

Safety Profile of SonoVue[®] **(Sulfur Hexafluoride Microbubbles)**

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SonoVue - Pharmaceutical Particulars

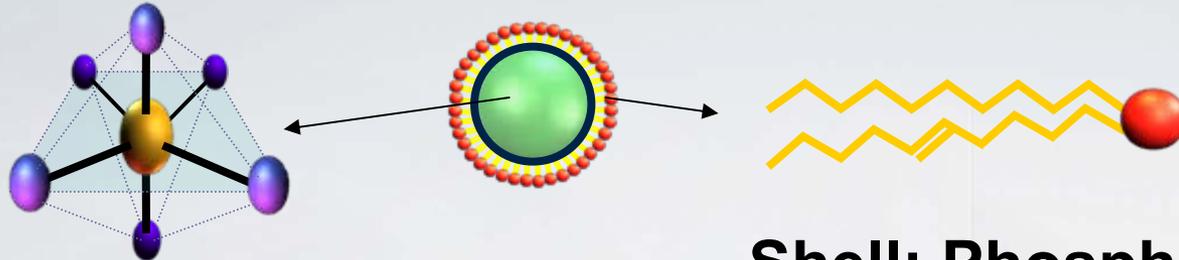
**Gas: 100%
Sulfur
Hexafluoride
(SF₆)**

**25 mg lyophilisate
powder containing
Macroglol 4000,
phospholipids and
palmitic acid**



**Solvent: 5 mL
sodium chloride
9 mg/mL (0.9%)
solution for
injection**

Structure of SonoVue Microbubbles



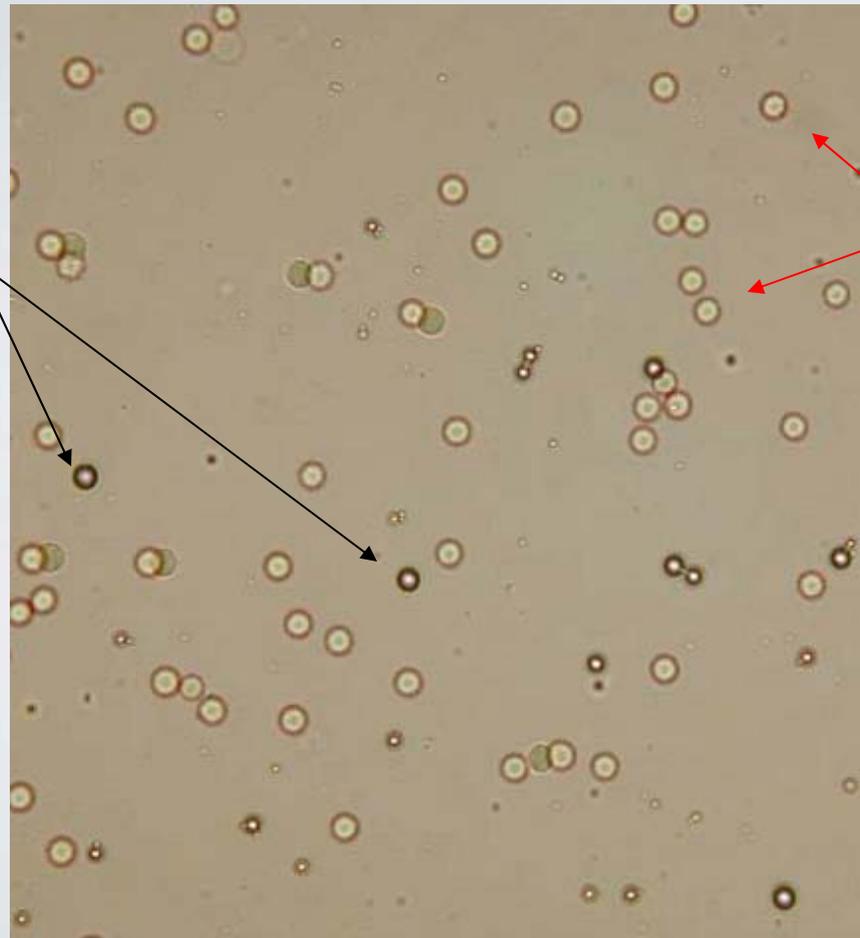
**Gas: 100% Sulfur
Hexafluoride (SF_6)**

Shell: Phospholipids

- **Mean microbubble diameter: 2.5 μm**
 - 90% < 6 μm
 - 99% < 11 μm
- **SF_6 volume in 1 mL is ~8 μl**
 - ~16 μl in a 2-mL dose (vs ~500 mL of pulmonary blood volume in an average person)

Microscopic Picture of SonoVue Microbubbles and Red Blood Cells

**SonoVue
Microbubbles**



Red Blood Cells

Regulatory Status [1]

- **SonoVue first approved in Europe in 2001**
 - Approved in 36 countries worldwide
 - Marketed in 25 countries in Europe and Asia
- **Approved Indications**
 - ***Echocardiography*** – to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation
 - ***Doppler of Macrovasculature*** – to increase the accuracy in diagnosis of abnormalities in cerebral, carotid, abdominal, peripheral arteries or portal vein by improving the Doppler signal-to-noise ratio
 - ***Doppler of Microvasculature*** – improves display of the vascularity of liver and breast lesions during Doppler sonography, leading to more accurate lesion characterization

Regulatory Status [2]

- **In the United States:**

- SonoVue is being **developed for use in echocardiography** to improve visualization of cardiac chambers and endocardial border delineation
- Clinical studies are being performed to assess the diagnostic performance (sensitivity and specificity) of SonoVue in characterization of focal liver lesions

Safety Database [1]

- **Completed Clinical Trials – Integrated Database**

Type of Study	N. of Patients
Clinical Pharmacology (Healthy Volunteers and Special Populations)	166
Echocardiography	1769
Doppler of Macrovasculature	555
Doppler of Microvasculature	2785
Total	5275

- **Ongoing Clinical Trials:** 1195 patients dosed as of end March 2011

Safety Database [2]

- **Post-Marketing Safety Surveillance**
 - Exposure of an estimated **1,651,451** patients from first approval in April 2001 until end December 2010
- **Literature Review**
 - Overall safety analysis involving **39,722** patients from 53 publications in peer-reviewed journals

Type of Safety Review [1]

- Assessment of **possible predictable untoward side effects on:**
 - **ventricular repolarization** in patients with coronary artery disease
 - **pulmonary hemodynamics** in patients with congestive heart failure and pulmonary hypertension
 - **oxygen saturation and pulmonary function** in patients with moderate to severe chronic obstructive pulmonary disease
 - pharmacokinetics and **oxygen saturation** in patients with diffuse interstitial pulmonary fibrosis

Type of Safety Review [2]

- Rate and type of adverse events:
 - reported from clinical trials
 - spontaneously reported during post-marketing surveillance
 - reported in the literature

Possible Predictable Untoward Side Effects

Joint Meeting of the Cardiovascular and Renal Drugs Advisory Committee and the
Drug Safety and Risk Management Advisory Committee 2 May 2011



Effects on Ventricular Repolarization

- Two prospective clinical pharmacology studies:
 - **Study BR1-112:** single-blind, placebo-controlled, randomized, 3-way crossover comparison of the effects on QTc interval of single IV injections of two bolus doses of **SonoVue (0.1 and 0.5 mL/kg) with matched volumes of placebo** in coronary artery disease (CAD) patients
 - **Study BR1-113:** single-blind, placebo-controlled, randomized, 4-way crossover study to compare the effects on QTc of **0.1 mL/kg IV bolus of SonoVue and placebo** in conjunction with **continuous heart insonation mode at mechanical index (MI) settings of 0.4–0.5 and 1.5–1.6 in CAD patients**
- 0.1 and 0.5 mL/kg correspond to 7 and 35 mL in a 70-kg person, i.e., approx. 3.5 and 17.5 times the recommended dose of SonoVue (2 mL, i.e., approx. 0.03 mL/kg in a 70-kg person)

Effects on Ventricular Repolarization ECG Assessments

- **Continuous 12-lead ECG monitoring** using the Mortara H-12, 12-lead Holter device from 3 hours prior to each dose through 12 hours postdose
- The data collected for each subject were recorded on ‘flash memory’ data cards and forwarded to the **ECG central laboratory** (eResearch Technology Inc., Philadelphia, PA – “eRT”).
- **Manual digitization of 3 beats from Lead II** for the RR, PR, QRS, and QT interval durations **at protocol-specified time points** was performed at eRT using the Sigma Scan digipad system
- Manual measurements were obtained by **trained specialists, board-certified cardiologists**, who were **blinded** to identity of the study agent, verified the **interval duration measurements** and **interpreted each ECG** (presence of pathological U waves, clinically significant T wave changes and postdose arrhythmia included)

