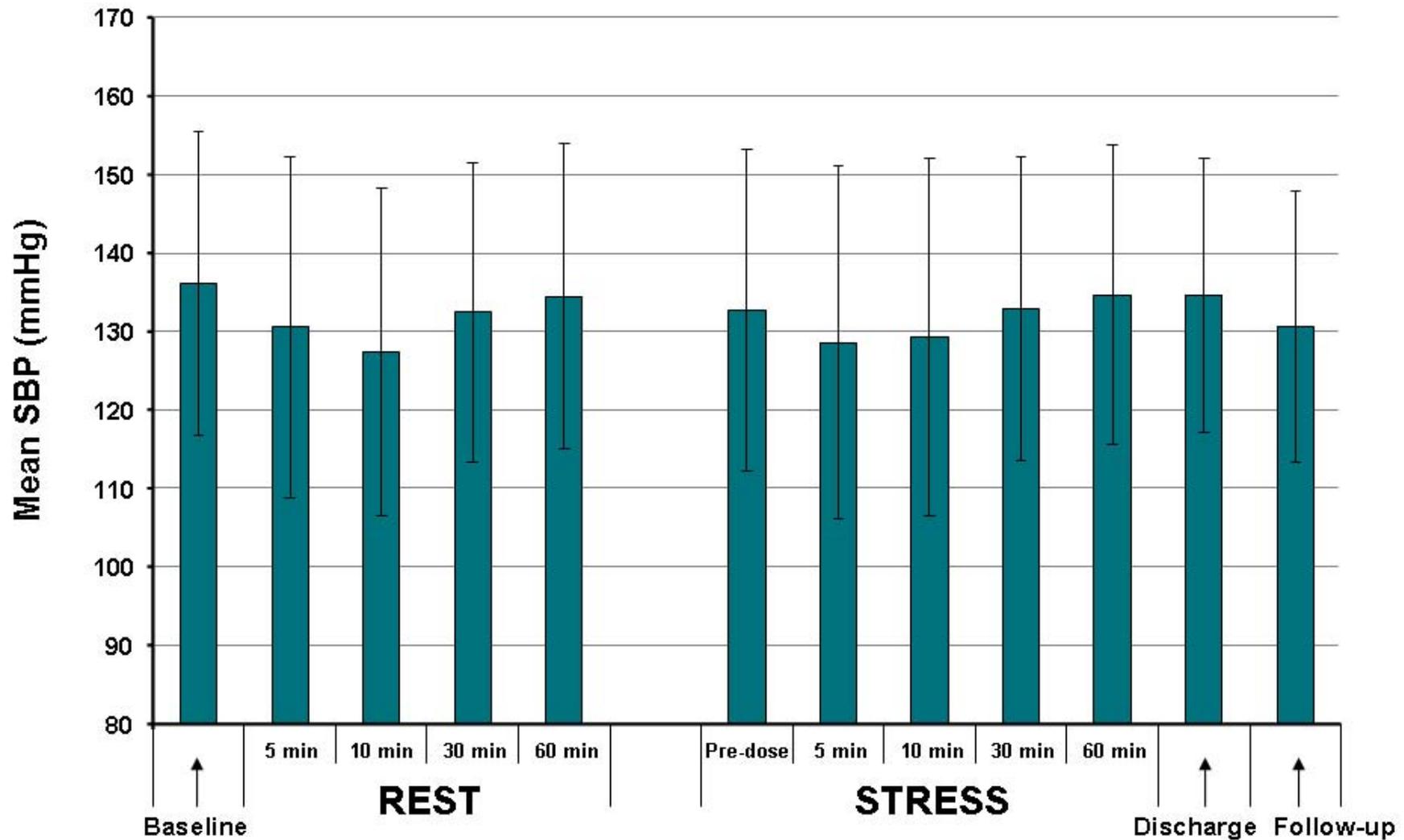
# Most Frequent AEs: Relationship to Drug Administration



# Most Common AEs in Patients Who Received 0.08 mL/kg AI-700 or Placebo and DIPY (Study AI-700-21)



# Mean Systolic Blood Pressure (AI-700-32 and -33; n=778)



# Hypotensive Adverse Events

- 41 hypotensive AEs in 38 of 911 patients (4%)
  - 12 AEs occurred at rest (n=12)
    - Lowest reported SBP (77 mmHg) decreased from Baseline (104 mmHg)
  - 28 AEs occurred at stress (n=25)
  - 1 AE occurred at follow-up (during ANGIO)
- All patients remained conscious
- Short duration
- Resolved without residual effects
- No subpopulation identified with increased risk

