ADVISORY COMMITTEE MEETING

BRIEFING DOCUMENT

DEFINITY® (Perflutren Lipid Microsphere)

NDA # 21-064

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1. OVERALL SUMMARY

Activated DEFINITY® is a second-generation lipid encapsulated perfluoropropane (PFP) filled microsphere suspension designed for intravenous (IV) administration. The product is provided in vials which contain a blend of three endogenous lipids (one conjugated to methoxypolyethylene glycol 5000) and PFP in the headspace. Upon activation by agitation with a Vialmix® (specialized mixer), the clear colorless liquid becomes a homogenous, opaque, milky white injectable suspension of PFP lipid microspheres of consistent number and size distribution. At the recommended maximum clinical dose for ultrasound imaging (0.01 mL/kg with possible administration of a second dose of 0.01 mL/kg), the average number of microspheres that are administered is low (~10^9 to a 70 kg individual: about the same as the number of cells in 1 mL of blood). The total amount of PFP administered at the clinical dose of activated DEFINITY® is small (≤ 350 μL to a 70 kg individual).

Extensive preclinical and clinical safety and efficacy assessment established the product was effective and safe as an ultrasound contrast agent and was approved in the US for left ventricular opacification and delineation of the left ventricular endocardial borders in 2001.

1.1 Preclinical

Safety pharmacology and toxicology studies were designed to support the clinical use of DEFINITY® as an intravenously administered agent used in the acute setting. Safety pharmacology studies in the dog demonstrated that doses up to 25 times the clinical dose (0.5 mL/kg) did not affect cardiopulmonary function. At higher doses marked increase in respiration rate and pulmonary arterial pressure were observed and fatalities were produced. However, even in a model where severe pulmonary compromise was induced, the highest dose tested (0.2 mL/kg [10 times the clinical dose]) did not affect cardiopulmonary function.

Similarly DEFINITY® (0.5mL/kg) did not affect cardiopulmonary function in mechanically ventilated dogs. Examination of the affect of activated DEFINITY®on the microcirculation indicated a very small fraction (1.2%) of the administered dose was retained and this transient retention did not have a detrimental effect on the systemic hemodynamics even at 40 times the clinical dose (800 μl/kg). Additional safety of activated DEFINITY® was demonstrated in the rhesus monkey where cardiovascular and ECG effects were not seen even at 50 times the clinical dose (1000 μl/kg). Studies with DEFINITY® in the primate also suggest cardiopulmonary effects are only produced at very high doses levels (≥150 fold the recommended clinical dose). The main toxicological findings in rats and primates relevant to clinical use were acute clinical signs seen with high dose levels that were consistent with the safety pharmacology studies and suggestive of a cardiopulmonary origin. Moderate changes in Pulmonary Artery Pressure (PAP) were reported for DEFINITY® in the pig. However, in the primate, the clinical signs were not associated with anaphylactoid response mediated by mast cell degranulation or activation of complement, suggesting that complement activation-related pseudoallergy (CARPA) was not involved in this animal model. Overall the no observable effect levels in the rat and primate were 5 and 15 fold the maximal recommended clinical dose.

1.2 Clinical

The safety pharmacology and toxicology assessments in rats and primates indicated DEFINITY® was likely to be well tolerated in man. Overall safety evaluation of data from 3985 subjects in 48 studies (26 in echocardiography and 12 in radiology) support this conclusion. Based on detailed safety analysis from the 40 studies submitted in the MAA, a total of 26% subjects had at least one new-onset adverse reactions and 7.6% of them judged to be treatment related AEs. The most common drug related AEs (reported >1%) are fatigue, headache, dyspnea, back pain, nausea, flushing, and dizziness. Less commonly reported AEs

(0.5%~1.0%) are dysgeusia, chest discomfort, pain NOS, altered sensation, and pain at the injection site. Several of the clinical studies involved stress testing which is known to produce adverse events. Furthermore, no dose relationship was identified for any individual new-onset AEs reported for DEFINITY®-treated subjects regardless of mode of administration.

In placebo-controlled studies, a total of 126 (56%) of 224 placebo subjects experienced a new-onset AE while 259 (48%) of 543 subjects receiving DEFINITY® experienced a new-onset AE. The profiles of the AEs were also similar. AE rates in rest-stress echocardiographic imaging studies were slightly higher (56%) than in rest-only studies (24%) attributable mainly to the use of stress agents and additional dosing to the stress subjects.

The rates of serious AEs and fatal outcomes were low. A total of 34 SAEs from 3985 subjects were reported. Out of 34 SAEs, eight events had fatal outcomes and the remaining 26 SAEs were non-fatal AEs that were classified as serious. None of the serious AEs was considered by the investigators to be related to the use of DEFINITY®. No fatal outcome occurred in placebo subjects. All of these fatal outcomes occurred at least one day after the administration of DEFINITY® and were considered to be not be related to drug.

Studies involving specific disease populations were also performed. A clinical study of 38 subjects on mechanical ventilation reported no clinically significant abnormalities in any of ventilation safety parameters. These findings are consistent with preclinical studies. A small pharmacokinetic study was performed in COPD subjects with a high (50uL/kg) dose of DEFINITY®. No serious adverse events were observed and the lung clearance of PFP in the COPD subjects was similar to the normal control. Some adverse events were reported for both the COPD (7/12) and Normal (4/12). In addition, based on reviewing the case report forms (CRFs) from the patients who had a history of COPD and received at least one dose of

DEFINITY® in other trials, a total of 10 (22%) of the 46 patients reported at least one new-onset of AE. It appears the nature of these events was similar and overall suggests pulmonary compromise did not change DEFINITY® effects. Interestingly, no clinically important changes were noted in most of the immunology parameters evaluated (CH50, immunoglobulins A, E, G and M, histamine, anti-double stranded DNA antibody, tryptase) with the exception of a transient elevation of C3a. Since the increase in C3a levels with this high DEFINITY® dose was not associated with the release of bioactive mediators there does not appear to have been mast cell or basophile activation or any anaphylactoid / anaphylactic reactions. The toleration of DEFINITY® in this limited evaluation of COPD subjects is consistent with no changes seen in cardiopulmonary parameters in the preclinical pulmonary compromised dog studies.

In placebo-controlled studies with subjects with CHF, the overall incidence of new-onset AE appeared to be slightly higher in DEFINITY®-treated subjects than placebo subjects (35% vs. 23%). Overall rate of new-onset AEs observed in subjects with acute MI was lower than that observed for DEFINITY®-treated subjects in overall echocardiography studies.

Retrospective analysis of ECG monitoring data for ventricular and atrial premature beats, indicates that use of DEFINITY® appears not to pose a risk related to premature beats because DEFINITY® is commonly used with a low-MI mode in clinical practice. (The maximum recommended Mechanical Index for ultrasound used with DEFINITY® is 0.8)

Based on the above study data from all clinical trials, DEFINITY® was safe and well tolerated in general both in echocardiography and radiology studies and also in high risk population of subjects with pre-existing cardiac conditions.

We identified 23,772 human subjects in 52 publications who were administered DEFINITY®. In general, all these publications state that DEFINITY® appears to be well

tolerated and treatment-related SAEs were rarely observed. They did not appear to show nephrotoxicity or cardiotoxicity and the incidence of hypersensitivity or allergic events appears much lower than in current X-ray or MR contrast agents (Blomley et al. 2007).

In summary, the overall rate of treatment-related AEs in clinical trials was low based on 3985 subjects who received at least one dose of DEFINITY® in clinical trials. The most frequent treatment-related AEs with DEFINITY® were fatigue, headache, dyspnea, back pain, nausea, flushing, and dizziness. They were mild-to-moderate in intensity, short duration, and did not require therapeutic intervention. In peer-reviewed publications, DEFINITY® was well tolerated and no significant safety issue was observed. Other safety parameters showed minor changes that were not clinically significant. Thus, the use of DEFINITY® provides a significant diagnostic benefit compared to a small and clinically insignificant risk.

1.3 Post-marketing Safety Experience

Small absolute numbers (<300) of serious adverse events including very small numbers fatalities have been reported following the use of DEFINITY® in routine clinical practice since its introduction in 2001. Most of these events have been identified through spontaneous post-marketing AEs reports. Over the same period, approximately two million doses of DEFINITY® have been administered to patients. We recognize that the rates of serious adverse events are difficult to estimate based upon post-market reports. However, for use of DEFINITY®, reporting rates of serious reactions may be improved since DEFINITY® is always administered in a clinically monitored environment and delivered in the presence of healthcare providers. Accordingly it appears that these serious events are rare or very rare.

We are not aware, beyond those already examined, of specific animal models that would easily predict such rare clinical events or allow patient subgroups that are predisposed to be prospectively identified. We do, however, intend to use model systems to further investigate possible effects if they are identified. The low frequency of events makes it difficult to

identify such reactions in the pre-market clinical studies as well. Eight fatal adverse events did occur in our pre-market clinical studies, though all fatal events occurred more than a day after DEFINITY® administration (most several days later), and were considered related to underlying medical conditions rather than to DEFINITY® administration. Given the spectrum of underlying cardiovascular disease prevalent at high rates in the intended target population for use of DEFINITY®, it is not surprising to see an association of serious cardiopulmonary reactions, including rare fatalities, in the post market experience. Main et al (2007) reviewed the issue and the authors argue that many such serious adverse cardiopulmonary reactions are likely "pseudo-complications", that is, events resulting from underlying disease regardless of any temporal association with DEFINITY® exposure. Subsequently, Kuznetsky et al (2008) reviewed short term in hospital mortality in over 18,000 patients in a single hospital experience, including those who had echocardiography performed with or without contrast administration. They concluded that there was no excess risk of fatality associated with DEFINITY®, while those exposed to DEFINITY® were actually sicker at baseline prior to exposure. Kuznetsky's results prompted our own large scale retrospective review of in-hospital mortality within 24 hours of DEFINITY®. In a cohort of over 50,000 patients that received DEFINITY® and over 4 million receiving echocardiography without DEFINITY®, we conclude that use of DEFINITY®, rather than increase the risk of fatality, may have sufficient benefit on diagnosis and patient management so as to improve survival.

Since the trigger mechanism underlying these adverse reactions is not clear, some reactions may be pseudocomplications and therefore unrelated to DEFINITY® use, and since the patients who often stand to benefit the most from diagnostic information produced by contrast echocardiography may be the most critically ill (Main et al, 2007), we conclude that specific contraindications for certain patient populations is not warranted at this time (except for those with known prior hypersensitivity reactions to perflutren-containing microbubble contrast agents). Lantheus Medical Imaging does agree that appropriate warnings about the

potential for serious hypersensitivity, cardiopulmonary and or central nervous system reactions are made, as well as directing the responsible physician to make a careful risk-benefit assessment in unstable patients.

1.4 Safety Surveillance and Safety Commitments

Our continuing commitment to gathering and understanding the safety profile of DEFINITY® as used in clinical practice. A multidisciplinary team ensures continuous proactive assessment of product safety throughout its life cycle. In addition to individual case and periodic aggregate spontaneous adverse event (AE) data review and reporting, we conduct review of adverse events reported in clinical trials sponsored by LMI, global literature review, and other signal detection activities as part of internal company procedures, utilizing close physician review and descriptive assessments to further facilitate early identification and characterization of emerging safety issues of specific importance, ongoing benefit/risk evaluation and timely updating of DEFINITY® labeling.

In summary, safety signal detection includes the integration of all reasonably available sources of safety information to identify and characterize unrecognized safety risks or changes in those which are currently expected adverse drug reactions. As needed, changes in the safety profile of DEFINITY® are addressed by appropriate adjustments to risk communication and risk minimization strategies, such as changes in Company Core Safety Information and Product Labels. Such changes in safety information are included with the aggregate reports and ad hoc communications with Health Authorities. The ultimate objective of signal detection is the early identification, characterization, and communication of clinically important adverse events to achieve patient benefit at the lowest risk.

This process is comprehensive, integrated risk management plan to ensure the maintenance of a favorable benefit-risk balance for DEFINITY®. The RMP has been designed to continually assess known or potential risks. The risk management plan also gathers further

information on risks among patients with pulmonary hypertension and other subgroups of patients with serious underlying cardiopulmonary disorders. The safety surveillance process has been designed to continually assess known or potential risks. As new safety data emerge, the information will be communicated to Health Authorities in scheduled aggregate reports or on an ad hoc basis as warranted.

1.5 Introduction

In spite of improving survival trends, Coronary Heart Disease (CHD) is the number one cause of death in the U.S. leading to 953,000 deaths per year. Furthermore, an estimated 1.2 million myocardial infarcts occur yearly of which 494,000 will be fatal. Also, Congestive Heart Failure (CHF) rates continue to increase as the population ages and therapies for acute coronary syndromes reduce mortality. Echocardiography is widely used for the diagnosis and management of these and other cardiopulmonary conditions.

Echocardiography is a practical, cost-effective and non-invasive imaging modality that has been used for over 30 years as the diagnostic procedure of choice for a wide variety of cardiac and vascular diseases, including the detection of coronary artery disease. However, physical factors limit the quality of left ventricular images in up to 10-20% patients, especially those with obesity, lung disease, and bodily structural abnormalities. Many times these patients are seriously ill or in critical care. In stress echocardiography, the proportion of technically limited studies may be as high as 33%. Poor images reduce the sensitivity and specificity of the tests and thus impair the quality of the diagnostic information obtained.

In response to these issues, echo contrast agents have been developed to provide greater left ventricular opacification and delineation of the left ventricular endocardial borders, so that the size, shape and motion of the ventricular walls can be better visualized. Because of the physics of ultrasound scattering, the contrast agents developed have generally been in the form of short-lived gas-filled microbubbles that are sufficiently small to pass through the

pulmonary capillaries and therefore appear in the left side of the heart after simple intravenous bolus injection or infusions. Microbubble contrast agents have been available in the US since the first approved agent in 1994 (Albunex MBI, California]).

So-called "second generation" perfluorocarbon-filled microbubble agents DEFINITY® (BMS, NJ) and Optison (GE Healthcare (UK)) were approved by the FDA after demonstrating superiority to placebo and Albunex, respectively (Main 2007). In addition both agents demonstrated acceptable safety profiles. These agents have greater bubble uniformity and longer intravascular persistence with resulting improvements in echocardiographic images (Bhatia 2008).

Prior to launching DEFINITY® an intensive physician and sonographer training program called DEFINITY®Value Assessment (DVA) was initiated to ensure optimal product use in accordance with the approved label. Medical Liaisons (ML) pre-qualified health care professionals in understanding product characteristics, product activation, administration, equipment settings, imaging protocols, and patient monitoring. A satisfactory assessment was required prior to making commercial product available. Ongoing support was then established with qualified customers. Medical Liaisons continue to provide physician and sonographer training and are critical professionals in communicating scientific and labeling information to customers.

Approximately 600,000 DEFINITY® contrast enhanced echocardiography procedures were performed in the year 2007 and more than 2,000,000 have been performed since the first product approval in the year 2000.

The American Society of Echocardiography (ASE) (asecho.org) has developed comprehensive guidelines for the use of contrast in Echocardiography procedures. Some of these guidelines include:

- "Guidelines for the Cardiac Sonographer in the Performance of Contrast Echocardiography: Recommendations of the American Society of Echocardiography Council on Cardiac Sonography"
- "Contrast Echocardiography: Current and Future Applications"
- "Stress Echocardiography: Recommendations for Performance and Interpretation of Stress Echocardiography"

These guidelines are useful in communicating the safe and effective use of contrast echocardiography. They recognize that contrast is a necessary option for the diagnosis of the cardiac patient since it provides important information for difficult to image patients and provides a portable bedside option for the critically ill patient.