Effects on Ventricular Repolarization

Study BR1-112: Design [1]

- Each subject randomized to receive three doses of study agent (SonoVue and placebo) according to one of the following 6 sequences:

Sequence	First Dose	Second Dose	Third Dose
1	SonoVue 0.1 mL/kg	SonoVue 0.5 mL/kg	Placebo
2	SonoVue 0.1 mL/kg	Placebo	SonoVue 0.5 mL/kg
3	SonoVue 0.5 mL/kg	SonoVue 0.1 mL/kg	Placebo
4	SonoVue 0.5 mL/kg	Placebo	SonoVue 0.1 mL/kg
5	Placebo	SonoVue 0.1 mL/kg	SonoVue 0.5 mL/kg
6	Placebo	SonoVue 0.5 mL/kg	SonoVue 0.1 mL/kg

- The volume of placebo (saline) administered was evenly distributed between 0.1 mL/kg and 0.5 mL/kg within each sequence
- There was a 48-hour safety assessment period between each administered dose

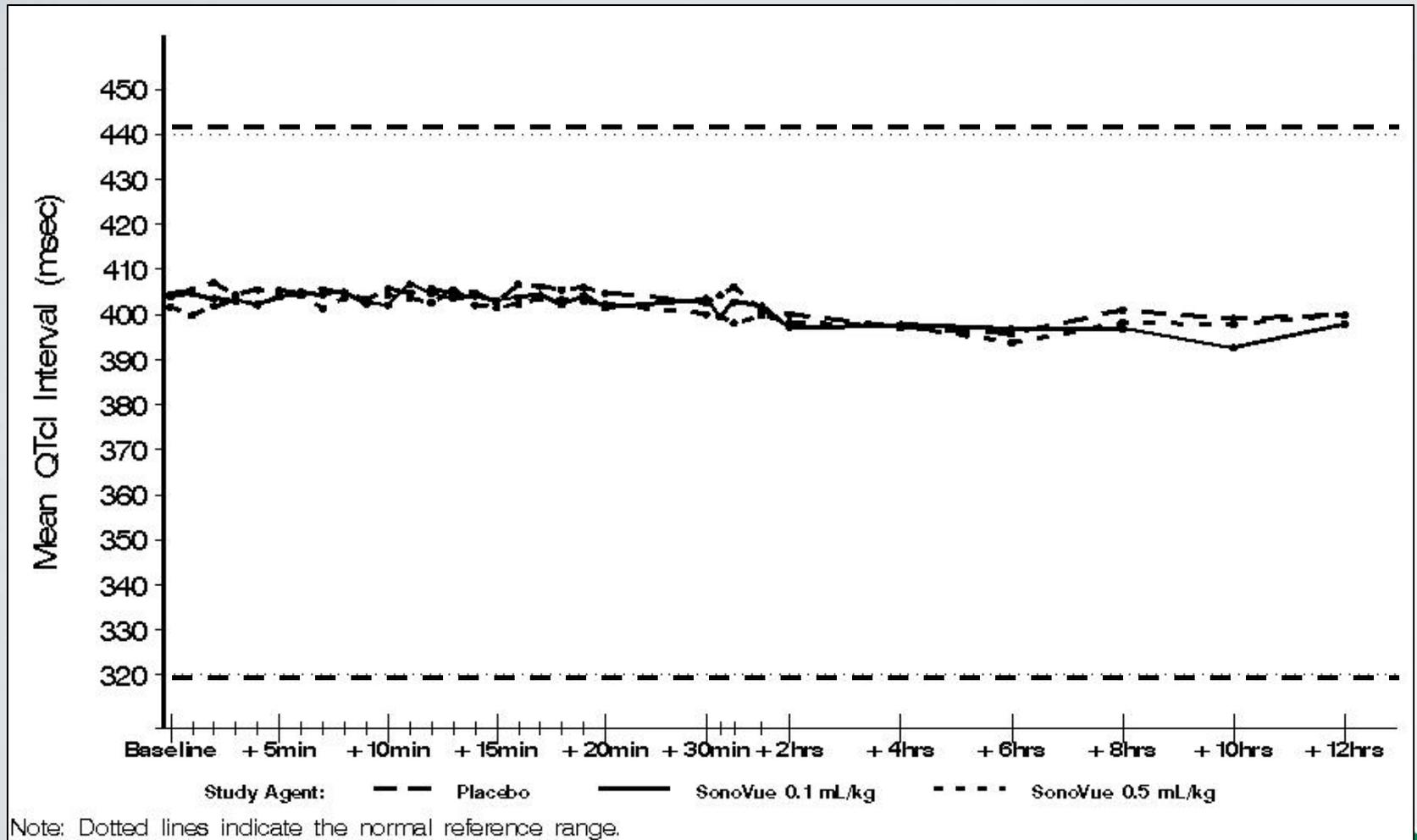
Effects on Ventricular Repolarization – Study BR1-112: Design [2]

Event	Screening	Dosing Periods (Days 1, 3 and 5)					Postdose
	Day -30 to -1	-3 hours	Predose	Postdose	12 hours	24 hours	48 hours
Written Informed Consent	X						
Adverse Events	→	→	→	→	→	→	X
Concomitant Medications	→	→	→	→	→	→	X
Medical History	X						
12-Lead Conventional ECG	X	X					X
12-Lead Holter Monitoring		X	→	→	X		
Physical Examination	X		X			X	X
Vital Signs/Pulse Oximetry	X	X	X	X	X	X	X
Laboratory Evaluation	X	X		X		X	X
Pregnancy Test	X						
Drug and Alcohol Screening	X						
MMSE / Neuro Exam	X		X	X		X	X
B-mode Echocardiography			X				

Effects on Ventricular Repolarization – Study BR1-112: Significant Medical History

Cardiovascular Finding		No. (%) of Subjects (N=49)
<i>Congestive Heart Failure</i>		44 (89.8)
NYHA class:	I	33 (67.3)
	II	11 (22.4)
	III	0
	IV	0
Arrhythmia		2 (4.1)
Mild		2 (4.1)
Moderate		0
Severe		0
Angina		1 (2.0)
At rest		0
During exercise		1 (2.0)
Unstable		0
Hypertension		35 (71.4)
Hypotension		1 (2.0)
Previous Cardiac Surgery		16 (32.7)

Study BR1-112: Corrected QT Interval (QTcI, msec) Mean Values Over Time



Study BR1-112: Study BR1-112: Corrected QT Interval (QTcI, msec) – Changes from Baseline [1]

Time Period / Change Criteria	Placebo	SonoVue 0.1 mL/kg	SonoVue 0.5 mL/kg
	(N=48)	(N=48)	(N=48)
0 min to 20 min postdose			
Increase ≤ 10 msec	9 (18.8)	15 (31.3)	8 (16.7)
Increase > 10 – ≤ 20 msec	24 (50.0)	17 (35.4)	24 (50.0)
Increase > 20 – ≤ 30 msec	9 (18.8)	13 (27.1)	11 (22.9)
Increase > 30 – ≤ 60 msec	4 (8.3)	2 (4.2)	3 (6.3)
Increase > 60 msec	0	0	0
0 min to 1 hr postdose			
Increase ≤ 10 msec	7 (14.6)	11 (22.9)	6 (12.5)
Increase > 10 – ≤ 20 msec	24 (50.0)	20 (41.7)	25 (52.1)
Increase > 20 – ≤ 30 msec	11 (22.9)	13 (27.1)	11 (22.9)
Increase > 30 – ≤ 60 msec	5 (10.4)	3 (6.3)	4 (8.3)
Increase > 60 msec	0	0	0

Effects on Ventricular Repolarization – Study BR1-112: QTcl Interval (msec) – Changes from Baseline [2]

Time Period / Change Criteria	Placebo	SonoVue 0.1 mL/kg	SonoVue 0.5 mL/kg
	(N=48)	(N=48)	(N=48)
0 min to 20 min postdose			
Decrease ≤ 10 msec	18 (37.5)	13 (27.1)	19 (39.6)
Decrease > 10 – ≤ 20 msec	16 (33.3)	21 (43.8)	15 (31.3)
Decrease > 20 – ≤ 30 msec	7 (14.6)	9 (18.8)	6 (12.5)
Decrease > 30 – ≤ 60 msec	2 (4.2)	3 (6.3)	5 (10.4)
Decrease > 60 msec	1 (2.1)	1 (2.1)	0
0 min to 1 hr postdose			
Decrease ≤ 10 msec	15 (31.3)	7 (14.6)	11 (22.9)
Decrease > 10 – ≤ 20 msec	18 (37.5)	24 (50.0)	16 (33.3)
Decrease > 20 – ≤ 30 msec	8 (16.7)	11 (22.9)	11 (22.9)
Decrease > 30 – ≤ 60 msec	3 (6.3)	5 (10.4)	7 (14.6)
Decrease > 60 msec	1 (2.1)	1 (2.1)	0