# Hypotensive AEs – Rest

Patient ID (age, gender)	CAD status (truth standard)	BP Baseline (mmHg)	BP Nadir (AE) (mmHg)	Time of Nadir (post dose)	AE Duration	Treatment and Resolution
23.34.003* (68 y/o, male)	Negative	<b>120/75</b>	<b>100/60</b>	30 min	40 min	Resolved w/o treatment
23.39.003 (49 y/o, male)	Positive (Prior MI)	<b>121/63</b>	<b>117/45</b>	10 min	20 min	Resolved w/o treatment
32.04.009 (59 y/o, female)	Negative	<b>123/80</b>	<b>95/57</b>	10 min	20 min	Resolved w/o treatment
32.12.003 (68 y/o, male)	Negative (ANGIO)	<b>145/90</b>	<b>90/60</b>	10 min	11 min	Resolved w/o treatment
32.16.021* (68 y/o, male)	Negative	<b>118/86</b>	<b>105/70</b>	30 min	15 min	Resolved with treatment
32.16.025 (66 y/o, female)	Negative	<b>156/92</b>	<b>108/63</b>	10 min	17 min	Resolved with treatment
32.18.008 (61 y/o, male)	Positive (ANGIO)	<b>127/76</b>	<b>90/52</b>	5 min	5 min	Resolved w/o treatment
33.35.071 (68 y/o, male)	Positive (ANGIO)	<b>135/81</b>	<b>89/49</b>	5 min	5 min	Resolved w/o treatment
33.35.074 (49 y/o, female)	Negative (CAD Review)	<b>93/62</b>	<b>80/50</b>	10 min	50 min	Resolved w/o treatment
33.35.085 (57 y/o, male)	Negative (ANGIO)	<b>105/66</b>	<b>87/49</b>	5 min	25 min	Resolved w/o treatment
33.48.047 (73 y/o, female)	Negative (ANGIO)	<b>115/50</b>	<b>102/52</b>	10 min	3 min	Resolved w/o treatment
33.49.062* (53 y/o, male)	Positive (ANGIO)	<b>104/62</b>	<b>77/50</b>	5 min	5 min	Resolved w/o treatment

\*Discontinuations of dosing

# Summary of Respiratory AEs

- 98 respiratory AEs reported in 79 of 911 patients
  - 2 patients treated with supplemental oxygen at rest
  - 6 patients treated with supplemental oxygen at stress
  - Resolved without residual effects
- Dyspnea was the most frequently observed respiratory AE [43 AEs in 40 (4%) patients]
  - 4 AEs at rest; 36 AEs at stress; 3 AEs at follow up
  - 13 AEs of dyspnea required treatment (all during stress)
  - Dyspnea AEs were of short duration and did not result in  $\text{SaO}_2 < 90\%$  at rest
- Other respiratory AEs occurred in  $\leq 1\%$  of patients