As noted above, the most frequent causes for non-evaluable procedures resulting from poor quality images have been related to patient obesity, lung disease and bodily structural abnormalities. Moreover it is worth noting practitioners ultimately view utilizing microbubble contrast agents, when baseline images (>2 endocardial segments poorly visualized) are sub-optimal and contrast will salvage an otherwise non-diagnostic procedure, as a standard of care (Lester 2008). Contrast then allows these otherwise non-evaluable patients to benefit from the diagnostic information obtained by a non invasive standard of care procedure without requiring further risk of radiation exposure during nuclear testing, exposure to gadolinium containing agents, iodinated contrast agents or invasive coronary angiography procedures (Lester 2008). In addition, the expense for other testing in time and procedural costs are avoided when a contrast echocardiography procedure provides the desired diagnostic information.

Echocardiography is also frequently used to assess the impact of coronary artery disease on ventricular size and systolic wall motion both with and without contrast (Bhatia 2008). This procedure combines the echo exam with either treadmill or pharmacological stress testing (typically dobutamine or dipyridamole) to determine coronary blood flow. Approximately 40% of the DEFINITY® procedures performed in 2007 were done utilizing stress protocols.

Again this stress echocardiography procedure obviates the need for further patient risk associated with other procedures.

While the safety and efficacy of DEFINITY® with exercise stress or pharmacologic stress testing have not been established from a regulatory perspective, in the studies we conducted there were 27 controlled clinical trials with a total of 823 patients that have been studied.

Contrast echocardiography procedures are especially useful in the critically ill patient where the clinical setting mandates the efficient and timely acquisition of diagnostic patient information and may be the only possible approach; patients to sick to undergo invasive radiological imaging undergo contrast echocardiography. In these sick patients the assessments include evaluation of hypotension, shock, post cardiac arrest status and tamponade (Bhatia 2008, Main 2007).

However, this may have lead to some of the concerns regarding product safety. As noted by Main et al in 2007, complications may occur after any medical procedure and may be either attributable to the procedure itself or may be due to progression of the underlying disease state. This phenomenon has been defined as pseudocomplication and was first characterized decades ago in evaluating patient safety before and after cardiac catheterization. In essence the patients are “sick enough” to have events including death.

A key question becomes how to differentiate between events that are merely temporally related to the procedure and with those that are caused by the procedure. Initial retrospective studies comparing non contrast and contrast procedures suggest there is no difference in serious adverse event rates and additional studies are underway to prospectively address this key question.

Nevertheless, even when fully discounting the impact of pseudocomplication and comparing contrast echocardiography with other common procedures contrast echocardiography has the most favorable benefit-risk result. Also, Main et al (2007) note that even if the four fatalities reported in the FDA Alert were attributable to contrast the reporting death risk would be 1:500,000. However, by comparison the mortality rate for diagnostic coronary angiography is approximately 1:1,000, and the risk of myocardial infarction or death with exercise treadmill testing is approximately 1:2,500. Furthermore the lifetime risk of fatal malignancy after SPECT imaging is estimated to be between 1:1,000 to 1:10,000 (Main 2007).

To summarize, contrast echocardiography is an important diagnostic tool that provides critical patient information in a cost effective manner and avoids subjecting patients to other procedures with greater overall risk (Bhatia 2008, Main 2007).

2. REGULATORY SUMMARY

Regulatory Milestones

A New Drug Application (NDA) was filed for DEFINITY® on 8 December 1998 for:

Echocardiography: imaging of cardiac structures (ventricular chambers and endocardial borders) and function (regional wall motion)

Abdominal Ultrasound: imaging of the liver and kidney to provide additional diagnostic information which improves the evaluation of pathology and is useful in patient management decision making.

Approval

Two Approvable Letters (8 October 1999 and 4 August 2000) that included comments on Clinical Pharmacology/Toxicology, Chemistry, Clinical and Statistical analysis. After Lantheus Medical Imaging Inc. provided response packages (7 February 2000 and 30 January 2001) DEFINITY® received approval from the agency on 31 July 2001. It was approved with the following indication:

"Activated DEFINITY® (Perflutren Lipid Microsphere) Injectable Suspension is indicated for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border."

The approval letter also contained details for the four post-marketing commitments and the completion dates agreed upon with the agency. These commitments were:

1. To perform non-clinical studies to determine the fate of the activated microspheres, characterizing the length of microsphere persistence and the potential for microsphere gas exchange. (Completed 16 September 2004)
2. To complete non-clinical studies of the effects of mechanical ventilation on microbubble characteristics and on the toxicity of activated DEFINITY®. (Completed 16 September 2004)
3. To evaluate the efficacy and safety of activated DEFINITY® in adults undergoing mechanical ventilation. (Study results of study DMP115-214 were submitted 29 June 2007. FDA rescinded requirement with the inclusion of the new contraindications in the package insert 10 October 2007)
4. To perform a surveillance study of adverse events in at least one thousand patients receiving marketed DEFINITY®. The goal is to capture post-marketing safety information on DEFINITY® as it is actually used in clinical practice.

Boxed Warning

On 10 October 2007 FDA approved the labeling supplement that included the addition of a boxed warning, additional contraindications and warnings. Included in this approval was the commitment by Lantheus Medical Imaging to conduct a safety registry study in at least 1000 patients using DEFINITY® in routine clinical practice, as it is actually used in the clinical setting. This study is currently enrolling. The final study report is due to the Agency January 2010.

Lantheus Medical Imaging recently (12 May 2008) received approval for revisions to the DEFINITY® Package Insert. This approval included revisions to the boxed warning and warning sections that focused the monitoring of patients on those with unstable

cardiopulmonary conditions and pulmonary hypertension. In addition the contraindications that were included in the 10 October 2007 labeling supplement approval that focused on specific patient characteristics were removed. The labeling contains the contraindications that were approved in the original application (known or suspected cardiac shunts and hypersensitivity to perflutren).

In addition to the label revisions, Lantheus Medical Imaging committed to two post-marketing studies.

- a study to evaluate the effects of DEFINITY® enhanced echocardiography on pulmonary artery (PA) hemodynamics. This would be a single arm clinical study in which pulmonary artery hemodynamics will be assessed before and after the administration of DEFINITY® and evaluable data obtained from at least 30 patients with know or suspected cardiac disease. At least 15 of these patients will have pulmonary artery hypertension documented on baseline pulmonary artery pressure assessment.
- an observational clinical study using an existing database(s) to compare in-hospital mortality in critically ill patients undergoing echocardiography with and without DEFINITY®.

Foreign Approvals

DEFINITY® has been submitted and approved in many countries around the world with different indications based on the data that was available at the time of review and approval.

In **Canada** the approved indication is:

"DEFINITY® (perflutren injectable suspension) is indicated for contrast-enhanced ultrasound imaging of cardiac structures (ventricular chambers and endocardial borders) and function (regional wall motion) in adult patients with suboptimal echocardiograms.

The safety and efficacy of DEFINITY® with exercise stress or pharmacologic stress testing have not been established."

Abdominal Ultrasound

"DEFINITY® is also indicated for contrast-enhanced ultrasound imaging of the liver and kidneys in adult patients to improve the evaluation of pathology."

In **Europe** were the product is marketed with the trade name LUMINITY™, the approved indication is:

"Luminity is an ultrasound contrast-enhancing agent for use in patients in whom non-contrast echocardiography was suboptimal (suboptimal is considered to indicate that at least two of six segments in the 4- or 2-chamber view of the ventricular border were not evaluable) and who have suspected or established coronary artery disease, to provide opacification of cardiac chambers and improvement of left ventricular endocardial border delineation at both rest and stress."

The following table provides an overview of the approved DEFINITY® indications by country.

DEFINITY® Worldwide Approvals by Indication

	Resting Echocardiography	Stress Echocardiography	Cardiac Wall Motion	Abdominal Ultrasound
United States	√			
Europe	√	√		
Canada	√		√	√
Australia	√	√	√	√
Israel	√		√	√
India	√			
Argentina	√			
Venezuela	√			
Chile	√			
Columbia	√			
Brazil	√			
Mexico	√	√	√	√
Singapore	√		√	
Korea	√			

Lantheus Medical Imaging is currently in the process of harmonizing the labeling worldwide based on the recent (12 May 2008) labeling supplement approval received from FDA.

3. DEFINITY® CMC

DEFINITY® is provided as a buffered, sterile, non-pyrogenic, hypertonic liquid that upon activation contains:

- 6.52 mg/mL PFP (also known as octafluoropropane or perfluoropropane) in the headspace, determined by GC
- 0.75 mg lipid blend consisting of, determined by HPLC with evaporative light scattering detection:
  - 0.401 mg of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC)
  - 0.045 mg of 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid monosodium salt (DPPA)
  - 0.304 mg of N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine monosodium salt (MPEG5000 DPPE)
- Formulation matrix composed of phosphate buffer, saline, glycerin and propylene glycol.

DEFINITY® is activated prior to use with Vialmix®, a modified dental amalgamator which shakes the vial for 45 seconds at a 4550 cycles per minute. Activation produces a homogeneous opaque, milky white, injectable suspension of perflutren (PFP) lipid microspheres that is tested for: particle size and concentration using light obscuration (Accusizer, Particle Sizing Systems, CA), absence of microspheres ≥ 20 μm by microscopy, and PFP/mL by GC. Activated DEFINITY® has:

- A mean microsphere diameter 1.1 μm to 3.3 μm
- A concentration of 6.0×10^7 to 1.2×10^{10} perflutren lipid microspheres per ml
- Not less than 90% of the microspheres with a 1μm ≤ diameter < 6 μm
- Not less than 98% of the microspheres with a 1μm ≤ diameter < 10 μm

- Not less than 99.8% of the microspheres with a 1μm ≤ diameter < 20 μm
- No microspheres ≥20 μm
- 150±100 μL PFP (octafluoropropane) per ml at the time of initial preparation (approximately 7.5% of the initial headspace gas)

Specialized studies of activated DEFINITY® by freeze fracture electron microscopy indicated the microsphere is composed of a gas surrounded by a phospholipid monolayer. Analysis of the volume distribution using a Coulter LS 230 indicated greater than 60% of the gas is contained in microspheres greater than 1 μm in diameter. Zeta potential was measured for three lots of DEFINITY® as -3.5 (± 0.3) mV and +1.4 (± 2.1) mV, using two independent capillary electrophoretic techniques. The differences between the mean values for the two studies are within the experimental error of 5 mV for the two techniques, and indicate the surface of the microsphere is near neutrality.

4. DEFINITY® NON-CLINICAL SAFETY

4.1 Introduction

Gas-filled microspheres are proposed as a contrast enhancing medium for clinical ultrasound to aid in the assessment of left ventricular wall motion abnormalities, myocardial perfusion and in monitoring flow through various normal and diseased (i.e. cancer) capillary beds. Procedures using this material are noninvasive and significantly simpler to perform compared to angiography and nuclear medicine and can be performed at the patient's bedside, emergency room or in an outpatient setting. Activated DEFINITY® is administered as an intravenous administration during an ultrasound imaging session to guide diagnosis.

During the product development the characteristics of activated DEFINITY® response to ultrasound were examined *in vitro* using an acoustic attenuation system. The effectiveness of activated DEFINITY® for enhancement of ultrasound imaging was then assessed in a number of animal models. In particular, the open chest dog was used to allow assessment of left ventricular opacification and myocardial perfusion. A number of studies with DEFINITY® have also been performed in tumor bearing animal models by academic groups (Fleischer, 2004; Maruyama et al.,2000, 2003, 2004, 2005; O'Brien et al., 2004; Pollard et al., 2002; Schlosser, 2001). These test systems established the feasibility and usefulness of activated DEFINITY®.

Safety pharmacology and toxicology studies were designed to support DEFINITY® administered intravenously as a single injection in an acute setting. Activation of DEFINITY® (mechanically shaken with Vialmix®) results in a consistent microsphere

number and size distribution. At the recommended maximum clinical dose of 0.02 mL/kg for ultrasound imaging (0.01 mL/kg with possible administration of a second dose of 0.01 mL/kg), the average number of microspheres that are administered is low (~10^9 to a 70 kg individual: about the same as the number of cells in 1 mL of blood). The total amount of PFP administered at the clinical dose of activated DEFINITY® is small (≤ 350 μL to a 70 kg individual). DEFINITY® safety was evaluated as the finished product (all components including the non-endogenous phospholipid, MPEG5000 DPPE, and the gas, perfluoropropane) following activation.

The effect of activated DEFINITY® on cardiopulmonary dynamics, electrocardiography, hematology, blood hemolysis and blood gases were studied in the anesthetized dog. The dog was selected as a species widely used for cardiovascular assessment with a cardiopulmonary system similar to the human. Studies were also performed to specifically examine the possible impact on vascular beds by injection of activated DEFINITY® into the cerebral vasculature of rats, microscopic examination in the capillary beds of the mouse cremaster and microscopic examination in the capillary beds of the rat spinotrapezius muscle.

A series of toxicology studies included acute (single dose) and subchronic (up to 1 month in duration) studies in rats and non-human primates, a battery of *in vitro* and *in vivo* genetic toxicology assays, and developmental reproductive studies in rats and rabbits. Additional toxicology studies in rats and non-human primates were performed to investigate clinical symptomatology observed following high doses of activated DEFINITY®. The species selected were based on early study findings and the historical usage of these species in toxicity testing. Other toxicology studies to evaluate the potential irritancy and antigenicity of activated DEFINITY® were also performed.

4.2 Safety Pharmacology

4.2.1 Microcirculation

To assess the potential of activated DEFINITY® to interfere with the microcirculation, a study was conducted utilizing intravital microscopy in the mouse cremaster muscle. Administration of fluorescently labeled activated DEFINITY® via the jugular vein allowed the transit of activated DEFINITY® through the normal capillaries to be observed as single microspheres. The velocity of the microspheres was essentially identical to that of red blood cells (RBC) within the arterioles, capillaries and venules and did not cause any obstruction. In addition, even at the 800 μL/kg dose (40 times the human dose) used in this study the microspheres were separated by hundreds of RBCs and no aggregation of microspheres was seen. Intravenous administration does however require passage of microspheres through the lung capillary beds prior to entering the cremaster muscle. A second study was performed to examine the effect of intra-arterial administration of activated DEFINITY® up to 800 μl/kg using the rat spinotrapezius muscle. The study results demonstrated that a very small fraction (1.2 %) of the injected microspheres are retained transiently (85% were dislodged by 10 minutes) within the microcirculation during the first pass. There were no detrimental effects of activated DEFINITY® microspheres following intra-arterial administration and systemic hemodynamics and regional microvascular perfusion were not altered, even at the highest bolus dose tested (800 μL/ml-40 times the maximum human dose). To determine the influence on cerebral vascular damage, a study was performed to assess the effects of activated DEFINITY® at 100 μL/kg (5 times the human dose) injected directly into the carotid artery of anesthetized rats. Histopathology of brains taken 5 minutes after administration indicated no adverse effect to the cerebral vasculature. A literature report (*Mychaskiw, 2000*) using high doses of a different contrast agent (Optison™), showed disruption of the blood-brain barrier based on Evans blue distribution that lasted at least 24

hours in rats. However, this study examined brain sections at 90 minutes, 180 minutes and at 24 hours, whereas, activated DEFINITY® was only examined at 5 minutes. The significance of these differences is unclear. However, in the pre-clinical repeat dose toxicology studies performed by the Sponsor in rats and cynomolgus monkeys, thorough histological evaluation of sections of the brain did not reveal any effect on the cerebral vasculature.