Effects on Ventricular Repolarization

Study BR1-113: Design [1]

- Each subject received all four treatments according to one of the following four sequences:

Sequence	First Treatment	Second Treatment	Third Treatment	Fourth Treatment
1	SonoVue 0.1 mL/kg MI 0.4	SonoVue 0.1 mL/kg MI 1.5	Placebo 0.1 mL/kg MI 0.4	Placebo 0.1 mL/kg MI 1.5
2	SonoVue 0.1 mL/kg MI 1.5	Placebo 0.1 mL/kg MI 1.5	SonoVue 0.1 mL/kg MI 0.4	Placebo 0.1 mL/kg MI 0.4
3	Placebo 0.1 mL/kg MI 0.4	SonoVue 0.1 mL/kg MI 0.4	Placebo 0.1 mL/kg MI 1.5	SonoVue 0.1 mL/kg MI 1.5
4	Placebo 0.1 mL/kg MI 1.5	Placebo 0.1 mL/kg MI 0.4	SonoVue 0.1 mL/kg MI 1.5	SonoVue 0.1 mL/kg MI 0.4

- There was a 48-hour safety assessment period between each administered dose

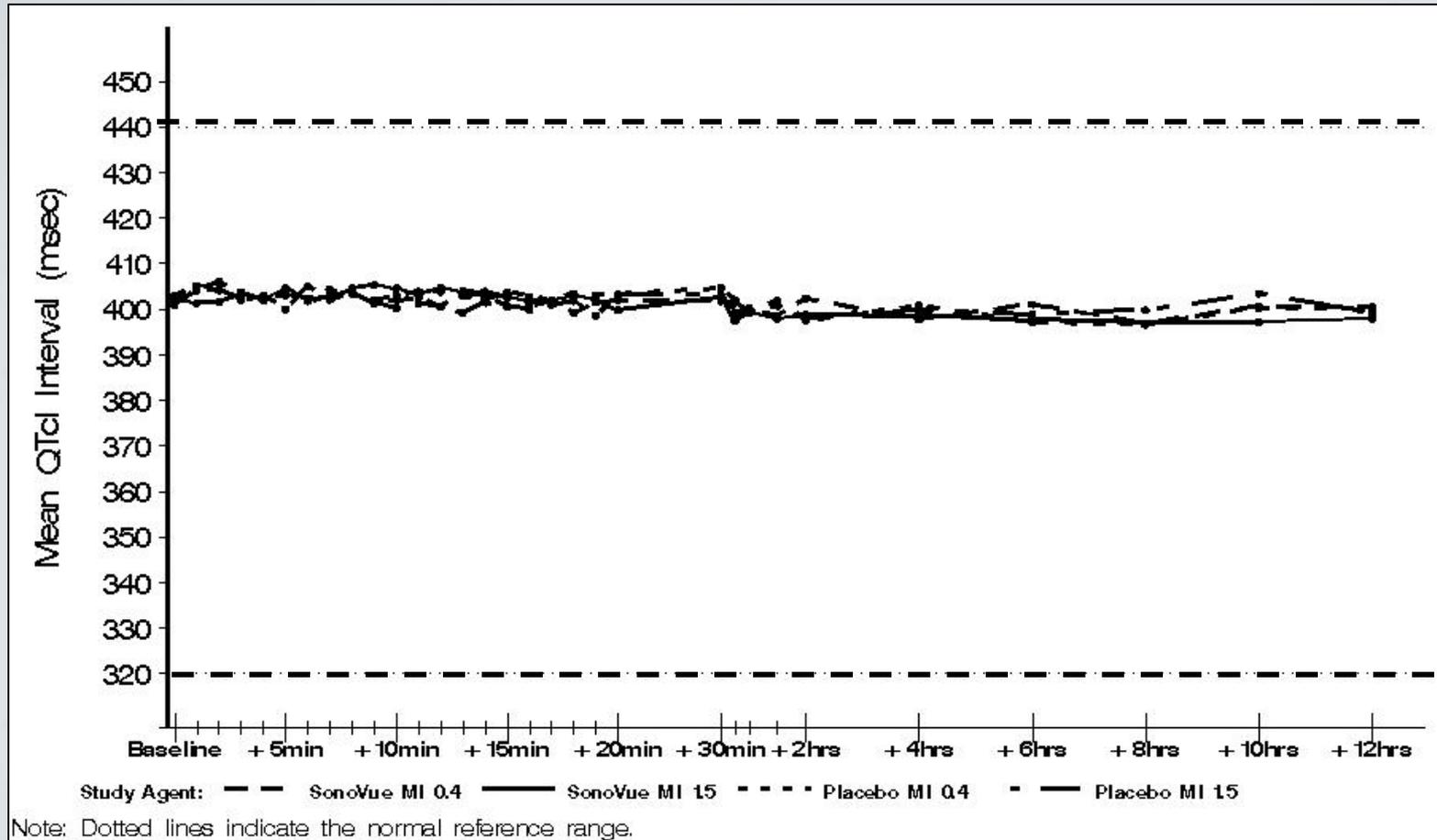
Effects on Ventricular Repolarization Study BR1-113: Design [2]

Event	Screening	Dosing Periods (Days 1, 3, 5 and 7)					Postdose	Follow-up
	Day -30 to -1	-3 hr	Predose	Postdose	12 hr	24 hr	48 hr	72 hr
Written Informed Consent	X							
Adverse Events	→	→	→	→	→	→	→	→
Concomitant Medications	→	→	→	→	→	→	→	
Medical History	X							
Standard 12-Lead ECG	X	X					X	
Continuous 12-Lead ECG		→	→	→	→			
Physical Examination	X		X			X	X	
Vital Signs/Pulse Oximetry	X	X	X	X	X	X	X	
Laboratory Evaluation	X	X		X		X	X	
Pregnancy Test	X							
Drug and Alcohol Screening	X							
MMSE / Neuro Exam	X		X	X		X	X	
B-mode Echocardiography			→	→				

Effects on Ventricular Repolarization – Study BR1-113: Significant Medical History

Cardiovascular Finding		No. (%) of Subjects
		(N=53)
Congestive Heart Failure		52 (98.1)
NYHA class:	I	40 (75.5)
	II	12 (22.6)
	III	0
	IV	0
Arrhythmia		0
Angina ^a		2 (3.8)
	At rest	2 (3.8)
	During exercise	0
	Unstable	0
Hypertension		42 (79.2)
Hypotension		0
Previous Cardiac Surgery		21 (39.6)

Effects on Ventricular Repolarization – Study BR1-113: QTcI Interval (msec) - Mean Values Over Time



Effects on Ventricular Repolarization – Study BR1-113: QTcl Interval (msec) – Changes from Baseline [1]

Time Period / Change Criteria	Placebo MI 0.4	Placebo MI 1.5	SonoVue MI 0.4	SonoVue MI 1.5
	(N=50)	(N=50)	(N=50)	(N=50)
0 min to 20 min postdose				
Increase ≤ 10 msec	12 (24.0)	9 (18.0)	8 (16.0)	11 (22.0)
Increase > 10 – ≤ 20 msec	20 (40.0)	25 (50.0)	24 (48.0)	21 (42.0)
Increase > 20 – ≤ 30 msec	11 (22.0)	11 (22.0)	13 (26.0)	11 (22.0)
Increase > 30 – ≤ 60 msec	6 (12.0)	3 (6.0)	4 (8.0)	4 (8.0)
Increase > 60 msec	0	0	0	0
0 min to 1 hr postdose				
Increase ≤ 10 msec	7 (14.0)	7 (14.0)	7 (14.0)	10 (20.0)
Increase > 10 – ≤ 20 msec	24 (48.0)	25 (50.0)	22 (44.0)	23 (46.0)
Increase > 20 – ≤ 30 msec	12 (24.0)	12 (24.0)	16 (32.0)	11 (22.0)
Increase > 30 – ≤ 60 msec	6 (12.0)	5 (10.0)	5 (10.0)	4 (8.0)
Increase > 60 msec	0	0	0	0

Effects on Ventricular Repolarization – Study BR1-113: QTcl Interval (msec) – Changes from Baseline [2]

