Joint Advisory Committee Meeting
24 June 2008

OPTISON™

Perflutren Protein-Type A Microspheres
Injectable suspension, USP

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

2D	Two-dimensional
ADE	Adverse device event
ADME	Absorption, Distribution, Metabolism and Excretion
AE	Adverse event
ALARA	As low as reasonably achievable
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ASE	American Society of Echocardiography
BP	Blood pressure
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CI	Confidence interval
c-IMT	Carotid intima media thickening
CT-Angio	Computerized tomography angiography
D	Diameter
DBP	Diastolic blood pressure
DTAF	Dichlorotriazinylaminofluorscein
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FITC	Fluorescein isothiocyanate
FPIA	Flow particle image analysis
FS069	Development name for OPTISON
GCP	Good Clinical Practice
GPV	Global Pharmacovigilence
HA	Human albumin
HB	Heart block
HE	Hematoxylin and eosin
HED	Human equivalent dose
HPS	Hepatopulmonary Syndrome
HR	Heart rate
HSA	Human serum albumin
IDE	Investigational Device Exemption
IRB	Investigational Review Board
LDH	Lactate dehydrogenase
LVH	Left ventricular hypertrophy
LVO	Left ventricular opacification

MAA	Macro aggregated albumin
MBI	Molecular Biosystems, Inc.
MI	Myocardial infarction
MRA	Magnetic resonance angiography
NDA	New Drug Application
NOAEL	No adverse effects level
NSAID	Non-steroidal anti-inflammatory drug
O_2	Oxygen
OFP	Octafluoropropane
OSA	Obstructive sleep apnea
PAC	Premature atrial contraction
PAF	Platelet aggravation factor
PF	Perflutren
PFP	Perfluoropropane
PK	Pharmacokinetic
PMA	Pre-market Approval
RBC	Red blood cell
SAE	Serious adverse event
SHAPE	Screening for Heart Attack Prevention and Education
TIA	Transient ischemic attack
TNF-α	Tumor necrosis factor-alpha
ULN	Upper limit of normal
US	United States
USCA	Ultrasound contrasting agent
USP	United States Pharmacopeia

1 BACKGROUND OVERVIEW

1.1 Introduction

This Briefing Package has been prepared for the joint Cardio-Renal/Drug Safety & Risk Management Advisory Committee and invited Special Government Employees to be held 24 June 2008 at the request of the Agency. In April and May of 2008 teleconferences were held between members of the Division of Medical Imaging and sponsors of microbubble ultrasound contrast agents to discuss the agency's plans to hold an advisory committee meeting to discuss these agents. It was noted by the Agency that the focus of the meeting would be a review and understanding of the experience with microbubble contrast agents to learn how to better develop new agents and new indications. It was emphasized that this would be a discussion meeting, not a decision meeting, to obtain advice from the advisory committee in response to questions from the Division of Medical Imaging and Hematology Drug Products with the goal being the understanding of how the Agency can assure safe development of these products.

The request for the advisory committee meeting was follow-up to a decision by the Agency to require that the package inserts for all microsphere contrast agents include a boxed warning and other language about the possibility of serious cardiopulmonary reactions. This action was based upon 4 fatal cardiac arrests and other non-fatal adverse events (AEs) uncommonly reported with these agents. In response to discussions with echocardiographers and sponsors of these agents, the Agency has since decided to modify this language; however, a boxed warning continues to be required. A key feature of this warning is a requirement for monitoring vital signs, electrocardiogram (ECG), and cutaneous oxygen saturation (O_2) for 30 minutes following administration in all subjects with pulmonary hypertension or unstable cardiopulmonary conditions. In addition to these class labeling requirements, the Agency has requested commitments from sponsors for comprehensive risk management plans composed of clinical studies and safety monitoring boards. The Agency has requested this Advisory Committee Meeting in the light of their safety concerns with these agents and a desire to better understand how they can be developed safely for new indications.

The Division requested a background package for and presentation to the Advisory Committee of the experience with OPTISON including a summary of chemistry, manufacturing and controls, preclinical and clinical information provided in the original New Drug Application (NDA); experiences from post-marketing surveillance; and a review of future indications for use including animal models for use in studying these agents. For future indications, GE Healthcare has focused on vascular imaging and the safety parameters to be assessed in future development and clinical trials. As agreed with the Division of Medical Imaging, other clinical areas of development will be addressed by other companies providing briefing packages and presentations at the meeting.

This briefing document presents information and data on the safety of OPTISON based on its current approved indication: for use in subjects with suboptimal echocardiograms to opacify the left ventricle and to improve the delineation of the left ventricular endocardial borders. Efficacy for this indication is not addressed in this document because efficacy has been established by agency approval. The section of this document on the development of future indications for vascular imaging focuses on safety parameters for investigational development; however, some efficacy and safety data are included from the literature and other sources to provide an estimated benefit/risk evaluation.

1.2 Regulatory History

OPTISON was considered, by the original Sponsor, Molecular Biosystems, Inc. (MBI), as a modification to the approved product, Albunex. Albunex was approved under a PMA application as a Class 3 medical device. Albunex contained microspheres filled with air and OPTISON was developed as a modified product containing perfluoropropane (PFP) gas.

OPTISON was filed in October 1996 as an amendment to the existing Albunex PMA, however, during the review process, CDRH decided that the modified product should be considered a new PMA and a new number was assigned.

During the OPTISON review, jurisdictional issues arose over microbubble products and a Citizen's Petition was filed requesting a determination of whether these products were to be considered medical devices or drug products. By September 1997, the FDA, in response to the Citizen's Petition, reclassified all microbubble products as drug products. As a result of this action, the file was transferred to the Center for Drug Evaluation and Research (CDER) and NDA number 20-899 was assigned. CDER continued the review process and approved the new product on 31 December 1997.

OPTISON was marketed by Mallinckrodt Inc. (which had purchased the product and its NDA from MBI) until December 2002 when the product was sold and the NDA transferred to GE Healthcare (formerly Amersham Healthcare). OPTISON remained on the market until November 2005 when it was voluntarily withdrawn as a result of certain observations documented during a Food and Drug Administration (FDA) inspection of the contract manufacturer. OPTISON returned to the marketplace 31 October 2007.

1.3 Basic Concepts of Ultrasound Contrast Agents

1.3.1 Principle

The concept of contrast for ultrasound imaging was discovered by [Gramiak and Shah 1968] who observed enhancement of echo during intra-cardial injections of indocyanide green, saline and dextrose. [Kremkau 1970] later confirmed that the observed contrast enhancement was due to formation of small gas bubbles during rapid injection of solutions. This is the principal

physical contrast creating mechanism of all ultrasound contrast agents (USCAs); the scattering of ultrasound from small envelopes of gas as they undergo volume oscillations in the sound beam. Due to the high compressibility of gases and their ability to resonate when insonated, a population of microspheres is very effective in scattering incident ultrasound compared to surrounding blood or tissue. During ultrasound scanning, body cavities and compartments containing microspheres will appear white compared to regions without contrast agent.

1.3.2 Effects of size

The main drive for the development of a safe and efficacious compound has been to produce stable microspheres of a pre-defined, biologically acceptable size, which will pass through capillary beds and thus allow for imaging throughout the cardiovascular system. The average diameter of the lung capillaries has been reported to be approximately 7 μm with approximately 95% being equal or larger than 4 μm [Hogg 1987]. To optimize for free flow and avoid potential capillary embolism, the size of microspheres in USCAs should preferably be smaller than 4 μm.

Another important aspect of microsphere size is its influence on product efficacy; the ability of any given microsphere to scatter ultrasound is strongly dependent on the diameter (D). For a typical microsphere the scattering efficacy per bubble volume (at 2 MHz) is a monotonically increasing function of size scaling as D^3 up to some 7 μm before dropping sharply scaling as D^{-2} [Sontum et al. 1999 and Sontum 2008]. Unfortunately these 2 effects (potential capillary retention and product efficacy) pull the optimal size in different directions. The bigger the microspheres, the more efficacious the product, but the greater the potential for capillary retention.

1.3.3 Effects of gas and shell type

Various formulation concepts have been explored in developing an USCA with suitable properties. Early products investigated the stabilization of air bubbles, either by release *in vivo* from micro-porous crystalline sugar structures as with Levovist® (Schering AG) or by stabilizing individual envelopes of air by a shell of denatured human serum albumin as with Albunex® (Mallinckrodt Inc.). Both concepts were successful in bringing microspheres through the capillary bed of the lungs, producing contrast in the bulk volume of the left heart chamber after an intravenous (*i.v.*) injection. Their stability *in vivo* (and hence the contrast persistency) was insufficient and their clinical utility limited. A primary cause for the poor stability of air-based USCAs is that the small molecules of the air easily diffuse across the stabilizing structure. As air is relatively soluble in the surrounding matrix (ie, blood), the microspheres dissolve too quickly after injection. To improve the stability of the contrast agents, a concept of encapsulating air with a more solid, polymer based, shell was investigated [Bjerknes et al. 1997]. In this case, however, the stiffness of the shell lowered the physical, *in vitro* acoustic efficacy of the compound to such a degree that the substance did not deliver the necessary imaging quality [Hoff et al. 2000]. An alternative approach for increasing the

stability of the contrast agent is based on the type of gas used. The life span of a non-stabilized bubble in a liquid matrix is proportional to the density, and inversely proportional to the solubility/diffusivity, of the gas in question. Using a denser, more slowly diffusing, low solubility gas increases the stability of microspheres, without the need for a rigid stabilizing structure [Dugstad et al. 1996]. Gases such as sulphur-hexafluoride or low molecular weight perfluoro-carbons in combination with various flexible stabilizing structures are now used in several (2^{nd} generation) products. The main components of 5 USCA`s are listed in Table 1.

Table 1: Principal Components of 5 Contrast Agents for Ultrasound Imaging

Product	Shell Material	Encapsulated Gas
Albunex®	Protein	Air
OPTISON	Protein	Octafluoropropane
Sonovue®	Lipids	Sulphur-hexafluoride
Definity®	Lipids	Octafluoropropane
Sonazoid™	Lipids	Perfluorobutane

Note: Only OPTISON and Definity® are available in the US market.

As apparent from the discussion above, for USCAs the *physical state* of the active ingredient may be more important to product performance than its mass content or chemical structure. In this context it may be more correct to refer to a contrast enhancing *system* rather than a *substance* (i.e., gas or shell) since the *in vivo* performance is related to a number of parameters of the formulation as a *physical entity*.

1.4 References

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2 CHEMISTRY, MANUFACTURING AND CONTROLS

2.1 Production and Structure

OPTISON is a diagnostic agent for the enhancement of contrast effect in echocardiography investigations (NDA 20-899). The active (gas) substance is Perflutren (octafluoropropane, OFP) in the form of microspheres stabilized by a thin shell of heat-modified human albumin, suspended in 1% human albumin solution. OPTISON is produced aseptically by colloid mill homogenization of OFP into a 1% albumin solution heated to approximately 70°C. During this homogenization small bubbles of OFP are formed around which albumin molecules denature to form a non-soluble shell approximately 15-nm thick. The observed shell thickness is consistent with a monomolecular layer of albumin packed with the long axis of the ellipsoid molecule perpendicular to the shell surface. The structure of the OPTISON microsphere is visualized in Figure 1 and a micrograph of reconstituted product is shown in Figure 2.

Figure 1 Structure of the OPTISON Microsphere: Octafluoropropane Encapsulated by an Approximately 15-nm Thick Shell of Heat Denatured Human Albumin

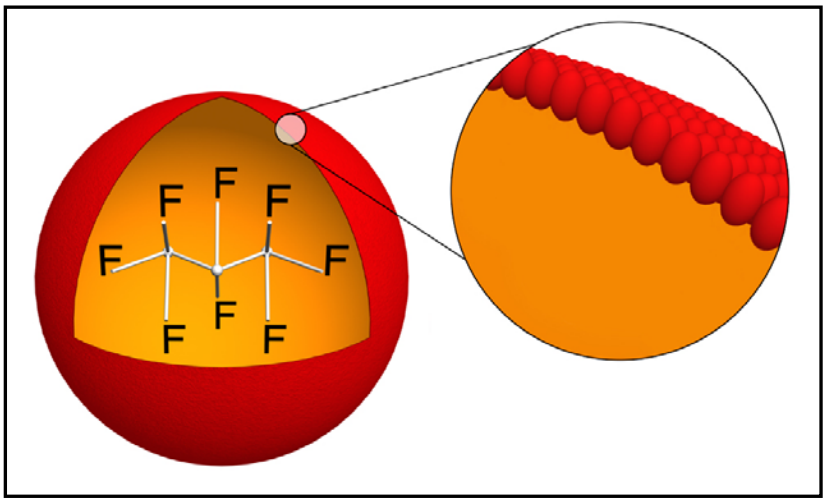
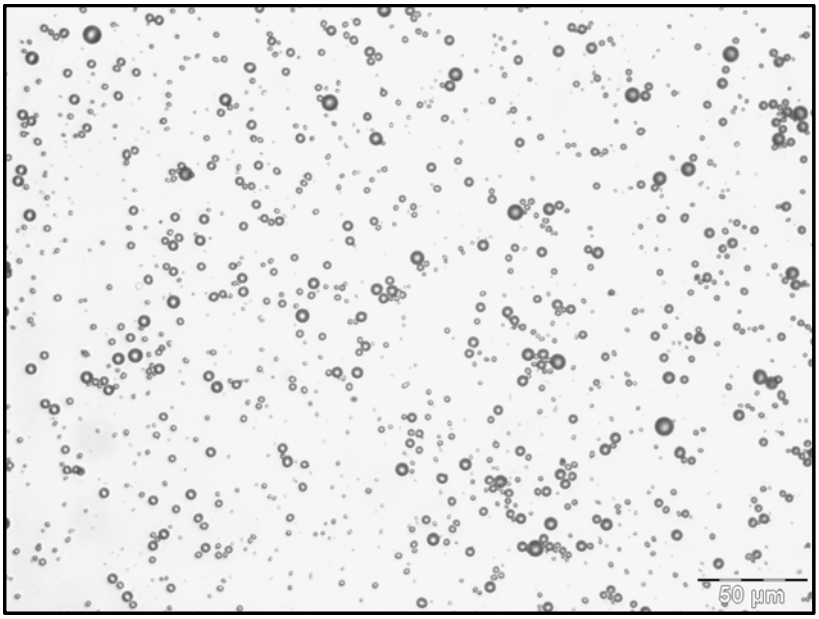


Figure 2 Microscopy of OPTISON Microspheres in Reconstituted Product



Note: Size bar is 50 µm.

2.2 Raw Materials

The albumin raw material used for production of OPTISON conforms to United States Pharmacopeia (USP). The plasma that supplies the albumin for manufacture is collected in accordance with all applicable regulations for plasma prescribed by the United States (US) Department of Health and Human Services, FDA, for the manufacture of biological products, as outlined in Chapter 1 of Title 21 of the Code of Federal Regulations (CFR), Subchapter F-Biologics.

The octafluoropropane (OFP) used for production of OPTISON is high purity grade with an assay greater than 98.8% OFP. The quality of all raw materials used during production of OPTISON is assured by extensive testing with properly validated analytical methods.

2.3 Product Properties

After primary production OPTISON is filled into 3-mL glass vials and the headspace is filled with OFP prior to stoppering. The quality of the product is assured by extensive testing with properly validated analytical methods. Main product specifications are listed in Table 2.

Table 2: Main Product Specifications for OPTISON

Parameter	OPTISON Specification
Microsphere Concentration and Size Number Concentration	$5.0\text{-}8.0 \times 10^8$/mL

Mean Diameter Number Size Distribution ≤10 µm	3.0-4.5 µm ≥ 95.0%
OFP Content of Suspension Layer	0.11–0.33 mg/mL
Protein Content	8-12 mg/mL *
pH	6.4–7.4
Sterility (USP)	Sterile
Bacterial Endotoxin (USP)	≤0.5 EU/mL

* 5-7% as shell protein.

The microsphere characteristics are ensured through control of concentration, mean size and size distribution. This testing hence assures a consistent performance of the product with regards to efficacy and bubble related toxicology.

The recommended dose of OPTISON is 0.5 mL/subject which may be repeated up to 5 mL in 10 minutes; the maximum dose for a single subject study is 8.7 mL.

3 PRECLINICAL DATA

3.1 Introduction

OPTISON consists of Perflutren (OFP; PF; PFP; C_3F_8) gas-filled, microspheres, stabilized by a thin shell of heat-modified human albumin, suspended in 1% human albumin solution.

The starting dose is 0.5 mL/subject or 0.007 mL/kg in a 70 kg subject, which can be increased up to 5 mL within a 10 minute period, and up to a total maximum imaging dose volume of 8.7 mL. The PF content (encapsulated gas) is nominally 0.22 mg/mL (specification range of 0.11-0.33 mg/mL) or about 2.8% by volume. Total PF in the maximum approved human dose is 0.003 mL/kg in a 70 kg subject.

3.2 Perflutren Gas

Perflutren is an inert, stable gas. It is sold as a device under the trade name ISPAN (Alcon Surgical Ltd., United Kingdom) as a surgical aid for use in the treatment of uncomplicated retinal detachment. Safety of intraocular use (0.3 mL dose) in humans has been demonstrated. [Chortkoff et al. 1994] have shown that inhalation of 65% Perflutren and 35% oxygen for 4 hours is without anesthetic effects.

The elimination of Perflutren after dosing of OPTISON was studied in 1 study in anesthetized dogs for OPTISON doses of up to 1.0 mL/kg. Perflutren was rapidly cleared via the exhaled air with an elimination $t_{1/2}$ of 40 seconds, in either blood or exhaled air. Approximately 100% of the injected dose was recovered in less than 15 minutes, suggesting that Perflutren residence in the body is very short.

3.3 Human Albumin

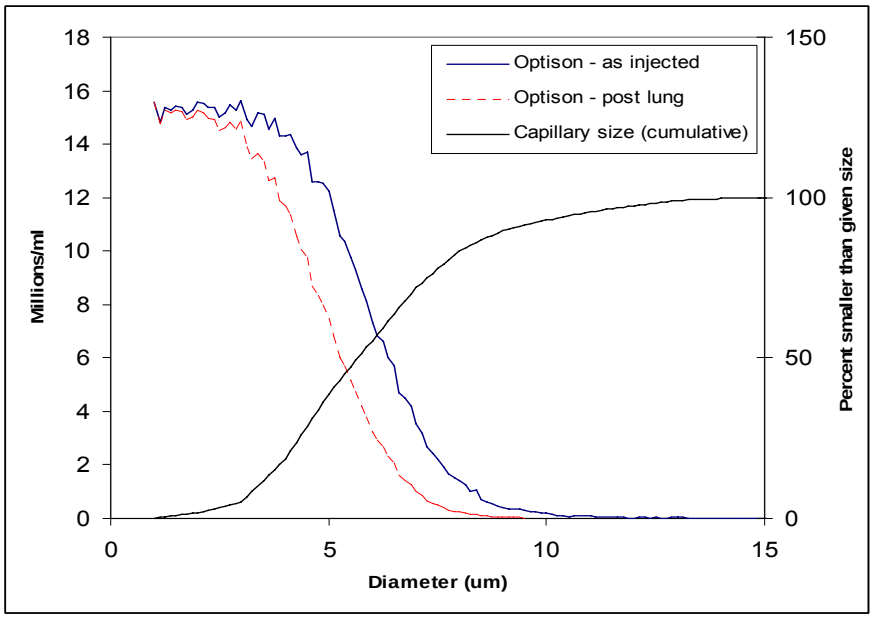
With regard to human albumin this is, in the form of 5% (w/v) aqueous solution, widely used in large quantities as a blood expander. Typical doses for i.v. infusion are in the range of 500-1000 mL compared to a typical dose of OPTISON of 1 mL containing 1% (w/v) albumin.

With regard to heat denatured (solid) albumin this is also widely used in the form of macro aggregated albumin (MAA) used for Tc^{99m} scintigraphy of the lungs (e.g., Pulmolite, Pharmalucence). These products contain solid particles of albumin, typically in the size range 10-90 μm, which are designed to be trapped in the lung microcirculation after an i.v. injection. A typical dose of MAA is approximately of 0.2 mg (as solid, insoluble albumin) compared to approximately 0.7 mg per mL OPTISON administered.

3.4 Particle Characteristics Relevant for Possible Microembolization

The upper tail of the microsphere size distribution of OPTISON (and other USCA`s) contains microbubbles that may be retained in capillary vessels. Figure 3 below shows a typical numerical size distribution of OPTISON. In addition, this figure contains the cumulative distribution of lung capillary diameters (data from [Hogg 1987]) and the calculated theoretical distribution of OPTISON after passage through this "capillary filter." As can be observed, while a fraction of microspheres could theoretically be retained in the lungs, the clinical significance of such retention, if any, would be expected to be minimal. The human lungs contain approximately 280 billion capillaries [Hogg 1987] thus the number of microspheres that are retained represents a fraction of the total number of vessels.

Figure 3 The Size Distribution of OPTISON Microspheres as Administered Compared to the Size Distribution of Lung Capillaries (Cumulative Percentage Smaller Than Stated Size) and the Theoretical Distribution of OPTISON After Passage Through the Lungs



Note: The particle size distribution was calculated by counting size observations of individual particles in bins of 0.125 µm along the horizontal axis.

The potential effect of any possible retention of the microspheres in the circulation will also be dependent on the stability of the microspheres after administration. If retention is transient and on a scale of minutes no permanent effects would be expected in the circulation. The stability of OPTISON in experimental systems mimicking the conditions after an i.v. injection has been extensively studied and the results from these studies are reviewed below.

Another factor in the evaluation of potential retention of OPTISON is whether the size as determined *in vitro* is an accurate reflection of the size after administration. Two effects could be considered in this regard; coalescence of microspheres and microsphere growth due to a net influx of gas from the surrounding matrix (i.e., blood).

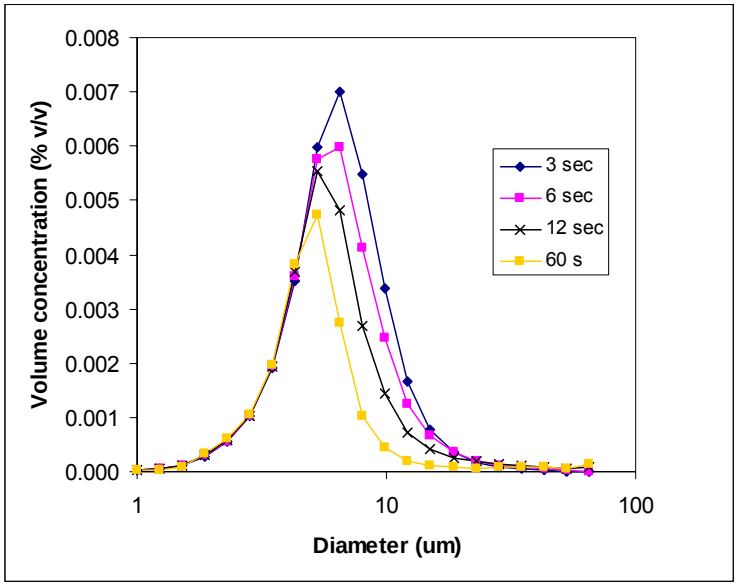
Non-encapsulated gas bubbles dispersed in a liquid tend to coalesce. This process is driven by the fact that the energy in maintaining a small radius of curvature is greater than maintaining a large radius. Thermodynamic force will drive 2 small bubbles in close contact to form 1 larger bubble. For an encapsulated microsphere such as OPTISON the shell structure prevents direct contact between the gas/liquid interfaces of 2 bubbles. The energy required to disrupt the solid albumin shell is larger than the energy potentially released by coalescence so the bubbles do not coalesce. Microscopy studies mimicking these *in vivo* conditions have confirmed that spontaneous coalescence between 2 OPTISON microspheres does not occur. This is also demonstrated by the storage stability of the product; the microsphere concentration and size distribution is maintained unchanged after 18 months.

Even though effectively preventing spontaneous coalescence, the albumin shell of the OPTISON microspheres is permeable to the gas molecules of the core, as well as to the gas molecules dissolved in the surrounding liquid. In the vial (under stable conditions), the Perflutren contained in the system is in diffusion equilibrium between the gas in the microsphere core, the gas dissolved in the liquid phase, and the gas in the vial headspace. In this case there is no net flux of gas to/from the microsphere and the system is thermodynamically stable. Upon administration into the blood stream, however, this equilibrium is shifted. In blood the microspheres are dispersed in a system which is void of Perflutren, but which is partially saturated with blood gases. The total tension of blood gases is approximately 93.2 kPa on the venous side and 101 kPa on the arterial side (difference mainly due to a difference in the partial pressure of oxygen). In combination with hydrostatic pressures at approximately 1 kPa on the venous side and 12.5 kPa on the arterial side, this makes the blood slightly under-saturated with gas at approximately 91.2% and 87.5% saturation at the venous and arterial sides, respectively. Under these conditions, thermodynamic forces will pull Perflutren out of the microsphere and blood gases will be pulled into the microsphere.