4.2.2 Cardiopulmonary

The impact of activated DEFINITY® on the cardiopulmonary systems was assessed following intravenous administration in the anaesthetized closed-chest dog. At dose levels up to 0.5 mL/kg (25 times the human dose) activated DEFINITY® had no effect on hemodynamic parameters (LVP, LVEDP, PAP, ECG including QTc interval, HR, MAP), myocardial contractility, respiration rates and arterial blood gases. At 1 mL/kg activated DEFINITY® produced a marked increase in respiratory rate (300%) and pulmonary arterial pressure (180%) and the death of one animal. Parameters in surviving animals had returned to normal by 30 minutes.

The possibility that activated DEFINITY® could induce toxicity by affecting pulmonary function was further examined in anaesthetized spontaneously breathing dogs. Dogs were administered sephadex microspheres to induce either moderate (+15mm Hg) or severe (+30 mm Hg) acute pulmonary hypertension. In this pulmonary compromised model activated DEFINITY® administered up to the highest dose tested (0.2 mL/kg –10 times the human dose) did not change pulmonary arterial pressure, myocardial contractility or systemic hemodynamics. In addition respiratory rate, heart rate, QTc interval and arterial blood gases were not affected.

The influence of mechanical ventilation was also examined in the anaesthetized dog. Mechanical ventilation did not influence the persistence of activated DEFINITY® (500 μL/kg; 25 times the maximum recommended human dose) in the circulation as measured by auditory signal amplitude using a Doppler flow probe on the femoral artery. Furthermore, at this dose, activated DEFINITY® did not cause any meaningful changes in heart rate, arterial blood pressure, left ventricular pressure, +dP/dt, pulmonary arterial pressure or femoral arterial blood flow.

A further study was performed by the Sponsor using telemetered rhesus monkeys. Animals were dosed with activated DEFINITY® via intravenous infusion (2 mL/min) up to 1000 μL/kg. No clinically observable effects were apparent and cardiovascular parameters (blood pressure, heart rate and ECG) were not affected.

Elevated pulmonary arterial pressure and decreased arterial oxygen saturation have been shown in pigs following intravenous administration of small particles including the ultrasound contrast agent, Albunex® (Miyamoto et al., 1988; Ostensen et al., 1992). The pig appears to be very sensitive to induction of pulmonary hypertension as a consequence of thromboxane release from the pulmonary intravascular macrophages that are specific to artiodactyla (cloven hoofed) species. Szebeni et al have proposed that the pig represents a highly sensitive testing model for assessing hypersensitivity reactions and have reported on the effects of various liposomes (Szebeni 2005, Szebeni et al 2007).

Hypersensitivity reactions have been categorized as types I to IV. The type I reactions are acute and mediated by IgE, whereas types II-IV are more prolonged changes mediated by IgG, immune complexes or lymphocytes (Coombs and Gell, 1968). However, recently it has been proposed that a portion of type I type reactions do not involve pre-existing IgE antibodies. These non-IgE-mediated “anaphylactoid, pseudoallergic or idiosyncratic” reactions may actually be a large proportion of all immune-mediated immediate

hypersensitivity reactions (Demoly et al., 1999, Szebeni, 2001). Known examples of pseudoallergy include the reactions caused by X-ray contrast media (XRCM), liposomes, and solvents such as cremophor EL (CrEL) (Szebeni, 2005). Recent reports suggest that the reactions caused by XRCM, liposomes and CrEL have a common trigger mechanism: complement activation. The hypersensitivity reactions where the allergen can activate complement have been tentatively named complement activation-related pseudoallergy (CARPA) (Szebeni et al., 1999, 2000 a,b, 2005).

The hemodynamic changes caused by high dosing regiment of some liposomes in pigs included massive rises in pulmonary arterial pressure (PAP) with declines of systemic arterial pressure, cardiac output and left ventricular end-diastolic pressure (LVEDP) (Szebeni et al., 1999, 2000a,b). The hemodynamic changes were associated with large, although transient ECG alterations including tachycardia, bradycardia, arrhythmia, ST segment and T wave changes, ventricular fibrillation and cardiac arrest, all attesting to severe myocardial ischemia and consequent functional disturbance (Szebeni et al., 2005).

Studies have been reported on the cardiovascular effects and ultrasound imaging of DEFINITY® in the pig. Grauer et al (1996) reported that activated DEFINITY® at dose levels ranging from 0.5 to 10 μL/kg produced no changes in heart rate, systemic systolic pressure, arterial pO2 or LV fractional shortening in the pig and allowed quantifiable myocardial opacification. However, a mild reversible increase in PAP was seen (12 and 16 mmHg with 5 and 10 μL/kg, respectively). The highest dose represents the recommended highest single bolus clinical dose. Chantal et al (2005) reported on cardiovascular effects and ultrasound imaging with a continuous intravenous infusion of DEFINITY®. At an infusion rate of 33.6 μL/min over 5 minutes (representing approximately 5 μL/kg total dose) optimal sustained myocardial opacification was achieved. At this dose a mild, transient increase in mean pulmonary pressure (11.6%) was seen without significant changes in either heart rate (HR) or systemic arterial pressures.

A study was performed in cynomolgus monkeys by the sponsor to better understand the acute response observed with high doses of DEFINITY® in the animal models. A single intravenous dose of 3 mL/kg of well-characterized DEFINITY® administered post activation at a rate ~1 mL/kg/min elicited clinical signs (including abnormal respiration and unresponsiveness) during or immediately following dosing.

In this study there were no DEFINITY®-related changes in plasma tryptase levels in control monkeys and monkeys given 3 mL/kg of DEFINITY®, when values were compared as group means at each time point (pre-dose, 1, 3, 10 and 30 min), or when individual monkeys were compared to their respective pre-dose values. Likewise, no differences were found in mean plasma histamine values between control monkeys and monkeys given DEFINITY®. Virtually all histamine values for monkeys given DEFINITY® were less than the highest level achieved in controls. Also, no DEFINITY®-related differences could be detected in SC5b-9 complement levels. Although there was high intra- and inter-animal variability in SC5b-9 levels among control and DEFINITY®-dosed monkeys, the range of values was similar in control (0-83 ng/mL) and DEFINITY® (0-135.8 ng/mL) monkeys and there were no consistent trends.

The absence of any meaningful change in hematology parameters or plasma levels of histamine, tryptase or complement (SC5b-9) in this animal model indicated that the acute response to activated DEFINITY® was not an anaphylactoid response mediated by mast cell degranulation or activation of complement. Abnormal electrocardiographic changes, including ST-T segment depression followed by cardiac arrhythmias within 1 minute of initiation of dosing, did suggest transient myocardial ischemia may be a factor following high dose administration of activated DEFINITY®, however the dose of 3 mL/kg is 150 times the maximum recommended bolus clinical dose.

In vitro studies using well-characterized lots of DEFINITY® were conducted to evaluate the effect of activated DEFINITY® on histamine release, production of reactive oxygen species and on platelet aggregation. These *in vitro* studies with activated DEFINITY® indicated it had no effect on histamine release from rat basophilic leukemia cell line (RBL-2H3), production of reactive oxygen species by rat peritoneal neutrophils or on aggregation of rabbit and human platelets.

4.2.3 Bioeffects

A concern with ultrasound has been the potential for induction of tissue damage associated with cavitation. The response to address this potential was the introduction of the mechanical index parameter and the restriction of clinical ultrasound instruments to a maximal MI of <1.9. Ultrasound contrast agents provide a nucleus source and reduce the ultrasound energy required to produce cavitation (Miller 2007). Work from several groups, using multiple testing models, have demonstrated all the ultrasound contrast agents can act similarly as a cavitation nucleus. Several extensive reviews have indicated that all the ultrasound contrast agents have the potential to induce cellular damage in vitro and microscale bioeffects in tissues in vivo when present at sufficient concentrations and exposed to sufficient ultrasound energy for sufficient time (Miller 2007, Miller 2008, Dalecki 2007). The medical significance of bioeffects with diagnostic ultrasound is not yet fully understood. Our approach taken with DEFINITY® is consistent with ALARA (as low as reasonably achievable) with a maximum recommended mechanical index (MI) for ultrasound used with DEFINITY® of 0.8 and a maximal recommended single dose of DEFINITY® of 10 μL/kg (with possible administration of a second dose of 10 μL/kg).

Several studies reported in the literature have specifically examined DEFINITY® in model systems for the potential to induce bioeffects. For example in vitro incubation of mouse

phagocytic cells (RAW-264.7)) with a high concentration (0.5%) of DEFINITY® for 30 minutes, followed by washing and exposure to ultrasound, demonstrated alterations of cell viability by Trypan Blue exclusion (Miller and Dou 2004).

A study by Kobayashi et al (2003) investigated the influence of DEFINITY®ultrasonography on microvessels. Rat mesentery was exposed to 1.8-MHz pulsed ultrasound with intravenous injection of high dose DEFINITY® (100 or 1000 µl/kg) and microvessel bleeding and endothelial cell injury was examined. Impaired endothelial cells were identified by the fluorescence of propidium iodide. Microvessel bleeding was examined also in the rat myocardium. DEFINITY®(100 µL/kg) with high energy (1.6 MI) ultrasound did not cause microvessel bleeding but did produce some endothelial cell damage. When the dose was increased to 1000 µL/kg, the combination of DEFINITY®and high energy ultrasound (1.6 MI) exposure resulted in capillary bleeding and increased endothelial cell injury in capillaries and venules. In the myocardium, microvessel bleeding was not observed under any conditions.

Chen et al. (2002) investigated the potential for myocardial damage in rats resulting from contrast echocardiography. Rats were administered DEFINITY®(400 µL/kg over 15-20 minutes) and exposed to diagnostic ultrasound with high MI values ranging from 0.8 to 1.6. No effects were observed on left ventricular size or function, nor was there evidence of damage from histology at any MI level. Troponin T levels were not changed at an MI of 0.8 even with the high DEFINITY®dose, however, levels of troponin T were elevated with higher MI and reached statistical significance at 1.6.

Li et al. (2004) examined the effects of ultrasound and DEFINITY®on premature beats and vascular permeability in rats *in vivo*. Effects were assessed by counting numbers of

premature contractions, counting petechiae (capillary rupture with erythrocyte extravasation) on the surface of the heart and measuring area of Evan's blue dye leakage as an indicator of increase in vascular permeability. With very high MI (1.5) petechiae, Evans Blue leakage and premature ventricular contractions were produced in the heart at all levels of DEFINITY®(10 to 1000 μL/kg) in a dose related manner. The magnitude of effects was also shown to be MI related when examined using a high dose of DEFINITY®(100 μL/kg); 0.41 and 0.77 MI did not significantly change PVC and 0.41 MI did no produce petechiae. In a second study in the rat (Miller et al 2005) examining Evans Blue leakage and premature heart beats a similar finding was made. High MI (0.9 or 1.6) with DEFINITY®(20 or 80 μL/kg) and long exposure (5 or 20 minutes in the same plane) produced dose, time and MI related effects. This paper concluded that limiting the MI and changing the plane of ultrasound imaging (as would be done in a clinical scan) would mitigate the potential for bioeffects in the clinic.

The rat model testing has been useful in demonstrating potential effects and identifying some dose/time/MI relationships. However there are some reservations concerning the relevance of findings in small animal testing to humans. For example, the ultrasound scan plane covers a substantial fraction of the entire rat heart. Miller et al. (2006) examined the microscale bioeffects in a canine model of myocardial contrast echocardiography. DEFINITY®was infused at 2 μL/kg/min for 10 min and imaged at 1.5 MHz (triggered every four beats at the end of systole) for 10 minutes in the same plane. Microvascular leakage and petechia were detected in the scan plane at an MI of 1.8. The effects were less apparent at an MI of 1.0 but still reached statistical significance. Premature complexes were detected on ECG records with the very high MI (1.8) but were not apparent with the sham or 1.0 MI studies. Similar findings in the dog were reported by Okazaki et al (2004) where MI <1 in combination with DEFINITY®as high as 100 μL/kg/min did not induce premature ventricular contractions.

The sponsor also performed a study in anesthetized open chest dog. Blood samples were taken from the left ventricle and carotid artery following high MI ultrasound exposure (fundamental freq=1.67, MI =1.2, gated second harmonic freq=1.67/3.34, MI=1.4) to the heart in the presence of clinically relevant (10 μL/kg/2min and 40 μL/kg/2min) dose levels of contrast agent. Whole blood cell count and plasma hemoglobin measurements indicated the absence of hemolysis

Overall studies with DEFINITY® and other ultrasound contrast agents indicate microscale bioeffects can be produced in some situations in animal models. Although the clinical relevance is not yet apparent the maintenance of a low dose of agent in combination with low MI and short durations of scan in any particular plane will mitigate the potential to produce microscale bioeffects.

4.3 Toxicology

4.3.1 Single Dose Toxicity

The clinical signs observed in rats and cynomolgus monkeys given single intravenous doses of activated DEFINITY® were similar in nature (decreased activity, increased respiration, change in heart rate), and occurred during or soon after intravenous dosing and, apart from mortality, typically resolved within 30 minutes. In studies conducted by the Sponsor with well-characterized DEFINITY® the no observable-effect dose (NOEL) for clinical signs were 0.1 mL/kg in rats and 1 mL/kg in cynomolgus monkeys given a single intravenous dose of activated product.

The no-effect doses for clinical signs in rats and monkeys are 5 and 50 times, respectively, the recommended maximum clinical dose of 0.02 mL/kg for ultrasound imaging (0.01 mL/kg with possible administration of a second dose of 0.01 mL/kg). When these no-effect doses are expressed in terms of the rate of dose administered, ~ 2 mL/kg/min in rats and ~ 1.2

mL/kg/min in monkeys, the margin is ≥ 60-fold compared to the injection rate for diagnostic use in humans (a discrete dose of 0.01 mL/kg administered in ~ 30-60 seconds or 0.01-0.02 mL/kg/min).

The effect of rate of activated DEFINITY® administration on clinical signs was more specifically examined by comparing bolus administration and infusion in the rat. Clinical signs were observed following a rapid single intravenous injection of 2 mL/kg (12 mL/kg/min) of DEFINITY® whereas, no clinical signs were observed following intravenous infusion of 2 mL/kg (0.2 mL/kg/min). Whether the difference in the rate of administration of DEFINITY® was solely responsible for the difference in clinical response in this study could not however, be conclusively determined because the perfluoropropane content and microbubble size distribution of activated DEFINITY® were not comparable under the conditions used for injection compared to those used for infusion.

4.3.2 Repeat Dose Toxicity

In general, the spectrum of clinical signs observed in rats and cynomolgus monkeys given single or repeated daily intravenous injections of activated DEFINITY® were similar. As with single doses the clinical signs (abnormal respiration, lying on side, and loss of consciousness) with repeat dosing occurred during or soon after intravenous administration and, apart from mortality, typically resolved within 30 minutes in both species. Regarding the cause of death of the rats and monkeys, a graded clinical response was observed with increasing doses of DEFINITY® Clinical signs were short lived and reversible and consisted of abnormal respiration, heart rate changes and decreased activity soon after administration of DEFINITY® at doses 0.3 mL/kg (rat) and 1.0 mL/kg (monkey) in single and repeated dose toxicity studies in both species. Higher doses of DEFINITY® 1.0 mL/kg (rat) and 3.0 mL/kg (monkey), resulted in more severe signs in both species including unresponsiveness, and

occasionally death. Although the cause of death in rats and monkeys were not evident from gross examination at necropsy or from microscopic evaluation of tissues, the nature of the acute response and the proximity to dose administration suggest altered cardiopulmonary function.