Time Period / Change Criteria	Placebo MI 0.4	Placebo MI 1.5	SonoVue MI 0.4	SonoVue MI 1.5
	(N=50)	(N=50)	(N=50)	(N=50)
0 min to 20 min postdose				
Decrease ≤ 10 msec	16 (32.0)	11 (22.0)	18 (36.0)	14 (28.0)
Decrease > 10 – ≤ 20 msec	17 (34.0)	20 (40.0)	16 (32.0)	23 (46.0)
Decrease > 20 – ≤ 30 msec	12 (24.0)	10 (20.0)	11 (22.0)	8 (16.0)
Decrease > 30 – ≤ 60 msec	3 (6.0)	6 (12.0)	1 (2.0)	4 (8.0)
Decrease > 60 msec	0	0	1 (2.0)	0
0 min to 1 hr postdose				
Decrease ≤ 10 msec	12 (24.0)	11 (22.0)	14 (28.0)	10 (20.0)
Decrease > 10 – ≤ 20 msec	18 (36.0)	19 (38.0)	17 (34.0)	22 (44.0)
Decrease > 20 – ≤ 30 msec	15 (30.0)	13 (26.0)	13 (26.0)	13 (26.0)
Decrease > 30 – ≤ 60 msec	4 (8.0)	6 (12.0)	3 (6.0)	5 (10.0)
Decrease > 60 msec	0	0	1 (2.0)	0

Conclusions From Studies BR1-112 and BR1-113

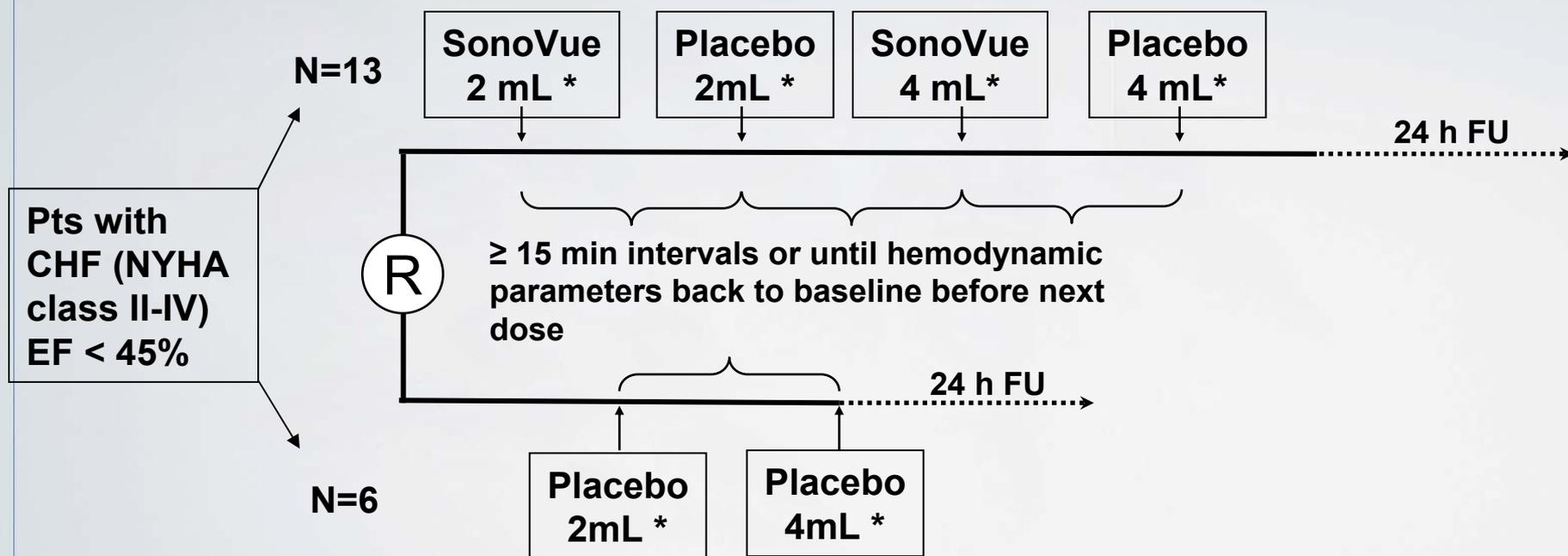
- For all ECG parameters, the magnitude of the changes from baseline was similar for SonoVue and placebo
- SonoVue did not show any significant effects on ventricular repolarization
 - at single, bolus IV doses 3.5-17.5 times higher than the recommended dose for endocardial border delineation
 - at high mechanical index and continuous insonation mode
- In general, no detrimental effects of SonoVue on cardiac electrophysiology were observed

Effects on Pulmonary and Cardiac Hemodynamics

Study BR1-016: Patient Population

- Phase II placebo-controlled, single centre, randomized study
- Eligibility criteria:
 - Pts with congestive heart failure (NYHA class II-III)
 - Ejection Fraction (EF) < 45%
 - Referred for right cardiac catheterization, echocardiography and multi-gated (MUGA) radionuclide angiography

Effects on Pulmonary and Cardiac Hemodynamics Study BR1-016: Design



* Sequence Order Randomized

Effects on Pulmonary and Cardiac Hemodynamics

Study BR1-016: Controls

- Parameters assessed 5,4,3,2,1 min prior to 1st dose and 30 sec, 2,4,6,10 min after dosing or at 3-min intervals until back to baseline values:
 - Right atrial pressure
 - Systolic, diastolic, and mean pulmonary artery pressure
 - Pulmonary capillary wedge pressure
 - Cardiac output
 - Stroke volume
 - Pulmonary and systemic vascular resistance
 - Heart rate and systemic blood pressure (systolic, diastolic, mean)
 - Oxygen saturation

Effects on Pulmonary and Cardiac Hemodynamics

Study BR1-016: Significant Medical History

Cardiovascular Finding	SonoVue + Placebo (N=13) N (%)	Placebo (N=6) N (%)
Previous Myocardial Infarction	7 (53.8)	3 (50.0)
Congestive Heart Failure NYHA Class:	13 (100.0)	6 (100.0)
II	9 (69.2)	3 (50.0)
III	4 (30.8)	3 (50.0)
Arrhythmia	3 (23.1)	3 (50.0)
Angina	3 (23.1)	4 (66.7)
During exercise	3 (23.1)	4 (66.7)
At rest	0	0
Ejection Fraction < 45%	12 (92.3)	6 (100.0)
Systolic PAP >30 mmHg and/or diastolic PAP > 15 mmHg	11 (84.6)	5 (83.3)
Mean PAP ≥ 25 mmHg	8 (61.5)	5 (83.3)

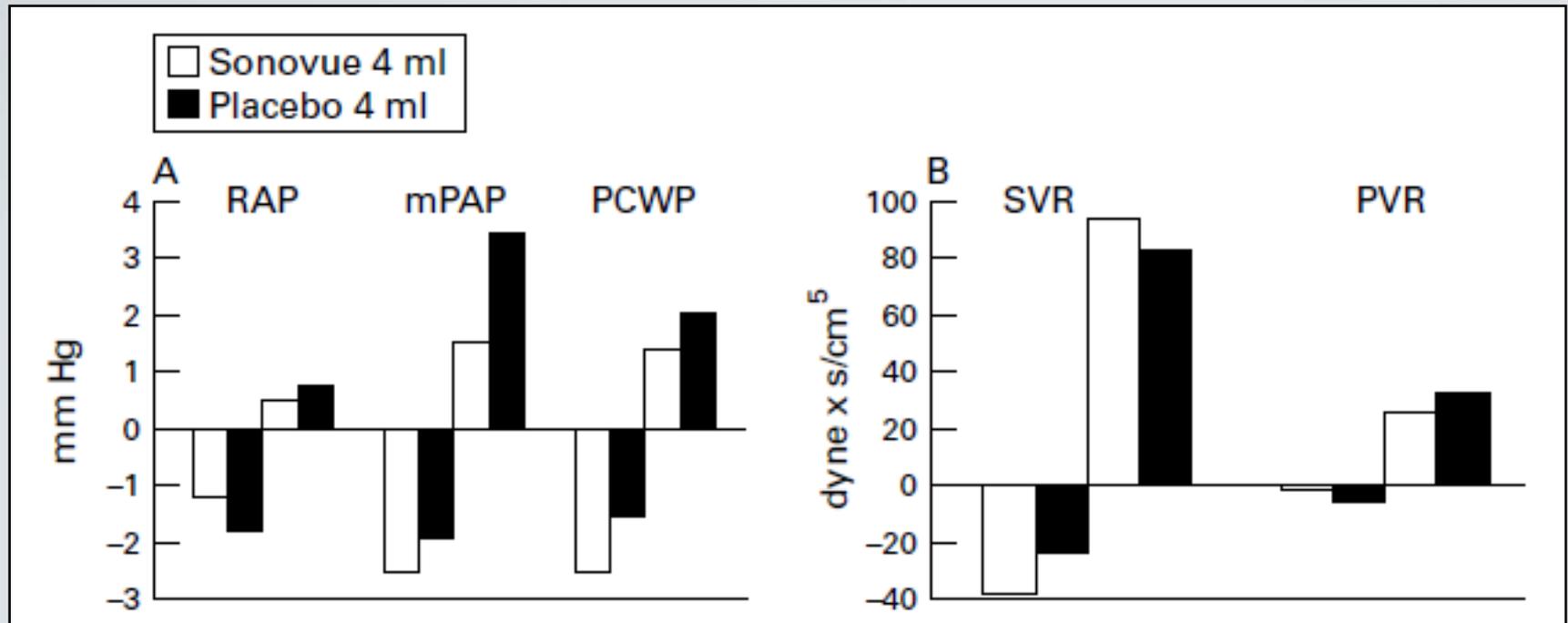
Effects on Pulmonary and Cardiac Hemodynamics