These theoretical considerations have been examined in several studies submitted to NDA 20-899 on the behavior of OPTISON microspheres in experimental systems mimicking the conditions after an i.v. injection. During these studies the microspheres were dispersed in human plasma at 37°C. The saturation of gas in the plasma was controlled to 85-95% to mimic the situation *in vivo*. In the first of these studies the volume concentration and volume size distribution of OPTISON microspheres was measured by laser diffraction. As the scattering efficacy of microspheres is proportional to their diameter in the 6^{th} power, this technique is inherently very sensitive to the large end tailing of the distribution. Results from this study are shown in Figure 4. As can be seen from this figure a monotonic decrease in concentration and size is observed. Sixty seconds after incubation the volume concentration

has decreased by approximately 50% and the mean diameter by approximately 20%. These results demonstrate that the microspheres do not expand significantly upon contact with blood under physiological conditions and that the Perflutren gradually leaks out into the matrix with a resulting decrease in size and concentration.

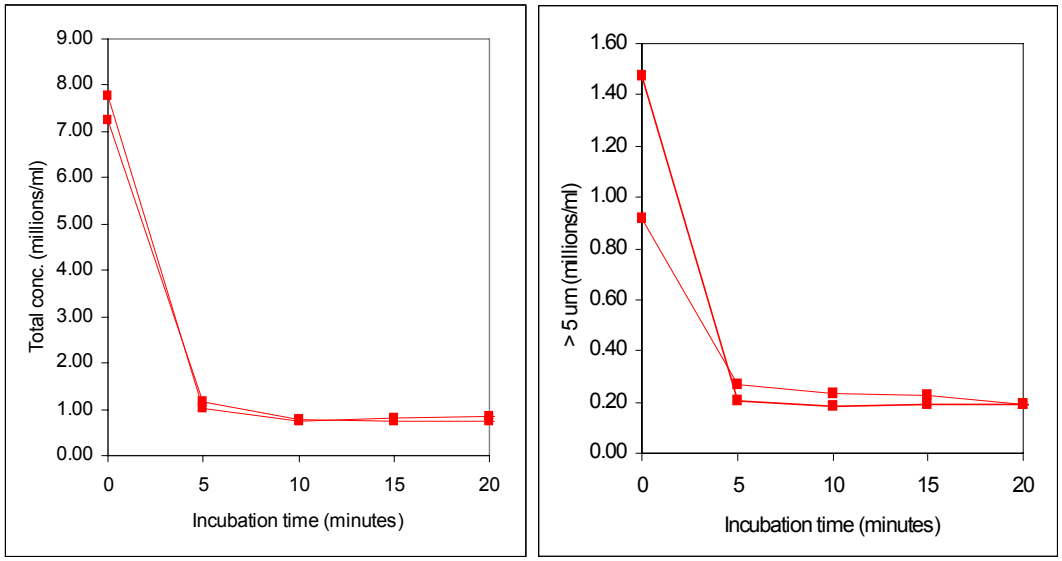
Figure 4 OPTISON in Plasma (37°C, Gas Tension Between 85-95% of Saturation)



Note: Volume concentration and distribution at various time points after incubation (as stated in legend above).

In a second study the number concentration and size distribution of OPTISON microspheres was determined by full automated image analysis utilizing Flow Particle Image Analysis (FPIA). To mimic *in vivo* arterial conditions further, these samples were continuously pressure cycled between 80-150 mmHg (80 cycles/minute). Results from this study are summarized in Figure 5. As can be seen from this figure both the total concentration of microspheres and the number of large microspheres decrease rapidly after dilution. Five minutes after incubation both the number concentration and the concentration of microspheres larger than 5 µm has decreased with more than 80% from initial values. The microscopy output from these analyses (not shown) demonstrated that the microspheres shrink immediately upon dilution and onset of pressure cycling.

Figure 5 OPTISON in Plasma (37°C, Gas Tension Between 85-95% of Saturation, Pressure Cycled Between 80-150 mmHg)



Note: Total number concentration (left) and number concentration of particles larger than 5 µm (right) versus time after incubation. The two curves in each graph are results from separate experiments.

In conclusion, both of these studies show no evidence of an increase in microsphere size upon dilution in an *in vivo* mimicking system.

3.5 ADME

The distribution and elimination of OPTISON with a shell labeled with ^{125}-I was studied in rats after an intravenous bolus dose of 0.25 mL/kg. The results indicate that most of the intact ^{125}I-OPTISON microspheres were cleared from the blood pool during the first few minutes following dosing. The activity found in plasma collected 5 minutes post-treatment, and in all of the tissue samples at necropsy are believed to represent radiolabeled albumin fragments and free ^{125}I, and not intact ^{125}I labeled microspheres. Since the ^{125}I activity recovered in the liver was highest of all the tissues at all time points, the liver is considered the major organ of uptake, metabolism, and elimination of the radiolabeled albumin shell via normal protein elimination pathways.

3.6 General Toxicology

The safety of OPTISON following a single administration was evaluated in rats, dogs and Rhesus monkeys. A single intravenous injection of OPTISON doses up to 20 mL/kg was well tolerated in these species. The minimum lethal dose in rats is 25 mL/kg (Human Equivalent Dose (HED) = 4 mL/kg), 32 times the maximum approved human dose. The No Adverse

Effects Level (NOAEL) in rats and dogs was 20 mL/kg (HED = 3.2 and 11.1 mL/kg, respectively or 26 and 89 times the maximum approved human dose). The NOAEL in Rhesus monkeys was 10 mL/kg (HED = 3.2 mL/kg, or 26 times the maximum approved human dose) based on increased incidence of thymic atrophy seen in female monkeys treated with 20 mL/kg that was considered possibly treatment-related. The occurrence of this change in some control animals precludes definitive interpretation of treatment relationship.

Repeat dose studies were performed in the rat and dog, respectively. Rats were dosed for 31 consecutive days with 0.25, 5 or 10 mL OPTISON/kg/day. There were no apparent findings for animals given 0.25 or 5 mL/kg/day. After dosing with 10 mL/kg/day, slightly higher beta globulin concentration, increased spleen weights, and slight lymphoid hyperplasia in the spleen of males was observed. This latter finding was reversible following a 2-week recovery. It is speculated that the observed effects are related to known effects of human albumin aggregates, known to result in lymphocyte stimulation and anaphylactoid responses in various animal species. The NOAEL in this study is 5 mL/kg/day (HED = 0.8 mL/kg/day, 6 times the maximum approved human dose).

Intravenous daily administration of OPTISON to dogs for 29 consecutive days at a dose of 0.25 mL/kg resulted in clinical chemistry findings of lowered serum albumin, protein, and calcium. Doses of 5 or 10 mL OPTISON/kg/day resulted in clinical findings of vomitus, excessive salivation, mucoid or nondeformed feces, hypoactivity and paleness; in clinical chemistry findings similar to those seen at 0.25 mL/kg/day and in microscopic findings of hypercellular marrow and increased extramedullary hematopoeisis in the spleen and liver. Remaining effects observed may be primarily immunological in origin due to the repeated intravenous administration of human albumin in the canine model. A NOAEL was not achieved in this study

Two other repeat dose studies in rats and dogs were also performed in which animals were administered 3 doses/week for 3 weeks. The NOEL in these studies was 5 mL/kg in rats (HED = 0.8 mL/kg, 6 times the maximum approved human dose) and 20 mL/kg in dogs (HED=11.1mL/kg, 92 time the maximum approved human dose). Pulmonary lesions observed at dosages of 10 and 20 mL/kg in the rat study were probably related to the infusion rate of a highly concentrated suspension of microspheres (due to floatation in the syringe).

No signs of toxicity or local irritation (intravascular, perivascular, intramuscular, dermal and ocular) which could be attributable to OPTISON, were detected in rabbit studies.

OPTISON did not show any adverse effects in the standard *in vitro* and *in vivo* test battery of genetic toxicology studies.

Carcinogenicity studies were not performed since OPTISON is a contrast agent intended for short-term clinical use only.

No embryonic or teratogenic effects were observed in rats at dosages causing significant maternal toxicity including death. Doses of 0.25, 5 and 10 mL/kg/day were used. The maternal NOEL is 0.25 mL/kg/day; the 5 and 10 mL/kg/day doses resulted in clinical observations, and reduced maternal body weight gain. The 10 mL/kg/day dose also caused deaths and gross tissue alterations. The developmental NOEL is greater than 10 mL/kg/day. In the rabbit teratology study doses of 0.25, 2.5 and 5 mL/kg were used. The maternal NOEL is less than 0.25 mL/kg/day and the developmental NOEL is 0.25 mL/kg. Increased abortions and premature deliveries, increased embryo-fetal deaths, reduced fetal body weight, and increased number of fetuses with dilated ventricles in the brain was observed in the groups dosed with 2.5 and 5 mL/kg/day. The clinical relevance of or the mechanism causing increased incidence of dilated ventricles in the brain of developing rabbit embryos is unknown.

The toxicological data presented support the conclusion that OPTISON is safe to use in humans for the indications as detailed in the package insert.

3.7 Particle-Related Studies

A blood compatibility study has been performed in which OPTISON (investigational name for OPTISON) was incubated with human blood *in vitro*. No hemolysis was detected. The study was performed without ultrasound exposure; this was addressed in an *in vivo* study in rabbits. The animals were dosed with OPTISON during imaging ultrasound exposure, including a latex particle group as a non-specific particle reference. Blood samples for measuring hemolysis (free hemoglobin and lactate dehydrogenase [LDH]) were taken. No hemolysis was seen, but reductions in white blood cell counts were observed in both the OPTISON and latex particle group, interpreted as a physiological reaction to particles.

The cardiovascular effects of OPTISON was studied in mongrel dogs, using 3 groups (vehicle, OPTISON, OPTISON + dipyramidole, a vasodilating stress agent) with 3 animals in each group. All animals were dosed with 0.25 mL/kg OPTISON or vehicle human serum albumin (HSA). Measurements were made of heart rate (HR), systemic arterial pressure, pulmonary arterial pressure, and central venous pressure. No effects that can be related to OPTISON were observed, but effects caused by dipyramidole were seen.

In order to investigate possible endothelial damage by the combined effect of OPTISON and ultrasound exposure, 3 dogs were injected with vehicle, and 3 dogs with repeated doses of 0.72 mL/kg OPTISON, all animals were exposed to high-power (mechanical index=0.8-1.8) ultrasound imaging of selected tissues. Tissue samples coinciding with the imaged volumes from jugular vein wall, aortic wall, myocardium and kidney were taken together with reference samples from sites not exposed to ultrasound. Histology (HE and immunohistochemical staining for albumin) showed no signs of endothelial damage.

3.8 Particle-Related Studies from the Literature

The microvascular behavior of albumin microspheres has been studied by [Yasu et al.]. Intravital fluorescence videomicroscopy of fluorescein isothiocyanate (FITC)-labeled microspheres given i.v. was performed, and inflammation of the endothelium was induced by local application of 10^{-8} M PAF. The microspheres move with the same velocity as erythrocytes without adhering to the endothelium. Inflammation caused some microspheres to adhere to venular endothelium without blocking red blood cell (RBC) passage.

Arterial and venous vessels 7-20 μm diameter were studied by [Dittrich et al. 1995] using intravital microscopy while OPTISON 1.25 and 2.5 mL/kg was injected i.v. No alterations in arterial pressure and microvascular blood flow were observed, and large microparticles or aggregates were not seen in the vessels.

The difference in microvascular properties of OPTISON injected i.v. and intraarterially was studied by [Skyba et al. 1996] in a combined dog and rat study. Cardiac function (hemodynamic recordings), regional myocardial perfusion (by radiolabeled microspheres), regional wall thickening (by ultrasound imaging), and pulmonary gas exchange (blood gas analysis) was observed in dogs dosed with OPTISON. No effect on any variable was observed after repeated bolus injections of OPTISON using doses of 0.3-0.8 mL/kg. Direct intracoronary injections resulted in persistence of contrast longer than after i.v. injection.

Intravital fluorescence videomicroscopy of exteriorized rat spinotrapezius muscle was performed using dichlorotriazinylaminofluorescein (DTAF)-labeled microbubbles injected intraarterially. Occasional blockage of capillaries caused by large bubbles was seen. This is consistent with the finding of prolonged contrast in myocardium observed after direct intracoronary injections.

In vivo interactions between leukocytes and OPTISON microbubbles have been studied by [Lindner et al. 2000]. No microbubble adherence to leukocytes seen in the microcirculation at baseline. Inflammation (by ischemia-reperfusion or TNF-α) cause bubble attachment to adherent leukocytes.

[Yamaya et al. 2002] performed a study in anesthetized, instrumented dogs (pulmonary arterial pressure, systemic arterial pressure, arterial blood sampling, and multiple inert gas elimination) in order to detect hemodynamic effects in the compromised pulmonary circulation. Injections of OPTISON at doses of 0.04, 0.12 and 0.2 mL/kg were given before and after partial pulmonary embolization by 0.625 mm polystyrene microspheres. No effects of OPTISON on pulmonary gas exchange parameters were observed. Before embolization, OPTISON caused a dose-dependent transient isolated rise in pulmonary vascular resistance up to 3.7-5.5 mmHg/l/min at the highest dose, peaking 3-4 minutes after injection. The authors speculate that this might be caused by release of vasoactive mediators from the endothelium. The response to OPTISON was not seen after embolization by polystyrene microspheres.

3.9 References

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4 ANIMAL MODELS

The following sections give a general overview with discussions of animal models for investigation of particle-related effects from ultrasound contrast agents, including effects of such agents that might arise in combination with ultrasound exposure. There is also a section discussing why pigs are not suited as experimental animals for evaluation of ultrasound contrast agents in general. These sections have been included in response to a request from the FDA for information on animal models that can be used to evaluate these agents.

4.1 Acute Particle-Related Toxicity, Hemodynamic Recordings

Rats and rabbits can be instrumented for measurements of arterial pressure, but the more complex measurements that are required to give information about causal relationships between cardiovascular variables are not possible in small animals for technical reasons.

Larger animals, such as the dog are far better suited for this purpose. An anesthetized dog can be surgically instrumented to measure and record:

- Arterial pressure
- Left ventricular pressure (Millar catheter)
- Central venous pressure
- Pulmonary arterial pressure
- Cardiac output (by flowmeter or thermodilution)
- Coronary blood flow (flowmeter on dissected coronary artery)
- Flow in peripheral arteries
- ECG

A range of variables can be derived from these measurements, including vascular resistance in several territories and indices of cardiac contractility. Such a collection of simultaneous measurements might provide input for mechanistic interpretations.

Emphasis has been put on observation of pulmonary arterial pressure in this model. When an increase in pulmonary pressure is seen, it commonly starts with a latency of about 20 seconds after ultrasound contrast injection, and reaches a peak within a few minutes before it gradually subsides [Yamaya et al. 2002]. The response in dogs is believed to be caused by release of vasoactive substances [Yamaya et al. 2002]. A self-limiting anaphylactoid reaction to OPTISON has been observed in a sub-population of dogs by [Yamaya et al. 2004], and the response was reproduced by injection of human serum albumin alone [Yamaya et al. 2004]. Other human albumin based microaggregates have also been shown to elicit a pulmonary hypertension response in dogs as well [Allen et al. 1973]. The magnitude and temporal

development pattern of the response to OPTISON does not support a mechanical trapping hypothesis. The number of capillaries in the pulmonary circulation in man is in the order of 480×10^9 [Hogg 1987]. An animal such as the dog is expected to have a somewhat lower number, a careful estimate would be 100×10^9, which is a factor of about 1000 more than the actual number of bubbles injected (100×10^6) in a clinical dose scaled to the dog's body weight.

A substantial number of bubbles would be required to be trapped in the lungs before mechanical blockage of vessels gives a noticeable increase in pulmonary arterial pressure. Given that there are no compensatory mechanisms, the relative increase in pulmonary flow resistance and pulmonary arterial pressure will be equal to the relative fraction of blocked capillaries. For this reason, the model is expected to be quite insensitive to mechanical trapping of small particles. If substantial trapping occurs, it is expected to have an immediate effect after a bolus injection, with a "step" increase in pulmonary arterial pressure and pulmonary vascular resistance coinciding with the first pass of the bolus through the lungs a few seconds after injection.

It can be concluded that observation of pulmonary arterial pressure in this model has no or low value for predicting mechanical trapping of bubbles in the microcirculation, but the model is considered to be a standard model for evaluation of hemodynamic safety.

4.2 Microcirculation, *in vivo* Microscopy

The behavior of ultrasound contrast particles in the microcirculation can be observed directly by intravital microscopy. Typical preparations are the mouse cremaster muscle and the rat spinotrapezius muscle. The latter allows intra-arterial injections via the retrograde carotid artery route.

A typical clinical dose of ultrasound contrast agent in man contains about 0.5×10^9 particles, giving a numerical concentration in blood of about 10^5 particles per mL. The numerical concentration of red blood cells is about 5×10^9 per mL, thus they outnumber the contrast agent by a factor of 50,000. Techniques such as fluorescent labeling of the contrast particles and automated video-based image analysis can be used for increasing the number of observed particles.

In order to be able to observe enough particles in the microcirculation, large doses are commonly used. The probability of inducing bubble interactions such as coalescence or aggregation will then increase dramatically; simple statistical considerations indicate that the rate of particle interactions depends on the square of their concentration. In a study performed by [Dittrich et al. 1995], doses of OPTISON up to 2.5 mL/kg were given. This is about 350 times the initial human dose, and the probability of aggregate formation is expected to be elevated by a factor of $(350)^2$ or more than 100,000. If bubble aggregates in this kind of experiments are observed, then this should be interpreted very carefully.

The data obtained from such studies will mostly be of a qualitative nature. Observations of particles that block the circulation and how long they persist can be made, interactions between cells (endothelium and white blood cells) and particles can be studied, and changes in the general blood flow pattern such as red blood cell velocity can be measured. The clinical relevance of findings in such models is difficult to assess.

4.3 Model for Detection of Endothelial Damage

Ultrasound contrast bubbles oscillate by radial expansion and compression when exposed to ultrasound. These oscillations might cause mechanical damage to surrounding tissue structures [Skyba et al. 1998], especially in confined spaces such as small vessels.

Large animals (dogs) can be used for studying the potential endothelial tissue damage caused by the combined effect of contrast agent bubbles and imaging ultrasound. Due to the focused nature of ultrasound, selected tissue volumes containing vessels can be exposed to ultrasound while injecting bubbles, and anatomical landmarks or fiducial markers can be used to identify locations of ultrasound exposure during necropsy. Neighbor tissue volumes can be used as references for determining the effect of ultrasound exposure in the presence of contrast agent compared to the effect of contrast agent alone. Gross endothelial damage with vessel wall rupture can be detected at necropsy by looking for macroscopic petechial bleedings. Histological sections can be stained for serum albumin and examined to detect leakage into the interstitium.

Smaller animals can also be used for such studies, but with increasing difficulties in assuring that ultrasound exposure correctly resembles the clinical situation due to mismatch between transducer footprint size, focal point depth, frequency and actual body size.

An in-house study in dogs with OPTISON and ultrasound exposure has been performed without any endothelial damage being detected, see section 3.7 above.

4.4 Arrhythmias, Triggered Imaging

It has been observed that ultrasound contrast agents during ultrasound imaging of the heart might trigger occasional single premature ventricular contractions in man. The combination of ultrasound scanner settings that cause such contractions is [Van der Wouw et al. 2000]:

- High emitted acoustic power at typical cardiac imaging frequencies
- Triggered image acquisition, meaning that the scanner is not running continuously, but instead acquires 1 image for each cardiac cycle (or any multiple of cycles)
- End-systolic triggering (delayed imaging with respect to the ECG QRS complex)

Analysis of the ECG from such contraction events indicates that the origin of the myocardial depolarization wave varies between individual contractions, and that patterns resembling both

left and right bundle block are seen [Van der Wouw et al. 2000]. The phenomenon might be related to the observations of microvessel rupture caused by ultrasound and contrast bubbles by [Skyba et al. 1998]. Mechanical irritation is known to cause premature contractions; this is commonly observed when intracardiac catheters touch the endocardial surface. The mechanistic explanation of the phenomenon strongly indicates that it is a class effect linked to the acoustic properties (bubble oscillations) that are required to make an effective contrast agent. It is possible to reproduce the conditions that cause such contractions in animal experiments using anesthetized dogs, a standard clinical scanner with contrast imaging presets and giving bolus doses or infusions of the contrast agent. The frequency of premature ventricular contractions in this kind of model is however quite low.

4.5 Acute Myocardial Ischemia or Infarction

Transient and permanent coronary arterial occlusion and stenosis can be created in dogs. This can be done using open surgery, or by using a percutaneous balloon catheter under fluoroscopic guidance. The animal should be instrumented for measurements of hemodynamic variables, as described above. The safety of ultrasound contrast agents in different phases of cardiac ischemia can be studied in such models. The effects on infarction size (measured by serial slicing of the heart, tetrazolium staining and planimetry) and arrhythmias can be studied as well as how the agent is tolerated in animals with a compromised central circulation.

4.6 Model of Lung Circulation Impairment

Pulmonary circulation impairment with regional ventilation / perfusion mismatch conditions can be induced in experimental animals by injecting large polystyrene (or other materials) particles intravenously. This creates a situation characterized by elevated pulmonary arterial pressure, reduced cardiac output (by a reduced left-side preload), reduced pulmonary diffusion capacity and ventilation/perfusion mismatch, evident as increased deviations between arterial and mixed alveolar O_2 and CO_2 partial pressures. Although an increase in pulmonary arterial pressure is seen, this is not a model that reproduces clinical pulmonary hypertension in man. It is however a model of increased sensitivity for detection of pulmonary microembolization, since the number of available vascular units for blood flow shunting is reduced.

4.7 Pigs as Experimental Animals

Almost all studies of ultrasound contrast agents in large animals are made in dogs, although the pig might seem to be a good alternative option.

It has been reported that pigs react to intravenous injections of ultrasound contrast agents with a drop in arterial pressure, pulmonary hypertension and circulatory collapse. Some controversies in the early days of Albunex development were caused by observation of this phenomenon [Østensen et al. 1992]. A similar response has been observed in sheep after injection of liposomes. [Miyamoto et al. 1988]. A general finding in pigs is also that the

ultrasound contrast effects observed in the left ventricle and the systemic arteries are weak, and that there is almost no recirculation of the contrast in the blood pool.

Some animal species, notably pig, goat, sheep and cow (all from the order *Artiodactyla*) have abundant pulmonary intravascular macrophages, while other species such as dogs, rats, mice, rabbits, baboons and man have very few such macrophages [Dehringer and Wismar. 1989]. The macrophages react to particles in the bloodstream by activation, phagocytosis and release of thromboxane A2, which in turn cause an increase in pulmonary vascular resistance, pulmonary hypertension, reduced left heart diastolic filling and a drop in systemic arterial pressure. This chain of reactions can in some cases be fatal. The hemodynamic response can be blocked by pre-treatment with indomethacine (Figure 6), or by using a thromboxane A2 receptor antagonist [Østensen et al. 1992]. The consequence of the phagocytosis activity is high first-pass retention of particles in the lungs after i.v. injection, with substantial loss of contrast effects in the left side of the heart and the systemic arterial circulation. The phagocytosis is not blocked by indomethacine.

In the absence of intravascular lung macrophages, the pig would have been an excellent animal model for evaluation of ultrasound contrast media, with a suitable overall body size, cardiac anatomy and coronary vascular supply and ease of ultrasound imaging of the heart.

Figure 6 Hemodynamic Recording From a Pig Given Albunex, Before and After Treatment With Indomethacine

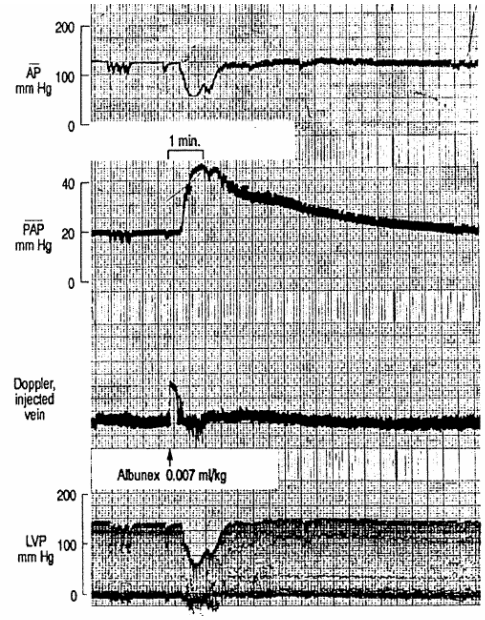

Fig. 1. Haemodynamic effects of intravenous injection of 0.2 ml Albunex[R] to a pig weighing 27 kg. The reflected energy from a 10 MHz pulsed Doppler flow velocity probe placed on the vein was used as an injection marker.