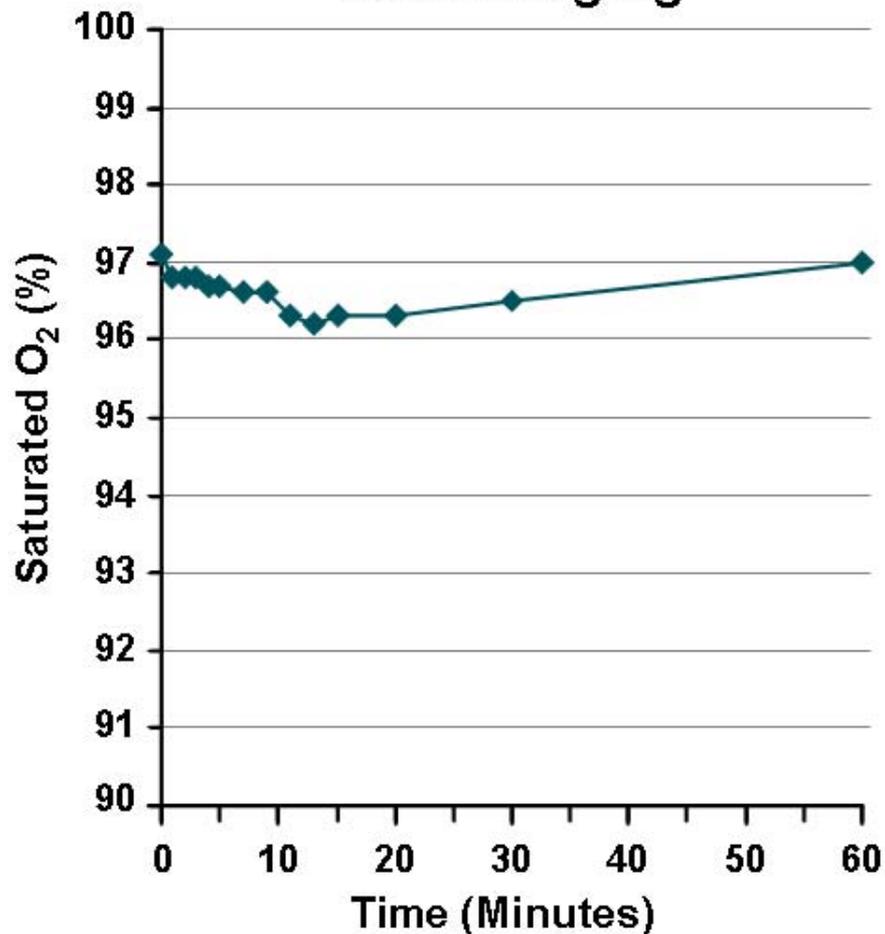
# Onset of Respiratory AEs\*

MedDRA Preferred Term	AI-700 Rest n (%)	DIPY Stress n (%)	AI-700 Stress n (%)	Discharge to Follow-up n (%)	Total n (%)
Respiratory, thoracic, and mediastinal disorders	12 (1.3)	37 (4.1)	27 (3.0)	12 (1.3)	79 (8.7)
Dyspnea	4 (0.4)	20 (2.2)	16 (1.8)	3 (0.3)	40 (4.4)
Throat tightness	0	7 (0.8)	3 (0.3)	1 (0.1)	10 (1.1)
Cough	4 (0.4)	2 (0.2)	2 (0.2)	0	7 (0.8)
Pharyngolaryngeal pain	0	5 (0.6)	1 (0.1)	1 (0.1)	7 (0.8)
Nasal congestion	1 (0.1)	0	3 (0.3)	0	3 (0.3)
Wheezing	1 (0.1)	0	1 (0.1)	1 (0.1)	3 (0.3)
Bronchospasm	0	0	2 (0.2)	0	2 (0.2)
Hypoxia	1 (0.1)	0	1 (0.1)	0	2 (0.2)
Sinus congestion	2 (0.2)	0	1(0.1)	0	2 (0.2)
Tachypnea	1 (0.1)	1 (0.1)	0	0	2 (0.2)

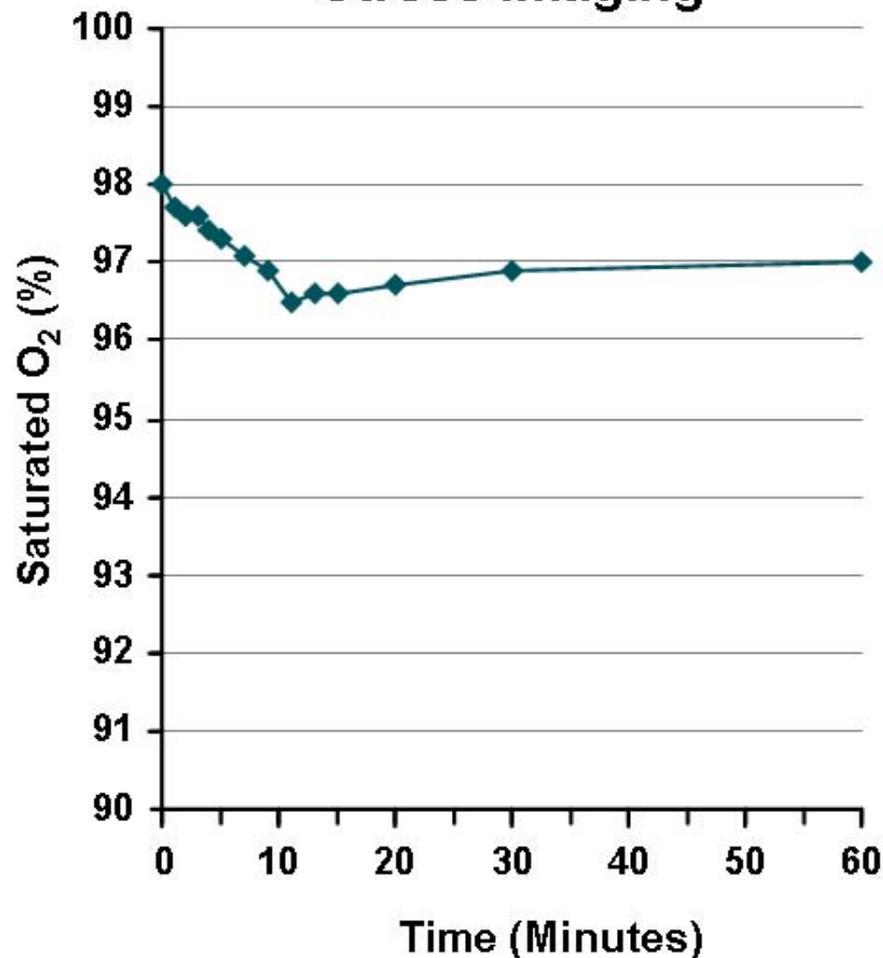
\*Respiratory AEs described here occurred in  $\geq 2$  (0.2%) patients.