Study BR1-016: Results [1]

- Oxygen saturation within normal in all cases
- No significant changes in cardiac output
- No signs of cardiac function worsening
- **No significant effects on pulmonary hemodynamics after either SonoVue or placebo:**
 - no difference between SonoVue and placebo
 - no difference between the 2-mL and 4-mL SonoVue doses

Effects on Pulmonary and Cardiac Hemodynamics

Study BR1-016: Results [2]



Mean deviation from baseline of the hemodynamic parameters (A) and systemic and pulmonary vascular resistance (B) measured after administration of SonoVue and placebo

RAP: right atrial pressure; mPAP: mean pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; SVR: systemic vascular resistance; PVR: pulmonary vascular resistance

Effects on Pulmonary Hemodynamics: New Study

- In study BR1-016, there were:
 - 11/13 patients with systolic pulmonary arterial pressure (PAP) > 30 mmHg and/or diastolic PAP > 15 mmHg administered with 6 mL SonoVue (predefined study criteria for pulmonary hypertension)
 - 8/13 patients with baseline mean PAP \geq 25 mmHg at rest administered with 6 mL SonoVue
- Bracco agreed with FDA to conduct a new study (BR1-133) to assess the effect of 4.8 mL SonoVue and matched volumes of placebo on pulmonary hemodynamics in approx. 34 patients:
 - undergoing right heart catheterization as part of their clinical care
 - having normal or elevated (at least 15 patients) baseline mean pulmonary arterial pressure (\geq 25 mmHg)
 - randomized to order of administration of SonoVue and placebo

Effects on Pulmonary Hemodynamics in Clinical Trials and Post-Marketing Surveillance

- No spontaneous reports of pulmonary embolism
- No spontaneous reports of any adverse event in patients with pulmonary hypertension
- One case of pulmonary embolism from an ongoing clinical trial in the United States:
 - in-patient with prostate cancer and deep venous thrombosis
 - abdominal pain, shortness of breath and right shoulder pain several hours (at least 10) after 2.4 mL SonoVue
 - bilateral upper lobe pulmonary embolism detected by CT on the following day
 - considered totally unrelated to SonoVue administration by the on-site principal investigator

Effects on Pulmonary Function

Study BR1-022

- Randomized, single-blind, crossover comparison of the effects on respiratory function of 4 mL IV bolus of SonoVue or placebo in patients with moderate-to-severe chronic obstructive pulmonary disease (COPD)
- 12 patients:
 - 6 with moderate COPD and 51% to 69% of predicted forced expiratory volume in 1 second (FEV_1)
 - 6 with severe COPD and 27% to 46% predicted FEV_1
- Controls: serial pre- and post-dose measurements of FEV_1 , forced vital capacity (FVC), forced expiratory flow 25-75% ($FEF_{25-75\%}$), oxygen saturation (+ ECG, vital signs, clinical lab investigations and AE monitoring)
- **Mean and individual changes in FEV_1 , FVC, $FEF_{25-75\%}$ and oxygen saturation superimposable after SonoVue and placebo**
- One case of supraventricular tachycardia after placebo
- No spontaneous or literature reports of worsening of pulmonary function

SonoVue PK, Oxygen Saturation and Safety in Patients with Diffuse Interstitial Lung Disease

- 12 patients with restrictive lung disorders, due to parenchymal lung diseases (autoimmune, occupational, infectious or connective tissue diseases), and reduced gas transfer
- Serial measurements of SF₆ in blood and expired air following IV injection of 0.3 mL/kg SonoVue
- Safety controls: oxygen saturation, vital signs, ECG, clinical lab investigations and AE monitoring
- **No changes or clinically meaningful trends in oxygen saturation or other safety parameters**
- The injected dose of SF₆ was completely recovered in the expired air / Dose adjustments do not appear necessary in patients with reduced gas transfer
- No spontaneous or literature reports of AEs in patients with restrictive lung disorders

Predictable Untoward Side-effects: Conclusions

- Clinical pharmacology studies did not show any significant effect on pulmonary hemodynamics, pulmonary function, blood pressure, oxygen saturation, vital signs, cardiac function, electrocardiographic parameters and laboratory test results even at high doses and continuous insonation at high mechanical index
- Overall safety data from clinical trials, post-marketing surveillance and published papers seem to confirm the absence of predictable untoward side effects of SonoVue on cardiac, systemic and pulmonary hemodynamics, cardiac function and pulmonary function

Safety of SonoVue From Clinical Trial Database

Joint Meeting of the Cardiovascular and Renal Drugs Advisory Committee and the
Drug Safety and Risk Management Advisory Committee 2 May 2011



LIFE FROM INSIDE

Rate of Adverse Events (AEs) From Completed Clinical Trials of Various Ultrasound Contrast Agents

	SonoVue®¹	Definity®²	Optison™³
Patients Dosed	5275	3985	279
% Pts with AEs	10.8%	26%	16.8%
% Pts with treatment related AEs	5.7%	7.6%	Not reported
% Pts with Serious AEs	0.4%	0.85%	0.4%
Most frequently reported AEs	(>0.5%) headache (2.1%) nausea (0.9%) chest pain (0.6%) chest discomfort (0.6%) injection site pain (0.5%)	(>1%) * fatigue, headache, dyspnea, back pain, nausea, flushing, dizziness	headache (5.4%) nausea/vomiting (4.3%) warm sensation or flushing (3.6%) dizziness (2.5%)

1. Completed clinical trials and integrated SonoVue database

2. 2008 Advisory Committee Meeting Briefing Package – Definity® (Perflutren Lipid Microsphere). From FDA website: <http://www.fda.gov/ohrms/dockets/AC/08/briefing/2008-4369b1-04.pdf>. Accessed on: April 26, 2011

3. 2008 Advisory Committee Meeting Briefing Package – Optison™ Perflutren Protein-Type A Microspheres Injectable suspension, USP. From FDA website: www.fda.gov/ohrms/dockets/AC/08/briefing/2008-4369b1-05.pdf. Accessed on: April 26, 2011

* Treatment-related AEs

Serious AEs with Fatal Outcome From Clinical Trials

- 10 deaths were reported from all clinical trials (since clinical development started in September 1993)
- All deaths were considered unrelated to study agent
 - 1 death was reported for a patient who died of acute myocardial infarction before receiving SonoVue
 - 2 patients had procedural complications during PCI hours after a well-tolerated echocardiography with SonoVue
 - 1 patient died 3 days after SonoVue administration and shortly after surgery for right hepatectomy
 - 6 patients died ≥ 10 days after exposure to SonoVue and none of them presented any shorter-term reaction/complication to the administration of SonoVue

Serious AEs From SonoVue Completed and Ongoing Clinical Trials

- Nr. of patients dosed:
 - completed clinical trials: 5275
 - ongoing clinical trials (as of 3/31/2011): 1195
 - total: 6470
- Serious AEs occurred in 33 patients (0.5%)
 - 28 serious AEs (including the 9 deaths) considered unrelated to SonoVue administration
 - 5 serious AEs (0.08%) related to SonoVue administration in the integrated database (relationship “unknown”, “probable” or “definite”)

Serious AEs From Clinical Trials [1]

Case No./ Study No./Site No./Patient No./Indication/ Date of Onset/Country	MedDRA System Organ Class/ Preferred Term	Relation to Investigational Product	Outcome	Narrative
BRO-006589/ BR1-066/14 /1403/ Stress Echo for CAD/ Jul 10, 2003 / France	General Disorders and Administration Site Conditions / Chest Pain	Unknown*	Recovered	The patient experienced severe chest pain at peak dose of dobutamine stress echo with ST elevation , hypotension and dizziness. Treated and transferred to CCU, recovered completely in 10 minutes. The patient had a prior history of anterior myocardial infarction.
	Investigations / Electrocardiogram ST Segment Elevation	Unknown*	Recovered	
	Vascular Disorders/ Hypotension	Unknown*	Recovered	
* Relationship 'unknown' in clinical trial database, however, subsequent information received from the Investigator indicated that events were clearly related to ischemia triggered by dobutamine.				