In definitive 1-month toxicity studies conducted by the Sponsor using well-characterized product, the no-observable-effect dose of activated DEFINITY® for clinical signs was 0.1 mL/kg/day in rats and 0.3 mL/kg/day in cynomolgus monkeys. The no-observable-effect doses (NOELs) for clinical signs after 1 month of treatment in rats (0.1 mL/kg/day) and monkeys (0.3 mL/kg/day) are 5 and 15 times, respectively, the recommended maximum clinical dose of 0.02 mL/kg for ultrasound imaging (0.01 mL/kg with possible administration of a second dose of 0.01 mL/kg). When these no-observable-effect doses are expressed in terms of the rate of dose administered, (2 mL/kg/min in rats and 1.2 mL/kg/min in monkeys), the margin is ≥ 60-fold compared to the injection rate for diagnostic use in humans (0.01 mL/kg administered in 30-60 seconds or 0.01-0.02 mL/kg/min; See Table 4.1 below). These safety margins are adequate to support the intended clinical use of activated DEFINITY®

Table 4.1 No-Observable-Effect-Dose (NOEL) for Clinical Signs – Interspecies Scaling of Administered Dose Based on Body Weight (Surface Area)

Species	DEFINITY® Dose mL/kg (mL/m^2)[a]	Dose Multiple	Rate of Dose Administration mL/kg/min (mL/m^{2}min)[a]	Dose Rate Multiple
Human	0.01x2 (0.37x2)[b]		0.01x2 (0.37x2)[b]	
Rat	0.1 (0.6)[c]	5 (0.8)	2.0 (12)[d]	100 (16)
Monkey	0.3 (3.6)[c]	15 (5)	1.2 (12.5)[e]	60 (17)

[a]FDA conversion factors (F) for interspecies scaling of body weight to surface area (mL/kg X F = mL/m^2) F = 37 (human); 6 (rat); 12 (monkey)

[b]The recommended maximum human dose for ultrasound imaging is 0.02 mL/kg (0.01 mL/kg in 30-60 seconds with possible administration of a second dose of 0.01 mL/kg). 30 sec / 60 sec/min =0.5 min. Hence 0.01 mL/kg / 0.5 min = 0.02 mL/kg/min.

[c]NOEL for clinical signs in 1-mo toxicity studies in rats and monkeys

[d]Dose administration in 1-month toxicity study in rats (T98-3-46) = ~3-5 sec. Therefore, 3 sec / 60 sec/min = 0.05 min. Hence 0.1 mL/kg / 0.05min = 2 mL/kg/min

[e]Dose administration in 1-month toxicity study in monkeys (T98-5-2) = ~1.2 mL/kg/min

In the 1-month toxicity study rats dosed with DEFINITY®≥ 0.1 mL/kg/day had lung lesions characterized by perivascular and peribronchiolar eosinophilic infiltrates, alveolar macrophage accumulation, bronchiolar goblet cell hypertrophy and hyperplasia, hemorrhage, and interstitial pneumonia. These changes were present in all males and females given 0.3 or 1 mL/kg/day, as well as in one male but no females dosed with 0.1 mL/kg/day of DEFINITY®. An increased incidence of minimal pulmonary eosinophil infiltration was seen in males and females given 0.1 mL/kg/day. Bronchiolar and mediastinal lymph node lymphoid hyperplasia seen in rats given ≥ 0.3 mL/kg/day of DEFINITY®and splenic extramedullary hematopoiesis seen in rats given 1 mL/kg/day of DEFINITY®were considered secondary to the pulmonary findings. There were no treatment-related pulmonary or lymph node changes in rats given 1 mL/kg/day of the formulation control. These lesions were seen to be reversible over a 28 day follow up. While pulmonary lesions were observed

in rats given ≥ 0.1 mL/kg/day of activated DEFINITY® for 1 month, no lung lesions were observed following a single intravenous dose ≤ 0.3 mL/kg in rats or at ≤ 1 mL/kg in cynomolgus monkeys (doses which are 15 and 50 times greater, respectively, than the recommended maximum clinical dose of 0.02 mL/kg for ultrasound imaging). The fact that the pulmonary changes were only observed after 1 month of dosing in rats indicates that repeated daily dosing with activated DEFINITY® is required to produce these lesions. Pulmonary lesions were not observed in cynomolgus monkeys even at the highest dose given (1 mL/kg/day) for 1 month. As activated DEFINITY® will not be given to patients on a repeated daily basis, the pulmonary lesions in rats following 1 month of treatment are not relevant to the clinical use of activated DEFINITY® Eosinophil infiltration has been reported following daily intravenous infusion of large volumes of saline in rats for a period of 1 month (*Morton et al., 1997*). This finding suggests that the eosinophil infiltration seen in the lungs of rats given repeated daily doses of activated DEFINITY® represents a non-specific response in this species.

4.3.3 Genotoxicity

Genetic toxicity assays (bacterial mutagenesis, *in vitro* chromosome aberration, and *in vivo* rat micronucleus assays) were conducted by the Sponsor with well characterized DEFINITY® All *in vitro* assay results were confirmed in replicate trials and activated DEFINITY® was negative for bacterial mutagenesis and *in vitro* chromosome aberration. In addition, activated DEFINITY® was negative in the rat micronucleus assays. It was, therefore, concluded that activated DEFINITY® is not genotoxic.

4.3.4 Reproductive and Developmental Toxicity

Studies demonstrated activated DEFINITY®had no effect on early embryonic development or on fetal growth, survival and morphological development and on pre-, postnatal development in rats and rabbits. However, high doses (≥ 0.3 mL/kg/day) did produce clinical signs in the pregnant rat, similar to those seen in previous toxicity studies in the non-pregnant rat. Similarly high doses (0.3 mL/kg/day) in the rabbit occasionally produced transient respiratory signs and doses of 1.0 mL/kg/day were sometimes associated with clinical signs of abnormal respiration, in-coordination, decreased activity and/or convulsion like motions with some deaths.

4.3.5 Other Toxicity Studies

The antigenic potential of activated DEFINITY®was investigated early in the development program in two separate studies in guinea pigs, each involving evaluation of active systemic anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) models. Only a weak response was noted in the ASA model, which was not paralleled by a positive result in the PCA model. Similarly, in another study, activated DEFINITY®was not shown to have antigenicity in mice or skin sensitizing potential in guinea pigs. Therefore, these studies indicate that there is little potential for immune-mediated hypersensitivity to activated DEFINITY®

4.3.6 Non-Clinical Conclusion

Safety pharmacology studies in the dog demonstrated that doses up to 25 times the clinical dose (0.5 mL/kg) did not affect cardiopulmonary function. At higher doses marked increase in respiration rate and pulmonary arterial pressure were observed. However, even in a model where severe pulmonary compromise was induced, the highest dose tested (0.2 mL/kg [10

times the clinical dose]) did not affect cardiopulmonary function. Similarly DEFINITY® (0.5mL/kg) did not affect cardiopulmonary function in mechanically ventilated dogs. Examination of the effect of activated DEFINITY®on the microcirculation indicated a very small fraction (1.2%) of the administered dose was retained and this transient retention did not have a detrimental effect on the systemic hemodynamics even at 40 times the clinical dose (800 µl/kg). Additional safety of activated DEFINITY®was demonstrated in the rhesus monkey where cardiovascular and ECG effects were not seen even at 50 times the clinical dose (1000 µl/kg). Studies with DEFINITY®in the primate also suggest cardiopulmonary effects are only produced at very high doses levels (≥150 fold the recommended clinical dose). The tolerance of DEFINITY® in rat and primate toxicity studies were consistent with the safety pharmacology studies. Moderate changes in PAP were reported for DEFINITY® in the pig, however, in the primate, the clinical signs were not associated with anaphylactoid response mediated by mast cell degranulation or activation of complement suggesting that complement activation-related pseudoallergy (CARPA) was not involved in this animal model.

Several extensive reviews have indicated that all the ultrasound contrast agents, including DEFINITY®have the potential to induce cellular damage in vitro and microscale bioeffects in tissues in vivo when present at sufficient concentrations and exposed to sufficient ultrasound energy for sufficient time (Miller 2007, Miller 2008, Dalecki 2007). The medical significance of bioeffects with diagnostic ultrasound is not yet fully understood. Our approach taken with DEFINITY®is consistent with ALARA (as low as reasonably achievable) with a maximum recommended MI for ultrasound used with DEFINITY® of 0.8 and a maximal recommended single dose of DEFINITY®of 10 µL/kg (with possible administration of a second dose of 10 µL/kg). The maintenance of a low dose of agent in

combination with low MI and short durations of scan in any particular plane will mitigate the potential to produce microscale bioeffects.

5. DEFINITY® CLINICAL SUMMARY

5.1 Introduction

The following section summarizes clinical studies in DEFINITY® prior to submission of the Marketing authorization Application (MAA) in 2004 and clinical studies conducted in post-MAA submission. In addition, a review of peer-reviewed publications was conducted to evaluate the use of DEFINITY® in perfusion and stress echocardiography and radiology indications.

5.2 Clinical trials of DEFINITY® (pre-MAA)

The clinical trials of DEFINITY® included 2951 subjects in 40 clinical studies. Twenty of the studies were echocardiography studies, 12 were radiology, and eight were studies assessing either the safety or other assessments (e.g., dose-ranging, etc) of DEFINITY®. The above 2951 subjects include 44 healthy subjects in various studies. In addition, small cohort of 185 subjects received placebo (16 healthy subjects, 163 echocardiography subjects, and 6 radiology subjects) primarily in early phase studies. All 40 studies were included in the evaluation of safety. The clinical studies are listed by study type category in Table 5.1.

Table 5.1 List of Clinical Studies by Study Type

Study Type	N (n)	Study Numbers
Echocardiography Studies	20 (1420)	DMP 115-004, -005,- 006,- 007,- 017, -027, -202, -303, -304, -902, -904, -018, -022, -024, -025, -205, -206, -209, -309, -310
Radiology Studies	12 (1122)	DMP 115-001, -008, -009, -010, -013, -016, -023, -026, -203, -214, -305, -306
Safety/Other Studies	8 (409)	DMP 115-011,-012, -014, -020, -211, -213, -903, -905

N=Number of studies; number of subjects

5.2.1 Summary of DEFINITY® Clinical Studies (pre-MAA)

5.2.1.1 Safety

DEFINITY® was administered either as an IV bolus or as an IV infusion. In general, earlier studies were performed using single or multiple short IV bolus injections followed by a saline flush. In nearly all studies, dosing was completed in a single day. Subsequent studies administered single or multiple infusions (or combinations of bolus and infusions) in order to optimize quality and durations of image enhancement.

In echocardiography studies, about one third of all subjects treated with DEFINITY® received the agent as bolus injections and two thirds as infusions. In the pivotal echocardiography studies, all 359 subjects received DEFINITY® as a bolus injection. In the supportive echocardiography studies, approximately 258 subjects received DEFINITY® as an infusion as compared to 62 subjects who received DEFINITY® as a bolus injection.

In the 28 studies conducted either in echocardiography indications or for safety assessment, 1513 subjects and 823 subjects, respectively, received DEFINITY® in conjunction with rest echocardiographic and stress echocardiographic imaging. Out of 823 subjects who underwent stress imaging, 498 (61%) subjects received a pharmacologic agent for the stress test (dipyridamole: 274 [33%] subjects; dobutamine: 156 [20%] subjects, and adenosine:

68 [8%] subjects); 300 (36%) subjects underwent exercise stress testing and the remaining 25 subjects (3%) underwent other procedures such as atrial pacing.

Overall, approximately 70% of echocardiography subjects who were administered a bolus injection of DEFINITY® received the agent in a dosage of 10-20 µL/kg. Only about 2% of the echocardiography subjects received a bolus dose in excess of 40 µL/kg. Infusion doses given in echocardiography studies ranged from ≤1.6 mL to >2.9 mL. The maximum permitted total infusion dose was 3.9 mL. Infusion doses >2.9 mL DEFINITY® were given in supportive studies DMP 115-209 and -211 and in other echocardiography studies DMP 11-011, -014, -020, -025, -202, -205, -206 and -904. In most of these latter studies, the investigator was permitted to titrate the dose or infusion rate as required or to give a second dose of 1.3 mL DEFINITY® if the first dose of 2.6 mL did not produce the desired enhancement.

In radiology studies, about two thirds of the DEFINITY®-treated subjects received the agent as a bolus injection and the reminder as an infusion. Bolus injection dosages of DEFINITY® given in radiology studies were generally higher than those used in echocardiography studies. Overall, 61% of subjects who received bolus injection received the agent in a dosage of >20 - 40µL/kg and the remaining subjects were administered 10 – 20 µL/kg (27%) or >40 µL/kg (10%). Only 3% of subjects were given less than 10 µL/kg.

Demographically, 61% of the subjects treated with DEFINITY® were male. The subjects had a mean age of 56 years and mean body weight of 83 kg. Three quarter of the subjects were white. There were no notable differences between the two indications (echocardiography and radiology) on the basis of pooled data per indication for age, gender, race and weight.

Adverse events (AE) data were collected in all studies with DEFINITY®. Most of the earlier studies coded the AEs using the World Health Organization Adverse Reaction Terms

(WHOART) coding thesaurus and the later studies coded the AEs using Medical Dictionary for Regulatory Activities (MedDRA). In the majority of studies, subjects were followed up to 24±8 hours post- DEFINITY® dose where as, in few studies; subjects were followed for longer duration (72±8 hours) or shorter duration (4±1 hours) than 24 hours. In the majority of studies, there was a 14-day reporting period of SAEs starting with completion of the DEFINITY® dosing.

The overall incidence of new-onset of AEs in DEFINITY®-treated healthy subjects was 25% (11 of 44 subjects). Treatment related new-onset AEs occurred in 5 (11.4%) of the 44 subjects. The most frequently reported new-onset AEs in DEFINITY®-treated healthy subjects were headache, nausea and flushing.

Regardless of indication, study design, DEFINITY® dose administered and mode of administration, approximately 26% of the 2951 DEFINITY®-treated subjects experienced at least one new-onset AE. Approximately 8% of the subjects experienced treatment-related AEs. The system organ classes (SOC) most frequently affected were general disorders and administration site conditions (11.5%); nervous system disorders (5.5%); respiratory, thoracic and mediastinal disorders (4.5%); gastrointestinal disorders (3.3%); vascular disorders (2.9%); and musculoskeletal and connective tissue disorders (2.8%).

The frequently reported new-onset AEs were fatigue (5.9%); headache (3.5%); dyspnea (3.2%) and chest pain (2.4%). The frequently reported new-onset AEs of fatigue, chest pain and dyspnea occurred primarily in the echocardiography studies, in particular those using exercise stress testing; such AEs occurred only rarely in the radiology studies. Back pain and abdominal pain were reported more frequently in the radiology studies. The overall percentage of DEFINITY®-treated subjects with a new-onset AE in echocardiography was 31% whereas the percentage in radiology studies was 18%. The higher AE rate in the echocardiography subjects was attributed mainly to the use of stress agents.

The frequency of non-completion due to AEs was low and was similar for echocardiography and radiology subjects (echocardiography: 0.7%, radiology: 0.8%). Most of the AEs resulting in study non-completion were transient, resolving within 1 minute to 2.5 hours. None of these AEs were serious.

In eight placebo-controlled studies conducted for the echocardiographic indication and one placebo-controlled study conducted for the radiology indication, a total of 767 subjects received DEFINITY® and 224 subjects received placebo. A total of 259 (48%) of the DEFINITY®-treated subjects experienced at least one new-onset AE while 126 (56%) placebo subjects experienced at least one new-onset AE.