# SaO<sub>2</sub> Changes Following AI-700 Administration (AI-700-32 and -33; n=778)

## Rest Imaging



## Stress Imaging



# SaO<sub>2</sub> Decreases of ≥5% from Baseline to <90% - Rest (AI-700-32 and -33; n=778)

Patient Number	Smoking History	Baseline SaO <sub>2</sub> (%)	Lowest SaO <sub>2</sub> (%)	Post-dose Time(s)	Post-Dose SaO <sub>2</sub> Range <sup>1</sup>	Concurrent AE
32.16.010 (64 y/o female)	Former	97	89	20m	89-99	Cough, lacrimation, flushing, wheezing
32.18.008 (61 y/o male)	Former	93	88	11m	88-96	Hypotension
33.34.003 (71 y/o male)	Former	97	89	7m	89-98	none
33.34.012 (68 y/o male)	--	95	87	11m	87-96	none
33.34.016 (77 y/o female)	--	95	89	15m	89-97	none
33.35.035 (61 y/o female)	--	99	89	3m	89-99	Allergy to contrast
33.35.047 (69 y/o male)	Former	97	89	15m	89-95	Hypoxia
33.37.008 (59 y/o female)	Former	95	89	20m	89-97	Syncope vasovagal
33.37.041 (34 y/o male)	Former	93	88	15m	88-94	none
33.37.046 (66 y/o male)	Former	95	83	15m	83-92	Facial flushing, SaO <sub>2</sub> decrease, cyanosis
33.37.053 (69 y/o male)	Former	94	89	20m	89-95	none
33.48.007 (62 y/o male)	Former	97	89	11, 13m	89-95	none

1. Post-dose range is any time after first AI-700 dose through Discharge.

## Moderate COPD Patients (Study AI-700-05, n=8)\*

- Patients had  $D_LCO < 75\%$ ,  $FEV_1/FVC < 75\%$  predicted, and  $FEV_1$  50% to  $< 70\%$
- Randomized to AI-700 (0.04mL/kg x2) or placebo in crossover design
- Decreases in  $FEV_1$  observed in all patients
  - Fatigue
  - Maximum mean  $FEV_1$  decrease was 22% at 15-20 minutes after 2nd AI-700 dose versus 8% for placebo
  - 2 patients had  $FEV_1$  decrease  $> 15\%$  at 15-20 minutes post-AI-700 dose
- 2 patients had  $SaO_2 < 90\%$  (Baseline: 92% & 94%, minimum: 88% & 89%, respectively)
  - $SaO_2$  decreases were asymptomatic
  - Occurred within 20 minutes post-dose and recovered to  $> 90\%$

\* Per-protocol population

# Pulmonary Function Test Interpretation (Study AI-700-05, COPD Cohort)

- Transient subclinical spirometric changes were observed
  - No AEs reported
- Most were mixed obstructive/restrictive changes;  
2 of 8 patients demonstrated primarily obstructive changes
- Mechanism for transient subclinical bronchoconstriction may involve vascular-airway interactions
- Pulmonary function tests returned to Baseline levels for all subjects

# Rigors

- 41 AEs occurred in 39 of 911 patients
  - 9 AEs at rest (3 treated)
  - 24 AEs at stress (4 treated)
  - 8 AEs at Follow-up (4 treated)
- Common treatments were acetaminophen (5 patients) and Benadryl (2 patients)
- 25 AEs mild, 14 AEs moderate, and severe for 2 AEs
- 2 patients discontinued AI-700 dosing due to rigors
- All resolved without residual effects

# ECG Evaluation Methodology (AI-700-32 and -33; n=778)

- 12-lead ECGs collected at:
  - Baseline, 5, 10, 30, 60 minutes post dose for both imaging sessions
  - Discharge and follow-up
- Continuous monitoring by ultrasound ECG
- 3-hour Holter monitoring performed in 109 consecutive patients at 9 sites
- Rhythm and ischemia assessment performed by investigators
- Core lab performed Holter interval analysis and over-read of abnormal 12-lead ECG QT/QTc intervals