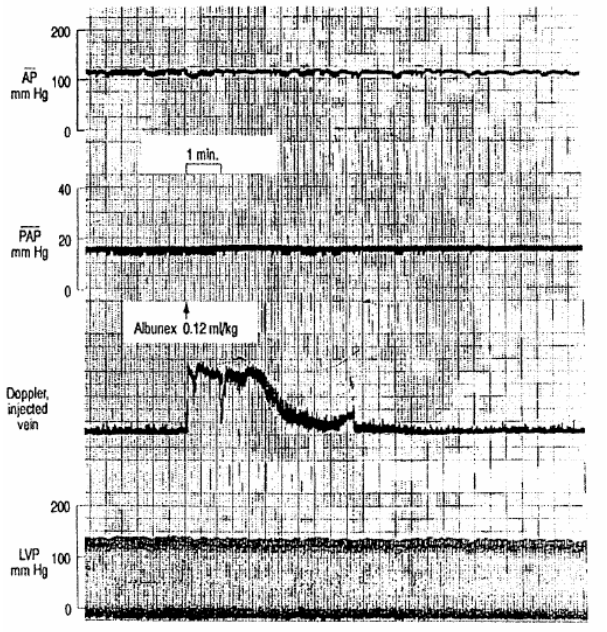

Fig. 2. Lack of haemodynamic effect of i.v. injection of 3.24 ml Albunex[R] to same pig as in Fig. 1 after treatment with indomethacin 10 mg kg^{-1} + 5 mg kg^{-1} h^{-1}.

Source: [Østensen et al. 1992]

4.8 References

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5 SUMMARY OF NDA CLINICAL SAFETY DATA

5.1 Introduction

Echocardiography is a clinically useful, noninvasive and safe modality which provides real-time tomographic imaging of the heart. This utilization allows the echocardiographer to obtain regional and global diagnostic information on cardiac function.

It is recognized that approximately 15% of subjects undergoing routine echocardiography have non-diagnostic baseline studies where all segments of the left ventricular endocardium cannot be adequately visualized. It is this group of subjects who will derive potential benefit from the addition of an echocardiographic contrast agent that will convert non-diagnostic to diagnostic echocardiographic evaluations. The American Society of Echocardiography (ASE) Committee on Standards stated that visualization of precise endocardial definition is required for the accurate assessment of regional wall motion, quantitation of left ventricular volume, area dimensions and derived indices of contractile function [Schiller NB, et al. 1990]. [ASE Committee on Standards, Subcommittee on Quantitation of 2-D Echocardiograms.1990]. The ASE Committee recommended that if greater than 20% of the endocardial border is not visible on the examination, the echocardiographer should not attempt to assess a continuous border as this will heighten the likelihood of error in assessments from the examination.

The Phase 3 clinical protocols required subjects to have a minimum of 33% inadequately visualized endocardium (2 of 6 left ventricular endocardial segments) in order to qualify for study enrollment, thereby evaluating a subject population in whom benefit would be derived from the administration of a contrast agent.

Later, in 2000, the ASE published a position paper on the clinical indications for the safe and efficacious use of ultrasound contrast agents [Mulvagh SL, et al. 2000]. These ASE guidelines have influenced the practice of cardiology with regard to the use of diagnostic ultrasound images. It is noted that if the left ventricular endocardial chamber cannot be visualized in 2 of 6 regions, the use of ultrasound contrast is indicated.

Figure 7 and Figure 8, highlight the use of ultrasound contrast agents for the identification of left ventricular chamber identification (left ventricular opacification i.e LVO). The images represent standard 2-dimensional (2D) echocardiographic images of a subject's heart. The images represent an apical 4-chamber view of the heart. The chambers that are identified on this view include the left atrium and left ventricle. The apex of the heart placed at top and base of the heart in at the bottom of the figure.

The 2 sets of images reveal the left ventricular chamber without ultrasound contrast (left sided images) and the left ventricle chamber following opacification (right sided images). Noted in the second set of images (Figure 8), the left ventricular endocardial surfaces have been outlined

(traced) and the 6 regions have been numbered (#1-6) according to the standards adopted by the ASE.

These images demonstrate the effect intravenous ultrasound contrast agents on cardiac chamber enhancement (LVO).

Figure 7 Left Ventricular Endocardial Border Detection

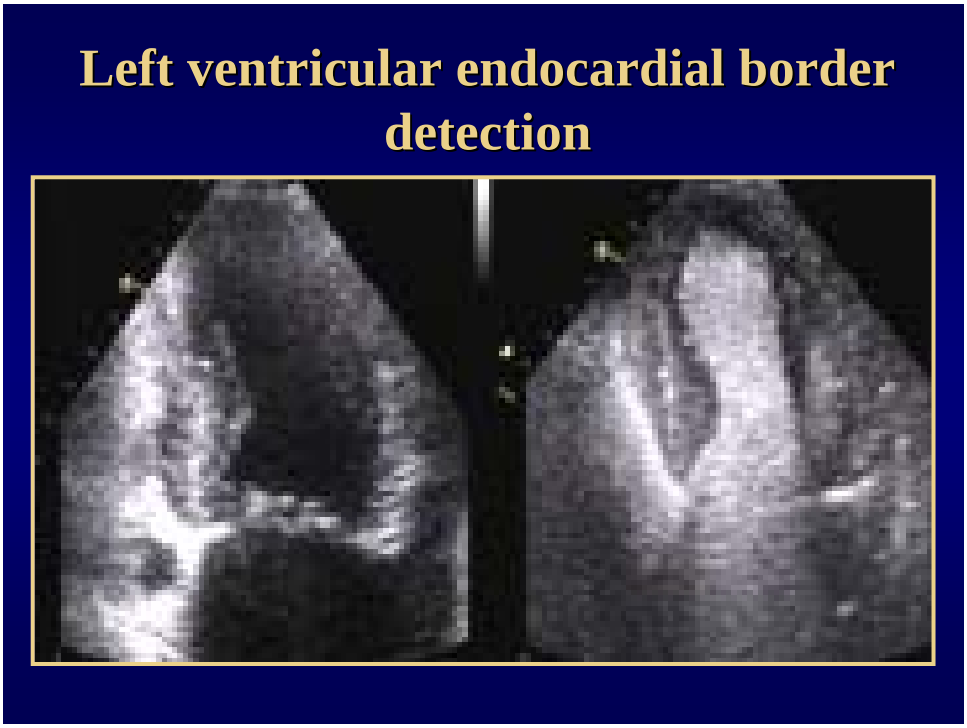
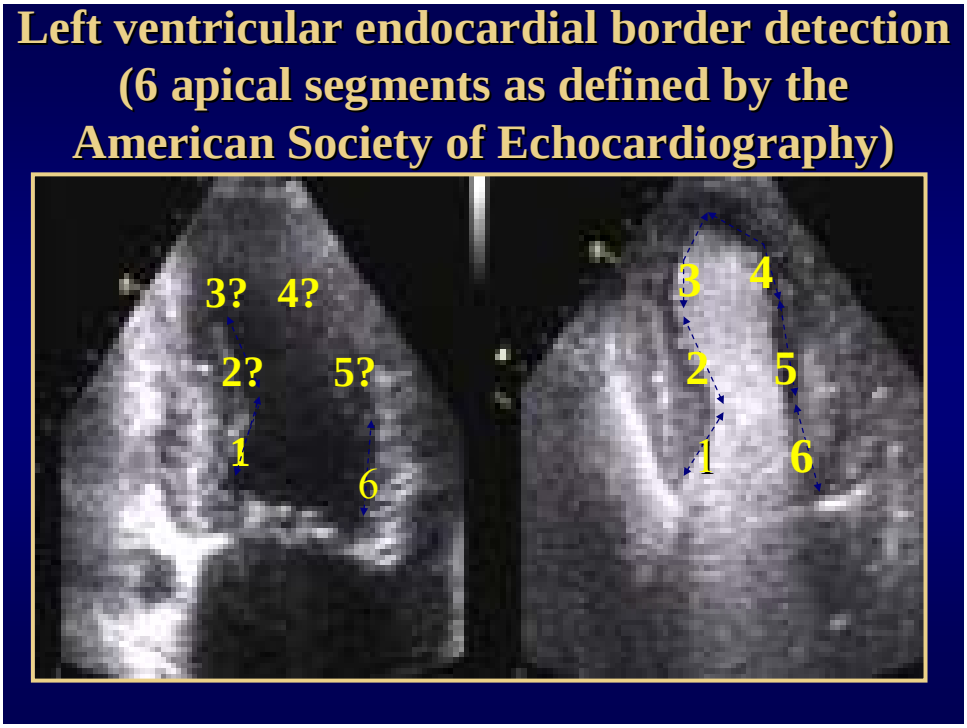


Figure 8 Left Ventricular Endocardial Border Detection (6 Apical Segments, as defined by the ASE)



5.2 Overview of Clinical Investigations

The Clinical Development Program for OPTISON consisted of 6 clinical trials evaluating a total of 308 subjects, 279 who received OPTISON. The safety and effectiveness of intravenous OPTISON were evaluated in these 6 studies (Table 3) between March 1995 and June 1996.

Table 3: Clinical Development Program for OPTISON: 6 Studies

Study No.	Phase	Type	Study Description	Number of Subjects
FS-1000	1	Safety Dose Ranging	Safety, dose-ranging, preliminary efficacy	40
FS-1250	1	Safety	Evaluate Immunological response to rechallenge – 1 year later (Patients from FS-1000)	5
FS-1500	1	Mass Balance	Measured PFP in blood and expired air as a measure of clearance; half-life; recovery in expired air	10
FS-6000	1/2	Safety Single-blind	Safety and immunological response in healthy volunteers and in subjects with cardiac, hepatic and respiratory diseases	50
FS-3000	3	Comparative vs. Albunex	Evaluate safety and efficacy for endocardial border delineation and left ventricular chamber opacification vs. Albunex and enhancement of Doppler signal vs. baseline, non-contrast signal	101
FS-3500	3	Comparative vs. Albunex	Evaluate safety and efficacy for endocardial border delineation and left ventricular chamber opacification vs. Albunex and enhancement of Doppler signal vs. baseline, non-contrast signal	102

In the Phase 3 clinical trials, FS-3000 and FS-3500, the investigational agent, OPTISON was directly compared to Albunex, which was a previously FDA approved 1st generation USCA. The difference between Albunex and OPTISON is the gas contained within the albumin microsphere. Albunex contains air whereas OPTISON contains perflutren. Notably, perflutren is relatively non-diffusible permitting a longer and more efficacious enhancement of the cardiac structures.

Protocol FS-1000 (Phase 1) evaluated 40 healthy volunteers with single OPTISON doses of up to 40.0 mL and cumulative dose volumes of up to 44.0 mL. Immunological testing was also performed in this protocol on 16 subjects (8 males and 8 females). One year later, 5 of these 16 subjects were selected for rechallenge with OPTISON and tested again for immunological response in Protocol FS-1250.

A mass balance study (Protocol FS-1500, Phase 1) was performed in 10 healthy volunteers to assess the elimination of perflutren in expired air and in blood. Single dose volumes of 20.0 mL were administered to all subjects in this protocol.

Protocol FS-6000 (Phase 1/2) was performed to evaluate the effect of administration of a placebo control as compared to intravenous OPTISON for ADE incidence rates and immunological response. In this trial, 20 healthy volunteers and 30 subjects with known disease (10 cardiac, 10 hepatic, and 10 respiratory subjects) were studied. All participants received a single 20.0 mL injection and were masked to the test agent, OPTISON or control (1% human albumin); 25 subjects received OPTISON, while the other 25 subjects received 1% human albumin. Assignment of either test agent was randomized in all subject groups.

Comprehensive immunological testing was performed on plasma samples from all 50 participants tested to 3 weeks post-study. The evaluations consisted of IgG, IgA, IgM, IgD and IgE immunoglobulins, and cytokine and complement activation analyses (Endogen IL-1, alpha, IL-2, TNF and C3).

The final 2 Protocols, FS-3000 and FS-3500, were considered pivotal Phase 3 studies and were identical comparative studies evaluating Albunex versus OPTISON for safety, endocardial border delineation, left ventricular opacification, and Doppler signal enhancement. A total of 203 subjects from 14 US centers were evaluated in the 2 protocols. Subjects received both Albunex (0.08 and 0.22 mL/kg) and OPTISON (0.2, 0.5, 3.0 and 5.0 mL) on separate days (minimum of 48 hours apart). A total of 199 subjects received OPTISON, while 200 received Albunex. An independent core laboratory masked to contrast agent identity, dose, and subject history read all non-contrast and contrast images for the 203 subjects. All claims were based upon the core laboratory efficacy assessments.

These 6 clinical studies of i.v. OPTISON were performed in the US at a total of 16 centers under an Investigational Device Exemption (IDE). All protocols were conducted according to Good Clinical Practices (GCPs) and sponsor operating procedures for conduct of clinical research studies, both of which support 21 CRF 860.7. Investigators were selected based upon expertise in the field of echocardiography and meeting qualification criteria required in the operating procedures. All subjects provided written Informed Consent prior to study participation and test agent administration. Institutional Review Board (IRB) approval was obtained at all study sites prior to study initiation.

5.3 Protocol Objectives

The objectives of the 6 clinical protocols are summarized below.

5.3.1 FS-1000 (Phase 1)

To evaluate the safety of intravenous OPTISON in 4 dose groups.

To evaluate the preliminary efficacy of intravenous OPTISON for left ventricular chamber opacification and endocardial border delineation.

To evaluate the preliminary efficacy of intravenous OPTISON for myocardial perfusion.

5.3.2 FS-1500 (Phase 1)

To determine the amount of PFP in parts per million over time in blood and expired air as a measure of clearance from the body.

To determine the half-life and recovery of PFP in expired air.

To evaluate the safety of i.v. OPTISON.

5.3.3 FS-6000 (Phase 1 and Phase 2)

To compare the safety profile of i.v. OPTISON ultrasound contrast microspheres to a control agent (1% human albumin) in both normal, healthy volunteers and in 3 subject populations.

To assess the immunological response of i.v. OPTISON as compared to a control agent (1% human albumin) in both normal, healthy volunteers and in 3 subject populations.

The 3 subject populations consisted of subjects with cardiac disease (e.g., cardiac dysfunction as documented by medical history, etc.), hepatic disease (e.g., cirrhosis, hepatitis, fatty liver, etc.) and respiratory diseases (e.g., COPD, emphysema, pulmonary hypertension, etc.).

5.3.4 FS-1250 (Phase 1)

To evaluate the immunological response of rechallenging subjects with OPTISON approximately 1 year later after FS-1000.

5.3.5 FS-3000 and FS-3500 (Phase 3)

The overall objectives of these trials were to determine whether intravenous OPTISON, when compared to Albunex, provided improved image enhancement of the left ventricle in diagnostic echocardiography and allowed a more complete echocardiographic examination. The specific hypotheses tested were:

- Primary Endpoint: Endocardial border delineation (EBD)
  H0: EBD with Albunex=EBD with OPTISON

- Secondary Endpoints:
  Left Ventricular (LV) Opacification
  H0: LV chamber opacification with Albunex=LV chamber opacification with OPTISON.
  Doppler Signal Enhancement
  H0: Doppler signal enhancement at Baseline=Doppler signal enhancement with OPTISON.

Protocols FS-3000 and FS-3500 were identical in all aspects including objectives, study design, inclusion/exclusion criteria, dosing regimen, imaging, safety tests and evaluations, follow-up intervals, and masked efficacy assessments.

5.4 Inclusion/Exclusion Criteria

The inclusion and exclusion criteria are provided in the following tables Table 4 a and b.

Table 4a: Inclusion Criteria

Criteria	FS-1000	FS-1500	FS-6000	FS-1250	FS-3000/ FS-3500
Male or female ages:	18-50	18-50	≥ 18	18-50	≥ 18
Normal cardiac function	X	X	X[1]	X	N/A
Subject body weight within 20% (+/-) of the ideal range based on height & frame size	X	X	X[1]	N/A	N/A
Negative serum pregnancy test within 24 hours of the procedure, if female and of child-bearing potential	X	X	X	X	X
Conscious and cooperative	X	X	X	X	X
Adequate baseline ultrasound images of the heart in the Apical 4- & 2-chamber views	X	N/A	N/A	N/A	N/A
Signed informed consent	X	X	X	X	X
Ability to return for the follow-up evaluations	X	X	X	X	X
Additional inclusion criteria for each protocol:					
FS-1500: nonsmoking participant; normal pulmonary and liver functions.					
FS-6000: [1]*Group I* only: normal, healthy volunteers; *Group II*: Significant cardiac disease as documented by medical history including, but not limited to, cardiac dysfunction: moderate to severe depressed cardiac function (ventricular ejection fraction ≤ 40% by 2-D echo), and/or symptomatic disease (New York Heart Association class II, III or IV). *Group III*: Hepatic dysfunction includes, but is not limited to, patients with fatty liver, fibrosis, cirrhosis, hepatitis, postoperative liver disease, vascular lesions and neoplasms. *Group IV*: Respiratory disease includes, but is not limited to, COPD, chronic bronchitis, emphysema, pulmonary hypertension, bronchiectasis, asthma, pulmonary vascular disease (diffusion capacity ≤ 70% predicted value); a minimum of one of the following: forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ≤ 0.70, forced expiratory flow 25-75% ≤ 70% predicted value, FVC ≤ 70% predicted value, or FEV_1 ≤ 70% predicted value.					
FS-1250: Received FS069 and tested for immunological response under Protocol FS-1000 conducted one year earlier.					
FS-3000/3500: Minimum of 2 of 6 EBD segments not seen from the Apical 4-chamber view. Subgroups: CPD includes, but is not limited to, patients with chronic bronchitis, emphysema, pulmonary hypertension, bronchiectasis and asthma; Congestive and dilated cardiomyopathy patients with a ventricular ejection fraction ≤ 40% ≥ 20% by 2-D echo.					

Table 4b Exclusion Criteria

Criteria	FS-1000	FS-1500	FS-6000	FS-1250	FS-3000/ FS-3500
Known or suspected hypersensitivityto blood, blood products or albumin	X	X	X	X	X
Known allergies	X	X[1]	X	X	N/A
Pregnancy or lactation	X	X	X	X	X
History of cardiac disease	X	X	X[2]	X	N/A
Abnormal CPK isoenzyme/ hematology/chemistryvalues	X	X	X	X	N/A
Abnormal physical examination	X[3]	X	X	X	N/A
Abnormal baseline 12-lead ECG	X	X	X	N/A	N/A
Elevated oral temperature (≥ 100°F).	NA	NA	X	NA	NA
Blood pressure > 145/95 as measured in two 30 min intervals	X	X	X	N/A	N/A
Total cholesterol over 240 mg/dL	X	X	X	N/A	N/A
Diabetes mellitus	X	X	X	N/A	N/A
Symptomatic adult asthma	X	X	X	X	N/A
Clinically significant liver & renal function tests: SGOT, SGPT, alkaline phosphatase, blood-urea-nitrogen(BUN) and creatinine	X	X	X[2]	X	N/A
Clinically significant anemia defined by the CBC	X	X	X[2]	X	N/A
Additional exclusion criteria for each protocol:					
FS-1500: [1]mild pollen allergies acceptable.					
FS-6000: [2]*Group I:* Drug or alcohol use. *Groups II, III, IV:* terminal illness (life expectancy < 1 month); end stage organ disease (awaiting transplant).					
FS-1000: [3]also included a neurological examination.					
FS-3000/FS-3500 recent (< 6 months) neurological event (CVA or TIA); doesn't require routine echocardiography; confined to ICU or receiving respiratory support via a continuous or intermittent ventilation machine; New York Heart Association Class IV Congestive Heart Failure patient; LVEF <20% by 2-D echo; severe liver disease based upon medical history.					

5.5 Study Population/Demographics

Table 5 summarizes the demographics and physical characteristics of the subjects and subjects who participated in the clinical trials.

Table 5: Demographics

Protocol	MALES							FEMALES						
	n	Age (yrs)		Height (in)		Weight (lbs)		n	Age (yrs)		Height (in)		Weight (lbs)	
		Avg	Median	Avg	Median	Avg	Median		Avg	Median	Avg	Median	Avg	Median
FS-1000	15	28	29	72	72	178	172	25	28	24	66	67	134	132
FS-1500	5	22	21	72	72	175	187	5	25	22	66	66	129	129
FS-6000	27	49	46	69	69	189	184	23	50	54	65	65	160	165
FS-1250	3	31	32	71	70	184	176	2	27	N/A	66	N/A	136	N/A
FS-3000	74	58	59	70	70	208	208	27	55	53	64	65	169	158
FS-3500	87	64	66	69	69	197	186	15	58	59	63	64	185	185

5.6 Safety Data Results

The summary of the NDA safety data has been prepared by Steve Feinstein, M.D., clinical cardiologist, senior medical attending and director of echocardiography at Rush Medical Center in Chicago, Illinois. Rush Medical Center personnel perform and interpret approximately 11,000 echocardiograms annually utilizing ultrasound contrast agents in nearly 35% of all studies following ASE practice guidelines.

Dr. Feinstein was primary investigator in the first clinical trial of Albunex and again in 1995-1996 when he was involved in the Phase 3 clinical trial of OPTISON (FS-3000).

For this review process, all OPTISON clinical trial reports were made available for review, without limitation.

This OPTISON Clinical Trials Safety Results Summary has been abstracted from the reports provided with additional comments focused on the present issues regarding the use of ultrasound contrast agents in the practice of clinical cardiology.

The following parameters were reviewed for each of the 6 clinical trials (Table 6):

Table 6: Safety Parameters included

	FS-1000* (n = 40)	FS-1500* (n = 10)	FS-6000** (n = 50)	FS-1250* (n = 5)	FS-3000/3500*** (n = 203)
12-lead ECG	X	X	X	X	X
Physical Examination	X	X	X	X	X
Neurological Examination	X				
Spirometry	X		X		
Vital Signs	X	X	X	X	X
O_2 Saturation	X	X	X	X	X
Chemistry Panel	X	X	X	X	X
Hematology	X	X	X	X	X
CPK Isoenzymes	X	X	X	X	X
Urinalysis			X		X
PT	X	X	X	X	X
PTT	X	X	X	X	X
ADEs	X	X	X	X	X

*All evaluations performed at baseline, and 2 and 24 hours post-study.
**All evaluations performed at baseline, 2 hours and 48 hours post-study.
***All evaluations performed at baseline, 30 minutes and 48 hours (up to 10 days) post-study for each Test Agent.
0_2 Saturation monitored every 2 minutes on study. Vital signs monitored at 5 to 20 minute intervals on study. A minimum of 48 hours and a maximum of 10 days were required between each Test Agent.

The subsections described include the following:

- 12 lead ECG
- Physical examination
- Neurologic examination
- Spirometry
- Vitals sign measurements
- O_2 Saturation

- Chemistry
- Hematology
- CPK enzymes
- Urinalaysis
- PT
- PTT
- ADEs

All safety data were evaluated for statistically and clinically significant changes. The statistically significant changes were found not to be clinically meaningful and were within the expected changes for the subject population. Study reports were reviewed for each of the described clinical significant changes and areas of particular interest are highlighted below.

5.6.1 12-lead electrocardiogram

The following parameters were evaluated:

- First degree heart block (HB)
- Second degree HB
- Complete HB
- Atrial arrhythmia
- Junctional rhythms
- Ventricular arrhythmia
- LVH (left ventricular hypertrophy)
- Other chamber enlargement
- Q-wave infarct
- ST-T wave changes; Ischemia suspected
- Nonspecific ST-T wave changes
- Other (Describe)

Each ECG assessment was scored as either normal or abnormal.

FS-1000 (n=40; all subjects received OPTISON)

Changes in the ECG scores from pre-study to 2 hours post-study and from 2 hours post-study to 24 hours post-study were evaluated. No statistically significant differences were found, either within each dose group or combining the 4 dose groups for the 40 subjects (subjects received up to a maximum of 44 mL of OPTISON. The current recommended dose is 0.5 mL, the maximum dose is 8.7 mL).

FS-1500 (n=10; all subjects received OPTISON)

Three subjects had minor rhythm changes between the pre-study and post-study measurement intervals. No other ECG changes were observed for any of the 10 subjects. The minor ECG irregularities are listed below (Table 7).