In the two bolus dosage categories most frequently used in echocardiography studies (10-20 µL/kg and >20-40 µL/kg), the overall new-onset of AEs were similar (27% vs. 27.3%) and comparable to that for subjects in the echocardiography studies as a whole. Only one of ten echocardiography subjects who received a DEFINITY® bolus dosage of >40 µL/kg experienced any new-onset AEs. The new-onset AE profile for echocardiography subjects receiving infusion doses of DEFINITY® was consistent with the overall profile of most frequent new-onset AEs observed in DEFINITY®-treated subjects in echocardiography studies. No clear dose relationship was detected with respect to the overall frequency of new-onset AEs in echocardiography subjects receiving doses of 10-20 µL/kg and >20-40 µL/kg of DEFINITY®. Similarly, no clear dose relationship was detected with respect to the overall frequency of new-onset AEs in radiology subjects receiving bolus doses of 10-20 µL/kg, >20-40 µL/kg and >40 µL/kg of DEFINITY®.

The AE data were not uniformly collected following rest and stress echocardiographic imaging across all studies. In some clinical studies, new-onset AEs were reported soon after each of rest and stress imaging procedures and in some other studies, new-onset AEs were reported cumulative of rest and stress echocardiographic imaging procedures. The AE data

were available from 516 subjects in eight rest-only echocardiography studies and from 423 subjects in eight rest-stress echocardiography studies. In addition, the AE data were also available from 56 rest-only and 168 rest-stress placebo subjects in eight placebo-controlled studies. One hundred twenty five (24%) of 516 rest-only study subjects treated with DEFINITY® experienced a new-onset AE where as 194 (56%) of 423 rest-stress subjects treated with DEFINITY® experienced a new-onset AE. However, 126 (56%) of 224 subjects treated with placebo experienced a new-onset AE. Based on the available data from these studies, AE rate in rest-stress subjects appeared to be higher than in rest-only subjects. However, AE rate was similar between rest-stress placebo subjects and rest-stress DEFINITY® indicating that the source of the majority of new-onset AEs was attributable to the stress agents.

Eight subjects (0.3%) of DEFINITY®-treated subjects in echocardiography and radiology studies experienced fatal outcomes. Table 5.2 summarizes the list of patients who experienced fatal outcomes in DEFINITY® studies. Three of eight fatal outcomes occurred in echocardiography studies and five in radiology studies. No fatal outcomes have been reported for any of the 185 placebo subjects during the clinical development of DEFINITY®. The eight deaths consisted of five men and three women. All AEs leading to death occurred at least one day after administration of DEFINITY® and were considered by the investigators as unlikely to be related to DEFINITY® dosing. All had serious cardiac illness or cancer. The one young subject who died was a 33-year-old black man with a history of CHF, idiopathic dilated cardiomyopathy and heart transplant.

Table 5.2 Listing of DEFINITY®-Treated Subjects Who Experienced Fatal Outcome

Study No.	Study Type	Site No./ Subject No.	Age/Gender/ Race	Adverse Event	Time of death after dosing	Dose of DEFINITY®
DMP 115-004	Echo	006/10	81/F/White	Cardiac arrest [a]	ca. 7 d	Bolus: 2 x 10 µL/kg
DMP 115-004	Echo	009/1	33/M/Other (black)	Cardiac arrest [b]	ca. 12 d	Bolus: 2 x 5 µL/kg
DMP 115-010	Rad.	001/107	66/M/Other (black)	Congestive heart failure	ca. 12 d	Bolus: 1 x 30 µL/kg 1 x 10 µL/kg
DMP 115-010	Rad.	002/104	66/M/White	Pulmonary embolus	ca. 5 d	Bolus: 1 x 30 µL/kg 1 x 10 µL/kg
DMP 115-023	Rad.	002/204	60/F/White	Dysrhythmia secondary to cardiac tamponade	35 h	Infusion of ca. 2 mL
DMP 115-305	Rad.	001/107	58/M/Other (filipino)	Coagulopathy with severe hemorrhage	ca. 4 d	Bolus: 3 doses Total: 2.4 mL
DMP 115-305	Rad.	002/203	93/F/White	Respiratory failure Cardiac arrest	ca. 5 d	Bolus: 5 doses Total: 2.2 mL
DMP 115-903	Echo.	003/301 [c]	78/M/White	Multiorgan system failure and sepsis	ca. 15 d	Bolus: 6 doses Total: 17.2 µL/kg

F=Female, M=Male; Echo=Echocardiography, Rad.=Radiology

[a] Preceded by chest pain (2 episodes), bradycardia (3 episodes), atrial fibrillation, and ventricular tachycardia and fibrillation

[b] Preceded by dyspnea, abdominal pain, atrial flutter and fibrillation, ventricular tachycardia (2 episodes), and ventricular fibrillation

[c] Identified as subject 3/1-A in study report

Overall, 33 (1.3%) of the DEFINITY®-treated subjects in echocardiography and radiology studies had SAEs. None of the SAEs were classified as treatment-related. One placebo subject in echocardiography study (DMP 115-304) experienced an SAE of chest pain. Twenty-five of the above 33 subjects experienced non-fatal AEs that were classified as serious, in nearly all cases secondary to hospitalization. Although there was only a small population of placebo subjects in these clinical trials, the SAE rate is similar between the placebo and DEFINITY®-treated subjects.

The ability of DEFINITY® to elicit an immune response was assessed in five studies with 128 subjects (116 healthy subject 12 COPD patients). Assessments included immunoglobulins A, E, G, and M, histamine (blood), anti-double stranded deoxyribonucleic acid antibody, tryptase, complement (CH_{50} and C3a), and lupus anticoagulant. No clinically

important changes were noted in the immunology parameters evaluated, with the exception of activated complement factor C3a. Measurements of C3a were obtained in study DMP 115-905, in which statistically significant increases in C3a were noted in an equal number (N=12) of healthy volunteers and COPD patients who received 50 µL/kg DEFINITY® (2.5 times the maximum recommended dose). C3a levels increased immediately after DEFINITY® administration then declined over 30 minutes after dosing. Based upon review of data and consultation with an immunology expert, it was believed that these increases in C3a levels were not associated with mast cell or basophile activation, or the release of bioactive mediators. An immunology expert concluded that the DEFINITY® does not appear to present an increased risk of anaphylactoid or anaphylactic reactions.

Post-treatment shifts and mean change from baseline in ECG parameters were analyzed in subjects with ECG data from 7 parallel-group controlled studies (191 control subjects and 481 DEFINITY®-treated subjects) for the MAA submission. For post-treatment shifts assessment, more control subjects than DEFINITY®-treated subjects had an absolute increase of ≥30 msec in QTc interval from baseline. QTc prolongation of ≥30 msec was noted in 78 (17.1%) of DEFINITY®-treated subjects as compared with 34 (18.6%) of control subjects. For mean change from baseline, no notable differences were observed between the treatments with respect to the number of subjects with percentage changes from baseline in any of the ECG parameters investigated. The ECG findings for the subsets of studies with resting ECG data and exercise stress studies were very similar to those for the group of all studies combined.

Hypertension or increased blood pressure was reported by investigators as a new-onset AE for 16 DEFINITY®-treated subjects, five of them in placebo-controlled studies. The frequency of hypotension reported as a new-onset AE was similarly low (13 subjects, 3 of them in placebo-controlled studies). Very few of the AEs were rated by the investigator as treatment-related, and no associated clinical symptoms were reported as AEs. Review of the

blood pressure data showed that the changes were transient, with blood pressure values returning to normal in all cases. No evidence was identified that the use of DEFINITY® was accompanied by an increased risk of increases or decreases in blood pressure.

Safety Exposure to DEFINITY® in Subgroups

The DEFINITY® safety profile has been evaluated in special patient populations such as chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), acute myocardial infraction (AMI) and subjects on mechanical ventilation.

A small pharmacokinetic study was performed in COPD subjects with a high (50uL/kg) dose of DEFINITY®. No SAEs were reported and the lung clearance of PFP in the COPD subjects was similar to the placebo control. AEs were reported for both the COPD (7/12) and placebo (4/12). In addition, based on reviewing the case report forms (CRFs) from the patients who had a history of COPD and received at least one dose of DEFINITY® in other trials, a total of 10 (22%) of the 46 patients reported at least one new-onset of AE. It appears the nature of these events was similar and overall suggests pulmonary compromise did not change DEFINITY® effects.

In the placebo-controlled studies, the overall incidence of new-onset AEs appeared to be slightly higher in DEFINITY®-treated subjects with CHF (35%) than in placebo subjects with and without CHF (28% and 23% respectively) or the DEFINITY®-treated subjects without CHF (27%). The distribution of new-onset AEs observed in the DEFINITY®-treated CHF subjects were not unexpected for this at-risk population with severe cardiac disease who are frequent candidates for contrast-enhanced echocardiography.

The AE rate observed in the echocardiography study (DMP 115-904) in subjects with acute myocardial infarction (14%) was lower than that observed for DEFINITY® in the

echocardiography studies overall. Only one cardiac AE (atrial tachycardia) was reported, and only one subject experienced a serious AE (mild chest pain).

Thirty eight subjects who were on mechanical ventilation received DEFINITY® intra-operatively during curative surgery of focal liver lesions in the DMP 115-214 study. Intravenous bolus doses of DEFINITY® were administered in the range of 0.4, 0.8, and 1.0 mL. No clinically significant abnormalities were noted during monitoring of FiO2, saturation %, ETCO2, temperature, BP, HR and CVP/PCWP. At no point in the study did any unexpected cardiovascular instability occur in relation to intra-operative administration of DEFINITY®.

DEFINITY® was safe and well tolerated in this high-risk population of subjects with pre-existing cardiac conditions. This study fulfilled the requirement of our post-approval commitment to evaluate the efficacy and safety of activated DEFINITY® in adults undergoing mechanical ventilation.

5.2.1.2 Efficacy

As discussed above, most studies were conducted in echocardiography and radiology indications. In echocardiography studies, main objectives of the studies were to evaluate ability of DEFINITY® to demonstrate adequate/full left ventricular cavity enhancement and to improve the delineation of the left ventricular endocardial border; ability of DEFINITY®-enhanced echocardiography in diagnosing perfusion abnormality with respect to nuclear perfusion or coronary angiography as truth standard; and to evaluate relative error of DEFINITY®-enhanced echocardiography in estimating ejection fraction (EF) in relation to MRI.

Independent blinded reading of echocardiography was conducted in approximately half of the studies. Echocardiography images were read at respective institutions where the studies

were conducted. Since the study objectives were different from study to study, the following list summarizes key efficacy findings from studies where blinded reads were performed.

Key efficacy findings:

- Percentage of subjects who demonstrated adequate/full left ventricular cavity enhancement were significantly higher in DEFINITY®-enhanced echocardiography imaging group than in placebo group (Studies DMP 115-004, -005, -017, -902)
- Use of DEFINITY® enhancement in echocardiography resulted in significantly higher percentage of subjects with one and two segment improvement compared with baseline (DMP 115-006, -007, -017)
- Ability of DEFINITY® enhancement in providing additional diagnostic information was significantly higher than unenhanced echocardiography (DMP 115-010)
- DEFINITY®-enhanced echocardiography imaging showed no difference between baseline and post-injection from MRI in the relative error measurement of EF (DMP 115-006, -007)
- Sensitivity, specificity and agreement estimates of DEFINITY®-enhanced echocardiography in diagnosing perfusion abnormality with respect to nuclear perfusion and coronary angiography varied in a wide range depending on type of study design, blinded/institutional image read and comparison truth standard (nuclear imaging vs. angiography) (DMP 115-904, -022, -209)

5.3 Clinical trials of DEFINITY® (Post-MAA)

Table 5.3 provides a brief overview of the post-MAA clinical trials by study type, including data regarding subject population primary objective, safety parameters, and a brief summary of efficacy and safety conclusions.

5.3.1 Tabulation of Post-MAA Clinical Study Data

Table 5.3 Post-MAA Studies in Perfusion Echocardiography - Overview

Study Identifier (Total Subjects Planned/ Enrolled)	Primary Objective(s)	Safety Follow-up	Conclusions
POST-MAA ECHOCARDIOGRAPHY STUDIES			
DMP 115-907 (100/106)	Compare agreement of DEFINITY® (bolus) enhanced rest and stress echo to nuclear perfusion to detect perfusion abnormalities	SAEs from start of DEFINITY® administration to end of enhanced imaging	Efficacy: Due to lack of sufficient number of subjects with coronary angiography and without disease, specificity estimates with respect to angiography were inconclusive. Safety: No SAEs reported in this study.
DMP 115-908 (30/9)	Compre agreement of infusion and bolus dosing of DEFINITY® - enhanced rest and stress echo to detect perfusion abnormality	SAEss from start of DEFINITY® administration to end of enhanced imaging	Efficacy: No efficacy evaluation was performed due to lack of sample size to make a comparison between bolus and infusion dosing. Safety: No SAEs reported in this study.
DMP 115-909 (100/100)	Compare agreement of DEFINITY® (infusion) enhanced rest and stress echo to nuclear perfusion to detect perfusion abnormalities	SAEs from start of DEFINITY® administration to end of enhanced imaging	Efficacy: No independent blinded read of images was conducted in the study, no conclusions on efficacy estimates were drawn. Safety: One subject reported bradycardia and hypotension following administration of Dipyridamole that was not related to DEFINITY®.

Study Identifier (Total Subjects Planned/ Enrolled)	Primary Objective(s)	Safety Follow-up	Conclusions
POST-MAA ECHOCARDIOGRAPHY STUDIES			
DMP 115-910 (75/75)	Assess the electrocardiographic safety of DEFINITY®-enhanced dipyridamole stress echocardiography	All ventricular and atrial premature beats related to high-MI	Efficacy: Not applicable Safety: The incidence of ventricular arrhythmias related to high-MI impulse during echo was low. When these occur, they usually only involved a single premature beat. Incidence of changes in 12-lead ECG occurring after microbubble infusion was also low.
DMP 115-401 (1200/560)	Compare the percentage of subjects requiring an additional follow-up diagnostic test due to limited investigator confidence in echocardiography results for subjects who have undergone unenhanced vs. DEFINITY®-enhanced exercise echo imaging	All AEs within 24±8 hours post-dose administration	Efficacy: DEFINITY® enhancement in exercise stress echocardiography significantly reduced need for additional diagnostic follow up test in the study. Safety: No SAEs reported. DEFINITY® was safe and well tolerated in the study.
DMP 115-402 (300/120)	Demonstrate the ability of DEFINITY®-enhanced dobutamine stress echocardiography performed in conjunction with automated edge detection software to improve correlation between novice and expert readers vs. unenhanced dobutamine stress echocardiography	All AEss within 24±8 hours post-dose administration	Efficacy: No efficacy analysis was performed. Safety: No SAEs reported. DEFINITY® was safe and well tolerated in the study.