# ECG Findings

## (Studies AI-700-32 and 33; n=778)

- No indication of ECG-related changes attributable to AI-700<sup>1</sup>
- No trend in QTc prolongation or other interval changes
- No increased rate of premature ventricular contractions (PVCs) when mechanical index  $\leq 1.0$
- ECG ST-T depression or ECG changes indicative of ischemia
  - Occurred during stress in all but 1 patient
  - Were reversible

[1] Pano et al., *Eur J Echo*. 2007.

# Clinical Laboratory Findings

- No shifts in clinical chemistries, coagulation or urinalysis
- Transient increases in WBCs and neutrophils
  - At discharge (2-4 hrs post dose), 17-30% of patients had increases in WBCs above the normal range
  - At discharge (2-4 hrs post dose), 43-57% of patients had increases in neutrophils above the normal range
  - Returned to normal range by follow-up ( $72 \pm 48$  hours)

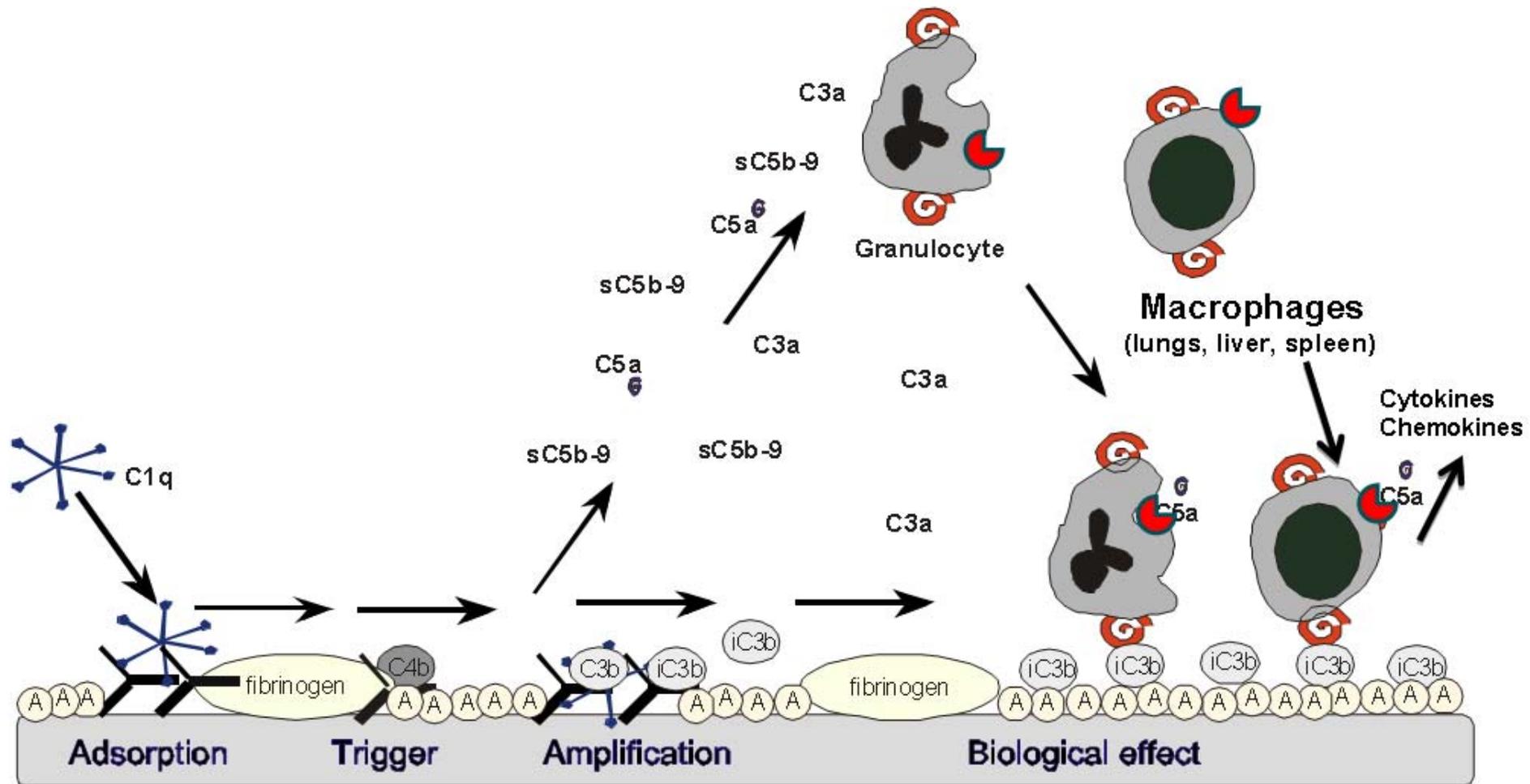
# Innate Immune Response to AI-700

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Department of Pathology & Laboratory Medicine  
University of Pennsylvania