Table 7: Electrocardiogram results

Subject Number	Cardiac Rhythm	Comment
101	Normal	
102	Normal	
103	Normal	
104	Normal	
105	Normal	Possible isolated premature ventricular contraction
106	Normal	Baseline artifact noted.
107	Normal	
108	Other	Normal Sinus Rhythm
COMMENT:	Isolated premature ventricular contractions noted prior to dose and continuing past dose.	
109	Normal	
110	Normal	

FS-6000 (n=50; 25 subjects received OPTISON and 25 subjects received 1% human albumin)

Four groups were studied: subjects categorized as normal, cardiac disease, lung disease and liver disease. There were no statistically significant differences between the OPTISON subjects (n=25) and the human albumin subjects (n=25) in rates of change from "not present" to "present" at 2 or 48 hours post-study in any of the ECG assessments.

FS-1250 (n=5; all subjects received OPTISON intradermally, 4 subjects received 20 mL injections)

One subject had sinus bradycardia and isolated premature atrial contractions (PACs) at baseline which changed to normal sinus rhythm 2 and 24 hours post-study. The remaining 4 subjects had normal ECGs at all intervals.

FS-3000 and FS-3500 (n=203; 199 subjects received OPTISON and 200 subjects received Albunex)

There were no statistically significant differences in rates of change from "not present" to "present" at 30 minutes post-study in any of the ECG assessments regardless of whether the subject received Albunex or OPTISON.

Subjects enrolled into both Phase 3 studies were scheduled for routine 2-D echocardiograms for evaluation of cardiac function. Baseline rhythm disturbances were expected in this subject population. Neither Albunex nor OPTISON increased the severity of their rhythm disturbances, the appearance of ischemic ST-T wave changes, ventricular arrhythmias or Q-wave myocardial infarctions (MI), which were distributed equally between both agents.

5.6.2 Physical examination

The following parameters were included in the physical examination:

- General appearance
- Skin
- Head
- EENT (eyes, ears, nose and throat)
- Heart
- Lungs
- Abdomen
- Lymph nodes
- Extremities

Each assessment was scored as either normal or abnormal.

FS-1000 (n=40)

Changes in the scores from pre-study to 2 hours post-study and from 2 hours post-study to 24 hours post-study were recorded. No statistically significant differences were found, either

within each dose group or combining all dose groups. Additionally, no clinically significant changes occurred in any of the 40 subjects.

FS-1500 (n=10)

There were no changes in physical examinations from baseline to 2 or 24 hours post-study.

FS-6000 (n=50)

There were no statistically significant differences between-OPTISON and 1% human albumin in rates of change from "not present" to "present" at 2 or 48 hours post-study in any of the physical examination assessments.

FS-1250 (n=5)

All assessments were normal and there were no changes from baseline to 2 or 24 hours post-study.

FS-3000 and FS-3500 (n=203)

There were no statistically significant differences between Albunex study days and OPTISON study days in rates of change from "not present" to "present" at 30 minutes or 48 hours post-study in any of the physical examination-assessments.

5.6.3 Neurological examination

A complete neurological examination was performed on the 40 subjects enrolled in Protocol FS-1000 at baseline (pre-study), 2 and 24 hours post-study. No statistically significant-differences were found, either within each dose group or combining all dose groups. Additionally, no clinically significant changes occurred in any of the 40 subjects.

5.6.4 Spirometry

FS-1000 (n=40)

Spirometry was performed in the 40 subjects enrolled in Protocol FS-1000. The spirometry variables of forced vital-capacity and forced expiratory volume were measured pre-study and 2 hours post-study. Repeated-measures ANOVAs found no significant differences in either of these variables when testing for differences in rates of change among the 4 dose groups. Additionally, no-clinically significant changes were observed in any subject.

FS-6000 (n=50)

Dynamic spirometry was performed in the 50 subjects enrolled in Protocol FS-6000 at the following intervals: 1 hour pre-study, immediately post-injection of OPTISON or HA, 15 minutes, 1 and 2 hours post-injection. The values obtained were not valid and did not meet American Thoracic Society Standards for test acceptability; therefore, no conclusions can be made from the spirometry data. However, if any respiratory AEs had occurred, such as the formation of emboli or immune-complexes, these would have been reflected by the consumption of complement and an increase in complement-split products. As demonstrated by clinical findings of ADEs and immunological testing, this clearly did not occur.

5.6.5 Vital sign measurements

Vital sign measurements include body temperature, heart rate, respiration rate, and blood pressure.

FS-1000 (n=40)

Repeated measures analyses of variance (ANOVA) were used to evaluate changes over time and differences among the 4 dose groups. There were no statistically significant differences in rates of change of vital signs among the 4 dose groups and there were no clinically significant out-of-range values for any of the 40 subjects. Body temperatures were recorded for the 40 subjects only at baseline and thus were not statistically analyzed.

FS-1500 (n=10)

Diastolic blood pressure (DBP) measurements had a significant t-test on changes from baseline to 2 hours post-study. Mean diastolic blood pressure dropped from 74 mmHg at baseline to 63 mmHg at 2 hours and increased slightly to 67 mmHg at 24 hours post-study, all within normal limits. Similarly, there were significant t-tests at 10 and 15 minutes post-injection and a significant repeated measures ANOVA. DBP increased from 63 mmHg just before injection to 66.5 mmHg 5 minutes post-injection, and to 70 mmHg at 10 and 15 minutes post-injection.

Body temperature measurements changed significantly by the repeated measures ANOVA. Mean body temperature declined from 97.6° F at baseline to 96.7 F 24 hours post-study. The slope was -0.0491, significantly different from zero due to small variabilities.

Mean HR changed from 70 bpm at baseline to 77 bpm at 2 hours post-study and to 67 bpm at 24 hours post-study, a significant slope of -0.1957 with the repeated measures ANOVA.

None of the changes in BP, body temperature or HR was considered clinically significant. Two subjects had 5 changes in vital sign measurements that were considered clinically significant. Subject 106 had elevated respirations of 22 breaths per minute. This measurement was taken in the 60-second interval between the 4-minute and 6-minute expired gas collection following injection. This slightly out of range value had returned to within normal limits at the

subsequent assessment (19 and 20 breaths per minute at 10 and 15 minutes, respectively, post-injection).

Subject 108 had multiple ADEs including nausea, tremulousness, lightheadedness, and flushing in the first 3 hours after the test agent administration. Concomitant, clinically significant increases in HR were noted at 5- and 10-minute and 2-hour measurements (114, 108, and 110 bpm, respectively) with a pre-injection heart rate of 86 bpm, along with an increase in body temperature from 96.4^{o}F to 101^{o}F at 2 hours. All values returned to normal at the 24 hour follow-up. The relationship between these events and the test agent was determined to be uncertain by the Investigator.

FS-6000 (n=50)

The ANOVAs testing for equality between OPTISON and human albumin found no statistically significant differences for any of the vital signs.

FS-1250 (n=5)

All vital sign measurements were within normal ranges. Changes were considered not to be clinically significant by the investigator.

FS-3000 and FS-3500 (n=203)

The ANOVAs testing for equality of changes between Albunex and OPTISON found no statistically significant differences for any of the vital signs.

5.6.6 Oxygen saturation

Oxygen saturation measured by pulse oximetry was performed for each of the 6 protocols at these intervals: 2 minutes prior to injection, 2, 4, 6, 8, 10, 20, and 40 minutes post-study.

FS-1000 (n=40)

The statistical significance observed in this study is due to the large number of time points and the small variability and is not considered clinically significant.

One subject (Subject 32) started to cough following the 40.0 mL OPTISON injection and her oxygen saturation decreased from 96% to 87% at 6 minutes post-injection while attempting to inflate the expired air collection bag. The subject stated she was attempting to minimize breathing while ultrasound imaging was performed, but was also being asked to breathe normally into the expired air collection bag. At 8 minutes post-injection, her oxygen saturation returned to 96%.

Table 8: FS-1000 Oxygen Saturations FS-1000

		Oxygen Saturation MIN															
		-2		2		4		6		8		10		20		40	
		MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD	MEAN	STD
Group	Injection																
A (0.5,5mL)	1	0.99	0.01	0.99	0.00	0.99	0.00	0.99	0.01	0.98	0.01	0.97	0.03	0.98	0.02	0.98	0.03
	2	0.99	0.01	0.98	0.01	0.98	0.01	0.98	0.01	0.98	0.00	0.98	0.01	0.98	0.01	0.98	0.01
B (1,10mL)	1	0.98	0.01	0.98	0.02	0.98	0.02	0.98	0.01	0.98	0.01	0.98	0.01	0.98	0.01	0.98	0.01
	2	0.98	0.01	0.98	0.01	0.98	0.02	0.98	0.01	0.98	0.01	0.98	0.01	0.99	0.01	0.98	0.01
C (2,20mL)	1	0.99	0.01	0.98	0.01	0.98	0.01	0.98	0.02	0.98	0.01	0.98	0.01*	0.98	0.01	0.98	0.02
	2	0.98	0.02	0.98	0.00	0.98	0.01	0.98	0.01	0.98	0.01	0.98	0.01*	0.97	0.02	0.98	0.01
D (4,40mL)	1	0.99	0.01	0.99	0.01	0.99	0.01	0.99	0.01	0.99	0.01	0.98	0.01*	0.98	0.01	0.98	0.01
	2	0.98	0.02	0.98	0.01	0.98	0.01	0.97	0.04	0.97	0.02	0.97	0.01*	0.98	0.01	0.98	0.01

FS-1500 (n=10)

There were no statistically significant changes (see table below).

Table 9: Oxygen Saturations FS-1500

	PERCENT	
Minute	MEAN	STD
-2	97.7	0.7
2	97.7	0.7
4	98.0	0.7
6	97.7	0.5
8	96.8	1.9
10	97.4	1.3
15	97.1	1.1

FS-6000 (n=50)

There were no statistically significant changes in oxygen saturations in any of these subjects after receiving 20 mL of OPTISON, which is particularly notable because 30 of these subjects had moderate to severe cardiac (n=10), liver (n=10) or pulmonary (n=10) diseases. It was noted, that in 1 "liver" disease subject, the baseline oxygen saturation was 76% and continued to be stable at 76% throughout the entire injection sequence. The low oxygen saturation level suggests a subject with undiagnosed hepatopulmonary syndrome (HPS); this implies that a significant right to left shunt may have existed within the pulmonary vasculature resulting in a baseline oxygen de-saturation. Of note, in the FS-6000 trial, is that there were no oxygen saturation changes observed in any of the "disease" subjects. The only de-saturation was noted in a "lung" disease subject that received 40 mL of 1% albumin. The subject received 1% serum albumin and experienced an 8.25% decline from pre-injection to 10 minutes post-injection (97-88%). The subject has a medical history of asthma, pneumonia, Legionella, and smoked 1 pack/day for 6 years.

Table 10: Oxygen Saturations FS-6000

		Oxygen saturation % for minutes after injection																					
		-2			2			4			6			8			10			20			40		
		N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD
Agent	Group																								
1% HA	Cardiac	5	94	1	5	95	3	5	96	2	5	94	3	5	94	2	5	93	1	5	95	3	5	95	2
	Hepatic	5	76	43	5	76	43	5	77	43	5	76	43	5	76	43	5	75	42	5	77	43	5	97	1
	Normal	10	97	2	10	98	2	10	98	2	10	97	2	10	97	2	10	97	2	10	96	2	10	98	2
	Resp.	5	95	3	5	95	3	5	95	4	5	94	5	5	77	0	5	93	5	5	94	4	5	94	3
FS069	Cardiac	5	94	1	5	94	1	5	95	2	5	93	1	5	90	7	5	92	3	5	94	3	5	93	3
	Hepatic	5	97	0	5	97	1	5	98	1	5	97	2	5	93	9	5	97	2	5	97	3	5	98	2
	Normal	10	97	2	10	97	3	10	98	3	10	97	3	10	97	3	10	96	3	10	97	2	10	98	2
	Resp.	5	94	3	5	94	2	5	93	1	5	95	3	5	81	9	5	93	3	5	94	3	5	93	2

FS-1250 (n=5)

All oxygen saturation levels were within normal ranges and there were no clinically significant changes from baseline (see table below).

Table 11: Oxygen Saturations FS-1250

	Oxygen Saturation %	
	MEAN	STD
Minutes after injection		
-2	97.00	2.55
2	98.20	1.30
4	98.20	1.30
6	98.00	1.22
8	98.00	1.22
10	98.20	0.84
20	98.20	0.84
40	98.20	0.84

FS-3000 and FS-3500 (OPTISON n=199 subjects and Albunex n=200 subjects)

Overall, there were no clinically relevant or statistically significant changes. Of the 3,636 oxygen measurements in FS-3000, 13 measurements changed (0.4%). Of the 3,672 oxygen measurements in FS-3500, 7 measurements changed (0.2%).

Of note, in 4 OPTISON subjects out of 199 (97 in FS3000 and 102 in FS3500), O_2 saturation dropped transiently and similarly in 4 of 199 OPTISON subjects, O_2 saturation increased. Two of the 4 OPTISON subjects with a transient drop in O_2 saturations were obese).

Table 12: OPTISON: Oxygen Saturation

Clinical Trial	Agent	Decreased Oxygen Saturation	Increased Oxygen Saturation	Comments
FS-3000				
	OPTISON	3117,		COPD, MI, hypertension, smoker
		3121		275lbs, DM, MI, nicotine and alcohol use
		3416		Nicotine and alcohol use, valve dx, hypertension, MI
			3113	228 lbs, DM, history of shortness of breath
	Albunex	3118		234lbs, pleural effusion
FS-3500				
	OPTISON	5319		COPD, pneumonia
			5111	221lbs, sleep apnea, DM,
			5305	83 years of age
			5410	Chronic bronchitis, pneumonia, CAD

The FS3000 and FS3500 clinical studies required the subjects to rest in the supine position for over 2 hours. It is now known that the prevalence of obstructive sleep apnea (OSA) is increased in obese subjects and estimated to be 38-50%. Overall in the US, the prevalence of OSA in the US is 6% with an additional 2% undiagnosed.[Richman RM, et al 1994] [Kripke DF et al. 1997]

Summary of FS-3000 and FS-3500

The observation that 4 OPTISON subjects and 1 Albunex subject transiently decreased their oxygen saturations (>7.5%), whereas, 4 OPTISON increased their oxygen saturations (>7.5%) are considered random fluctuation that may be related to the undiagnosed presence of OSA and/or variability of oxygen saturation measurements determined in subjects that are required to rest in the supine position for over 2 hours.

Table 13: Summary of Oxygen Saturation Values FS-3000

	Oxygen saturation % for minutes after in																							
	-2			2			4			6			8			10			20			30		
	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD
Agent (Dose)																								
ALBUNEX (0.08 ml/kg)	102	96	3	101	96	3	101	96	3	101	96	3	101	96	3	0	.	.	0	.	.	0	.	.
ALBUNEX (0.22 ml/kg)	101	96	2	101	95	3	101	96	3	101	96	3	101	96	2	101	96	3	101	96	3	101	96	2
FS069 (0.20 mL)	97	96	3	97	96	3	97	96	3	97	96	3	96	96	3	0	.	.	0	.	.	0	.	.
FS069 (0.50 mL)	97	96	3	97	96	3	97	96	3	97	96	3	96	96	3	0	.	.	0	.	.	0	.	.
FS069 (3.0 mL)	97	96	2	97	95	3	97	96	3	97	96	3	97	96	3	0	.	.	0	.	.	0	.	.
FS069 (5.0 mL)	96	96	3	96	95	3	96	95	3	96	96	3	96	96	3	96	96	3	96	96	2	95	96	2

Table 14: Clinically Significant Oxygen Saturation Changes FS-3000

PATIENT	TX	INJ	MINUTE	BASE	VALUE	PCCHG
3113	F	4	30	87.00	94.00	8.05
3117	F	3	8	95.00	87.00	8.42
3117	F	4	2	84.00	96.00	14.29
3117	F	4	4	84.00	96.00	14.29
3117	F	4	6	84.00	96.00	14.29
3117	F	4	8	84.00	95.00	13.10
3117	F	4	10	84.00	95.00	13.10
3117	F	4	20	84.00	95.00	13.10
3117	F	4	30	84.00	95.00	13.10
3118	A	2	2	92.00	85.00	7.61
3118	A	2	6	92.00	85.00	7.61
3121	F	3	2	88.00	80.00	9.09
3416	F	4	4	94.00	86.00	8.51

Table 15: Summary of Oxygen Saturation Values FS-3500

	Oxygen saturation % for minutes after in																							
	-2			2			4			6			8			10			20			30		
	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD
ALBUNEX (0.08 ml/kg)	99	96	3	99	95	3	97	96	3	98	96	3	95	95	3	0	.	.	0	.	.	0	.	.
ALBUNEX (0.22 ml/kg)	99	95	3	99	95	3	99	96	3	99	96	3	99	96	3	99	96	3	99	96	3	99	96	3
FS069 (0.20 mL)	102	95	3	102	95	3	102	95	3	99	95	3	100	95	3	0	.	.	0	.	.	0	.	.
FS069 (0.50 mL)	102	95	3	102	95	3	102	95	3	102	95	3	102	95	3	0	.	.	0	.	.	0	.	.
FS069 (3.0 mL)	102	95	3	102	95	3	102	95	3	102	95	3	102	95	3	0	.	.	0	.	.	0	.	.
FS069 (5.0 mL)	102	95	3	102	95	3	102	95	3	102	95	3	102	95	3	102	96	3	101	96	3	102	96	3

Table 16: Clinically Significant Oxygen Saturation changes FS-3500

PATIENT	TX	INJ	MINUTE	BASE	VALUE	PCCHG
5111	F	4	2	88.00	95.00	7.95
5305	F	2	6	92.00	99.00	7.61
5319	F	4	30	94.00	85.00	9.57
5410	F	4	4	90.00	97.00	7.78
5410	F	4	6	90.00	97.00	7.78
5410	F	4	10	90.00	97.00	7.78
5410	F	4	30	90.00	97.00	7.78

5.6.7 Clinical Laboratory Evaluation

Clinical Laboratory

There were no clinically significant changes noted in any of the laboratory values in any of the 6 clinical trials.

The following clinical laboratory tests were performed for all-protocols: CPK isoenzymes, hematology, chemistry, PT, PTT, and a microscopic examination of the smear. For Protocols FS-1000, FS-1500, and FS1250, these laboratory parameters were evaluated pre-study, 2 and 24 hours post-study. For Protocols FS-6000, FS-3000 and FS-3500, a routine urinalysis was also included and the intervals measured were pre-study, 30 minutes and 48 hours post-study.

FS-1000 (n=40)

Forty clinical laboratory variables were tested by repeated measures ANOVA; there were no clinically meaningful changes in any of the 40 subjects.

FS-1500 (n=10)

There were 2 subjects (Subject 101 and Subject 110) in whom 2 clinically significant but not clinically meaningful changes in laboratory values occurred. Subject 101 had a normal baseline total bilirubin (0.8 mg/dL), which rose to 1.3 mg/dL at 24 hours. This was within 0.1 mg/dL of the ULN (normal range 0.1-1.2 mg/dL). No other liver function tests in this subject were abnormal.

Subject 110 had a slightly elevated Total CPK at 2 hours (157 U/L), the normal range for females being 26-140 U/L. This subject also reported 5 ADEs including nausea, dizziness and mild retching which may have resulted in this slight increase in Total CPK. No myocardial (MB) CPK was detected. All symptoms resolved completely. The Total CPK at 24 hours was within normal limits.

FS-6000 (n=50)

All of the differences were small and not clinically meaningful, and demonstrate that neither OPTISON nor 1% human albumin were associated with large changes or trends in laboratory values.

FS-1250 (n = 5)

No changes in any of the laboratory measurements were clinically significant.

FS-3000 and FS-3500 (n = 203)

All of the differences were small and not clinically meaningful, and demonstrate that neither OPTISON nor Albunex were associated with large changes or trends in laboratory values.

5.7 Immunology

Immunological testing was performed on a total of 71 subjects from 3 protocols, FS-l 000 (n=16), FS-1250 (n=5) and FS-6000 (n=50). Forty-six (46) of the subjects received i.v. OPTISON and 25 subjects received intravenous 1% human albumin. All immunological analyses were performed by an independent, expert reviewer: Calvin A. Saravis, Ph.D., Associate Professor of Surgery, Harvard Medical School, Boston, Massachusetts.

All participants received an intradermal injection of 0.02 mL OPTISON or HA (skin test) approximately 24-hours prior to the study injection. Clinical observations were made for erythema, indurations, and pruritus. Blood samples were drawn for purposes of immunological testing at the following intervals:

- 24 hours pre-study (prior to intradermal skin test)
- 1 hour prior to intravenous study injection
- 2 hours post-study
- 1 week post-study
- 2 weeks post-study
- 3 weeks post-study

Plasma samples were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique for the following immunoglobulins: IgG, IgA, IgM, IgD and IgE in the 3 protocols.

FS-l000 (n=40)

A group of 16 of the 40 subjects (2 male and 2 female subjects from each of the 4 dose groups) were tested. None of the 16 subjects demonstrated an immunological response to administration of OPTISON for any of the immunoglobulins.

FS-1250 (n=5)

Protocol FS-1250 was designed to test the feasibility of OPTISON re-injection. Five of the 16 subjects tested in Protocol FS-1000 were enrolled into Protocol FS-1250 approximately 1 year later. Re-injection within a short period of time is not as challenging as re-injection after a period of time has passed. With time, if there are specific antibody producing cells, they tend to have a higher avidity and affinity, and give greater immunological findings. In addition to the immunoglobulins tested, cytokines (Interleukin- l alpha, Interleukin-2 and TNF-α) and complement activations (iC3b and SC56-9) were also tested in this protocol.

Subject 103 had a mild skin reaction to the 0.02 mL OPTISON intradermal injection and did not receive the 20.0 mL intravenous injection. He did complete all remaining safety evaluations and blood draws for immunological testing to 3 weeks. Immunological testing showed Subject 103 had no specific antibodies to OPTISON. Cytokine and complement activation concentrations in this subject did not significantly increase. Further skin testing in this subject with saline and histamine gave normal reactions. The cause of the mild skin test reaction is unknown, but is not associated with OPTISON related reactions, cytokine release or complement activation.

No significant increases of the individual immunoglobulins tested were found in any of the 5 subjects. Additionally, no significant increases above the normal ranges for cytokines and complement activations were seen in any of the 5 subjects.

The laboratory findings of this study clearly demonstrate, there were no cells making specific antibodies, of any class, to OPTISON.

FS-6000 (n=50)

A total of 20 healthy volunteers and 30 subjects with known disease (10 cardiac, 10 hepatic, and 10 respiratory subjects) were enrolled into this study. Of the 50 enrolled subjects, 25 received 20.0 mL OPTISON and 25 received 20.0 mL 1% human albumin according to a randomization schedule. In addition to the immunoglobulins tested, cytokines (Interleukin-l alpha, Interleukin-2 and TNF-α) and complement activations (iC3b and SC5b-9) were also tested. There was no evidence of antibody production to OPTISON, cytokine production or complement activation in any of the 50 subjects.

5.8 Expired Air

FS-1000 (n=40)

Pharmacokinetics (PK) analysis of PFP elimination in expired air was performed in all 40 subjects following the second OPTISON injection (5.0, 10.0, 20.0 or 40.0 mL) in each of the 4 dose groups. Analysis of the elimination constant (KEL), volume of distribution (L/kg) and elimination half-life ($t_{1/2}$) were performed by an independent, expert reviewer: Roger W. Jelliffe, M.D., Professor of Medicine, Director. Laboratory of Applied Pharmacokinetics, University of Southern California, Los Angeles, California.

The mean elimination constant was 0.8 minutes for the 40.0 mL dose group and 1.0 minute for the 5.0, 10.0 and 20.0 mL dose groups. Mean volume of distribution was 0.4 L/kg for the 40.0 mL dose group and 0.3 L/kg for the other dose groups. Mean elimination half-life was 0.93 minutes for the 40.0 mL dose group and 0.68 minutes for the other dose groups.