Study Identifier (Total Subjects Planned/ Enrolled)	Primary Objective(s)	Safety Follow-up	Conclusions
POST-MAA ECHOCARDIOGRAPHY STUDIES			
DMP 115-409 (1500/31)	Determination of DEFINITY® safety profile when administered to special population. Comparison of the adverse event rates observed in special population subjects versus the adverse event rate included in the prescribing information	All AEs within 24±8 hours post-dose administration	Efficacy: No efficacy analysis was performed. Safety: No AEs were reported in this retrospective study.
DMP 115-410 (108/108)	Compare diagnostic accuracy of DSE in detecting coronary artery disease compared to non-contrast imaging, using coronary angiography as the standard for comparison.	All AEs within 24±8 hours post-dose administration	Efficacy: No efficacy analysis has been performed by the sponsor. Safety: No SAEs were reported in this study. DEFINITY® was safe and well tolerated in the study.
DMP 115-407 (500/400)	Determine prognostic value of stress echo as a screening examination in peri- and post-menopausal females with an intermediate pre-test likelihood of CAD to identify subjects at a higher risk of experiencing future cardiac events.	All AEs within 24±8 hours post-dose administration	Efficacy: This study is ongoing and no data are available. Safety: This study is ongoing and no data are available.
DMP 115-412 (110/129)	Demonstrate the ability of DEFINITY®-enhanced versus unenhanced echocardiography to improve the accuracy of left ventricular ejection fraction (LVEF) when compared to cardiac magnetic resonance imaging (MRI) in a blinded assessment	Monitoring for AEs for 1 hour ± 30 minutes following completion of DEFINITY® dosing	Efficacy: This study has been completed and data review is ongoing. Safety: This study has been completed and data review is ongoing.

Study Identifier (Total Subjects Planned/ Enrolled)	Primary Objective(s)	Safety Follow-up	Conclusions
POST-MAA ECHOCARDIOGRAPHY STUDIES			
DMP 115-415 (ongoing - 1600/107)	Determine serious adverse event profile in subjects within 30 minutes following DEFINITY® administration (deaths or life threatening cardiopulmonary events)	All AEs within 24±8 hours post-dose administration	Efficacy: This study is ongoing and no data are available. Safety: This study is ongoing and no data are available.

5.3.2 Summary of DEFINITY® Clinical Studies (post-MAA)

5.3.2.1 Safety

The safety of DEFINITY® was studied in 11 post-MAA studies conducted in subjects with either suboptimal echocardiographic evaluation of ventricular function, or subjects with known or suspected CAD scheduled for myocardial perfusion imaging. Out of 11 studies, overall safety evaluation has been performed for eight completed studies to date (Studies DMP 115-907, -908, -909, -910, -401, -402, -409 and -410). Detailed safety analyses are currently on-going. The remaining three studies are either ongoing or have completed enrollment of subjects (DMP 115-407, -412 and -415). Approximately 1034 subjects were enrolled in the eight completed studies, out of which 848 subjects were treated with DEFINITY® and the remaining 184 subjects were treated with placebo. Approximately 593 subjects were enrolled and treated with DEFINITY® in the three ongoing studies and results are not available.

In most of the above studies, safety monitoring for AEs was conducted for 24±8 hours post-DEFINITY® administration. In some studies, only SAE data were collected. One retrospective study (DMP 115-910) was conducted in subjects enrolled previously in studies DMP 115-907, -908 and -909 to follow ECG safety, especially ventricular and atrial premature beats related to high Mechanical index (MI). Full safety analysis of all completed studies will be included in the supplemental NDA submission.

From 848 subjects treated with DEFINITY®, one subject reported the SAE, bradycardia and hypotension six minutes following administration of dipyridamole. The SAE resolved within 12 minutes of onset and was treated with aminophylline, atropine and sodium chloride. The SAE was reported as not related to DEFINITY®. In studies that collected all AEs, the AEs reported included: headache, ventricular bigeminy, chest pain, angina pectoris, dyspnea,

hypotension, nausea, and neck pain. All of the above AEs were of mild to moderate intensity, occurring in <1% of subjects and most were not deemed by the physician as related to study drug.

In a retrospective study (DMP 115-910) to follow ECG safety, especially ventricular and atrial premature beats related to high Mechanical Index (MI), two ECG readers observed two premature beats which were related to a single high-MI impulse for one subject. In clinical practice, DEFINITY® is most commonly used with a low-MI mode so it should not pose a risk related to premature beats.

Adverse event data were also collected from a retrospective study of 31 subjects in special populations (i.e., COPD, CHF, cardiac shunts, pulmonary hypertension, renal disease and hepatic disease). No AEs were reported, and no ECG or vital signs abnormalities of clinical significance were reported in these studies. No clinical laboratory data were collected in these studies. DEFINITY® was safe and well tolerated in the Post-NDA & MAA clinical studies.

5.3.2.2 Efficacy

The use of DEFINITY®-enhanced rest and stress echocardiography as a myocardial perfusion agent was evaluated in 3 of the 8 completed post-NDA & MAA clinical studies. These studies compared DEFINITY®-enhanced rest and stress echocardiography imaging to nuclear perfusion and coronary angiography in a subset of subjects. Images from one study were read by independent blinded readers, whereas the images were read by the institutions in other two studies. There was no standardization or consistency of image reading between studies which explains the considerable variation in the results of sensitivity, specificity and predicted values across studies.

One study (DMP 115-401) was conducted with an objective to compare the percentage of subjects requiring additional follow-up diagnostic testing due to limited investigator confidence in echocardiography in a parallel group design. The study showed a significantly smaller percentage (12.2%) of subjects with DEFINITY®-enhancement were recommended follow-up tests than subjects (32.6%) without DEFINITY® contrast.

5.4 DEFINITY® Literature Summary

The primary goal of this literature review was to obtain published clinical study data documenting the usefulness of DEFINITY® with echocardiography and radiology, and in particular its use as a perfusion and stress echocardiography contrast imaging agent. In addition, any published safety data for DEFINITY® used in radiology applications are summarized. The search focused primarily on prospective studies but also on the following publications:

- Meta-analyses
- Systematic review data
- Prospective and retrospective single-center studies
- Relevant radiology safety considerations

5.4.1 Methods

The search used databases dedicated to peer-reviewed papers, providing thorough and overlapping coverage of the biomedical literature. DEFINITY® citations were obtained for the following four search categories:

- Overall echocardiography
- Perfusion echocardiography
- Stress echocardiography
- Radiology

For all four categories, a broad search was performed of the Dialog databases as presented in Table 5.4.

Table 5.4 Search Criteria Databases By Study Category

Clinical Study Type	Databases
Overall Echocardiography	Medline, Embase, EMCare, Biosis, Scisearch, Inspec, Pascal, and Chemical Abstracts.
Perfusion Echocardiogrpahy	Inspec, Biosis Previes, El Compendex, Scisearch, EMCare, Biobase, Embase, International Pharmaceutical Abstracts, Pascal, Medline, Toxfile, Adverse Reactions Database, Periodical Abstracts
Sress Echocardiography	Inspec, Biosis Previes, El Compendex, Scisearch, EMCare, Biobase, Embase, International Pharmaceutical Abstracts, Pascal, Medline, Toxfile, Adverse Reactions Database, Current Contents, Periodical Abstracts
Radiology	Biosis, Scisearch, Embase, International Pharmaceutical Abstracts, Medline, Chemical Abstracts, and IMS R&D

The search included terms commonly used in the literature for describing DEFINITY® (e.g., Perflutren, DMP 115, etc.). Results were restricted to clinical data in English-only articles. The search was designed to include only those citations that were relevant to the five study categories by excluding citations in which DEFINITY® was used in ancillary indications (e.g., optical, drug delivery, etc.). Duplicate citations were also excluded.

Following review of the publications for each study category, 22 overall echocardiography, 21 perfusion and stress and 9 radiology were identified as containing relevant clinical data. The relevant overall echocardiography publications are discussed in Section 5.4.3. A tabulation of the overall echocardiography publications is not included as an in-text table because of the significant number of citations identified during the search. A complete listing of the overall echocardiography citations is available in Section 7.2. Copies of the publications are available upon request.

Because the relevant publications for perfusion and stress echocardiography had significant data overlap, the two study categories were tabulated together in Table 5.5. The radiology studies are summarized in Table 5.6; these study categories are also discussed in Section 5.4.3.

5.4.2 Tabulation of Published Literature

Table 5.5 Tabulation of Published Literature - Perfusion/Stress Echocardiography Studies

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Published Perfusion/Stress Echocardiography Studies					
Elhendy et al., 2004 (Stress)	J Am Coll Cardiol	Prospectively studied 1,318 subjects with known CAD referred for DSE by real-time MCE combined with WMA. A subset of 179 subjects had coronary angiography. Objective compared accuracy of MCE and wall motion abnormalities (WMA) during submaximal and peak DSE for the diagnosis of CAD. Evaluable population was 170 subjects (DEFINITY®=30; Optison=140).	CAD detected in 127 (75%) subjects. For MCE versus WMA, Sn of maximal stress=91% vs 70%, Sn of intermediate stress=84% vs 20%, Sp=51% vs 74%, accuracy=81% vs 71%, and CAD Sn=67% vs 28%.	No safety issues presented.	Majority of inducible perfusion abnormalities occur at intermediate phase of the stress test without wall motion abnormalities. MCE provides better sensitivity than WMA, especially in submaximal stress and subjects with multivessel CAD.
Elhendy et al., 2005 (Stress)	Diabetes Care	128 diabetic subjects referred for evaluation of CAD by DSE real-time myocardial contrast perfusion imaging (MCPI). Objective was to assess accuracy of MCPI (DEFINITY®=41; Optison=87) during dobutamine stress in the diagnosis and localization of CAD.	CAD detected in 101 (79%) by coronary angiography. For MCPI, Sn=89%, Sp=52%, and accuracy 81%. Reversible abnormalities detected in 44\56 subjects with multivessel CAD and 8\63 without. Regional sensitivity was 75% for left anterior descending CAD, 71% for left circumflex, and 67% for right CAD.	No safety issues presented.	MCPI is a useful noninvasive technique for the diagnosis and localization of CAD in diabetic subjects. The extent of perfusion abnormalities can identify subjects with multivessel CAD with moderate sensitivity and high specificity.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Elhendy et al., 2006 (Stress)	J Am Soc Echocardio-graphy	64 coronary artery bypass graft (CABG) disease subjects referred for evaluation of myocardial ischemia by dobutamine stress MCPI (DEFINITY®=26; Optison=38) and coronary angiography.	Significant stenosis (≥70% by quantitative angiography) in ≥1 grafts was detected in 49 (77%) subjects. Reversible abnormalities evident in 44\49 subjects (Sn=90%). Significant stenosis detected in 74 of 176 bypass grafts (42%).	Study demonstrated that MCPI with dobutamine stress echo was safe. No deaths or MI occurred during or immediately after the stress test.	Dobutamine stress MCPI is a useful technique for the evaluation of CABG.
Kirkpatrick et al., 2004 (Perfusion)	J Am Coll Cardiol	16 subjects with intracardiac or pericardial masses underwent perfusion imaging with echocardiographic contrast (DEFINITY® or Optison).	7/16 subjects, contrast enhanced resulted in greater pixel intensity in the mass than in the adjacent myocardium. Masses were malignant (n=6) or benign and vascular (n=1). 9 masses demontrated decreased pixel intensity compared with the myocardium and diagnosted as myxomas (n=2) or thrombi (n=5), or resolved with anticoagulation.	No safety data presented.	Quantification of echocardiography perfusion imaging aids in the differentiation of cardiac masses. Compared with the adjacent myocardium, malignant and highly vascular tumors hyper-enhanced whereas stromal tumors and thrombi hypo-enhanced.
Main ML et al., 2003 (Perfusion)	Am J Cardiol	Study enrolled 11 subjects with significant LAD disease amenable to percutaneous coronary intervention (PCI), normal LV systolic function in the distribution of LAD, and adequate apical echocardiographic windows. Subjects underwent myocardial contrast echocardiography with DEFINITY® during PCI.	Quantitative analysis demonstrated significant difference in myocardial contrast intensity by flow state (p=0.0001 for occluson vs reperfusion). Qualitative assessment demonstrated a high rate of correct classification (92%). Real-time myocardial perfusion imaging using MCE acurately differentiates coronary occlusion and reactive hyperemia by qualitative and quantitative assessment.	No safety data presented.	This technique may be clinically useful in assessing the efficacy of thrombolytic therapy in ST-segment elevation AMI and in clinical trial assessment of new drugs and devices aimed at limitation of infarct size.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Moir S et al., 2004 (Stress)	Circulation	Prospective study of 85 subjects in 2 groups: 70 with suspected CAD undergoing coronary angiography and 15 with low-risk of CAD. Examined whether addition of DEFINITY® myocardial contrast echo (MCE) with combined dipyridamole- exercise stress echo provides incremental benefit in the evaluation of CAD.	Significant stenoses (50%) present in 43 subjects involving 69 coronary territories. Addition of MCE improved sensitivity for the detection of CAD (91% vs. 74%) and extent of disease (87% vs. 65% territories).	No safety issues presented.	Addition of low-mechanical index MCE to standard imaging during combined dipridamole-exercise stress echo improves detection of CAD and extent of disease.
Mor-Avi V et al., 2006 (Stress)	Eur J Echocardio-graphy	117 subjects with poorly visualized endocardium undergoing dobutamine stress tests. Assessed if real time color-encoding of DEFINITY®-enhanced images would allow objective detection of stress-induced wall WMA.	20/67 subjects had resting WMA and 13/67 developed WMA at peak stress. Automated technique detected stress-induced WMA in at least 1 vascular territory with Sn=0.80, Sp=0.65, and accuracy=0.69. Level of agreement 0.62, 0.91 0.85, respectively.	No safety issues presented.	Analysis of color-encoded, DEFINITY® images allows objective, accurate, automated detection of stress-induced WMA in subjects with poor accoustic windows.
Noll DR et al., 2001 (Stress)	JACC	14 subjects with intermediate risk of CAD received myocardial contrast replenishment (MCR) using DEFINITY® to test the feasibility of pulse inversion doopler (PID) during an adenosine stress test (AST) .	MBF abnormalities due to >50% diameter stenoses were evident visually during AST by a delayed contrast replenishment (9.0±3.2 seconds vs 2.2±0.8 seconds in normal regions). SPECT identified 9 defects while delayed MCR with PID identified 13 defects.	No safety issues presented.	Confidence interval of DEFINITY® produced bright MCE in 12\14 subjects allowing rapid real-time acquisition of MCR.
Plana JC et al., 2008 (Stress)	JACC	101 subjects referred for stress testing underwent 2 dobutamine stress echo studies: 1 DEFINITY® and 1 unenhanced at least 4h apart in a randomized order during a 24h period.	DEFINITY® improved the percentage of segments visualized at baseline (72±24% to 95± 8%) and more at stress (67±28% to 96±7%). Interpretation of wall motion with high confidence increased 36% to 74% ($p<0.001$) with DEFINITY®.	No serious adverse events reported. Adverse events reported at 10% in both groups (contrast and noncontrast).	DEFINITY® administration improves endocardial visualization at rest and even more at stress, leading to a higher confidence of interpretation and greater accuracy in evaluating CAD.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Porter TR et al., 2001 (Perfusion)	J Am Coll Cardiol	Pulse inversion doppler (PID) imaging was performed in 117 subjects with intermediate pretest probability of significant CAD. Subjects underwent dobutamine stress echocardiography (DSE) with contrast (DEFINITY®=19; Optison=98) to determine myocardial perfusion defects.	Absense of signal noted from myocardium before contrast injections for all subjects. Regional myocardial contrast defects at peak stress observed in all 30 subjects with >50% stenosis in at least 1 vessel. Contrast defects observed in 17 territories subtended by >50% diameter stenosis that had normal wall motion at peak stress. Agreement between quantitative angiography and MCE on territorial basis was 83% vs 72% for wall motion.	No safety data presented.	PID detects myocardial perfusion abnormalities in real-time during stress echo and adds quality and sensitivity to this test.
Toledo E et al., 2005 (Perfusion)	J Am Soc Echocardiogr	Adenosine-induced changes in perfuson were measured in 8 healthy volunteers. Study explored a new approach for MCE quantification by brief interruptions of DEFINITY® to overcome the limitations of existing techniques.	Adenosine increased peak contrast inflow rate to 278±123% of baseline ($P < .05$).	No safety data presented.	The interruption of DEFINITY® is a sensitive tool for accurate quantification of myocardial perfusion, which may consititute an alternative to currently used techniques.
Toledo E et al., 2006 (Perfusion)	Eur J Echo	34 subjects referred for coronary angiography for suspected CAD received DEFINITY®. Subjects were randomized to the "study group" (receiver operating characteristics analysis) or "test group" (automated detection). Automated detection of perfusion defects based on parametric perfusion images was validated against coronary angiography.	LAD and non-LAD stenosis >70% was found in 19 (study group) and 17 (test group). In the study group (n=17) for LAD, Sn=83%; Sp=67%; and accuracy=75%. Flow reserve index was LAD=0.95 and non-LAD 0.68. In the test group (n=17), sensitivity, specificity, and accuracy were 75%, 67%, and 71% in LAD and in non-LAD, 75%, 75%, and 75%, respectively.	No safety data presented.	Automated quantitative analysis of DEFINITY® parametric perfusion images is feasible and may aid in the objective detection of CAD.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Toledo E et al., 2006 (Perfusion)	J Am Coll Cardiol	Adenosine-induced changes in perfusion were measured in 8 normal volunteers. Tested feasibility of real-time 3-dimensional echocardiography (RT3DE).	Adenosine increased peak contrast inflow rate to 198±57% of baseline ($p < 0.05$).	No safety data presented.	DEFINITY®-enhanced RT3DE imaging provides the basis for volumetric imaging and quantification of myocardial perfusion.
Tsutsui JM et al., 2005 (Stress)	Am J Cardiol	36 subjects with known or suspected CAD underwent SPECT and DEFINITY® MCE (TRI and RTI) at baseline and after dipyridamole administration. A subset of 16 subjects had quantitative angiography.	Agreement detecting perfusion defects in SPECT and TRI in 26 subjects (72%) and SPECT and RTI in 27 subjects (75%). Agreement localizing coronary territories between MCE and SPECT. Accuracy of RTI for detecting >50% diameter stenoses by angiography 79%, TRI 71%, and SPECT 65%.	No safety issues presented.	Data indicated different low-MI imaging techniques are equivalent to SPECT in accurately detecting diseased coronary artery territories during vasodilator stress.
Tsutsui JM et al., 2005 (Stress)	J Am Coll Cardiol	Large cohort safety study of 1,486 subjects with known or suspected CAD who underwent stress real-time contrast echocardiography (RTCE) with low-mechanical index pulse sequence schemes after contrast agents (DEFINITY® 35%; Optison 65%).	Myocardial perfusion imaging (MPI) was considered feasible for analysis in 94% of the baseline walls and 95% at peak stress. MPI with RTCE had a higher accuracy for detecting subjects with angiographically significant CAD than analysis of wall motion (84% vs 66%, respectively).	No deaths or myocardial infarction occurred during RTCE. No difference in the incidence of nonsustained ventricular tachycardia (VT), sustained VT, or supraventricular tachycardia between RTCE and DSE.	Dobutamine stress RTCE appears to be a safe and feasible technique for evaluating subjects with known or suspected CAD.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Tsutsui JM et al., 2005 (Stress)	Echocardio-graphy	158 subjects with chest pain and possible acute coronary syndrome. Objective to determine accuracy and prognostic value of WMA and myocardial perfusion analysis (MPA) with dobutamine-atropine stress RTMCE (DEFINITY®=25; Optison=133).	For MPA, Sn=92%, Sp=77%, and accuracy 88%. For WMA, 62%, 85%, and 67%, respectively.	Three-year event free survival was 87% in subjects with negative WMA and MPA, 49% with positive WMA and MPA, and 51% in subjects with negative WMA but positive MPA.	RTMCE improved the accuracy of DSE for detecting CAD and was an independent predictor of outcome.
Tsutsui JM et al., 2006 (Stress)	Liver Transpl	Examined WMA and MPI in 230 subjects with advanced liver disease (ALD) subjects to determine value of MPI during dobutamine stress RTMCE (DEFINITY® 55; Optison 175) for predicting prognosis of ALD.	MPI obtained by RTMCE appears to be a useful tool in predicting mortality in ALD subjects.	Of 85 liver transplant subjects, 4 had abnormal MPI and 81 normal perfusion. Hospital mortality rates were 50% in subjects with abnormal MPI and 2% in subjects with normal MPI. Two-year mortality was 24% for normal MPI and 45% with abnormal MPI.	A normal MPI is associated with a low perioperative mortality after transplant, and shows significantly better overall mortality at 2-year follow-up. Abnormal MPI is an independent predictor of mortality in subjects with ALD.
Tsutsui JM et al., 2005 (Stress)	Circulation	Retrospective study of 788 subjects with real-time contrast echocardiography (RTCE) during DSE using either DEFINITY® (n=213) or Optison (n=575). Study objective was to determine the value of myocardial perfusion imaging and wall motion analysis during DSE in predicting the outcome of subjects with known or suspected CAD.	During 20-month follow-up, 75 events (9.6%) occurred (58 deaths, 17 nonfatal myocardial infarctions). Abnormal myocardial perfusion had significant incremental value over clinical factors, resting ejection fraction, and wall motion responses in predicting events (P=0.001).	No safety issues presented.	Myocardial perfusion (MP) imaging during dobutamine stress RTCE provides incremental prognostic information in subjects with known or suspected CAD. Subjects with normal MP have a better outcome than subjects with wall motion.