Past President of International Complement Society

# Innate Immunity Response to AI-700



C5a: Hypotension, Neutropenia, Neutrophilia

# Investigation of AI-700 Induced Immune Response (Study AI-700-04, n=12)

- Assessed in healthy volunteers
  - AI-700 (0.08 mL/kg) or placebo (IV single injection)
  - Percutaneous skin prick tests (SPT) prior to re-challenge >1 year later
  - Measured C3a, C3, tryptase (surrogate of histamine release), and CRP
- No hypersensitivity to AI-700 (no typical allergic response/negative SPT)
- C3a increases within 10 minutes of AI-700 dosing
  - Temporally related to decreases in neutrophils and WBCs
- C3a, neutrophils, WBC counts normalized at 30 minutes post-dose
- Neutrophils and WBCs increased above Baseline levels at 2-8 hours post dose and normalized by Follow-up
- No changes in vital signs or SaO<sub>2</sub>

# Innate Immune Response to AI-700

- Nonclinical findings support clearance of AI-700 microspheres by reticuloendothelial macrophages
- Decreases in BP and SaO<sub>2</sub> may reflect normal clearance of AI-700 microspheres from bloodstream
  - Within 10-15 minutes of dosing, similar to expected peak of complement activation
  - Transient, similar to duration of complement activation
  - Complement activation associated with hypotension<sup>1</sup>
- Increases in neutrophil levels and inflammatory events (rigors, pyrexia) may be due to cytokines which:
  - Affect temperature control (hypothalamus)
  - Increase release of neutrophils from bone marrow

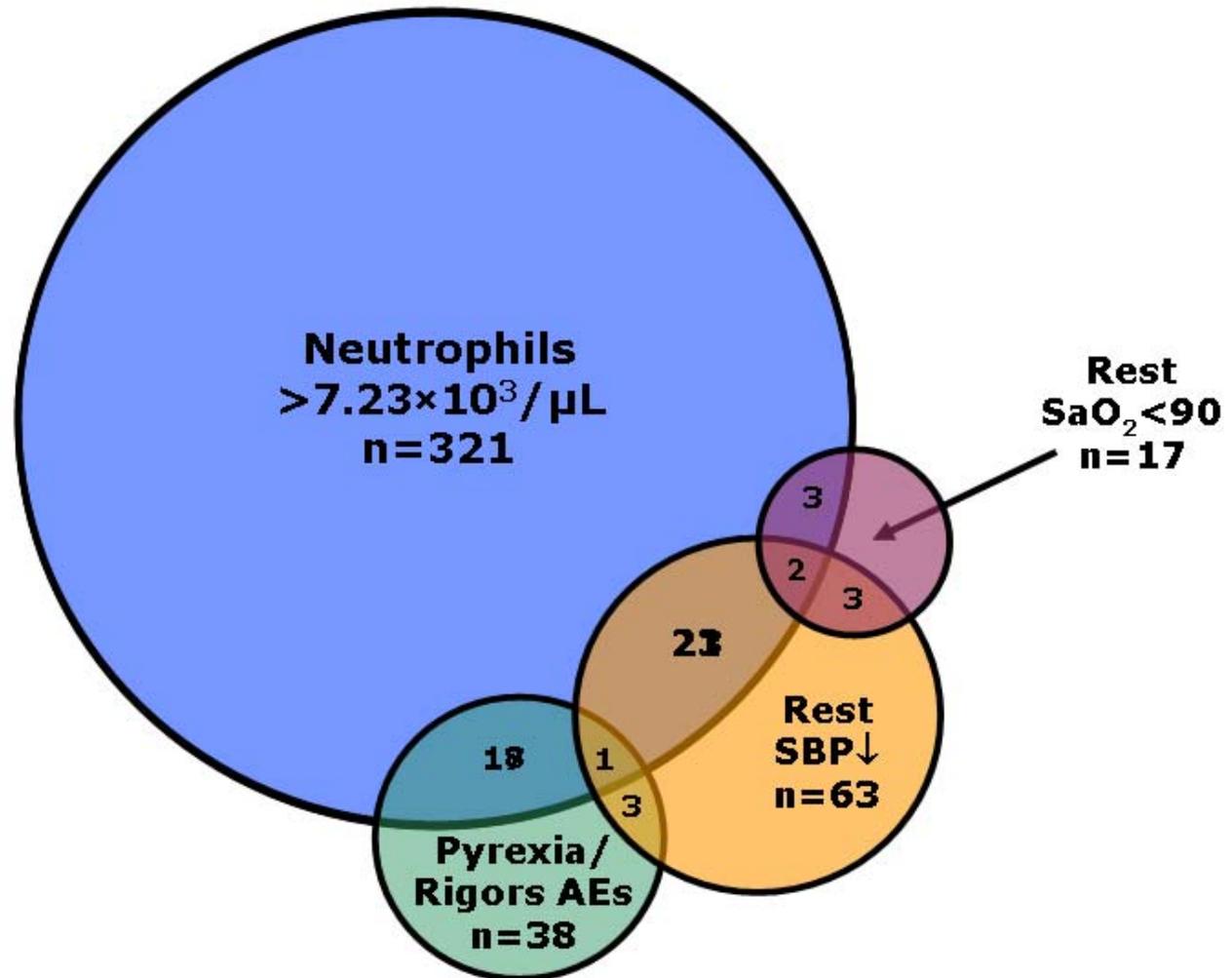
[1] Shiga et al. *Artif Organs*. 1998.