FS-1500 (n=10)

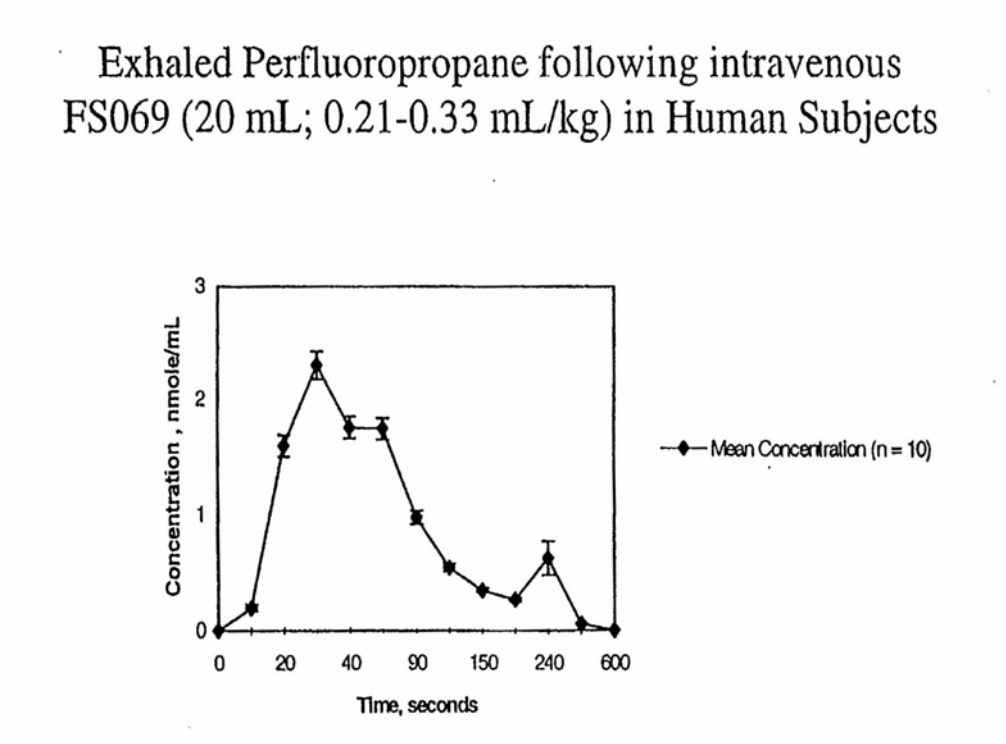
Protocol FS-1500 was a mass balance study performed in 10 healthy volunteers each receiving 20.0 mL of OPTISON. The objectives of the trial were to determine the amount of PFP in parts per million over time in blood and expired air as a measure of clearance from the body and to determine the half-life and recovery in expired air. Expired air (20 mL) was collected upon exhalation and PFP concentrations were measured at approximately the following intervals: 180 seconds prior to injection and 10, 20, 30, 40, 60, 90, 120, 150, 180, 240, 360, and 600 seconds following the start of the OPTISON injection. Blood samples (l0 mL) were collected and PFP concentrations were measured at approximately the following intervals: 180 seconds prior to injection and 20, 60, 120, and 600 seconds following the start of the OPTISON injection. Calculation of the percent recovery and rate of elimination of PFP in expired air was analyzed by an independent, expert reviewer: Joachim Grevel, Ph.D., President of BAST, Inc., 609 Cross Park Drive, Austin, Texas.

Subject 103 had leaky air container bags for the samples taken at 20 and 30 seconds. Calculations were made both with and without Subject 103.

The 90% confidence interval (CI) calculated for recovery using the log-transformed data including all subjects was 80.45% to 108.37% with a point estimate of 93.88%. The 90% confidence interval calculated for log-transformed recovery data without Subject 103 was 86.74% to 111.80% with a point estimate of 98.47%. From a clinically relevant maximal intravenous dose of OPTISON (20 mL at 1 mL/second, 0.21 to 0.33 mL/kg) in normal human subjects, the PFP component of OPTISON was rapidly and nearly completely eliminated in less than 10 minutes with a pulmonary elimination half-life of 1.3 minutes. The PFP levels detected in blood following this dosage were so low and transient that PK parameters could not be accurately determined, even though the sensitivity of the assay was 2 ppm.

Figure 9 presents PFP elimination following 20.0 mL OPTISON in exhaled air.

Figure 9 PFP Elimination Following 20.0 mL OPTISON in Exhaled Air



5.9 Adverse Events and Complications

There were no SAEs or serious complications observed in any of the 6 clinical trials. There were no deaths associated with OPTISON or Albunex; however, 2 subjects died post-study and the details are provided below.

One subject in study FS-3000 presented with a medical history of congestive heart failure and Hodgkin's lymphoma. The series of 4 OPTISON injections was completed prior to the series of 2 Albunex injections. The subject expired 2 months and 5 days following the final injection of Albunex, secondary to a pulmonary thromboembolism. Immediately prior to death, the subject had undergone pulmonary surgery for recurrent plural effusion. The investigator attributed the death to the underlying severe pathology.

One subject in study FS-3500 died from head and neck cancer 2 days after study completion. The subject was diagnosed with head and neck cancer 5 years prior to study start, which was treated with surgical resection and radiation therapy. He was receiving radiation therapy for metastatic lesion to the spine. The Investigator noted that the subject had no adverse effects and no changes in vital signs, oxygen saturation or laboratory values that could be attributed to

OPTISON. The investigator considered the death unrelated to OPTISON and related to the subject's terminal, metastatic disease.

5.9.1 Overview of adverse events

Table 17 summarizes the AEs experienced by subjects in all clinical trials. The data presented in the table (Costart system) represent the number of subjects reporting 1 or more events following the administration of OPTISON and are pooled for those events experienced immediately following to 48 hours following OPTISON administration (on study and post study events). The character of AEs was mild or moderate in severity and transient in nature, and frequently determined to be unrelated to OPTISON.

Table 17: Adverse Events In All Participants

Adverse Events in All Participants Exposed to Optison				
Body System	**Phase III Trials (n = 199)**		**All Trials (Phase I, II & III) (n = 279)**	
	No.	**%**	**No.**	**%**
Body as a whole	13/199	6.5	47/279	16.8
Cardiovascular System	8/173	4.6	11/182	6.0
Digestive System	6/70	8.6	14/85	16.5
Endocrine System	3/73	4.1	3/77	3.9
Hemic and Lymphatic System	1/17	5.9	2/20	10.0
Musculoskeletal System	4/55	7.3	15/78	19.2
Nervous System	4/34	11.8	6/39	15.4
Respiratory System	8/79	10.1	14/92	15.2
Skin and Appendages	3/30	10.0	7/36	19.4
Special Senses	4/50	8.0	19/72	26.4
Urogenital System	2/56	3.6	9/74	12.2

5.9.2 Subjects with impaired pulmonary or cardiac function

Subjects with impaired pulmonary function and impaired cardiac function were enrolled in various studies. The chronic pulmonary disease subgroup included subjects with chronic bronchitis, emphysema, pulmonary hypertension, bronchiectasis, and asthma. The congestive and dilated cardiomyopathy subgroup included subjects having a left ventricular ejection fraction between 20 and 40% by 2-D echocardiography. Table 18 presents the AEs reported by subjects in these subgroups and the rates are considerably lower than in the total population allowing one to conclude that the safety profile of OPTISON is very good even in high risk subject groups.

Table 18: Adverse Events in all Impaired Function Patients

Adverse Events in All Impaired Function Patients Exposed to Optison								
Body System	**Phase III Trials**				**All Trials (Phases I, II & III)**			
	Impaired		**Not Impaired**		**Impaired**		**Not Impaired**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
CARDIAC IMPAIRMENT								
Body as a whole	1/ 34	2.9	12/165	7.3	1/ 34	2.9	46/245	18.8
Cardiovascular System	1/ 32	3.1	7/141	5.0	1/ 32	3.1	10/150	6.7
Digestive System	0/ 15	0.0	6/ 55	10.9	0/ 15	0.0	14/ 70	20.0
Endocrine System	1/ 16	6.3	2/ 57	3.5	1/ 16	6.3	2/ 61	3.3
Hemic and Lymphatic System	0/ 6	0.0	1/ 11	9.1	0/ 6	0.0	2/ 14	14.3
Musculoskeletal System	0/ 13	0.0	4/ 42	9.5	0/ 13	0.0	15/ 65	23.1
Nervous System	0/ 8	0.0	4/ 26	15.4	0/ 8	0.0	6/ 31	19.4
Respiratory System	1/ 30	3.3	7/ 49	14.3	1/ 30	3.3	13/ 62	21.0
Skin and Appendages	0/ 5	0.0	3/ 25	12.0	0/ 5	0.0	7/ 31	22.6
Special Senses	0/ 11	0.0	4/ 39	10.3	0/ 11	0.0	19/ 61	31.1
Urogenital System	0/ 10	0.0	2/ 46	4.3	0/ 10	0.0	9/ 64	14.1
PULMONARY IMPAIRMENT								
Body as a whole	2/ 47	4.3	11/152	7.2	5/ 53	9.4	42/226	18.6
Cardiovascular System	2/ 47	4.3	6/126	4.8	2/ 49	4.1	9/133	6.8
Digestive System	1/ 17	5.9	5/ 53	9.4	3/ 19	15.8	11/ 66	16.7
Endocrine System	2/ 22	9.1	1/ 51	2.0	2/ 24	8.3	1/ 53	1.9
Hemic and Lymphatic System	n/a*	n/a	1/ 17	5.9	0/ 1	0.0	2/ 19	10.5
Musculoskeletal System	0/ 10	0.0	4/ 45	8.9	3/ 15	20.0	12/ 63	19.0
Nervous System	1/ 9	11.1	3/ 25	12.0	1/ 10	10.0	5/ 29	17.2
Respiratory System	2/ 21	9.5	6/ 58	10.3	5/ 27	18.5	9/ 65	13.8
Skin and Appendages	0/ 8	0.0	3/ 22	13.6	0/ 10	0.0	7/ 26	26.9
Special Senses	0/ 10	0.0	4/ 40	10.0	3/ 13	23.1	16/ 59	27.1
Urogenital System	0/ 13	0.0	2/ 43	4.7	2/ 18	11.1	7/ 56	12.5

*n/a = not applicable (e.g., no subjects with underlying condition)

AE rates are displayed in Table 19 for impaired cardiac function subjects grouped by severity of disease as categorized by ejection fraction. In the subjects with more severely reduced left ventricular function, AE rates following OPTISON are significantly lower than in subjects with normal function (0.5% vs. 19.2%).

Table 19: Adverse Events in All Participants by Baseline Ejection Fraction

Adverse Events in All Participants Exposed to Optison Grouped by Baseline Ejection Fraction												
Body System	**All Trials (Phases I, II & III)**											
	< 20%		**20-30%**		**31-40%**		**41-50%**		**> 50%**		**Unknown†**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Body as a whole	0/ 5	0.0	1/ 20	5.0	0/ 8	0.0	0/ 4	0.0	45/234	19.2	1/ 8	12.5
Cardiovascular System	0/ 5	0.0	1/ 20	5.0	0/ 8	0.0	0/ 4	0.0	6/128	4.7	1/ 8	12.5
Digestive System	0/ 2	0.0	1/ 7	14.3	0/ 3	0.0	n/a	n/a	5/ 55	9.1	0/ 3	0.0
Endocrine System	n/a	n/a	1/ 9	11.1	0/ 5	0.0	0/ 2	0.0	1/ 52	1.9	1/ 5	20.0
Hemic and Lymphatic System	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1/ 17	5.9	n/a	n/a
Musculoskeletal System	0/ 2	0.0	0/ 1	0.0	0/ 3	0.0	0/ 1	0.0	4/ 46	8.7	0/ 2	0.0
Nervous System	n/a	n/a	1/ 3	33.3	0/ 3	0.0	0/ 1	0.0	3/ 26	11.5	0/ 1	0.0
Respiratory System	0/ 2	0.0	1/ 8	12.5	0/ 4	0.0	0/ 2	0.0	6/ 59	10.2	1/ 4	25.0
Skin and Appendages	0/ 1	0.0	0/ 2	0.0	0/ 1	0.0	0/ 2	0.0	3/ 23	13.0	0/ 1	0.0
Special Senses	n/a	n/a	0/ 5	0.0	n/a	n/a	0/ 2	0.0	4/ 41	9.8	0/ 2	0.0
Urogenital System	0/ 1	0.0	0/ 5	0.0	0/ 3	0.0	0/ 1	0.0	2/ 44	4.5	0/ 2	0.0

* n/a = not applicable (e.g., no subjects were eligible for inclusion for analysis)

† This group contains 8 patients from the impaired cardiac function subgroup in whom a quantitative EF was not provided on the echocardiogram report to allow subcategorization into this table.

Table 20 presents AE rates in impaired pulmonary function subjects grouped by severity of disease as categorized by baseline oxygen saturation. Subjects with more severe pulmonary dysfunction have lower AE rates than the total population following OPTISON administration.

Table 20: Adverse Events in All Participants by Percent O2 Saturation

Adverse Events in All Participants Exposed to Optison by Percent of O_2 Saturation												
Body System	Phase III Trials						All Trials (Phase I, II & III)					
	80-89		90-95		>95		80-89		90-95		>95	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Body as a whole	0/ 5	0.0	6/ 74	8.1	7/120	5.8	0/ 5	0.0	11/ 85	12.9	36/189	19.0
Cardiovascular System	0/ 5	0.0	5/ 69	7.2	3/ 99	3.0	0/ 5	0.0	6/ 74	8.1	5/103	4.9
Digestive System	0/ 1	0.0	4/ 33	12.1	2/ 36	5.6	0/ 1	0.0	7/ 37	18.9	7/ 47	14.9
Endocrine System	0/ 2	0.0	3/ 36	8.3	0/ 35	0.0	0/ 2	0.0	3/ 38	7.9	0/ 37	0.0
Hemic and Lymphatic System	0/ 1	0.0	1/ 8	12.5	0/ 8	0.0	0/ 1	0.0	1/ 10	10.0	1/ 9	11.1
Musculoskeletal System	n/a	n/a	2/ 27	7.4	2/ 28	7.1	n/a	n/a	5/ 33	15.2	10/ 45	22.2
Nervous System	n/a	n/a	2/ 19	10.5	2/ 15	3.3	n/a	n/a	2/ 20	10.0	4/ 19	21.1
Respiratory System	0/ 4	0.0	5/ 44	11.4	3/ 31	9.7	0/ 4	0.0	7/ 49	14.3	7/ 39	17.9
Skin and Appendages	0/ 3	0.0	3/ 13	23.1	0/ 14	0.0	0/ 3	0.0	3/ 15	20.0	4/ 18	22.2
Special Senses	0/ 3	0.0	2/ 19	10.5	2/ 28	7.1	0/ 3	0.0	7/ 24	29.2	12/ 45	26.7
Urogenital System	0/ 2	0.0	0/ 27	0.0	2/ 27	7.4	0/ 2	0.0	1/ 32	3.1	8/ 40	20.0

5.9.3 Phase 3 studies

FS-3000

Fourteen of the 101 subjects (13.9%) enrolled in this Phase 3 clinical trial experienced ADEs. A total of 33 ADEs were experienced, with 20 ADEs occurring with the administration of OPTISON and 13 occurring with the administration of Albunex.

Subject 706 experienced transient altered taste after each of the 4 OPTISON injections. Eosinophilia was listed as an ADE (uncertain if related) for Subject 209 during 2 concurrent laboratory draws after OPTISON administration. Finally, discomfort at injection site occurred twice for Subject 502. The Investigator considers this ADE related to the injection procedure because of the volume injected.

The most frequently experienced ADEs were transient altered taste (4 events reported with 3 related to the injection, 1 related to the procedure) and headache (3 reported with 1 related to the injection and 2 uncertain). ADEs considered not related and experienced once following OPTISON administration include: atypical chest pain, dyspnea, and left elbow cut. ADEs experienced only once with OPTISON administration and considered related to the injection included: flushing, warmth, and warm sensation in arm.

The ADEs experienced and attributable to the Albunex injection include: transient altered taste (4 events), ecchymosis at i.v. site (2 events; 1 was related to the procedure), and mouth dryness (1 event). ADEs considered not related to Albunex administration and experienced once include: cellulitis lower extremities, chest pain, conduction disorder, headache, and worsening heart failure.

FS-3500

Twelve of the 102 (11.7%) subjects experienced AEs. Five subjects experienced AEs with the OPTISON administration that included 2 AEs attributed as related to the injection, abnormal taste (1 event) and flushing (1 event). One event of headache was considered related to the procedure. One event of dyspnea was considered uncertain if related.

Eleven AEs were experienced with Albunex administration. Two events were considered uncertain if related and included: chest discomfort and flu-like symptoms with nausea and vomiting. Four AEs were experienced with Albunex administration and were considered not related. They include: chest pain, lethargy, decreased urinary stream, and gross hematuria. The remaining 5 AEs were considered related to the injection: pain at injection site (2 events); flushing (1 event), nausea (1 event), and warmth at injection site (1 event).

Table 21 provides a combined summary of the subjects experiencing adverse events in the Phase 3 studies (FS-3000 and FS03500) and compares those events to the adverse events reported for 370 subjects in the Albunex PMA.

Table 21: Summary of Overall Adverse Events for OPTISON and ALBUNEX Protocols FS-3000/3500 and ALBUNEX PMA studies

	3000/3500 (199 Patients) FS069 Overall		3000/3500 (200 Patients) ALBUNEX Overall		ALBUNEX PMA (370 Patients) On-Study		ALBUNEX PMA (370 Patients) Post-Study	
Description	n	%	n	%	n	%	n	%
Transient altered taste	5	2.5	4	2.0	16	4.3	3	0.8
Headache	4	2.0	1	0.5	1	0.3	7	1.9
Warm sensation/ flushing	4	2.0	2	1.0	3	0.8	1	0.3
Dyspnea	2	1.0	0	0	0	0	2	0.5
Eosinophilia	1	0.5	0	0	0	0	0	0
Discomfort at injection site	1	0.5	0	0	0	0	0	0
Chest pain	1	0.5	1	0.5	1	0.3	1	0.3
Left elbow cut	1	0.5	0	0	0	0	0	0
Pain at injection site	0	0	2	1.0	1	0.3	0	0
Arthralgia	0	0	1	0.5	0	0	0	0
Asthenia	0	0	0	0	0	0	0	0
Body/ muscle aches	0	0	0	0	1	0.3	3	0.8
Calming sensation	0	0	0	0	1	0.3	0	0
Cellulitis/ lower extemities	0	0	1	0.5	0	0	0	0
Chest discomfort	0	0	2	1.0	0	0	2	0.5
Chills and fever	0	0	0	0	0	0	0	0
Conduction disorder	0	0	1	0.5	0	0	0	0
Cont. dermatitis	0	0	0	0	2	0	0	0
Decreased urinary stream	0	0	1	0.5	0	0	0	0
Depression	0	0	0	0	0	0	1	0.3
Diaphoresis	0	0	0	0	0	0	2	0.5
Diarrhea	0	0	0	0	0	0	1	0.3
Dry mouth	0	0	1	0.5	0	0	0	0
Ecchymosis I.V. site	0	0	2	1.0	0	0	0	0
Epigastric burning	0	0	0	0	1	0.3	0	0
Flatulence	0	0	0	0	0	0	0	0
Flu-like symptoms	0	0	1	0.5	0	0	0	0
Gross hematuria	0	0	1	0.5	0	0	0	0
Hand cramping	0	0	0	0	1	0.3	1	0.3
hypoglycemia	0	0	0	0	0	0	1	0.3
I.V. infiltration	0	0	0	0	2	0.5	0	0
Increased thirst	0	0	0	0	0	0	1	0.3
Lightheaded/ dizzy	0	0	0	0	4	1	6	1.6
Malaise,weakness,fatigue	0	0	0	0	1	0.3	3	0.8
Mild hematoma	0	0	0	0	0	0	1	0.3
Nausea	0	0	1	0.5	0	0	3	0.8
Numbness distal to I.V.	0	0	0	0	0	0	2	0.5

Table 21 Cont'd Summary of Overall Adverse Events for OPTISON and ALBUNEX Protocols FS-3000/3500 and ALBUNEX PMA studies

	3000/3500 (199 Patients) FS069 Overall		3000/3500 (200 Patients) ALBUNEX Overall		ALBUNEX PMA (P900059) (370 Patients) On-Study			Post-Study
Description	n	%	n	%	n	%	n	%
Pruritis/ rash	0	0	0	0	0	0	2	0.5
Palpitations	0	0	0	0	0	0	4	1.1
Skin eruptions	0	0	0	0	0	0	3	0.8
Lethargy	0	0	1	0.5	0	0	0	0
Tachycardia	0	0	0	0	0	0	1	0.3
Tingling at injection site	0	0	0	0	1	0.3	0	0
Tingling sensation in chest	0	0	0	0	1	0.3	0	0
Tremor	0	0	0	0	1	0.3	0	0
Vasodilatation	0	0	0	0	0	0	0	0
Visual changes/ blurring	0	0	0	0	1	0.3	3	0.8
Worsening heart failure	0	0	1	0.5	0	0	0	0

n = Number of patients reporting each adverse device event listed above. (A subject experiencing the same event more than once is only listed once.)

Table 22 summarizes the number of ADEs for the 6 protocols. The incidence rates for ADEs are higher in Protocols FS-1000, FS-1250, FS-1500 and FS6000 than for Protocols FS-3000 and FS-3500. We believe this is most likely due to the higher single volumes (20 to 40 mL) studied in the first 4 studies while in the Phase 3 studies (FS-3000 and FS-3500) the highest single volume was 5.0 mL

Table 22: Summary of Adverse Events Incident Rate by Protocol

Protocol	Number of Patients	Test Agent (n)	Incidence Rate (%)
FS-1000	40	FS069 (40)	37.5
FS-1500	10	FS069 (10)	50.0
FS-6000	50	FS069 (25) Human Albumin (25)	44.0 32.0
FS-1250	5	FS069 (5)	60.0
FS-3000	101	FS069 (97)* ALBUNEX (101)	9.3 8.9
FS-3500	102	FS069 (102) ALBUNEX (99)**	3.9 9.1

*Four patients did not return for FS069 injection.
**Three patients did not return for ALBUNEX injection.

The initial recommended dose volume for OPTISON in the Package Insert is 0.5 to 3.0 mL. The ADE incidence rates reported in the package insert are from the combined results of Protocols FS-3000 and FS-3500. Even though these protocols included single doses of 0.2 and 5.0 mL in series, it is not possible to separate out ADEs by single dose.

In the 2 identical Phase 3 studies, the overall incidence rates of ADEs were 6.5% for OPTISON and 9.0% for Albunex. The Albunex ADE rate from the Phase 3 trials (9.0%) is very similar to the ADE rate from the Albunex PMA clinical trials (9.5%). In general, the ADEs were minor, transient and resolved without medical intervention. Based on these results, OPTISON and Albunex are safe for intravenous use in the recommended doses.

The incidence rates for Protocols FS-l 000, FS-1500, FS-6000 and FS-1250 include ADEs reported for single OPTISON doses of 20 and 40 mL (6-14 times the recommended dose for cardiac function).

5.10 Independent Review of OPTISON

The original OPTISON submission included a report from a review done by an Independent Safety Review Committee. The committee members included the following:

Robert M. Califf, MD

Eric D. Peterson, MD, MPH

Karen Patton Alexander, MD

Leslee J. Shaw, PhD

Their conclusion was "overall, the experience with OPTISON in 279 subjects shows it to be safe and comparable in mild side effects to other contrast agents like Albunex. A majority of the ADEs recorded were clearly unrelated to this agent and were likely due to anxiety or to the procedure. Based upon our review of summary data on 308 subjects (279 of whom received OPTISON), we have found the experience with OPTISON to be safe and acceptable for use in the general medical community. By avoiding subjects with a known hypersensitivity to albumin, and using OPTISON in a medical environment, we feel sure that the continued experience will be positive and safe for subjects."

5.11 Overall Discussion

Three protocols evaluated the immunological response of i.v. OPTISON in both healthy volunteers and subjects. Results in all subjects consistently demonstrated a lack of any immunological response to administration of OPTISON for the immunoglobulins IgG, IgA, IgM, IgD and IgE. Additionally, there was no evidence of antibody production to OPTISON, cytokine production or complement activation in any of the subjects evaluated.

A mass balance study evaluating the recovery and rate of elimination demonstrated the PFP component of OPTISON is rapidly and nearly completely eliminated in less than 10 minutes with a pulmonary elimination half life of 1.3 minutes. The PFP levels detected in blood were so low and transient that PK parameters could not be accurately determined. This study was performed with a 20.0 mL dose of OPTISON which is over 2 times the maximum dose recommended for clinical use in the approved package insert.

Comprehensive safety evaluations including 12-lead ECGs, physical examination, vital signs, oxygen saturation, hematology, chemistry panel, CPK isoenzymes, PT, and PTT were performed in the 6 clinical studies pre and post administration of OPTISON. The Phase 3 trials had 5 intervals of safety evaluations covering pre-study, 30 minutes and 48 hours following administration of both Albunex and OPTISON. Although statistically significant changes were observed, none were considered clinically meaningful. Few clinically significant changes occurred in the 308 subjects, none of which required the Investigator to discontinue dosing or discontinue the subject from additional study days in the Phase 3 trials.

ADEs were assessed in all protocols. Incidence rates in the Phase 1 and Phase 2 trials were higher than in the Phase 3 trials as the doses administered were up to 5 times higher (20.0 to 44.0 mL) than the doses recommended for clinical use (0.5 mL). For the Phase 3 trials, the overall ADE incidence rate for OPTISON was 6.5% following a cumulative administration of 8.7 mL (0.2, 0.5, 3.0, 5.0 mL). For Albunex, the overall ADE incidence rate was 9.0% following cumulative administration of 0.30 mL/kg (0.08, 0.22 mL/kg).