Lead Author Year (Study Category)	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Ward RP et al., 2004 (Perfusion)	J Am Soc of Echocardiogr	In 23 subjects, fundamental and harmonic imaging (HI) was performed in the transgastic short-axis (TSAX) view. In 14 additional subjects, perfusion imaging was performed in TSAX view with DEFINITY® administration.	HI demonstrated improved endocardial visualization in anterior and lateral segments ($P<.004$) in TSAX view and lateral segments in midesophageal 4-chamber view. Salvage rate was 8.3% in TSAX view and 12.6% in midesophageal 4-chamber view. Myocardial perfusion was confirmed for interior (86%), posterior (100%), and lateral (79%) segments but rarely in septal (21%), anteroseptal (0%), and anterior (14%) segments.	No safety data presented.	Use of HI with transesophageal echo improves endocardial visualization over fundamental imaging and allows partial assessment of myocardial perfusion with MCE.
Xie F et al., 2007 (Perfusion)	Echocardio-graphy	Comparison of real time perfusion echo (RTPE) with SPECT to detect significant CAD during adenosine infusion in 40 intermediate to high-risk CAD subjects.	SPECT and RTPE were in agreement 105/119 (88%) in coronary artery territories. In coronary angiography subjects, 3 subjects had normal appearing SPECT with adenosine but subendocardial perfusion defects with RTPE. Coronary angiography confirmed in all 3 cases the presence of a >50% diameter stenosis in the abnormal territory.	No safety data presented.	Adenosine stress imaging with RTPE is an accurate method of detecting CAD. The higher resolution of RTPE may identify subendocardial defects that would have gone undetected with SPECT.
Yu EH et al., 2004 (Perfusion)	J Am Coll Cardiol	Comparison study with 46 subjects to assess myocardial perfusion by myocardial parametric quantification (MPQ) to Tc-99m sestamibi-gated SPECT imaging. Each subject was assessed for visual cine-loop (VIS); myocardial parametric quantification (MPQ); combined VIS + MPQ.	Segmental rates of agreement for perfusion with SPECT were 83%, 89%, and 92% for VIS, MPQ, and VIS+MPQ, respectively. Agreement for presence or absence of moderate to severe perfusion defect were 92%, 97%, and 97%, respectively.	No safety data presented.	Myocardial parametric quantification demonstrates good agreement with SPECT and incremental agreement with visual cine-loop assessment. Analysis strategies that incorporate MPQ demonstrate better agreement with SPECT than visual analysis alone.

Table 5.6 Tabulation of Published Literature - Radiology Studies

Lead Author Year	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Published Radiology Studies					
Brannigan M et	Radiographics	Comparison of 3 microbubble contrast agents (DEFINITY®=275) used in ultrasound (US) of 400 patients to characterize focal liver masses.	During the portal venous phase, benign (e.g., hemangioma, focal nodular hyperplasia) lesions typically enhance more than the liver, whereas malignant (e.g., heptocellular carcinoma, metastases) lesions enhance less.	No safety data presented.	Initial studies suggest that liver US performed with contrast agents provides enhancement information comparable to contrast CT and MRI, with the added benefit of real-time morphologic evaluation of lesion vascularity.
Burns PN et al.,	Radiology	Study assessed the concordance of DEFINITY® -enhanced ultrasonographic (US) scans (mass n=144) with contrast-enhanced computed tomographic (CT) scans (n=116) or magnetic resonance (MRI) images (n=33) or both CT and MRI (n=5). The study enrolled 135 patients diagnosed with liver masses.	The highest concordance of 92% (132\144), with 95% confidence interval (CI) of 86% and 95% (κ> 0.84), was for the presence of peripheral pools and centripetal progression. Concordance in the portal venous phase was lower, with agreement for predominant enhancement of the lesion in 61% (86\142), with 95% CI of 52% and 68% (κ>0.83). Portal venous phase washout occurred in 75% (106\142), with 95% CI of 67% and 81% (κ>0.81).	No safety data presented.	US shows high concordance with CT or MRI especially for the arterial phase. Discordance in the portal venous phase may reflect the tendency of CT and MRI contrast agents, unlike microbubbles, to diffuse into interstitium.

Lead Author Year	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Halpern EJ et al., 2002	AJR	Evaluation of the value of biopsy in 40 patients for the detection of prostate cancer during DEFINITY®-enhanced endorectal sonography (n=40).	Prostate cancer identified in 30 sites in 16 (40%) patients. A suspicious site identified during contrast sonography was 3.5 times more likely to have positive biopsy findings than an adjacent site not suggestive of malignancy ($p<0.025$).	No safety data presented.	Contrast-enhancement transrectal sonography improves the detection of malignant foci in the prostate. The performance of multiple biopsies of suspicious enhancing foci significantly improves cancer detection.
Halpern EJ et al., 2001	Radiology	Examination of 60 patients with conventional gray-scale, harmonic gray-scale, and power Doppler sonography. Evaluation was repeated with DEFINITY® -enhanced imaging (n=60).	Prostate cancer present in 37 biopsy sites from 20 patients. Baseline imaging showed 14 sites in 11 subjects. Enhanced sonography showed 24 sites in 15 patients.	No safety data presented.	Enhanced transrectal sonography improves sensitivity for the detection of malignant foci within the prostate without substantial loss of specificity.
Hyun-Jung J et al., 2007	Radiology	Retrospective comparison of the arterial and portal venous phase enhancement patterns of hepatocellular carcinoma (HCC) with DEFINITY®-enhanced ultrasonography in 112 patients with histologically confirmed HCC.	In arterial phase, 97\112 (87%) HCCs showed hypervascularity, with a significantly higher proportion in moderately-differentiated HCCs (74\77, 96%) when compared with well- (14\23, 61%) and poorly-differentiated HCC (9\12, 75%). Dysmorphic arteries were seen in 81 (72%) HCCs. Of 97 hypervascular tumors, only 42 (43%) showed typical washout by 90 seconds. Late washout appeared in 25 (26%) HCCs in the 91-180 seconds phase and 21 (22%) in the 181-300 seconds phase. The remaining 9 showed no washout up to 300 seconds and 7 (78%) were well-differentiated.	No safety data presented.	Moderately-differentiated HCC generally shows classic enhancement features, while well- and poorly-differentiated tumors account for most atypical variations. Extended observation in the portal phase is important as late washout occurs with slightly more frequency than washout in the conventionally defined portal venous phase.

Lead Author Year	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Lanka B et al., 2007	J Ultrasound	Review of 1,040 DEFINITY® -enhanced US of the liver for mass characterization to determine their source, accuracy, and clinical impact.	DEFINITY® -enhanced US was accurate in 233 (89.2%) of 261 with histologic proof, including 208 malignant lesions. Clinical impact showed reduced referrals for other imaging in 226 (21.7%) of 1,040 patients, decreased time to diagnosis in 390 (37.5%), and successful guidance for ablation therapy in 26 (2.5%).	No notable adverse events occurred during the study.	Contrast-enhanced US has a positive impact on clinical management, providing rapid, accurate diagnosis of incidentally detected masses and resolving nodules on surveillance scans and indeterminate masses on other imaging.
Murphy-Lavalle J et al., 2007	J Ultrasound	Retrospective study of 50 patients metastases to describe enhancement (DEFINITY®=50) and vascularity characteristics of liver metastases with real-time low-mechanical index contrast US.	44\50 (88%) metastases showed arterial hypervascularity with dysmorphic vessels in 21 (42%) of 50. The pattern enhancement was rim in 21\50 (42%) and diffuse in 29\50 (58%). Time to peak arterial enhancement ranged from 8-27 seconds and the beginning of wash-out ranged from 13-50. All lesions (50\50) showed uniform complete wash-out in the portal phase.	No safety data presented.	Most hepatic masses including those thought to be hypovascular, show transient arterial hypervascularity on DEFINITY® -enhanced US followed by rapid and complete wash-out initiated within the conventional arterial phase.

Lead Author Year	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Wilson SR et al., 2006	AJR	Single-center, open-label, nonrandomized Phase 2 study enrolled 92 patients with liver lesions. Study objective was to develop an algorithm for liver mass diagnosis using Definity-enhanced pulse-inversion sonography. The resulting algorithm was based on responses to a questionnaire in a blind review format.	Portal phase enhancement is 1st step in algorithm with positive or sustained enhancement identifying 48 (92%) of 52 benign lesions and negative enhancement or washout present in 41 (93%) of 44 malignancies. Sustained portal phase enhancement with arterial phase peripheral nodularity and centripetal progression predicted 24 (92%) of 26 of the hemangiomas; diffuse arterial phase enhancement greater than the liver identified 19 (95%) of 20 of the focal nodular hyperplasias.	Safety monitoring limited to vital signs up to 120 minutes after sonography procedure. No adverse events were reported during the study.	A simple algorithm for interpretation of Definity-enhanced sonography provides sensitive and accurate diagnosis of commonly encountered liver masses.

Lead Author Year	Journal	Subject Population	Efficacy Results	Safety Results	Conclusions
Wilson SR et al., 2007	J Ultrasound	Comparison of the diagnostic accuracy, confidence level, and recommended management of focal liver masses in 156 patients with 167 masses. Study evaluated DEFINITY® -enhanced (n=156) versus ultrasonography alone.	Two readers could not determine benignancy or malignancy in 77 (46.1%) and 46 (27.5%) of 167 unenhanced scans compared with 2 (1.2%) and 1 (0.6%) of 167 DEFINITY®-enhanced US. For diagnostic accuracy, unenhanced scans agreed with the correct diagnosis in 85 (50.9%) and 63 (37.7%) of 167, and contrast agreed in 133 (79.6%) and 142 (85%) of 167 for readers 1 and 2, respectively. Regarding diagnosis, the confidence level on the unenhanced was 128 (82.1%) and 79 (65.3%) of 167 for the 2 readers. Further imaging decreased from 166 (99.4%) and 147 (88%) of 167 on the unenhanced scans to 30(18%) and 5 (3%) of 167 on contrast for readers 1 and 2.	No safety data presented.	Contrast-enhanced US improves the accuracy and confidence of diagnosis of focal liver lesions and reduces recommendations for further investigations.