# Innate Immune Response to AI-700

- Decreases in BP and SaO<sub>2</sub> may reflect normal clearance of AI-700 microspheres from bloodstream
- Increases in neutrophil levels and inflammatory events (rigors, pyrexia) may be due to cytokines

# Relationship Between SBP, SaO<sub>2</sub> Decreases, Neutrophil Increases and Inflammatory Response AEs

n=778



# Key Safety Findings

- Pulmonary
  - Transient effect on SaO<sub>2</sub>
  - Minimal rate of transient respiratory AEs
- Cardiovascular
  - Transient effect on blood pressure
  - Few cases of hypotension at rest
- Potential Mechanism for these findings
  - Transient activation of complement as a part of innate immune response
- No evidence that effects of AI-700 are additive to DIPY or ischemia (dominant variables in stress ECHO)

# AI-700 Studies Compared with Literature Results From Pharmacologic Stress Studies

	AI-700+DIPY Phase 3 Safety Population			Literature Results From Non-Contrast Cardiac Imaging Studies		
	1 <sup>st</sup> dose to DIPY %	DIPY to 2 <sup>nd</sup> dose %	Total (n=911) %	DIPY Imaging (n=3911) % <sup>1</sup>	Adenosine SPECT (n=1421) % <sup>2</sup>	Dobutamine ECHO (n=1012) % <sup>3</sup>
Chest pain	0.8	12.3	20.0	19.7	40.0	30.5
Flushing	1.9	5.8	9.3	3.4	44.0	10.3
Headache NOS	3.0	18.7	34.8	12.2	18.0	13.6
Dizziness (excl vertigo)	0.8	3.0	5.7	11.8	12.0	n/a
Dyspnea NOS	0.4	2.2	4.4	2.6	28.0	12.2
Nausea	0.5	3.8	10.4	4.6	13.0	8.0
Ventricular extrasystoles	0.2	0.3	1.2	5.2	n/a	12.0
ECG abn. /ST-T changes	0	0.3	0.4	7.5	n/a	n/a
Arrhythmia	0.3	0.7	2.0	3.2	1.0	7.0
AV Block	0.1	0.3	1.2	0.1	6.3	n/a

[1] Dipyridamole (package insert); [2] Adenoscan (package insert); [3] Dakik et al. *J Nucl Cardiol*. 1996.

The adverse events presented are those that were present at a rate >5% for any pharmacologic stress study.