An Independent Review of the safety data on all 308 subjects enrolled in clinical trials included in the NDA was conducted by a team from Duke University headed by Robert Califf, M.D. Their conclusions were that intravenous OPTISON, in the doses recommended for clinical use, was safe as evidenced by the type, severity and lack of significant ADEs, the majority of which required no intervention and resolved spontaneously. Additionally, their review concluded that the changes observed in the safety evaluations were within the expected range of variation for cardiac subjects, without statistically significant difference from Albunex which had been used in over 25,000 subjects. Finally, the overall ADE incidence rate for OPTISON is less than Albunex and the recommended dose is approximately half (8.7 mL OPTISON) of that for Albunex (15.0 mL).

Overall, in the 6 clinical trials, OPTISON was found to be safe in the dose ranges evaluated. Additionally, re-injection with intravenous OPTISON 1 year later was demonstrated to be safe as there were no cells making antibodies, of any class, to OPTISON.

5.12 References

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6 OTHER SAFETY DATA

6.1 OPTISON Post-Marketing Surveillance

6.1.1 Introduction

OPTISON was introduced in the US market in December 1997 by Mallinckrodt. The NDA was acquired by GE Healthcare in December 2002, at which time the company assumed pharmacovigilance obligations. This report provides an overview of safety data from initial marketing through 07 May 2008.

6.1.2 Method

GE Healthcare Global Pharmacovigilence (GPV) retrieved all OPTISON ADE reports from the global Safety Database.

6.1.3 GE healthcare pharmacovigilence database

GPV uses an electronic database software to record, store, analyze, and report, ADE reports for all company products, hereafter referred to as the GPV database. After acquiring the NDA, GE Healthcare received all post-marketing ADE reports from Mallinckrodt and entered them into the GPV database. This database was searched using the company drug name " OPTISON " to identify cases for inclusion in this report.

6.1.4 World-wide published literature

GPV subscribes to on-line literature alerting services. These alerting services automatically send GPV product-specific lists of citations by company-defined search criteria. These lists are then reviewed to identify relevant publications concerning Safety Data for the subject product. If an ADE report is identified, GPV obtains a copy of the published article and enters the case into the global PV Database. Such cases are included in the search criteria for this report.

6.1.5 Results

The global PV database contains 57 ADE reports for OPTISON. Details of serious ADEs are provided in the narratives, below. Non-serious ADEs are listed in Table 23.

Manufacturer UCN	Date received	Country
Reaction terms (MedDRA Preferred Term)		
EVENT DESCRIPTION		
optn-pr-0206s-0001	**19 June 2002**	**USA**
Nausea Vomiting Vasodilation Pulmonary edema Hypoxia Hypotension Chest pain Anaphylactic shock		
The following was reported by Amersham Health: "This incident occurred at Abbott Northwestern Hospital on Thursday, 23 May 2002. An in-patient was given Optison for LVO and within 5 minutes became diaphoretic. Once returned to her room, she became nauseated, vomited, and her BP plummeted to a systolic reading of 60 mmHg. Immediately she was taken to the cath lab where her coronaries were normal, but the study indicated she was vasodilated with increased cardiac output. A spiral CT scan was negative. She was returned to her room on a dopamine drip and within 12 hours began to feel better."		
Subject was contacted by Mallinckrodt on 05 June 2002: the subject was a 64 year old female originally hospitalized for a near syncopal episode on 22 May 2002. Subject has known atrial fibrillation. The subject had an ACD to control the atrial fibrillation, but the ACD had discharged and the subject had cardioverted to normal sinus rhythm on admission. On 31 May 2002, subject had Optison (lot number unknown) for an echocardiogram. Twenty minutes after the subject had returned to her hospital room, subject did not feel well; she had nausea and vomiting. She had a drop in BP and oxygen saturation (59%), she had evidence of flash pulmonary oedema (lung crackles) she was sent to cardiac cath as MI was suspected. Her arteries were clear but there was evidence of a systemic reaction with vasodilated vessels. She was given dopamine on 24 May 2002, she was feeling better and was sent to have a spiral CT scan with contrast to rule out PE. Study was negative. Subject was sent home on 27 May 2002 with a diagnosis of allergy to Optison (anaphylactic shock). Subject is not known to have blood product allergy.		
optn-pr-0206s-0002	**25 January 2001**	**USA**
Chest pain Electrocardiogram abnormal		
One hour after administration, subject developed ST elevation and chest pains. Unknown treatment.		
optn-pr-0206s-0003	**08 February 2001**	**USA**
Chest pain Hyperhidrosis Electrocardiogram abnormal Nausea		
Subject was having a stress echo using Dobutamine increased at 5 mcg/kg, 10 mcg/kg, 20 mcg/kg, and 30 mcg/kg to a final level of 40 mcg/kg. At the increase to 40 mcg/kg she became nauseated and diaphoretic associated with a vagal heart response. HR went from 125 to 71 bpm. HR increased to 136 bpm after 0.4 mg of Atropine. Sent to ER and admitted to ICU.		

Manufacturer UCN	Date received	Country
Reaction terms (MedDRA Preferred Term)		
EVENT DESCRIPTION		
optn-pr-0206s-0006	**08 February2001**	**USA**
Dyspnea Agitation Pulmonary embolism Hypoxia Laboratory test abnormal		
30-year-old obese female subject, 10 days post C-section, developed difficulty breathing after the injection of 3 mL Optison for an echocardiogram. Subject was seeing a cardiologist in a private office for chest discomfort. Subject had an oxygen saturation of 70% before she was injected with the Optison. Her right heart pressure was measured during echocardiography (which was suboptimal before Optison injection) as being 60. After the Optison was injected, she developed shortness of breath (SOB) and subsequently became very agitated. The oxygen saturation fell to 40% and the right heart pressure increased to 112. The reporter stated the right heart pressure value change was likely result of more optimal echo measurement after Optison was injected. Subject was sent by ambulance to ER and admitted to hospital. Subject was treated with heparin overnight and subject continued on heparin for 2 days. Subject remained hospitalized until 16 May 2001. Subject has recovered.		
optn-pr-0207s-0009	**12 August 1998**	**USA**
Convulsion Abdominal pain BP decreased		
An adult male subject presented in the ER with chest pain and ventricular tachycardia. He was cardioverted to a normal sinus rhythm and an echocardiogram was ordered for abnormal wall motion. On 01 August 1998, the subject was administered an i.v. dose of 1 to 3 mL of Optison for an ultrasound. After the administration, the subject complained of dizziness and abdominal pain. He experienced syncope and his BP dropped. The subject became incontinent of urine and stool and appeared to experience a temporal lobe seizure that lasted several minutes. The subject awoke and remained confused for several hours. No treatment was given. Several hours later, the subject appeared to be okay.		
optn-pr-0207s-0010	**24 February 2000**	**USA**
Bradycardia Hyperhidrosis		
On 01 February 2000, 72-year-old male subject received an i.v. administration of 0.5 mL of Optison for an echocardiogram because of dyspnea upon exertion. During the "rest study" portion of the examination the subject experienced profound bradycardia, faintness, cold/clammy skin, and diaphoresis. After being treated with i.v. bolus 250 mL of normal saline and 1 mg of atropine, the symptoms resolved. The subject completed the "stress" part of the examination without allergic reaction and was released.		
optn-pr-0207s-0011	**13 April 2000**	**USA**
Electrocardiogram change		
On an unknown date, a 68-year-old male subject received an i.v. administration of an unknown dose of Optison due to segmental abnormalities. The subject tolerated the procedure without incident. He was then sent to the EKG lab for a previously scheduled examination. During the EKG, changes were noted and thought serious enough to request a cardiac catheterization. The cardiac catheterization demonstrated multiple coronary artery disease. The reporting physician felt that the subject's EKG changes were not symptomatic or caused by the Optison.		
optn-pr-0207s-0027	**06 August 1999**	**USA**

Manufacturer UCN	Date received	Country
Reaction terms (MedDRA Preferred Term)		
EVENT DESCRIPTION		
Paraesthesia Urticaria Face edema Dyspnea		
One hour after the procedure, the subject complained of numbness in the mouth, urticaria, and face edema was observed. Treatment was initiated, but 2 hours later the symptoms still persisted and mild respiratory distress occurred. The subject was taken to the ICU for additional treatment where she recovered. The subject was initially treated with diphenhydramine (Benadryl), methylprednisone sodium succinate (Solu-Medrol), and cimetidine HCl (Tagament).		
optn-pr-0402s-0002	**17 February 2004**	**USA**
Anaphylactic reaction		
On 13 February 2004 a 57-year-old female subject received an i.v.administration of 1 mL Optison for a cardiac ultrasound to evaluate left ventricular function. The subject's medical history included previous blood transfusion without problems, acute coronary syndrome, diabetes, hypercholesterolemia, percutaneous transluminal coronary angioplasty*, unstable angina* and several allergies (contrast media, shellfish, codeine, ibuprofen and nuts). Forty-five minutes after the administration the subject experienced an anaphylactic reaction (face edema, face erythema, chest heaviness, SOB, and drop in oxygen saturation (85%). The subject received a subcutaneous administration of 0.3 mg of epinephrine and i.v. administrations of 50 mg prednisone, 50 mg diphenhydramine (Benadryl), and famotidine (Pepsid)*. The symptoms abated.* Seven to 8 hours later, the symptoms recurred and the subject was administered another 0.3 mg of epinephrine. The symptoms resolved later that day and the subject was hospitalized overnight for observation.* The reporter stated that this AE was life-threatening because treatment was required. Additional information was received on 17 February 2004 and has been incorporated into the narrative above. An asterisk * represents new information to the case.		
optn-pr-0402s-0003	**25 February 2004**	**USA**
Hypoxia		
On 25 February 2004, an obese male subject received an i.v. administration of 5 mL of diluted Optison (3 mL of Optison diluted in 7 mL of saline) for an echocardiogram due to poor endocardial visualization. The subject was hypoventilating* prior to the Optison administration and had undergone coronary artery bypass surgery 1 day earlier, 24 February 2004. He was taking the following concomitant medications at the time of the procedure: pantoprazole, atropine, cefazolin, morphine sulfate, dopamine, glyceryl trinitrate, nitroprusside, and propanolol. Fifteen minutes following Optison administration, the subject developed hypoxemia. The subject was intubated. At the time of this report, the subject was improving, but had not been extubated*. The reporter stated that the AE was not related to Optison as the subject was deteriorating and hypoventilating prior to the Optison administration*. Additional information was received on 27 February 2004 and has been incorporated into narrative above. An * represents new information to the case.		
optn-0508s-0006	**31 August 2005**	**USA**
Dyspnea exacerbated		

Manufacturer UCN	Date received	Country
Reaction terms (MedDRA Preferred Term)		
EVENT DESCRIPTION		
On 31 August 2005 a 78-year-old female subject received an i.v. administration of 4 mL of diluted Optison (3 mL of Optison diluted in 17 mL of saline) for stress echocardiogram to investigate SOB, fatigue, and ischemia. The subject also received an i.v. administration of dobutamine as part of the procedure. The subject's medical history was significant for pulmonary hypertension; cardiomyopathy; ejection fraction 40%; severe chronic obstructive pulmonary disease; emphysema; asthma; acute/chronic SOB with cough and yellow sputum; exertional epigastric pain (presumed ischemic in origin); hyperlipidemia; depression; osteoarthritis; recurrent soft-tissue infection of the right buttock; drug sensitivities including morphine, Percocet, Seldane, and NSAIDs. Concomitant medications were: dobutamine (procedural); methadone; prednisone; glyceryl trinitrate; metoprolol; salmeterol (Serevent); famotidine (Pepcid); monteleukast (Singulair); clavulin (Augmentin); ramipril (Altace); furosemide (Lasix); atorvastatin (Lipitor); albuterol; lisinopril; potassium chloride; sucralfate; aspirin. Immediately after the Optison administration the subject developed an increase in SOB. She was given oxygen (5 L/min) via nasal cannula and used her own nebulizer to help her breath. The hospital's Respiratory Team was called. She received administrations of hydrocortisone sodium succinate (Solu-Cortef) and furosemide (Lasix), and salbutamol. Intubation was considered, but not necessary. The subject was admitted to the critical care unit. The reporter stated that the subject was discharged, but the date and outcome was unknown.* Additional information was received on 14 September 2005 and has been incorporated into the narrative above. An asterisk * represents new information to the case.		
optn-uk-0507s-0005	**26 July 2005**	**United Kingdom**
Cardiac arrest		
A 58-year-old male subject was admitted on 18 July 2005 with a 1-week history of exertional chest pain culminating in a severe episode 2 nights prior to admission. His last pain had been on 17 July 2005. His ECG on admission confirmed an established inferior MI. He had no pain following admission and his in-patient course had been relatively uncomplicated. At around 09:00 on 21 July 2005, he underwent a low-dose dobutamine echocardiography (maximal dose 10mcg/kg/min) and a myocardial contrast echocardiography using Optison 6 mL. During his research echocardiography study he remained well with no chest pain, no other symptoms to suggest any problems. Approximately 1 hour later he was found collapsed in his room by a staff member. He had pulseless electrical activity and an emergency echocardiogram at this time revealed a pericardial effusion, which had not been present during his research study. A prolonged cardiopulmonary resuscitation attempt was made including attempts to drain the effusion emergently. Unfortunately this was unsuccessful. The cause of death was assumed to be myocardial rupture following acute MI, which was already present prior to the echocardiography. No post mortem examination was performed.		

Table 23: Non-serious ADE Reports

AER NO	Initial Received Date	Country of Occurrence	Age	Gender	ADE	Rxn-level Outcome
OPTN-NO-0207C-0013	26 February 1999	AUSTRIA	NR	Male	Headache	Resolved
OPTN-NO-0207C-0014	12 February 1999	GERMANY	78 yr(s)	Female	Pyrexia	Resolved
OPTN-NO-0207C-0015	12 February 1999	GERMANY	55 yr(s)	Male	Dysgeusia Salivary hypersecretion	Resolved
OPTN-NO-0207C-0016	10 January 2000	GERMANY	57 yr(s)	Male	Tinnitus	Resolved
OPTN-NO-0207C-0017	10 January 2000	GERMANY	69 yr(s)	Male	Headache	Resolved
OPTN-NO-0207C-0018	10 January 2000	FRANCE	73 yr(s)	Female	Dysgeusia	Resolved
OPTN-NO-0207C-0019	28 March 2000	GERMANY	56 yr(s)	Female	Headache	Resolved
OPTN-NO-0207C-0022	07 October 2000	AUSTRIA	69 yr(s)	Female	Vertigo	Resolved
OPTN-NO-0207C-0023	28 March 2000	GERMANY	68 yr(s)	Male	Dysgeusia	Resolved
OPTN-NO-0207C-0028	03 November 1998	GERMANY	64 yr(s)	Male	Left ventricular end-diastolic pressure increased	Resolved
OPTN-NO-0207C-0029	08 December 1999	GERMANY	65 yr(s)	Female	Flushing	Resolved
OPTN-NO-0207C-0030	25 November 1998	GERMANY	65 yr(s)	Female	Flushing	Resolved
OPTN-PR-0206S-0005	23 August 2001	USA	57 yr(s)	Male	Ventricular tachycardia	Resolved
OPTN-PR-0207S-0007	05 March 1998	USA	51 yr(s)	Male	Deafness	Resolved
OPTN-PR-0207S-0008	24 June 1998	USA	82 yr(s)	Male	Hyperglycaemia Nausea Vomiting	Not reported
OPTN-PR-0207S-0012	21 September 1998	USA	(blank)	Male	Visual disturbance	Unknown
OPTN-PR-0207S-0025	15 December 1998	USA	(blank)	Female	Visual disturbance	Unknown
OPTN-PR-0207S-0026	13-May-1999	USA	37 yr(s)	Female	Dyspnoea Flushing Oedema	Resolved
OPTN-PR-0209L-0031	09 September 2002	USA	72 yr(s)	Male	Dysgeusia	Resolved
OPTN-PR-0210L-0034	09 September 2002	USA	79 yr(s)	Male	Dysgeusia	Resolved
OPTN-PR-0210L-0035	09 September 2002	USA	56 yr(s)	Male	Nausea	Resolved
OPTN-PR-0210S-0033	03 January 2001	USA	36 yr(s)	Female	Pruritus Rash	Resolved

AER NO	Initial Received Date	Country of Occurrence	Age	Gender	ADE	Rxn-level Outcome
OPTN-PR-0211S-0036	02 August 2002	USA	55 yr(s)	Male	Urticaria	Not reported
OPTN-PR-0212S-0037	25 September 2002	USA	75 yr(s)	Male	Anxiety Chest pain Confusional state Cough Nausea Retching Vomiting	Resolved
OPTN-PR-0212S-0038	08 March 2002	USA	30 yr(s)	Female	Wheezing	Resolved
OPTN-PR-0212S-0039	08 March 2002	USA	40 yr(s)	Female	Wheezing	Resolved
OPTN-PR-0301S-0001	21 January2003	USA	(blank)	Female	Hypotension	Resolved
OPTN-PR-0302S-0002	28 February 2003	USA	54 yr(s)	Male	Dysuria	Not resolved
OPTN-PR-0306S-0003	06 June 2003	USA	54 yr(s)	Female	Bradycardia Chest pain Dizziness Dyspnoea Flank pain	Resolved
OPTN-PR-0307S-0004	17 July 2003	USA	(blank)	Female	Extravasation No adverse effect	Resolved
OPTN-PR-0308S-0005	27 August 2003	USA	35 yr(s)	Male	Dysgeusia	Resolved
OPTN-PR-0309S-0006	15 September 2003	USA	(blank)	Male	Oxygen saturation decreased	Resolved
OPTN-PR-0310S-0008	09 October 2003	USA	61 yr(s)	Female	Back pain Headache	Resolved
OPTN-PR-0310S-0009	20 October 2003	USA	56 yr(s)	Male	Hyperhidrosis Asthenia Nausea	Resolved
OPTN-PR-0402S-0001	10 February 2004	USA	(blank)	Female	Feeling abnormal Dizziness Vision blurred	Unknown
OPTN-PR-0403S-0004	26 March 2004	USA	77 yr(s)	Male	Extravasation Vessel puncture site bruise	Resolved
OPTN-PR-0403S-0005	31 March 2004	USA	75 yr(s)	Male	Hypersensitivity	Resolved

AER NO	Initial Received Date	Country of Occurrence	Age	Gender	ADE	Rxn-level Outcome
OPTN-PR-0409S-0007	07 September 2004	USA	(blank)	Female	Abdominal pain Nausea	Resolved
OPTN-PR-0409S-0008	07 September 2004	USA	(blank)	Female	Abdominal pain Nausea	Resolved
OPTN-PR-0409S-0009	07 September 2004	USA	(blank)	Female	Abdominal pain Nausea	Resolved
OPTN-PR-0409S-0010	07 September 2004	USA	(blank)	Female	Abdominal pain Nausea	Resolved
OPTN-PR-0412S-0011	13 December 2004	USA	63 yr(s)	Female	Injection site extravasation Injection site swelling	Unknown
OPTN-PR-0503S-0002	04 March 2005	USA	45 yr(s)	Female	Accidental exposure Ocular hyperaemia Eye pain	Resolved
OPTN-PR-0505S-0003	06 May 2005	USA	81 yr(s)	Female	Dizziness Dyspnoea Visual disturbance	Resolved
OPTN-PR-0505S-0004	27 May 2005	USA	59 yr(s)	Female	Hypersensitivity	Resolving

6.1.6 Global pharmacovigilence safety evaluation

6.1.6.1 Post-marketing ADE reporting

The global PV database contains 57 reports concerning OPTISON. These comprise 27 serious reactions in 12 patients and 83 non-serious reactions in 45 patients (see narratives and table, above).

6.1.6.2 Serious reactions

The most frequently reported serious reactions are chest pain (3 events), ECG abnormality/change (3 events), and hypoxia (3 events). Considering the underlying cardiovascular conditions of the subject population, this is a low rate of reporting and does not indicate a safety concern. Where outcome information was available, all reactions resolved.

One fatal ADE of cardiac arrest was reported in a subject in the post-MI phase (OPTN-UK-0507S-0005). The reporter attributed it to the subject's underlying medical condition and not to OPTISON administration. (See narrative in preceding section 6.1.5)

6.1.6.3 Non-serious reactions

The most frequently reported non-serious reactions are nausea (9 events), dysgeusia (6 events), and abdominal pain (5 events). These are isolated incidents and do not indicate a safety concern.

6.1.6.4 Subject exposure

Analyzing the world-wide safety data in 57 ADE reports and comparing the number of subjects experiencing adverse reactions with more than 1 million doses of OPTISON administered in routine use, and considering the nature of these adverse reactions, confirms OPTISON can be regarded as well tolerated when used for approved indications.

6.1.7 Published safety evaluation

Continuous survey of the world-wide scientific literature identified 1 publication with important safety information for OPTISON [Van der Wouw et al. 2000] and 1 publication with new information for the clinical use of OPTISON [Dalecki 2007]. The articles provide recent evidence to indicate that, in addition to using a low mechanical index as recommended, also the phase in which the ultrasound pulse is delivered seems to be of importance for biological side effects like endothelial cell injury and/or capillary rupture. Premature cardiac contractions have been shown to occur in both laboratory animals and humans when contrast agents are present in blood and end-systolic triggering is applied. The effect increases with increasing pressure amplitude and contrast agent dose. Accordingly, using a low mechanical index and triggering is recommended [Van der Wouw et al. 2000 and Dalecki 2007].

6.1.8 Company remarks

Because post-marketing AEs are reported voluntarily from a population of uncertain size, these reports do not reflect the true incidence of such events. Furthermore, a causal association between the product and an AE cannot be definitively made and the event may have been caused by the subject's concurrent medical conditions, a concomitant medication, or have occurred by chance at that time.

OPTISON was recalled from the world-wide market in November 2005 and letters requesting the return of unused product within its expiry date were sent to practitioners on 21 November 2005. The company believes that by January 2006 no product remained in use and no further administrations occurred. No ADE reports were received by the company during the period in which the product was not marketed.

The product was re-launched in the US in October 2007; no new ADE reports have been received by the company since re-launch.

6.1.9 Overall conclusions

Review and analysis of the accumulated safety data does not indicate a safety concern or risk with OPTISON administration in the target subject population. The sponsor believes that the risk/benefit profile of OPTISON remains favorable.

6.2 Review of Clinical Literature

6.2.1 Literature search process

A literature search was performed by Nerac, Inc. The databases used were Multi-database search with unique results from BIOSIS, Medline, Embase, Engineering Index, Inspec, Life Science Collection, and Defense Technical Information. The intent of this search was to compile safety information from Investigator-initiated clinical trials in the published literature. Because of the limited nature of any safety information in these publications there is insufficient subject information available to reach the threshold for collection and reporting to regulatory authorities; any information that reaches reporting thresholds is captured in the OPTISON GPV database. These publications; however, do give a general overview of safety experience in published Investigator initiated clinical trials.

6.2.2 Search results

The search covered published studies containing the keywords OPTISON, OPTISON or Perflutren or perfluoropropane linked with microspheres, human albumin or type A and the terms randomized, double-blind clinical trials, and multicenter. The search covered 1998 to the present.

The search resulted in 146 references of which, a number were excluded because they were review articles, publications on preclinical studies or veterinary use, non-English publications, articles based on results from the NDA clinical trials, etc. A total of 56 articles covering 9,250 subjects were reviewed for safety information related to clinical studies and the results are included in Table 24. The subject population covers a mix of subjects referred for echocardiography including known or suspected CAD, stress echo subjects, various types of ICU subjects including subjects with ongoing chest pain as well as subjects with other non-cardiac diseases.

6.2.3 Overall conclusions

An overview of clinical studies published on OPTISON from 1998 until current is presented in Table 24. The summary covers 56 published papers and inclusion of about 9,250 subjects dosed with OPTISON. The publications from the clinical trials are excluded from this overview. The subject population covers a mix of subjects referred to echocardiography including known or suspected CAD, stress echo subjects, various types of ICU subjects including subjects with ongoing chest pain as well as subjects with other non-cardiac diseases. Overall, none of the studies raise a concern about adverse events or changes in any other safety parameters after OPTISON administration.