Serious AEs From Clinical Trials [2]

Case No./ Study No./Site No./Patient No./Indication/ Date of Onset/Country	MedDRA System Organ Class/ Preferred Term	Relation to Investigational Product	Outocme	Narrative
BRO-002936/ BBG-001/15/ 1504/ Echo for Determination of CAD/ Jan 25, 2000/ France	Skin and Subcutaneous Tissue Disorders / Rash Nervous System Disorders / Syncope Vasovagal	Probable Probable*	Recovered Recovered	Severe rash approximately 1 minute after 2 mL SonoVue. Patient received dexchlorpheniramine and 2 min later methylprednisolone. Immediately after methylprednisolone (and about 10 min after SonoVue), the patient developed a severe vagal reaction with nausea, syncope and complete atrioventricular block , which resolved immediately after atropine.

* Relationship 'probable' in clinical trial database, however, subsequent information received from the Investigator indicated that **the vasovagal event was not directly related to SonoVue administration**

Serious AEs From Clinical Trials [3]

Case No./ Study No./ Site No./Patient No./Indication/ Date of Onset/Country	MedDRA System Organ Class/ Preferred Term	Relation to Investigational Product	Outcome	Narrative
BRO-000044/ BR1-014 / 09/0317/ Doppler for Cerebral Artery Abnormality/ Jul 1, 1997 / Switzerland	Nervous System Disorders / Paresis	Unknown	Recovered	The patient with occlusion of the left internal cerebral artery. 20 hrs after last SonoVue administration the patient experienced sensory motor paresis of right arm, which was treated, and resolved. This patient had been hospitalized 9 days prior to SonoVue administration for a left abducens nerve paresis.

Serious AEs From Clinical Trials [4]

Case No./ Study No./ Site No./Patient No./Indication/ Date of Onset/Country	MedDRA System Organ Class/ Preferred Term	Relation to Investigational Product	Outcome	Narrative
BRO-011949 / BRA013 / 01/0107/ Echo for Endocardial Border Delineation / Aug 9, 2007/ Germany	Immune System Disorders/ Anaphylactic shock	Definite	Recovered	About 1 to 2 minutes after the intravenous injection of 2 mL of SonoVue during a rest examination, the patient experienced anaphylactic shock, which consisted of heat sensation, asystole, loss of consciousness and hypotension. The patient was treated with atropine, adrenaline, corticosteroids, fluids, dimethindene maleate, and ranitidine. The patient was hospitalized in the ICU for further observation. Patient recovered in 30 minutes. The patient had no history of allergy.

Serious AEs From Clinical Trials [5]

Case No./ Study No./ Site No./Patient No./Indication/ Date of Onset/Country	MedDRA System Organ Class/ Preferred Term	Relation to Investigational Product	Outcome	Narrative
DE-000297 / BR1-125 / 11/1101/ Myocardial Perfusion Echo /Jan 14, 2009 / Germany	Cardiac Disorders / Asystole	Probable	Recovered	The patient received 6 mL SonoVue and underwent echocardiography. Once the procedure was completed, 50 mg of dipyridamole was infused in 4 minutes time, immediately followed by 3 mL of SonoVue for the stress portion of the echocardiography. The patient was reported to have increased heart rate followed by extrasystole, bradycardia, and short term asystole (duration of 30 seconds). The reaction resolved approximately 1 hour after the patient was treated with atropine, methylprednisolone, ranitidine hydrochloride, dimethindene maleate, and hydroxyethyl starch solution.

New Safety Clinical Trial Retrospective Cohort Study [1]

- **Purpose:** To compare in-hospital mortality (same day of echo procedure and/or the following calendar day) of critically ill patients undergoing contrast echocardiography with and without SonoVue
- **Study Design:** Retrospective observational study
- **Patient Population:**
 - Defined as critically ill according to a list of unstable cardiopulmonary conditions
 - Underwent echocardiography within 7 days from admission to hospital for the unstable cardiopulmonary condition
 - Source records available for patients from European centers

New Safety Clinical Trial Retrospective Cohort Study [2]

- ***Analysis Population:***
 - SonoVue Group:
 - Hospitalized patients from Europe from September 2001 to May 2010
 - Control Group Pool:
 - Hospitalized patients from Europe from September 2001 to May 2010
 - Hospitalized patients from Premier Perspectives Database
 - Propensity Score Match of Control Group Pool with individual SonoVue patients included in final analysis

Protocol currently under FDA review

Safety of SonoVue From Post-Marketing Surveillance Database

Joint Meeting of the Cardiovascular and Renal Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee 2 May 2011