# AI-700 ECHO

## Cardiopulmonary Risk Mitigation

- Risk mitigation strategies for AI-700 specified in the proposed label are similar to recommendations for rest/pharmacologic stress imaging studies<sup>1,2</sup> and include:
  - Contraindicated for acute coronary syndrome (including unstable angina), respiratory failure, severe COPD, high degree A-V block
  - Monitoring of vital signs, ECG, and oxygen saturation
  - Readily available resuscitation equipment, medications and trained personnel<sup>3,4</sup>
- These strategies are adequate to mitigate cardiopulmonary risk with AI-700 ECHO with the following addition:
  - Proposed labeling specifies that the mechanical index should not exceed 1.0

[1] Henzlova et al. *J Nucl Cardiol.*, 2006. [2] Pellikka et al., *J Am Soc Echocardiogr.*, 2007. [3] Part 7.4: Monitoring and Medications. *Circulation.* 2005. [4] Minimum Crash Cart Supplies and Drugs 2007 From: <http://www.ttuhscc.edu/som/clinic/forms/ACForm2.03.A.pdf>

# Safety Database Considerations Regarding Potential Life-Threatening SAEs

- In a safety population of this size (n=911), event rates of >0.4% could be excluded
- Post-marketing safety study is needed to assess the potential for deaths or life-threatening SAEs
- Meeting of the Cardiovascular and Renal Drugs Advisory Committee, June 24, 2008:
  - *“The committee suggested that there was a need for infrequent serious events to be obtained in well designed, post-marketing observational studies.”*
- Acusphere to discuss with FDA post-marketing studies
  - Patient safety registry for acute events
  - AI-700 pharmacologic stress ECHO compared to other pharmacologic stress tests

# Comparison of AI-700 ECHO Risk

- Risk assessment compared with other imaging techniques that provide diagnostic information in the same patient population
  - Two methods recommended<sup>1</sup> and used routinely in stable chest pain patients who cannot exercise
  - SPECT myocardial perfusion using adenosine agonist stress
  - ECHO non-contrast wall motion using dobutamine stress
- Literature provides guidance on comparable risk profiles
  - As low as 1:1600 life threatening SAE with pharmacologic SPECT stress (dipyridamole)<sup>2</sup>
  - As low as 1:140 life threatening SAE with pharmacologic ECHO stress (dobutamine)<sup>3</sup>

[1] Gibbons et al. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina. 2002.

[2] Lette et al. *J Nucl Ccardiol*, 1995

[3] Sicari et al. Stress echocardiography expert consensus statement. *Eur J Echo*. 2008

# Conclusions for AI-700 ECHO – Safety

- Overall well-tolerated
  - Small, marginal difference compared to DIPY SPECT
  - No ionizing radiation risk
- AI-700 produces transient cardiopulmonary safety signals
  - Fewer than 3% of patients at rest
  - Effectively managed with standard measures
- Risk of serious adverse experiences mitigated by:
  - Label restriction and risk mitigation strategies have been proposed which are similar to those of current pharmacologic stress imaging procedures
- Post-marketing safety study will be conducted

# AI-700: Concluding Remarks

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Senior VP, Clinical Research

Acusphere, Inc.  
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# The Clinical Need for a New CAD Stress Test

- Comprehensive evaluation of myocardial structure, function, and perfusion
- Safe procedure without biohazard to patients or healthcare providers
- Readily available procedure with potential for cost saving

# Limitations of Current Pharmacologic Stress Procedures

- Vasodilatory SPECT

- High ionizing radiation dose
  - Risk of fatal malignancy
  - Environmental impact and biohazard
- Major contributor to escalating cardiac imaging cost
- Less convenient and less available than ECHO
- Limited anatomic information

- Dobutamine non-contrast ECHO

- More complex and less safe than vasodilatory SPECT imaging
- No information on perfusion
- Non-diagnostic quality images in of patients due to poor acoustic window quality

# Efficacy of AI-700 ECHO

- Provides diagnostic performance that is clinically comparable to DIPY SPECT
- Is a strong independent predictor of CAD in chest pain patients
- Improves quality of ECHO images thus increasing value of stress ECHO

# Safety of AI-700 ECHO

- Overall well-tolerated with no ionizing radiation risk
- AI-700 produces transient cardiopulmonary safety signals
  - Fewer than 3% of patients at rest
  - Effectively managed with standard measures
- Risk of serious adverse experiences mitigated by:
  - Label restriction and risk mitigation strategies have been proposed which are similar to those of current pharmacologic stress imaging procedures
- Post-marketing safety study will be conducted

# AI-700 DIPY ECHO Benefits Outweigh the Risks

- First effective imaging agent to conveniently provide assessment of perfusion and wall motion
- Comparable safety to DIPY SPECT
  - Safety signals of AI-700 during DIPY ECHO over DIPY SPECT are offset by lack of ionizing radiation exposure
- Literature data indicate DIPY ECHO is safer than dobutamine ECHO
- In conclusion, the acute safety risk of AI-700 ECHO is offset by its unique added benefits

**Cardiovascular and Renal Drugs  
Advisory Committee  
December 10, 2008**

**Imagify™  
(Perflubutane Polymer Microspheres)**

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