Table 24: Literature Search Results

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
Cardiac				
1999 Miller	Optison 0.5 ml and 5 infusions of 3 ml each Total: 15.4 ml	6	Normals	1 subject with lethargy and headache reported 2 h post study
2000 Finkelhor	Optison 0.5 ml Several injections Dobutamin Exercise	1330 Optison: 212	CAD	No mentioning of AEs or safety concerns.
2000 Kornbluth	Optison 2.0 – 3.0 ml	50	ICU: All mechanically ventilated	No effect on PO_2. Safe
2000 Mor-Avi	Optison 0.3 ml Or Definity infusion	22 Optison: 6	CAD	No mentioning of AEs or safety concerns.
2000 Reilly	Optison 0.5 – 1.0 ml	70	Mixed ICU: post MI or unstable angina 31%, resp.failure/pulm.edema 10%, postcardiac arrest 7%, mechanical ventilation 31%	No mentioning of AEs or safety concerns.
2001 Daniel	Optison 2 injections	50	Mixed ICU patients: 48% on mechanical ventilation	No contrast-related side effects
2001 Desco	Optison Dipyridamol	80	CAD	No mentioning of AEs or safety concerns.
2001 Janerot-Sjoeberg	Optison: 2.7 ml	12	Ischemic heart disease	No effect on BP and HR, and no AE.
2002 Thanigaraj	Optison : Multiple 0.5 ml inj. 53% Dobutamin 47% Exercise	277	Stress echo for known or suspected CAD: 62% prior MI, 81% CAD, 95% chronic pulmonary disease, 9% CHF…more	No mentioning of AEs or safety concerns.
2002 Hagendorff	Optison: 1.4 - 2.0ml or Levovist 400 mg/ml infusion at 3.5- 5.0 ml/min for 60s for 2 times	32 Optison: 16	16 CAD and 16 non-CAD	No mentioning of AEs or safety concerns.
2001 Rainbird	Optison : 0.5 ml followed by several 0.2-0.3 ml injections	300	Known or suspected CAD. HTN 68%, previous MI 18%, history of dyspnea	No AEs related to Optison

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
	Dobutamin		48%	
2002 Shimoni	Optison Infusion rate 12-16 ml/h	20	CAD. Prevoius MI 25%, symptoms of HF 75%, angina 55%	No mentioning of AEs or safety concerns.
2002 Swinburn	Optison 0.3 ml 6-9 injections Dubutamin	19	CAD. Prevoius MI 21%, diabetes 21%, HTN 37%	No mentioning of AEs or safety concerns.
2002 Ward	Optison: 0.6- 1.0 ml	26	Suspected apical hypertrophic cardiomyopathy (ACM). 6 patients with suspected ACM, 10 patients with normal ECG and no HTN, 10 patients with HTN and pos ECG for LVH	No mentioning of AEs or safety concerns.
2002 Yong	Optison 0.3 - 1.0 ml. Total dose < 3 ml	32	Mixed ICU: 32% COPD 32%, mechanical ventilation 69%, recent cardiothoracic surgery 59%, CHF 19%, 16% stroke 16, HTN 59%	No sign. Changes in HR, BP, Oxygen saturation., One patients with nausea after Optison.
2003 Castello	Optison 0.5 ml one or more times	791	Mix echogardiography: out-patients 32%, in-patients 40%, ICU 28%, COPD 11%, mechanical ventilation 5%, obese 58% and overweight 19%.	No mentioning of AEs or safety concerns.
2003 Olszowska	Optison 0.3 – 0.5 ml Dobutamin	44	High probability of CAD. previous MI 48%, HTN 45%	No mentioning of AEs or safety concerns.
2003 Shimoni	Optison infusion 12-16 ml/h Dobutamin	20	CAD and ventricular dysfunction scheduled for CABG	No mentioning of AEs or safety concerns.
2004 Espinola	Optison 0.4 ml Several injections Dobutamine	13	Post surgery of congenitally corrected transposition.	No mentioning AEs or safety concerns.
2004 Smith	Optison 0.5-2.5 ml	20	Significant aortic stenosis	No AE.
2005 Elhendy	Optison : 0.2- 0.3 ml or Definity: 0.1- 0.15 ml Dobutamin	128 Optison: 87	Diabetic patients. Previous MI 22%, HTN 77%	Contrast referred to as safe. No mentioning of contrast AEs or safety concerns.
2005 Knebel	Optison: 3 ml Diluted with 10 ml saline and	20	Mixed echo population. Clinically stable. Exclusion: ACS, cardiogenic	There are no clinically relevant increases in serum markers for micro-necrosis, inflammation and

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
	infused over 3 min.		shock. Goal is to measure markers of myocardial necrosis, inflammation and oxidative stress after contrast.	oxidative stress.
2006 Makaryua	Optison	213	Mixed ICU patients	No mentioning of AEs or safety concerns.
2005 McMahon	Optison Weight < 20 kg: 0.3 ml Weight >20 kg: 0.5 ml	20	Children (9-18 years) for echocardiography	No hemodynamic effects, nausea or flushing. Three patients experienced transient headaches. One patient had transient change in taste.
2005 Tsutsui	Optison: 2.8 +/- 0.8 ml or Definity: 1.0 +/- 0.3 ml Dobutamin	1486 Optison: 963	Known or suspected CAD. Diabetes 34%, HTN 63%, previous MI 15%, previous PCI 22%. The hemodynamic and adverse effects of contrast were compared with 1,012 patients who underwent conventional dobutamine stress echocardiography (DSE) without contrast	Arrhythmias and AEs similar for all 3 groups. No increased incidence of chest pain. No MI and no death.
2005 Tsutsui	Optison 2.8+/- 0.9 ml Or Definity: 1.1 +/- 0.4 ml Dobutamin	788 Optison: 575	Known or suspected CAD. 3 years post contrast follow up.	No mentioning of contrast AEs or safety concerns.
2005 Xie	Optison: 0.2- 0.3 ml /inj Several injections Dobutamin	27	Chest pain and intermediate probability of CAD. HTN 74%,diabetes 67%, PCI 38%	No mentioning of AEs or safety concerns.
2006 Cianciulli	Optison 1 ml injection followed by 1 ml/min infusion	56	28 patients: post reperfusion 10 normals 18 patients with AMI	No AE. Well tolerated.
2006 Elhendy	Injection of Optison : 0.2- 0.3 ml or Definity: 0.1- 0.15 ml Followed by inf. 0.04ml/kg diluted in 100 ml saline. Inf usion rate 120-180 ml/h Dobutamin	56 Optison: 38	Re-stenosis in previous PCI patients. Previous MI 38%, HTN 59%, angina 39%, atypical chest pain 22%, dyspnea 19%	No deaths or MI during or immediately after. No mentioning of AEs or safety concerns.
2006 Elhendy	Optison : 0.2- 0.3 ml or Definity: 0.1- 0.15 ml	56 Optison: 46	Detect coronay artery bypass graft disease in previous CABG patients. Previous MI 34%, HTN 77%, angina	No serious complications. No mentioning of AEs or safety concerns.

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
	Dobutamin		36%, atypical chest pain 16%,dyspnea 14%	
2005 Espinola	Optison Dipyridamole	15	Takayasu's arthritis HTN 73%, shortness of breat 80%, headache 46%, angina 40%, dizziness 33%.	No mentioning of AEs or safety concerns.
2006 Malm	Optison 1,1 ml	53	Mixed cardiac echo population. 56% MI, 26% HTN Stable hemodynamics	No significant changes in BP, HR and rhythm. No AE
2006 Nesser	Optison: 3 – 9 ml Diluted to 100ml with saline and infused. Adenosine	64	Known or suspected CAD.	All AE due to adenosine were transient and none required intervention. No mentioning of AEs or safety concerns.
2006 Olszowska	Optison: 0.3- 0.5 ml Multiple injections	86	3 days post AMI.	No mentioning of AEs or safety concerns.
2006 Ressner	Optison 2.7 ml	12	Known ischemic heart disease	No AE. No change in HR or BP
2006 Roberts	Optison: 6 ml Diluted to 30 ml with saline and unfused at 120-180 ml/h Dobutamin	38	CAD patients. Diabetes 34%, HTN 29%, obesity 37%	No mentioning of AEs or safety concerns.
2007 Firschke	Optison 3.0 ml diluted to 40 ml with saline. Infusion rate 150-200 ml/h Adenosine	57	CAD. Previous MI 35%, previous PCI 30%, HTN 56%, diabetes 32%	No mentioning of AEs or safety concerns.
2007 Hodge	Optison 3ml diluted with saline to total of 60 ml. Infused at 4 ml/h	18	Postoperative evaluation of endovascular grafts for AAA exclusion.	No AEs
2007 Hu	Optison: 0.25- 0.5 ml or SonoVue:1.25- 2.5 ml Dobutamin	62 Optison: not defined	Known or suspected CAD in overweight and obese patients. Previous MI 24%, HTN 87%	8% headaches, 8% palpitations and 3% flush. All related to dobutamin.
2007 Park	Optison 0.3-0.5 ml Exercise	104 Optison: 89	Known or suspected CAD. 91 patients and 15 normals.	No mentioning of AEs or safety concerns.

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
2007 Sobkowicz	Optison 1.0 ml Several injections	44	End-stage renal disease. HTN 100%, diabetes 55%, previous MI 21%	2 cardiac arrests related to coronary angio. Not related to Optison. No mentioning of AEs or safety concerns.
2008 Henriksen	Optison: 0.2 ml	15	Athletes	No mentioning of AEs or safety concerns.
2008 Herzog	Optison Definity	16025 Optison: 3044	Mixed echo population: 54% rest and 46% stress, 18% ICU, 34% in-patient non-ICU, 48% out-patients	20 AE (0.12%) of which 4 SAE (0.031%). All after Definity. Back pain, transient vasodepressor episodes, pruritus, serious but nonfatal cardiopulmonary events, back and chest pain with transient arterial desaturation, dyspnea with chest and back pain, acute bronchospasm. 65% of patients with AE had history with allergy.
2008 van der Heide	Optison: 0.2-0.3 ml inj. Followed by infusion: 25ml/h. Total dose ~ 20 ml	20	Recent MI (7.1+/- 4.9 months)	No mentioning of AEs or safety concerns.
2005 Tong	Optison: 3.0 ml diluted with saline to 60 ml. Infused at 3 ml/min	975	ICU patients with chest pain and no S-T elevation. 16% of patients had ongoing chest pain. Study within 12h of onset of symptoms. HTN 66%, diabetes 28%	No mentioning of contrast AEs or safety concerns.
Cerebrovascular				
2000 Wiesmann	Optison 0.5 and 1.5 ml	13	Normals. Brain perfusion study.	No mentioning of AEs or safety concerns.
2004 Kono	Optison 0.5- 1.0 ml Several injections. Max: 8.7 ml	20	Carotid artery disease	No mentioning of AEs or safety concerns. Contrast was well tolerated.
2005 Holscher	Optison 0.75 ml	32	15 normals and 17 patients with cerebrovascular disease.	No mentioning of AEs or safety concerns.
2007 Shah	Optison 3.0 ml diluted with saline to 10 ml. 0.5- 1.0 ml	17	Symptomatic cerebrovascular disease. HTN 76%, diabetes 41%	No mentioning of AEs or safety concerns.
2008 Herzig	Optison	28 Optison: 14	Occluded internal carotid artery.	No mentioning of AEs or safety concerns.
Abdominal				

Publication Year, Main author	Product Dose Stress protocol	# Patients Total Optison*	Patient population and characteristics	Contrast safety findings
2000 Strobel	Optison 2 injections	Not defined 101 liver lesions	Liver tumors: Metastasis 18, focal nodular hyperplasia 23, hemangioma 32, HCC 13, cirrhotic nodules 6, additional liver lesions 9.	No mentioning of AEs or safety concerns.
2005 Jung	Optison 0.5 ml Diluted in 20 ml saline. 1 or 2 injections	20	Confirmed Hepatocellular carcinoma	No mentioning of AEs or safety concerns.
2006 Clevert	Optison:0.5 ml diluted in 20 ml saline.	100	Breast tumors. Not palpable.	No mentioning of AEs or safety concerns.
2006 Jung	Optison. 0.5 ml Diluted in 20 ml saline	60	Liver tumors: HCC 15, metastasis from colorectal cancer 35, mammary carcinoma 54, hemangioma 10.	No complications and no allergic reactions.
2006 Schwenger	Optison 3 ml	26	Renal transplant recipients. HTN 89%, CAD 31%, diabetes 43%	No effect of Optison on HR, BP. No significant changes in S-creatinine after 24h. No hematuria or local pain.
2006 Tsutsiu	Optison: 2.6 +/- 0.5 ml or Definity: 1.0 +/- 0.4 ml Dobutamin	230 Optison: 175	Advanced liver disease Diabetes 25%, HTN 30%	No mentioning of contrast AEs or safety concerns.

* If not the same as total # patients

6.3 References

Optison Research Report Number: 10538114-1 View Report Strategies April 25, 2008 Project Leader: M. Denise Ryan | Read Bio Report Wrap-up

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7 FUTURE USES (VASCULAR)

7.1 Background

With approximately 1.2 million heart attacks and 700 000 strokes afflicting an aging population each year, cardiovascular disease remains the number 1 cause of morbidity and mortality in the US [Rosamond et al. 2007]. Arterial occlusive disease commonly affects the supra-aortic arteries and results in cerebral infarction and stroke. Although it may affect the intracranial vessels themselves, 88% of subjects with transient ischemic attacks (TIAs) have atherosclerotic disease of the carotid arteries [Eisenberg et al. 1977]. Results of various studies show the effectiveness of carotid endarterectomy with a clear benefit for preventing stroke in symptomatic subjects with significant carotid stenosis [European Carotid Surgery Trialists' Collaborative Group 1991; North American Symptomatic Carotid Endarterectomy Trial Collaborators 1991; Executive Committee for the Asymptomatic Carotid Atherosclerosis Study 1995].

Vascular imaging is used for detection and localization of arterial disease. It aids decision making regarding therapy selection, monitoring, and follow-up. Carotid angiography has served as the standard diagnostic method for evaluation of the carotid arteries and is an established diagnostic method capable of reliably differentiating highly stenosed from near occluded vessels. However, because angiography outlines the arterial lumen in limited angular projections and does not demonstrate the arterial wall or the plaque itself, it is unreliable in depicting ulceration [Van Damme and Viavario 1993; Edwards et al. 1979]. Moreover, the invasiveness of the technique adds a 2.5% risk of TIAs and a <1% risk of permanent neurological deficit, making it impractical for screening of asymptomatic subjects or monitoring disease progression [Harley and Haynor 1995].

Duplex Doppler ultrasonography has changed the diagnostic algorithm in subjects with arterial occlusive disease such that invasive angiography is increasingly being reserved for intervention or for cases in which noninvasive test results are equivocal. Ultrasonography has become the primary examination in subjects considered for endarterectomy or suspected of having carotid artery disease. It is readily accessible, noninvasive, and relatively inexpensive. It was shown to be highly effective in the detection of flow-limiting disease [Moneta et al. 1993] but has been limited in the depiction of the echogenicity of plaques and ulcerations [Widder et al. 1990 and Young et al. 1992]. Also for precise determination of the degree of stenosis [Hood et al. 1996; van Everdingen et al. 1998; and Grant et al. 2000], and differentiation of critical stenosis from complete occlusion where a Duplex Doppler ultrasonography study is suboptimal [Bornstein et al. 1988 and AbuRahama et al. 1997], subjects are being referred for Computerized tomography angiography (CT-Angio) or magnetic resonance angiography (MRA) [Bates et al. 2007].

Gray-scale ultrasonography alone has been used to assess carotid intima media thickening (c-IMT), which is associated with an increased risk of cardiovascular events, and to determine

whether carotid plaques are soft, mixed, or hard [Feeley et al. 1991]. However, it cannot be used reliably to assess plaque ulceration [ECPSG 1995 and O'Leary et al. 1987] and detect neo-vascularization or to directly assess luminal narrowing [Barry et al. 1990]. In fact, the appearance of plaque surface at preoperative gray-scale ultrasonography showed no correlation to intra-operative findings in a 270-subject multi-centre study [ECPSG 1995].

7.2 Clinical Applications for the use of OPTISON in Carotid Vascular Imaging

Recent clinical studies have indicated that the use of contrast-enhanced ultrasonography for carotid imaging is useful for the assessment of occlusive disease. The applications primarily focus on 3 areas of clinical importance:

Carotid artery stenosis: For subjects with pre-existing, stable cerebrovascular disease, it is important to identify and quantify the presence of near-total stenosis and the presence of severe disease. The quantification of carotid artery stenosis along with subjects' symptoms provide a threshold determination for surgical or mechanical intervention (carotid endarterectomy or carotid stenting procedure). A enhanced, non-invasive method that could be used to detect the presence of near-occlusions or severe stenosis would have clinical value. Therefore, it is a reasonable goal to pursue enhancement of the carotid lumen using ultrasound contrast agents [Kono et al. 2004; Delcker and Diener 1994; Sirlin et al. 2001; and Martin and Lerakis 2004].

Carotid artery intima-media-thickness (c-IMT): The c-IMT is an accepted standard for the non-invasive assessment of cardiovascular risk in population studies and is an accepted surrogate marker for atherosclerosis by the FDA. Therefore is it anticipated that the use of contrast agents for carotid c-IMT will provide additional information on cardiovascular risk assessment in those subjects that have diabetes mellitus, metabolic syndrome, or impaired fasting glucose. With the use of ultrasound contrast agents, it is possible to more fully interrogate the entire vascular lumens and includes the anterior wall, bifurcation and internal carotid artery [Kono et al. 2004 and Macioch et al. 2004].

Identification of atherosclerotic-related neovascular-changes within the adventitial vasa vasorum and plaques morphology. For nearly 150 years, pathologists, vascular surgeons and experimentalists recognized that 1 of the earliest markers of systemic atherosclerosis was the development of arterial wall neovascularization (vasa vasorum). Recently [Feinstein 2004] noted that these early markers of atherosclerosis could be identified following the use of USCA during routine carotid ultrasound imaging. These initial observations have now been validated using histopathology studies from 2 laboratories [Shah et al. 2007 and Coli et al. 2008]. Subsequent to these initial observations there have been numerous publications confirming these initial observations, including safety [Rajaram et al. 2004; Feinstein 2004; Feinstein 2006; Granada 2008; and Shah et al. 2007].

7.3 Clinical Research Findings

7.3.1 Carotid artery stenosis

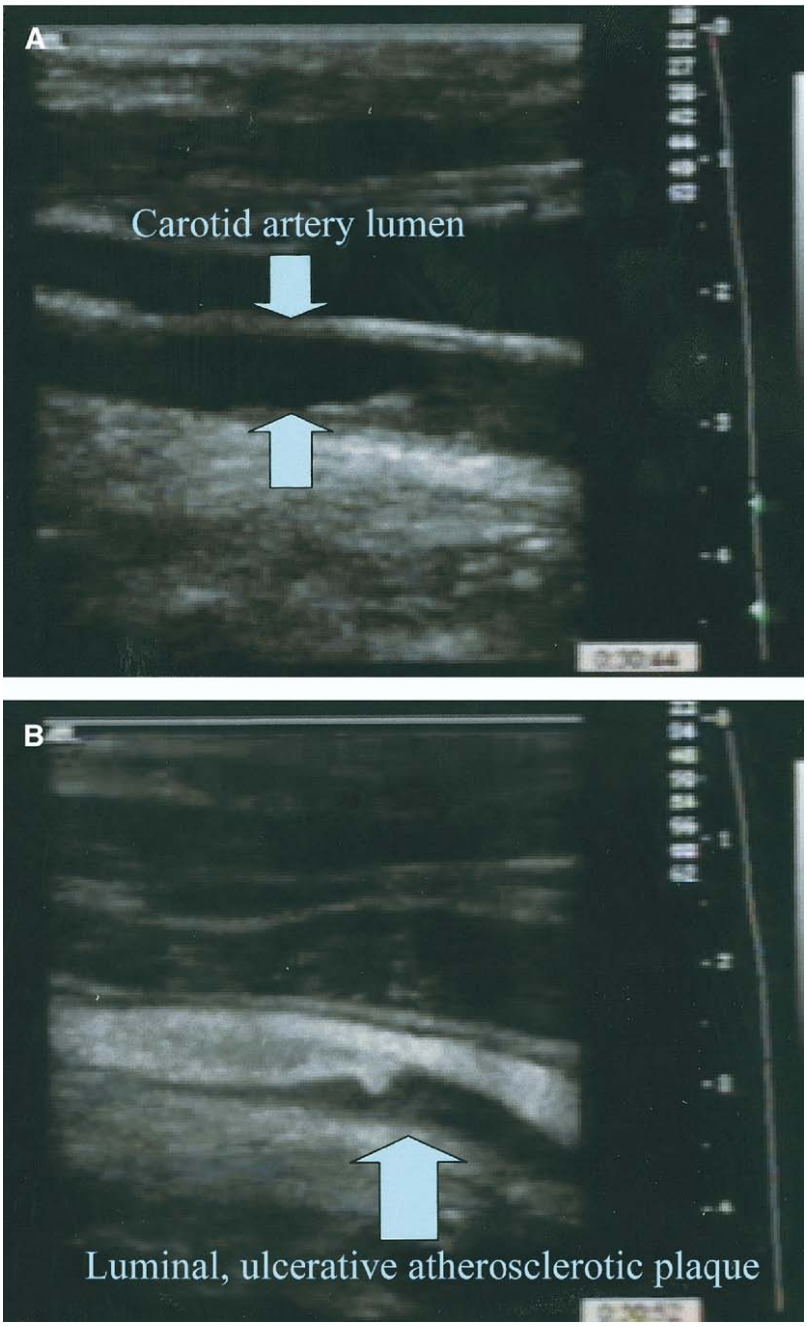
Carotid artery stenosis in subjects suspected of having carotid artery stenosis.

See Figure 1A and 1B. The use of blood pool agents permit a more accurate determination of the luminal aspects of the carotid arterial tree [Kono et al. 2004]. The detection of luminal irregularities (ulcers, plaque formation) provides valuable information on the early detection of atherosclerosis. If identified, additional aggressive medical therapy would be advised per the Screening for Heart Attack and Prevention Education (SHAPE) taskforce guidelines. From a review of the literature, it is apparent that USCAs have been efficiently used to enhance the carotid artery lumen. The earliest report was published by Mattrey in which he demonstrated the efficacy in an *in vitro* model and subsequently in an animal model (rabbit). Subsequently [Kono et al. 2004], published their results in humans and wrote in their abstract of their article the following:

B-mode ultrasonographic angiography enhanced with a microbubble-based USCA (OPTISON) was evaluated in human subjects with carotid artery disease. Results at contrast material-enhanced ultrasonographic angiography and duplex ultrasound were compared with those at conventional angiography. Both ultrasonographic angiography and duplex ultrasound accurately depicted stenoses of 70% or more compared with those depicted at conventional angiography. The percentage diameter stenosis of the internal carotid artery measured at ultrasonographic angiography strongly correlated with that measured at conventional angiography (r=0.988). The percentage area stenosis measured at ultrasonographic angiography strongly correlated with ex vivo measurements of the respected carotid plaque at magnetic resonance imaging (r=0.979). Ultrasonographic angiography depicted unsuspected wall irregularities, ulceration, and dissection.

Figure 10 A revealed a standard ultrasound longitudinal axis of the common carotid artery. The arrows highlight the lumen of the common carotid. Figure 10 B revealed the enhancement of the common carotid lumen in the same subject as displayed in Figure 10 A. The subject received an intravenous injection of OPTISON. This image revealed a more clearly defined carotid vessel lumen and luminal irregularities including a plaque with an associated ulcer.

Figure 10 (A and B) Standard Ultrasound Longitudinal Axis and Enhancement of the Common Carotid Artery



7.3.2 Carotid artery intima-media-thickness

c-IMT in subjects considered to be at high risk for premature cardiovascular disease (ie, subjects with diabetes mellitus or the metablic syndrome) and/or with suspected or known

cardiovascular disease. The measurement of the c-IMT of the vessel wall, is a well-established, commonly utilized surrogate marker of atherosclerosis Pignoli et al, first described the c-IMT in 1986 [Pignoli et al. 1986]. Thus, the ultrasound detection of the c-IMT has become a stable diagnostic tool to use as a surrogate marker for atheroslcerosis. The layers have been defined as follows:

- Intima layer. This layer is the first cellular layer bordering on the vessel lumen. Typically, this layer contains the luminal border-endothelial cells, foam cells, raised lesions, plaques, accompanying neovascularization and vasa vasorum, and complex luminal morphologic anatomy (see examples);

- Media layer. The media of the arterial wall is composed of smooth muscle cells, monocytes, fibroblasts, monocyte-derived macrophages, foam cells, collagen and calcium deposition, and complex plaque formations including vasa vasorum neovascularization.

The combined intima and media layer are described as carotid IMT. The literature is replete with clinical trials illustrating the close association of c-IMT and the progression and regression of atherosclerosis [Lorenz et al. 2007; Rajaram et al. 2004; and Patel et al. 2004]. The tables below provide additional information

Table 25, Table 26, and Table 27 provide a summary of the clinical utility of using ultrasound for the detection of c-IMT as a surrogate marker for atherosclerosis.

Table 28 and the ensuing discussion will focus on the use of USCAs for the enhancement of the c-IMT.

Table 29 identifies additional non-invasive, comparative imaging modalities (non-ultrasound-based).