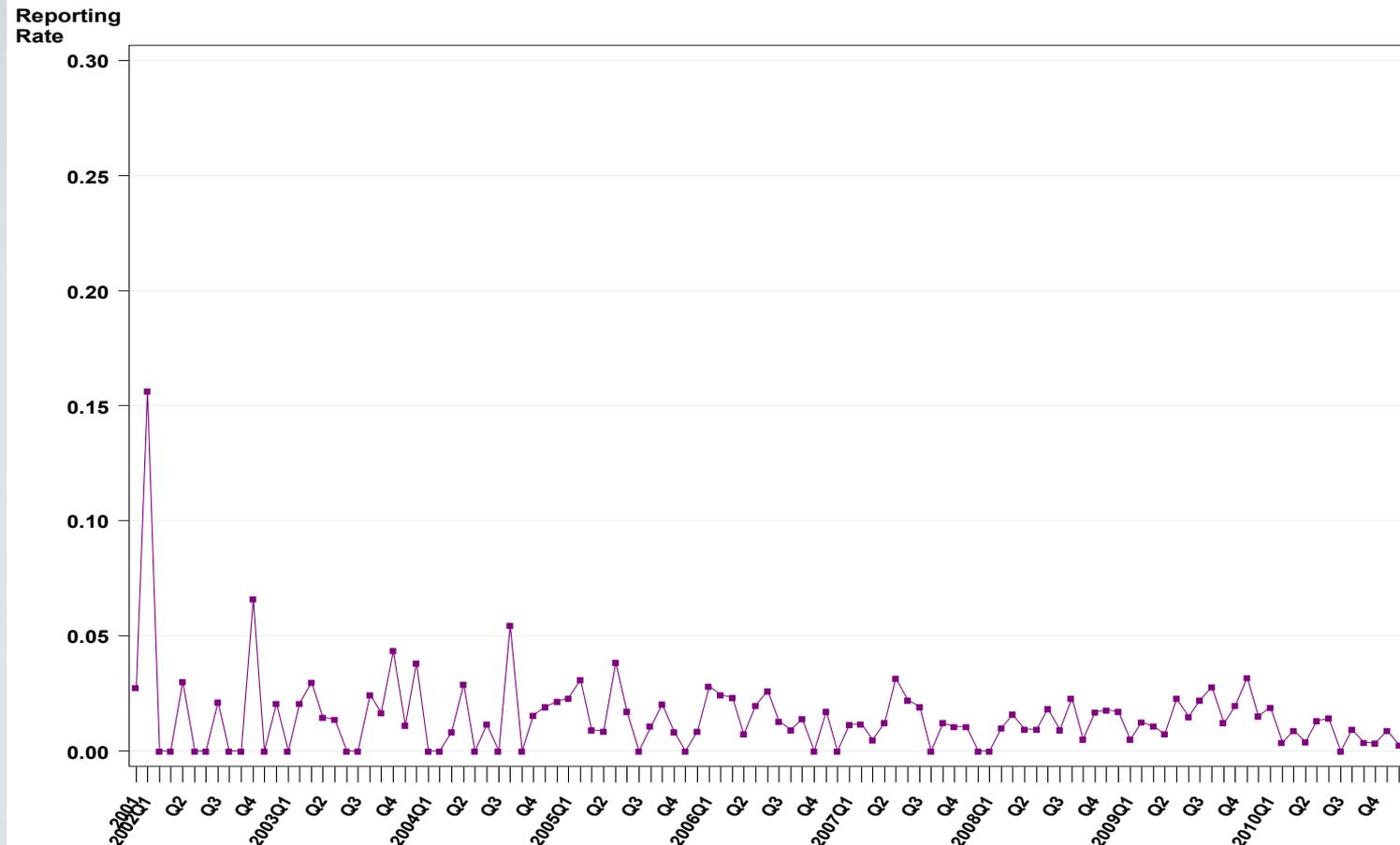


LIFE FROM INSIDE

Reporting Rate of Serious AEs

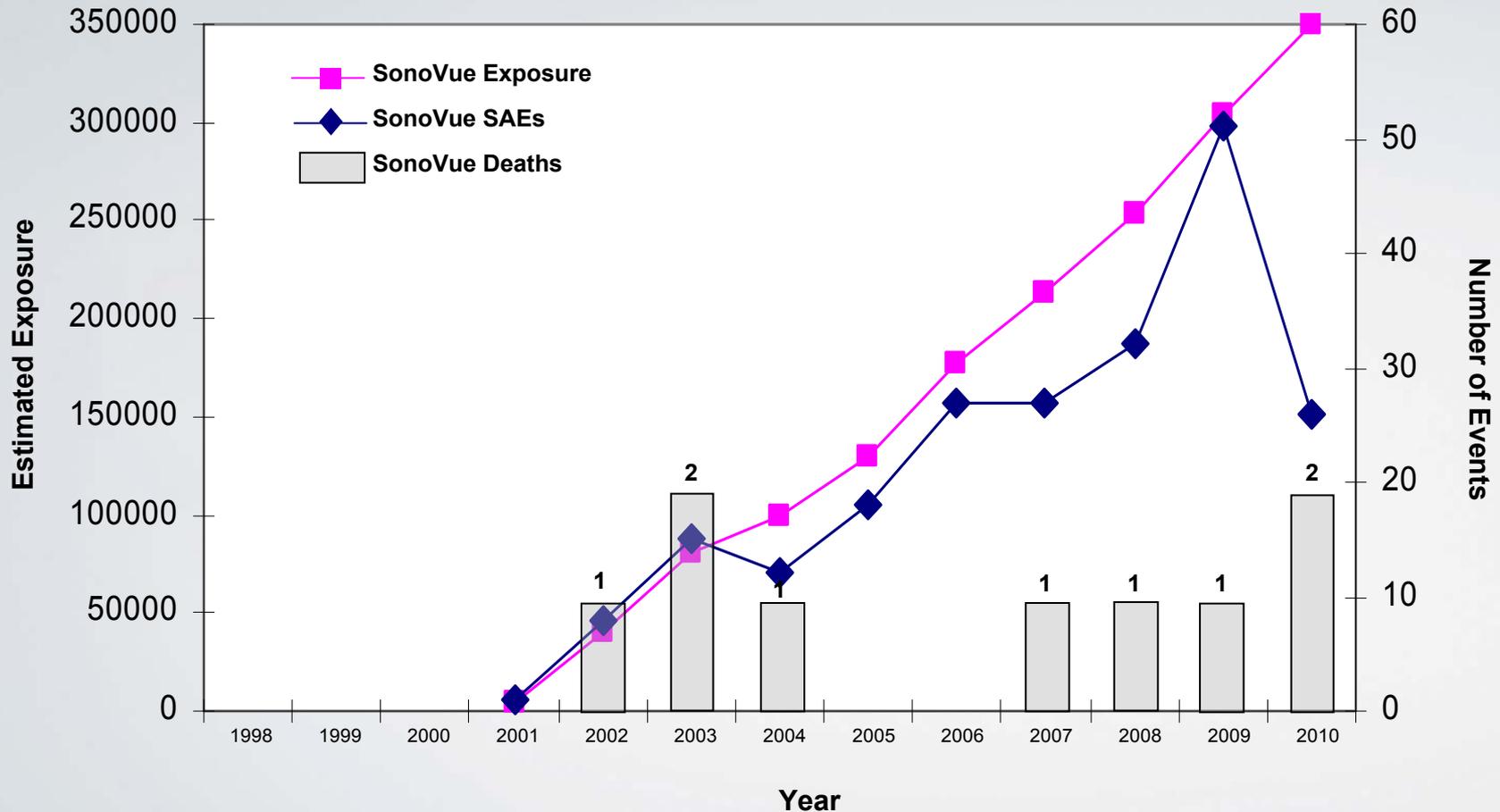
- 1,651,451 estimated patients exposed to SonoVue since its initial approval until December 31, 2010
- 217 cases of serious AEs (reporting rate: 0.013% or approx. 1:10,000)
 - 4 cases clearly unrelated to SonoVue
 - 213 patients – some kind of relationship with SonoVue administration
 - 162 cases (0.0098%) were hypersensitivity reactions

Post-Marketing Safety Surveillance: Incidence of Serious AEs by Quarter*



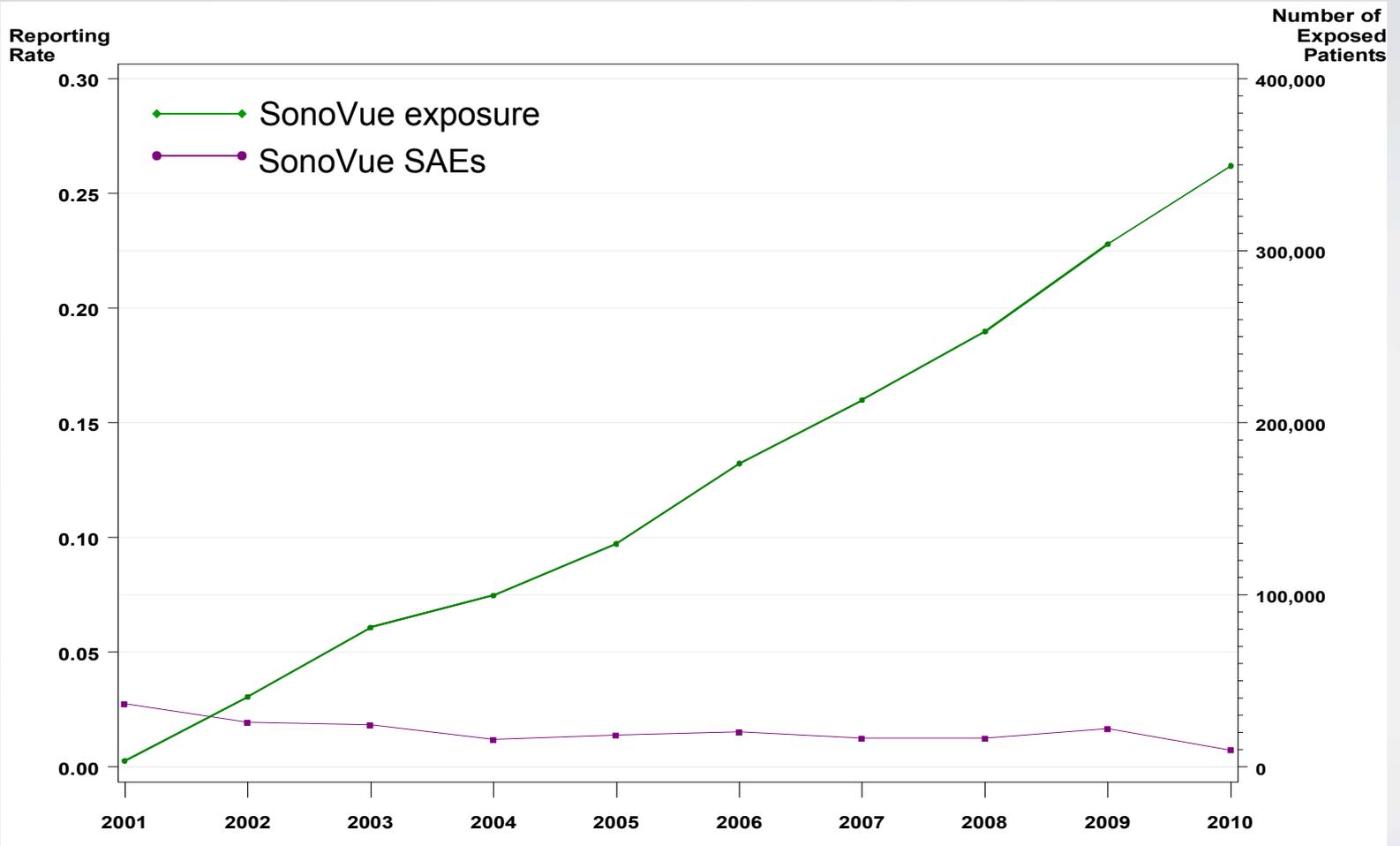
* From launch (April 1, 2001) until December 31, 2010

Post-Marketing Safety Surveillance: Serious AEs with estimated exposure to SonoVue*



* From launch (April 1, 2001) until December 31, 2010

Post-Marketing Safety Surveillance: Serious AEs with estimated exposure to SonoVue*



* From launch (April 1, 2001) until December 31, 2010

Comparison of Safety Data from Post-Marketing Surveillance for US Contrast Agents

	SonoVue[®] 1	Definity[®] 2	Optison[™] 3
Timeframe	Apr 2001 to Dec 2010	Oct 2001 to Dec 2007	1998 to 2008
Estimated Exposure	1,651,451	≈ 2million	> 1 million
Serious AEs	217 (0.013%)	277 (0.014%)	12 (< 0.001%)
Fatal cases	9	14	1

1. Bracco post-marketing surveillance database.
2. 2008 Advisory Committee Meeting Briefing Package – Definity® (Perflutren Lipid Microsphere). From FDA website: <http://www.fda.gov/ohrms/dockets/AC/08/briefing/2008-4369b1-04.pdf>. Accessed on: April 26, 2011
3. 2008 Advisory Committee Meeting Briefing Package – Optison™ Perflutren Protein-Type A Microspheres Injectable suspension, USP. From FDA website: www.fda.gov/ohrms/dockets/AC/08/briefing/2008-4369b1-05.pdf. Accessed on: April 26, 2011

Comparison of Safety Data from Post-Marketing Surveillance for US Contrast Agents

	SonoVue[®] 1	Definity[®] 2	Optison[™] 3
Timeframe	2008-2010	2008-2010	2008-2010
Estimated Exposure	906,634	1,083,000	55,000
Serious AEs	109 (0.012%)	169 (0.016%)	6 (0.011%)
Fatal cases	4 (0.0004%) *	10 (0.0009%) *	0

1. Bracco post-marketing surveillance database.

2. 2011 Advisory Committee Meeting Briefing Package – Definity[®] (Perflutren Lipid Microsphere).

3. 2011 Advisory Committee Meeting Briefing Package – Optison[™] Perflutren Protein-Type A Microspheres Injectable suspension, USP.

* As of 8 March 2011

Fatal Cases From Spontaneous Reporting*

- 9 deaths (0.0005%, i.e., 1:184,000) occurred during spontaneous reporting since initial approval of SonoVue
 - 3 cases considered unrelated by the reporter
 - 3 cases still unassessable
 - 3 cases for which a causal role played by SonoVue cannot be excluded
- As for cases with fatal outcome observed with X-ray, MRI and other ultrasound contrast agents, the poor underlying clinical conditions of the patients may have played the major role in the outcome of all these cases

* From launch (April 1, 2001) until December 31, 2010

SonoVue Post-Marketing Safety Surveillance: Conclusions

- When compared to other intravascular imaging agents:
 - The observed pattern of serious AEs is similar, especially for serious hypersensitivity reactions
 - The underlying disease/condition plays an important role in the severity and outcome of most serious AEs
- No major trends or significant changes in the reporting rate of serious AEs have been observed over time

Safety of SonoVue From Published Papers

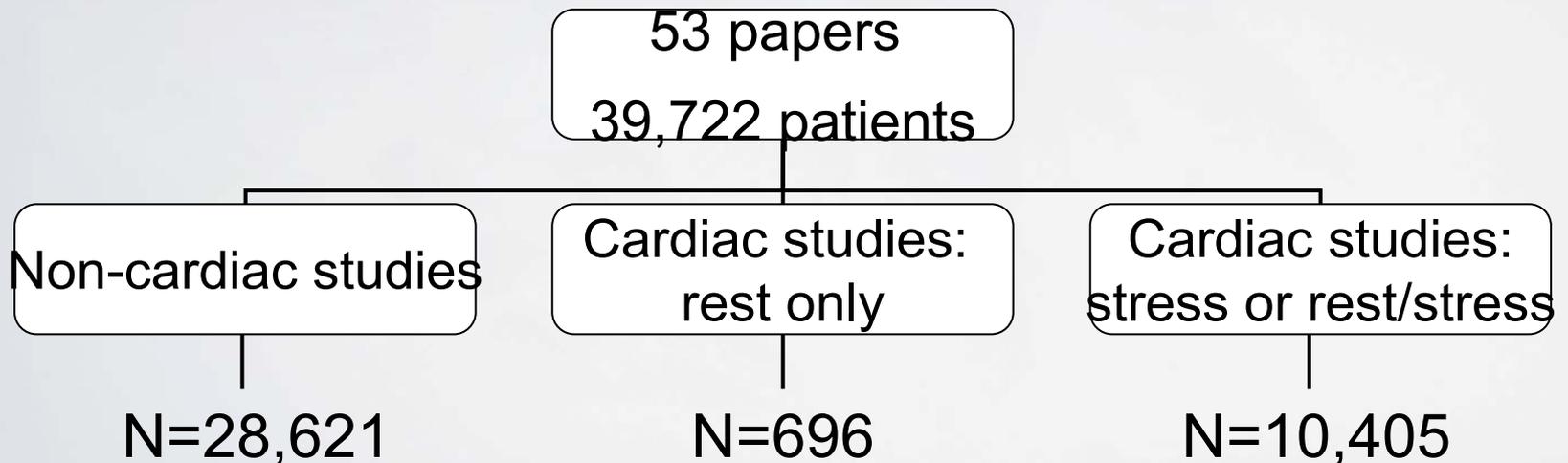
Joint Meeting of the Cardiovascular and Renal Drugs Advisory Committee and the
Drug Safety and Risk Management Advisory Committee 2 May 2011



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Literature Search

- Systematic and extensive search of large literature databases with predefined search strategy
- Time period: 1990-2010



Literature Review:

Safety of SonoVue in Abdominal Applications*

- *Purpose:* To assess the incidence of AEs after SonoVue administration when used in abdominal U/S examinations
- *Study Design and Patient Population:*
 - Independent, retrospective, multi-center study
 - 23,188 abdominal CEUS investigations from registries available at 28 centers
 - Patients from sponsored clinical trials excluded
- *Results:*
 - 29 adverse events reported (0.125%)
 - 27 non serious AEs (intensity: 23 mild, 3 moderate, 1 severe)
 - 2 serious AEs (hypersensitivity reactions, 0.0086%)
 - No fatal events occurred

* Piscaglia F, et al. *Ultrasound Med Biol* 2006;32(9):1369-1375

Literature Review:

Stress Echo w/ and w/o Contrast (SonoVue)*

- *Purpose:* To compare the safety of stress-echocardiography with and without contrast in patients with recent (less than 5 days before) chest pain and suspected cardiac ischemia
- *Study Design and Patient Population:*
 - Independent, retrospective, single-center study
 - 500 consecutive pts with recent chest pain undergoing SonoVue-enhanced stress-echo exams (5mL of SonoVue at 0.8-1.0 mL/min)
 - Historical group of 500 pts who underwent stress echo w/o contrast
- *Results:*
 - No death, myocardial ischemia, sustained arrhythmias or other life-threatening events
 - No allergic reactions
 - AEs were not significantly different between SonoVue and control group

* Gaibazzi N, et al. *Eur J Echocardiogr* 2009;10(6):726-732

Literature Review: Stress echo w/ and w/o Contrast (SonoVue or Optison)*

- *Purpose:* to compare safety of stress echo procedures with and without contrast
- *Design:* Independent retrospective analysis of 751 consecutive stress echo procedures
 - **419 w/ contrast**
 - 299 w/ SonoVue (infusion at 0.7 mL/min; max dose of 10 mL)
 - 120 w/ Optison (0.3 mL IV boluses; max dose of 3 mL)
 - **332 w/o contrast**
- *Results:*
 - No deaths, MI, allergic reactions
 - No differences in frequency, type, and seriousness of AEs in the 3 groups, with exception of premature atrial contractions (higher incidence in Optison group)

* Timperley J, et al. *J Am Soc Echocardiogr* 2005;18(2):163-167

24-hour Mortality Rate in Critically ill Patients

Article	Patient Population	No. of Patients	No. of Patients with Fatal Outcomes Within 24 Hours
SonoVue Group			
Agati et al, 2002	CAD patients with AMI	23	0
Galiuto et al, 2006	Patients within 12 hr onset of STEMI referred for PCI	50	0
Galiuto et al, 2008	Patients with AMI undergoing PCI	110	0
Guiducci et al, 2005	Patients with anterior AMI undergoing PCI or thrombolysis	62	0
Korosoglou et al, 2004	Patients with first occurrence of chest pain	100	0
Serra et al, 2005	Patients with AMI undergoing PCI	49	0
Soman et al, 2000	Patients with severe left ventricular dysfunction and congestive heart failure	13	0
Streb et al, 2008	Patients with first MI undergoing angioplasty	39	0
Winter et al, 2005	Patients with acute coronary syndrome	36	0
Wita et al, 2009	Patients with first AMI treated with PCI with and without thromboaspiration	46	0
Subtotal		528	0
Control Group			
Exuzides et al, 2009	Critically ill patients	11,600	129
Subtotal		11,600	129

Overall Summary [1]

- In clinical trials:
 - No significant effect on pulmonary hemodynamics, pulmonary function, blood pressure, oxygen saturation, vital signs, cardiac function, electrocardiographic parameters and laboratory test results were observed
 - The overall incidence of AEs and the type of most frequent AEs is similar among SonoVue, Optison and Definity

Overall Summary [2]

- Experience from post-marketing surveillance showed that:
 - The reporting rate of serious AEs is approx. 1:10,000 exposures
 - Serious hypersensitivity reactions were observed in fewer than 1:10,000 exposures
 - No major trends or significant changes in the reporting rate of serious AEs have been observed over time

Overall Summary [3]

- Peer-reviewed literature showed:
 - No significant differences in safety between SonoVue and controls
 - No increase in 24-hour mortality following the use of SonoVue in critically ill patients