Table 25: Clinical Trials Showing the Effect of Lipid-Lowering Therapy on Carotid Intima-Media Thickness

Study	Population Demographics	# of Subjects	Treatment	F/Up (Years)	Results	Conclusion
ARBITER (2002)	Subjects that met NCEP II criteria for lipid-lowering therapy	161	Atorvastatin 80mg vs. Pravastatin 40mg	1	Change in c-IMT (mm) Pravastatin: 0.025 ± 0.017 Atorvastatin: -0.034 ± 0.021	LDL reduction correlated with regression of c-IMT
ASAP (2001)	Subjects with familial hypercholesterolemia	325	Atorvastatin 80mg vs. Simvastatin 40mg	2	Change in c-IMT (mm) Atorvastatin: -0.031 Simvastatin: 0.036	Atorvastatin linked to regression of c-IMT
MARS (1996)	Subjects with CAD	188	Cholesterol-lowering diet plus placebo or Lovastatin 80mg	4	Progression of c-IMT (mm/y) Lovastatin: -0.028 ± 0.003 Placebo: 0.015 ± 0.005	Lovastatin reduces progression of early atherosclerosis
CAIUS (1996)	Subjects aged 45-65 with elevated LDL, no CAD, and ≥ 1 carotid lesion	305	Pravastatin 40mg vs. placebo	3	Progression of c-IMT (mm/y) Pravastatin: -0.0043 ± 0.0028 Placebo: 0.009 ± 0.0027	Progression of c-IMT beneficially affected by pravastatin
KAPS (1995)	Men aged 44-65	447	Pravastatin 40mg vs. placebo	3	Progression of c-IMT (mm/y) Pravastatin: 0.017 Placebo: 0.031	Pravastatin shows antiatherogenic effect
PLAC-II (1995)	Subjects with CAD	151	Pravastatin 10-40mg vs. placebo	3	Progression of c-IMT (mm/y) Pravastatin: 0.0295 ± 0.0058 Placebo: 0.0456 ± 0.0057	35% reduction in c-IMT progression with Pravastatin
REGRESS (1995)	Males with elevated cholesterol and CAD	255	Lovastatin 40mg vs. placebo	2	Change in c-IMT (mm) Pravastatin: -0.02 ± 0.01 Placebo: -0.03 ± 0.01	No correlation between change in c-IMT and CAD
ACAPS (1994)	Subjects with carotid atherosclerosis and elevated LDL	919	Lovastatin 20-40mg vs. placebo	3	Progression of c-IMT (mm/y) Lovastatin: -0.009 ± 0.003 Placebo: 0.006 ± 0.003	Regression of c-IMT; reduction in CV events/mortality
CLAS (1993)	Nonsmoking men with previous coronary bypass surgery	78	Cholesterol-lowering diet plus placebo or colestipol/niacin	4	Reduction in c-IMT at 2 (p=0.0001) and 4 years (p=0.0001) with treatment	Colestipol-niacin treatment reduces c-IMT

ARBITER=Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; ASAP=The Atorvastatin vs. Simvastatin on Atherosclerosis Progression Study; MARS=Monitored Atherosclerosis Regression Study; CAIUS=Carotid Atherosclerosis Italian Ultrasound Study; KAPS=Kuopio Atherosclerosis Prevention Study; PLAC-II=Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries; ACAPS=Asymptomatic Carotid Artery Progression Study; REGRESS=Regression Growth Evaluation Statin Study; CLAS=Cholesterol Lowering Atherosclerosis Study
Source: [Patel et al. 2004].

Table 26: Clinical Trials Showing the Effect of Beta-Blockade on Carotid Intima-Media Thickness

Study	Population Demographics	# of Subjects	Treatment	F/U (Years)	Results	Conclusion
ELSA (2002)	Subjects with mild-moderate hypertension	2334	Atenolol 50mg vs. Lacidipine 4mg	4	Progression of c-IMT (mm/y) Atenolol: 0.0145 Lacidipine: 0.0087	Greater efficacy of lacidipine on c-IMT progression when compared to atenolol
ELVA (2002)	Subjects with asymptomatic hypercholesterolemia	92	Metoprolol CR/XL 100mg vs. placebo	3	Progression of c-IMT reduced by metoprolol after 3 yr (mm) (-0.06 vs. +0.03)	Antiatherosclerotic effect of metoprolol in addition to statins
BCAPS (2001)	Subjects with carotid plaque	793	Metoprolol CR/XL 25mg, Fluvastatin 40mg, and placebo	3	Progression of c-IMT_{max} Carotid bulb reduced by metoprolol: -0.023 mm/y Progression of c-IMT_{mean} CCA reduced by fluvastatin: -0.009 mm/y	Beta-blockers can reduce the rate of progression of c-IMT

ELSA=European Lacidipine Study on Atherosclerosis; ELVA=Effect of Long-Term Treatment of Metoprolol CR/XL on Surrogate Variables for Atherosclerotic Disease; BCAPS=Beta-Blocker Cholesterol-Lowering Asymptomatic Plaque Study
Source: [Patel et al. 2004].

Table 27: Technical Aspects of Selected Studies Using Manual Measurement of Carotid Intima-Media Thickness

Study	Subject Population (n)	Transducer	Location of Measurement	Baseline c-IMT (mm)	Reproducibility
Baldassare et al. (2000)	Random consecutive subjects (n=963)	Biosound 2000 II SA 8 MHz	CCA/Bifurc/ICA (near/far wall)	0.85 ± 0.33 (total) 0.77 ± 0.29 (women) 0.92 ± 0.36 (men)	Variability Intra: r=0.95 Inter: r=0.96
O'Leary et al. (1999)	Subjects 65 yrs or older from CHS (n=4476)	Toshiba SSA 270A 6.7 MHz	CCA/ICA (near/far wall)	CCA: 1.03 ± 0.20 ICA: 1.37 ± 0.55	Spearman correlation between baseline and at 3 yr CCA: 0.75 ICA: 0.86 Combined: 0.84
Kronmal et al. (1996)	Subjects from CHS (n=3409)	Toshiba SSA 270A 6.7 MHz	CCA/ICA (near/far wall)	CCA: 0.99 ± 0.21 ICA: 1.57 ± 0.92	
Burke et al. (1995)	Subjects aged 45-64 from the ARIC study (n=13870)	Biosound 2000 II SA 8 MHz	CCA/Bifurc/ICA (far wall)	Mean difference of 0.06 mm in far wall c-IMT for subjects with and without cardiovascular disease	
Ferrara et al. (1994)	Subjects with primary hypertension (n=70)	Biosound 2000 II SA 8 MHz	CCA (far wall)	HTN vs. control Right: 0.71 ± 0.4 vs. 0.56 ± 0.2 Left: 0.83 ± 0.3 vs. 0.58 ± 0.2	Correlation index r=0.994
Riley et al. (1992)	Subjects from ACAPS (n=858)	Biosound Phase II 10 MHz	CCA/Bifurc/ICA (near/far wall)	1.31 ± 0.21 (w/in observer) 1.32 ± 0.22 (b/n observer)	Variability Intra: r=0.75 Inter: r=0.79

CHS=Cardiovascular Health Study; ARIC=The Atherosclerosis Risk in Communities Study; ACAPS=The Asymptomatic Carotid Artery Plaque Study; CCA=Common Carotid Artery; Bifurc=Bifurcation; ICA=Internal Carotid Artery
Source: [Patel et al. 2004].

Enhancement of the c-IMT with the use of USCAs

In 2004, [Macioch et al. 2004], described the use of i.v. USCAs enhance the carotid artery vessel wall lumen highlighting vessel wall luminal morphology including plaques, ulcers, and irregular luminal surfaces. In addition, the use of an ultrasound contrast agent provided an improved ability to detect the c-IMT of the traditionally difficult to image near wall of common carotid c-IMT. [van Swijndregt 1996].

Similarly, in 2004, [Kono et al. 2004], had performed clinical studies in subjects using USCAs for lumen and carotid artery stenosis identification.

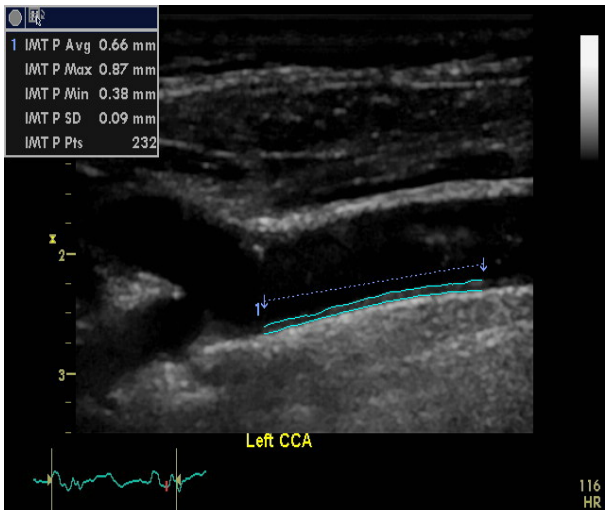
Table 28: The Effect of Contrast-Enhanced Ultrasound Imaging on Measurement of Carotid Artery Intima-Media Thickness

	Non-Contrast	Contrast	P-value
Near Wall c-IMT (mm)	0.62 ± 0.13	0.74 ± 0.18	<0.0001
Far Wall c-IMT (mm)	0.66 ± 0.14	0.66 ± 0.20	0.90
P-value	0.02	<0.001	

Source: [Macioch et al., 2004]

In Figure 11, the common carotid artery in imaged in the long axis view. The blue line located on the far wall (posterior) of the common is a computer-derived measurement of the c-IMT of the far wall of the common carotid artery. The numbers noted in the left upper corner of the figure are computer derived measurements of the c-IMT parameters (average, maximum, minimum, mean standard deviation, and number of points).

Figure 11 Longitudinal Axis of the Common Carotid Artery



In Figure 12, the common carotid artery in imaged in the long axis view as seen in Figure 11 above. The lumen is filled with ultrasound contrast material (white) which permits an enhanced view of the near (anterior) wall c-IMT of the carotid artery. The blue line located on the far wall (posterior) of the common is a computer-derived measurement of the c-IMT of the far wall of the common carotid artery. The numbers noted in the left upper corner of the figure are computer derived measurements of the c-IMT parameters (average, maximum, minimum, mean standard deviation, and number of points).

Figure 12 Enhancement of the Common Carotid Artery

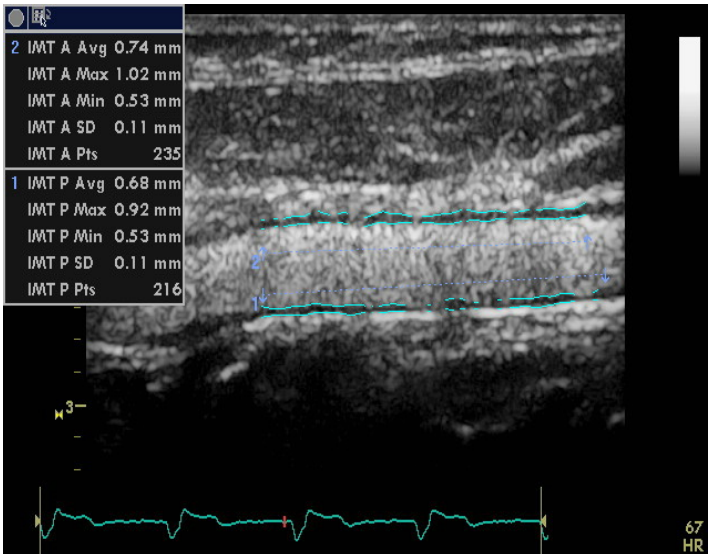


Table 29: Comparative Non-invasive, Surrogate Markers of Atherosclerosis

	Availability	Contribution to Office-based risk stratification	Reproducibility	Image Enhancing Techniques	Newer Developments
Carotid ultrasound Intima-Media Thickening (c-IMT)	Widely available	Yes	5-11% for CCA	Use of contrast agents	Imaging of vulnerable plaque and plaque vascularity
Coronary calcium imaging (EBCT)	Limited to large centers	Not proven	24-49%	Use of EKG trigger; decreased motion artifact; repeat measurements	3D reconstruction
Cardiac Magnetic Resonance Imaging (CMR)	Not widely available	No data	4-6%	Better image acquisition; improved external coils	Use of contrast agents; demonstration of plaque composition
Brachial Artery Reactivity Testing	Available	No data	Affected by many factors, including smoking, caffeine and temperature	-----	Edge detection software to delineate vessel wall interfaces (lumen-intima, media-adventitia)
Ankle-Brachial Index (ABI)	Widely available	Yes	10% for multiple measures	-----	-----

Adapted from the 34th Bethesda Conference: Task Force #3.
Source: [Patel et al. 2004].

7.3.3 Identification of atherosclerotic-related neovascular changes

Identification of atherosclerotic-related neovascular-changes within the adventitial vasa vasorum and plaques morphology in subjects with known carotid artery stenosis

In 2004, [Feinstein 2004] described the presence of the carotid arterial vasa vasorum in subjects with cerebral vascular disease as noted in the Figure 13. Subsequent clinical histopathology validation studies have been performed by 2 independent laboratories [Shah et al. 2007 and Coli et al. 2008]. Using MRI technologies, the presence of the angiogenesis within the arterial wall atherosclerotic plaque has been similarly identified [Kerwin et al. 2003].

Based on the several experimental studies [Heistad and Armstrong 1986; Wilson et al. 2002; and Mouten 2001] the presence of vessel wall angiogenesis (vasa vasorum) appears to precede the thickening of the IMT. Of note, in 1985, Heisted demonstrated the vasa vasorum regressed following the withdrawal of an inciting inflammatory noxious stimulus (cholesterol feeding in primates). Similarly, Wilson in 2002, induced vasa vasorum regression following the use of statins in swine fed high cholesterol diets. Using experimental models, the authors have shown that the earliest manifestation of atherosclerosis may be the earliest presence of the arterial vasa vasorum, through pathology studies have linked plaque angiogenesis and plaque vulnerability [Fleiner et al. 2004 and Moreno et al. 2004]. Historically, the observed connection between the tumor growth and the vasa vasorum was proposed by Judy Folkam in 1971. Folkman and Moulten proposed that atherosclerosis, similar to caner tumor growth stimulated the growth of angiogenesis, and the development of vasa vasorum [Folkman 1971 and Moulten et al. 2003].

Recent presentations at national meetings (including the American College of Cardiology [ACC] and Arteriosclerosis, Thrombosis and Vascular Biology [ATVB]) revealed the clinical utility of performing quantitative contrast ultrasound examination of the vasa vasorum in subjects with diabetes [Coli et al. 2007; Adam et al. 2008; and Coll et al. 2008].

Overall, the novel observation of detecting the presence of angiogenesis within the human atherosclerotic plaque using contrast ultrasound methods was considered a highlight in 2006 and described by the editors of the Journal of the American College of Cardiology [DeMaria et al. 2006].

Figure 13 is a longitudinal axis of a contrast-enhanced ultrasound examination of a carotid artery in a subject with significant luminal atherosclerotic plaques (arrow). Note the lumen is highlighted (white) with the contrast agent, and as shown by the arrows, there exists intra-plaque neovascularization as identified by the presence of the small white reflectors indicating OPTISON transit through the intra-plaque vasa vasorum.

Figure 13 Longitudinal Axis of a Contrast-enhanced Ultrasound Examination of a Carotid Artery in a Subject with Significant Luminal Atherosclerotic Plaques

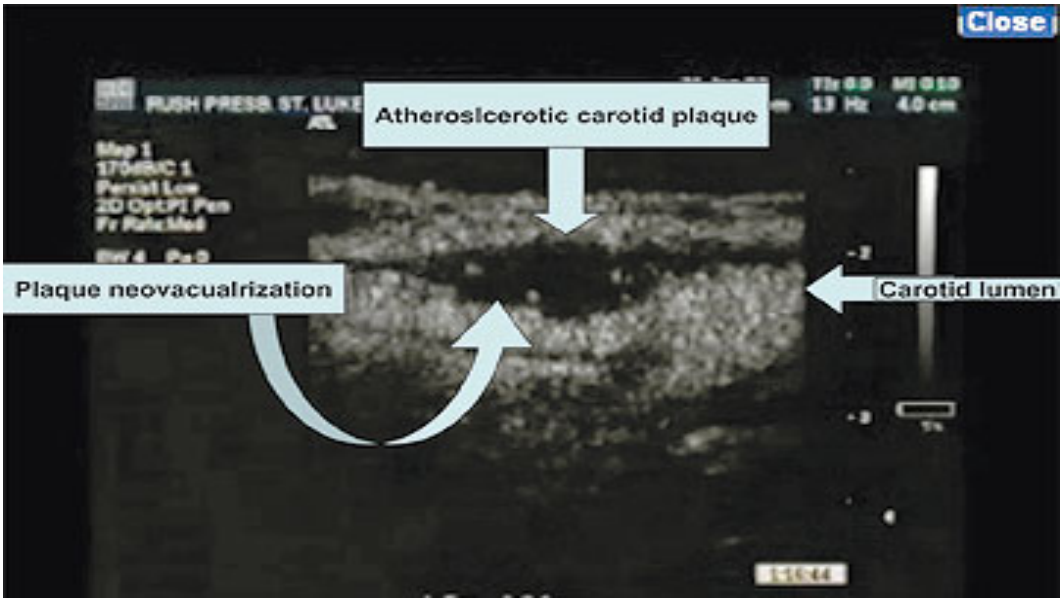
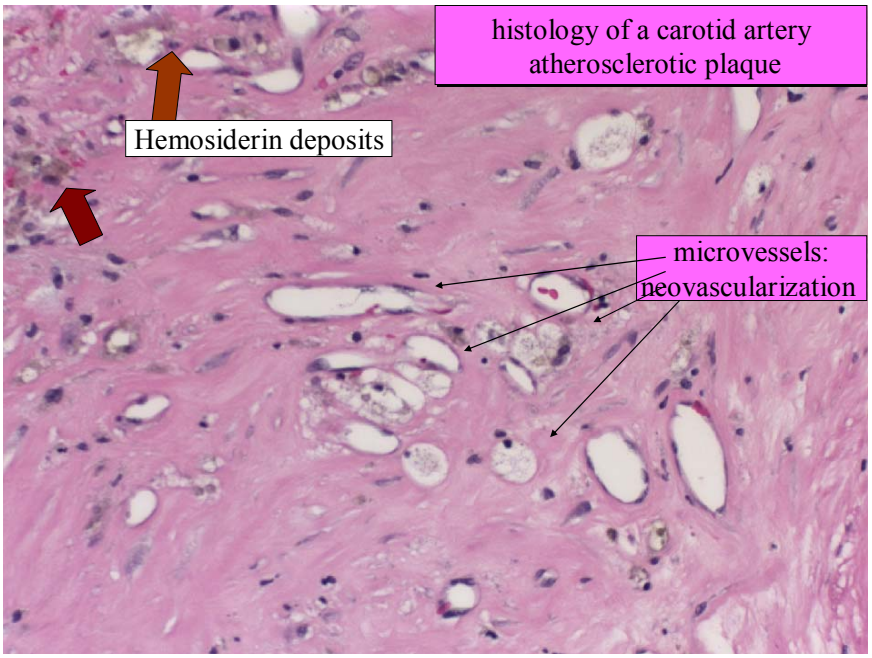


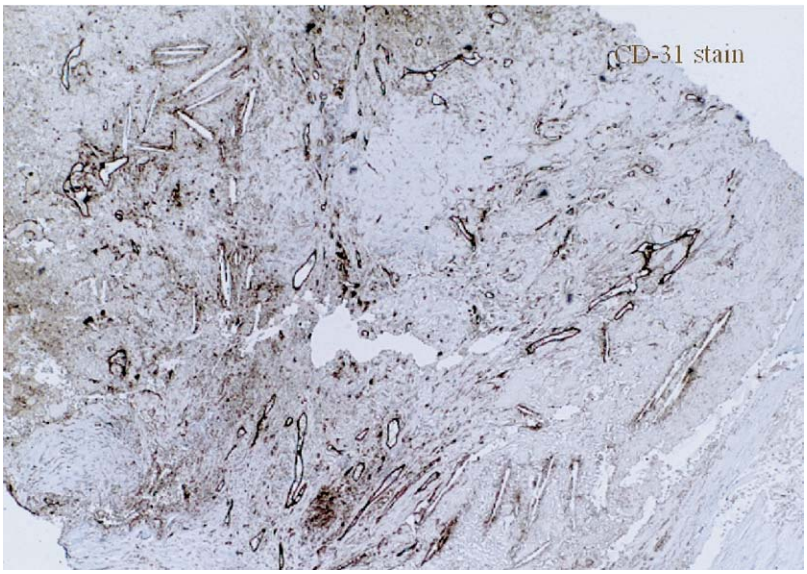
Figure 14 Representative Histology Slide from Subject who Underwent Carotid Endarterectomy Surgery



Note: Notice the presence of numerous intra-plaque vascular channels (H&E stain).

As noted in Figure 14 above, this tissue specimen displayed below in Figure 15 was obtained at the time of a surgical carotid endarterectomy. The intra-plaque neovascularization is highlighted by the use of CD-31 stain (mouse auto-antibody to human endothelial cells).

Figure 15 Representative Histology Slide from Subject who Underwent Carotid Endarterectomy Surgery



7.4 Safety Considerations

7.4.1 Dose of ultrasound contrast agent

It is anticipated that the ultrasound contrast dose for carotid vascular imaging will be reduced, or at most, stay within the same dose range as for approved indications and consequently there is no need to document safety for higher doses of these agents.

7.4.2 Acoustic energy, Mechanical Index

The acoustic energy required for visualization of the contrast material in the carotid artery is considerably less than that used in cardiac imaging (i.e. cardiac imaging includes Doppler and B-mode). Routine clinical practice in cardiac imaging with contrast utilizes the ALARA and consequently the operator uses a mechanical index value below 1.5. Mechanical index is defined as the peak negative pressure divided by the square root of the frequency and is expressed in log values. The mechanical index required for carotid imaging ranges between 0.06 and 0.40 and may vary depending on the on the manufacturer of the ultrasound equipment.

7.4.3 Population to be studied

The proposed study population could include the following subjects:

- Subjects with suspected or established carotid artery disease.
- Stable cerebrovascular disease (no recent stroke or TIA, < 6 weeks prior to enrollment).
- Stable cardiovascular disease (no recent MI, unstable angina of cardiac intervention within 6 weeks of enrollment).
- Stable subjects who are at increased risk of CV diseases with the diagnosis of metabolic syndrome, diabetes, impaired fasting glucose, and elevated lipids.
- Pre-operative evaluation: Subjects undergoing cardiovascular surgery are often at risk for post-operative strokes; therefore, it is often recommended that these subjects undergo a non-invasive technique to assess the carotid artery vasculature.
- Subjects with stable cerebrovascular disease who cannot undergo other non-invasive imaging techniques for detection of CV disease (i.e. MRI, CT scan).
- Subjects who cannot undergo additional invasive testing (i.e. dye allergy) and are otherwise stable cardiovascular subjects.

7.4.4 History and physical examination

All subjects should have a relevant history and physical examination prior to enrolling in the study. The history should include a history of allergy to blood products, recent history of MI, TIA, or CVA (within the last 6 weeks), history of unstable congestive heart failure. Physical examinations port injection should be performed as well.

7.4.5 Monitoring parameters

On study parameters should include: vital signs (BP, HR, and respiration), oxygen saturation, and 12-lead ECGs. Vital signs should be recorded prior to injection and frequently post injection (e.g., -2, 2, 4, 6, 8, 10, 20, and 30 minutes). ECGs and oxygen saturation should be performed prior to injection and twice post-injection (e.g., 10 and 30 minutes post injection).

Background safety data (current clinical experience and published literature)

Investigators at Rush University Medical Center in Chicago, Illinois, have performed 792 contrast-enhanced, carotid ultrasound examinations initiated in 2001 (Rush Medical Center, ORA# 01062001, initiated in 2001, PI Steven Feinstein). Since 2001, 692 studies have been performed. There have been no reported deaths or other SAEs.

[Jung et al. 2002] reported on the use of OPTISON in 88 subjects with significant pre-existing cerebrovascular disease (severe carotid artery stenosis). Both Doppler and B-flow imaging methods were used. There was no description of untoward safety events.

[Ohm et al. 2005], used contrast ultrasound imaging of the carotid artery in 10 subjects with severe advanced, cerebrovascular diseased subjects. There was no description of untoward safety issues.

[Macioch et al. 2004] reported on the use of OPTISON for carotid artery enhancement in 26 subjects with pre-existing cerebrovascular disease. No deaths or other SAE's were reported.

[Kono et al. 2004] reported on the use of OPTISON for the diagnostic evaluation of carotid artery disease. No deaths or other SAE's were reported.

7.5 Summary

It is anticipated that clinical trials for the use of ultrasound contrast agents in subjects will be a safe and efficacious non-invasive method that can be implemented to evaluate the presence of cerebrovascular disease in subjects considered to be at risk.

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