5.4.3 Discussion of the Published Literature

5.4.3.1 Safety

A total of 23,772 subjects were identified who received at least one dose of DEFINITY® from peer-reviewed publications; 21,573 in echocardiography studies and 2,199 in radiology. In echocardiography publications, at least 1417 subjects were identified as having undergone stress procedures with dobutamine; 168 with dipyridamole; 70 with adenosine and 85 with exercise.

Among the identified publications, a total of five publications have provided safety data. Four of them are in echocardiography and one remaining is in radiology. Two reports have provided retrospective safety analyses that were based on hospital in-patient database. The detailed discussion of retrospective reports is covered in the section on Summary of Post-market Experience below. In addition, eight additional clinical reviews have discussed safety information.

A total of 1835 patients were enrolled in the studies discussed in the above five publications, 1827 patients dose with DEFINITY® and eight were placebo subjects. A total of 520 patients from one study received pharmacological stress agent. From these studies, there were no clinically significant changes observed in physical examination, vital signs, ECG and clinical laboratory tests. One case report article reported a serious adverse event. A 64 year old woman with aneurismal subarachnoid hemorrhage (SAH) after administrated DEFINITY®, developed transient ST elevation on lateral ECG leads and elevated cardiac enzymes. An emergency coronary angiogram was performed. The report did not mention when this event happened after dosing DEFINITY®. The patient has recovered and confirmed recovery in one week and four month follow up. The AE rates reported in these publications appear to be similar to that of our clinical trials.

Based on the data available from original publications and overall reviews, DEFINITY® administration appears to be safe and, as expected, demonstrated a safety profile similar to that observed during clinical development. They did not appear to show nephrotoxicity or cardiotoxicity and the incidence of hypersensitivity or allergic events appear to be much lower than that in current X-ray or MR contrasts agents (Blomley et al. 2007).

5.4.3.2 Efficacy

5.4.3.2.1 Perfusion Echocardiography

Based on the systematic literature search presented above, published data came mainly from nine investigator-sponsored studies conducted in a single center or few study centers. Most studies were small in size, ranging from 8 to 117 subjects. The majority of the studies that used DEFINITY® were conducted in myocardial perfusion echocardiography, whereas one study (Kirkpatrick et al., 2004) used DEFINITY® as the imaging contrast agent in subjects with a cardiac mass. While the objectives varied, the results of the perfusion echocardiography publications were promising. Some publications reported the ability of DEFINITY® to provide improved image quantification, while other publications reported results demonstrating the ability of DEFINITY® as a perfusion echocardiography contrast agent compared to nuclear perfusion or coronary angiography.

In a study of 40 subjects with intermediate pretest probability of significant CAD, Porter et al.(2001) reported that Pulse Inversion Doppler (PID) detects myocardial perfusion abnormalities in real-time during stress echocardiography and adds quality and sensitivity to this test. In a study of 11 subjects with significant LAD disease amenable to percutaneous coronary intervention (PCI), Main et al. (2003) reported that real-time myocardial perfusion imaging using MCE with DEFINITY® accurately differentiates coronary occlusion and reactive hyperemia by qualitative and quantitative assessment. Toledo et al.(2006), in a randomized study of 34 subjects who were referred to coronary angiography for suspected

CAD, reported sensitivity of at least 75% and specificity of 67% in MCE with respect to coronary angiography in all subjects. Xie et al.(2007), in a study of 40 subjects with intermediate to high-risk CAD, reported that SPECT and DEFINITY® RTPE were in agreement in 105/119 (88%) coronary artery territories with adenosine stress imaging. The above studies are too small in size to provide statistical conclusion; however, they do provide a strong basis for the ability of DEFINITY® in detecting perfusion abnormalities. Large statistically powered clinical studies are needed to confirm the ability of DEFINITY® MCE in detecting perfusion abnormalities as compared to coronary angiography in subjects with CAD.

5.4.3.2.2 Stress Echocardiography

Thirteen publications contained data for stress echocardiography using DEFINITY® as the contrast agent, out of which 1510 subjects were treated with DEFINITY®. The majority of the subjects (94%) received a pharmacological stress agent in these studies, with dobutamine being the most commonly used agent.

Elhendy et al. (2004), in a study of 179 subjects with known CAD referred for DSE by real-time MCE, reported better sensitivity of MCE in detecting perfusion abnormalities than WMA. In another study of 128 diabetic subjects referred for CAD by DSE, Elhendy et al. (2005) reported sensitivity of 89% of detecting CAD using coronary angiography as the truth standard. Moir et al. (2004) reported that the addition of low mechanical index MCE during dipyridamole/exercise stress echocardiography improved the detection of CAD and extent of disease. In a large study of 1486 subjects with known or suspected CAD, Tsutsui et al.(2005) reported that MPI with RTCE has a higher accuracy for detecting subjects with angiographic significant CAD than WMA (84% vs. 66%). In another study of 158 subjects with chest pain and acute coronary syndrome, Tsutsui et al (2005) reported improvement of MCE accuracy for detecting CAD and was an independent predictor of outcome. Plana et al. (2008), in a study of 101 subjects referred for stress testing with two DEFINITY®-enhanced

dobutamine stress echocardiograms, reported improvement of endocardial visualization at rest and stress leading to higher confidence of accuracy in evaluating CAD. The literature review of stress echocardiography using DEFINITY® appears to provide a strong basis for improved accuracy in detecting CAD in subjects with known or suspected disease.

5.5 Literature Review Summary

The majority of clinical experience using DEFINITY® as contrast agent came from the both overall echocardiography as well as radiology. However, there is a reasonably large body of literature documenting the use of DEFINITY® as a myocardial perfusion agent for detecting perfusion abnormalities both at rest and in conjunction with exercise and pharmacological stress echocardiography. Although perfusion echocardiography studies were small and primarily investigator sponsored, they do provide a strong basis for the ability of DEFINITY® in detecting perfusion abnormalities. The literature review of stress echocardiography using DEFINITY® also appears to provide a strong basis for improved accuracy in detecting CAD in subjects with known or suspected disease.

Based on the data available from original publications and overall reviews, DEFINITY® administration appears to be safe and, as expected, demonstrated a safety profile similar to that observed during clinical development. They did not appear to show nephrotoxicity or cardiotoxicity and the incidence of hypersensitivity or allergic events appear to be much lower than that in current X-ray or MR contrasts agents (Blomley et al. 2007).

5.6 Overall Conclusion

The use of DEFINITY® in healthcare management has increased over recent years since its approval by FDA in 2001. Data from clinical studies provide the most recent evidence that DEFINITY® improves the diagnostic efficacy of echocardiography. Recently the FDA raised some concerns regarding the safety aspects on the use of contrast agents in clinical practice.

To further evaluate the safety profile of DEFINITY®, we summarized the safety results based on data from all completed clinical trials and reviewed the information based on the peer-reviewed publications.

Safety Exposure to DEFINITY® in All Clinical Trials

The evaluation of the safety of DEFINITY® is based on data from 3985 subjects from 48 completed studies that are basis of regulatory approvals and post-NDA/MAA clinical experience. The remaining three post-approval studies are ongoing. Approximately 593 subjects were enrolled in those studies and the results are currently not available. The clinical studies were conducted mainly in the USA, UK, Germany and Canada from 17 March 1995 to present. The 48 completed studies comprise of 36 echocardiography/safety studies and 12 radiology studies.

The total of 3985 subjects comprises 60 healthy subjects and 3925 patients. Of the 3985 subjects: 369 subjects received placebo (16 healthy subjects, 353 patients mostly in echocardiography studies). A total of 3616 subjects received at least one dose of DEFINITY®.

From the 40 studies submitted in the MAA for which detailed safety analysis has been completed, a total of 26% subjects had at least one new-onset adverse reactions and 7.6% of them were treatment related AEs. The most common drug related AEs (reported >1%) are fatigue, headache, dyspnea, back pain, nausea, flushing, and dizziness. Less commonly reported AEs (0.5%~1%0.) are dysgeusia, chest discomfort, pain NOS, altered sensation, and pain at the injection site. In all placebo-controlled studies, a total of 126 (56%) of 224 placebo subjects; 259 (48%) of 543 subjects receiving DEFINITY® experienced a new-onset AE indicating similarity in AE rates between placebo and DEFINITY®-treated subjects. The profiles of the AEs were also similar. No dose relationship can be identified for any

individual new-onset AEs reported for DEFINITY®-treated subjects regardless of mode of administration.

The rates of serious AEs and fatal outcomes were low. A total of 34 SAEs from 3985 subjects were reported (including one in a placebo subject and one in post-MAA study). Out of 34 SAEs, eight events were fatal outcomes and the remaining 26 SAEs were non-fatal AEs that were classified as serious, in nearly all cases due to the need for hospitalization. None of the non-fatal serious AEs was considered by the investigators to be related to the use of DEFINITY®. No fatal outcome occurred in placebo subjects. All of these fatal outcomes occurred at least one day after the administration of DEFINITY® and were considered to be not related to drug by the investigators.

Based on additional safety analysis conducted in overall subject populations and subgroup of disease populations, following are some of the safety conclusions:

- AE rates in DEFINITY®-treated subjects (48%) were similar or even lower than in placebo subjects in placebo-controlled clinical studies (56%). The profiles of the AEs were also similar.

- AE rates in rest-stress echocardiographic imaging studies were higher (56%) than that in rest-only subjects (24%) attributable mainly to the use of stress agents and additional dosing to the stress subjects.

- No clinically important changes were noted in the immunology parameters evaluated (CH50, immunoglobulins A, E, G and M, histamine, anti-double stranded DNA antibody, tryptase) in 128 subjects with the exception of activated complement factor C3a. However, the increase in C3a levels were not associated with mast cell or basophile activation or the release of bioactive mediators. An immunology expert reviewer

concluded that the DEFINITY® does not appear to present an increased risk of anaphylactoid or anaphylactic reactions.

- No notable differences in mean change from baseline in ECG parameters were observed between DEFINITY®-treated and placebo subjects in placebo-controlled studies.

- The frequencies of hypertension and hypotension reported as a new-onset AE were low and very few of the AEs were rated by the investigators as treatment-related and no associated clinical symptoms were reported as AEs.

- A small pharmacokinetic study was performed in COPD subjects with a high (50uL/kg) dose of DEFINITY®. No SAEs were reported and the lung clearance of PFP in the COPD subjects was similar to the placebo control. AEs were reported for both the COPD (7/12) and placebo (4/12) groups. In addition, based on reviewing the case report forms (CRFs) from the patients who had a history of COPD and received at least one dose of DEFINITY® in other trials, a total of 10 (22%) of the 46 patients reported at least one new-onset of AE. It appears the nature of these events was similar and overall suggests pulmonary compromise did not change DEFINITY® effects.

- In placebo-controlled studies in subjects with CHF, the overall incidence of new-onset AE appeared to be slightly higher in DEFINITY®-treated subjects than placebo subjects (35% vs. 23%). Overall rate of new-onset AEs observed in subjects with acute MI was lower than that observed for DEFINITY®-treated subjects in overall echocardiography studies.

- A clinical study of 38 subjects on mechanical ventilation reported no clinically significant abnormalities in any of ventilation safety parameters.

- In retrospectively monitoring ventricular and atrial premature beats, use of DEFINITY® appears not pose a risk related to premature beats because DEFINITY® is commonly used with a low-MI mode in clinical practice.

Based on the above study data from all clinical trials, DEFINITY® was safe and well tolerated in general both in echocardiography and radiology studies and also in high risk population of subjects with pre-existing cardiac conditions.

Review the Safety Information from Peer-Reviewed Publications

We identified 23,772 subjects in 52 publications who administrated DEFINITY®. In general, all these publications stated that DEFINITY® appears to be well tolerated and treatment-related SAEs were rarely observed. They did not appear to show nephrotoxicity or cardiotoxicity and the incidence of hypersensitivity or allergic events appears much lower than in current X-ray or MR contrast agents (Blomley et al. 2007).

In summary, the overall rate of treatment-related AEs in clinical trials was low based on 3985 subjects who received at least one dose of DEFINITY® in clinical trials. The most frequent treatment-related AEs with DEFINITY® were headache, flushing, back pain, nausea, dysgeusia, and dizziness. They were mild-to-moderate in intensity, short duration, and did not require therapeutic intervention. In peer-reviewed publications, DEFINITY® was well tolerated and no significant safety issue was observed. Other safety parameters showed minor changes that were not clinically significant. Thus, the use of DEFINITY® provides a significant diagnostic benefit compared to a small and clinically insignificant risk.

6. SUMMARY OF POST MARKET EXPERIENCE

6.1 Product Distribution

As referenced in Section 2 (Regulatory Overview) of the briefing document, DEFINITY® was first approved for sale in Canada in December 2000 and in the USA since 2001. It is also currently available in the EU, Australia, and various countries in Latin America and Asia. Data available for the time period October 2001 through 30 December 2007 indicated that approximately two million patients, worldwide, had received DEFINITY® (source: Arlington Medical Resources, Inc) over that period. The majority of global use was from 2005 onwards.

6.2 Safety Surveillance Methods and Process

A Medical Safety Team (MST) was initially constituted by DuPont Pharmaceuticals prior to DEFINITY®'s entry into Phase 3 trials, then transitioned to Bristol-Myers Squibb (BMS), upon sale of the Company in 2001. Since the sale of BMS Medical Imaging to Lantheus Medical Imaging, Inc. (LMI) in January 2008, pharmacovigilance activities have been performed by LMI in conjunction with BMS. A multidisciplinary team ensures continuous proactive assessment of product safety. Individual case and periodic aggregate spontaneous adverse event (AE) data review and reporting are conducted. In addition, the pharmacovigilance team reviews adverse events reported in clinical trials sponsored by LMI, conducts global literature review, and other safety "signal detection" studies and data analysis activities (see below) as part of internal company procedures. Physician review further facilitates early identification and characterization of any potential safety issues of importance.

Lantheus is also committed to appropriate and timely healthcare provider education regarding important changes to the DEFINITY® label, especially concerning safety issues.

We provide this through direct healthcare provider letters describing any labeling changes, web casts associated with major label changes, and provision of continuous medical information through our central medical affairs service and through our US field medical affairs liaisons. All our staff that interacts with healthcare providers are fully trained on any changes to recommended prescribing information.

6.3 Adverse Events/Adverse Reactions

We recognize the uncertainties of adverse event (AE) rates based upon estimates derived from primarily spontaneous reports from physicians in clinical practice. However, reporting rates may be higher than over the counter or outpatient therapeutics since DEFINITY® is always administered in an authorized clinical facility such as hospital or outpatient clinic, and patients are attended by sonographers and/or physicians in all cases. In addition, there are no comparable spontaneously reported data on adverse events reported in similar populations of patients receiving echocardiography or ultrasound exams without DEFINITY®, that may be used to assess the intrinsic rates of similar events in similar populations that receive ultrasound but not DEFINITY®.

6.3.1 Overall Post-Marketing Experience

The most frequently reported AEs for patients treated with DEFINITY® during the post-marketing experience (Date range: 28 December 2000 to 27 December 2007) are presented below, regardless of indication, dose of DEFINITY® administered, or mode of administration (bolus injection vs. infusion). While these data provide an overall frequency of AEs reported in patients who received DEFINITY® during the post-marketing experience, there are no comparisons to a control group. Consequently, there is uncertainty regarding the AEs that may be attributable to DEFINITY®.

A total of 3113 events, representing 1632 individual patient reports or cases, have been received during the post-marketing experience. The MedDRA (Medical Dictionary for Regulatory Activities) System Organ Classes (SOCs) with the most frequently reported AEs appear below in Table 6.1.

Table 6.1 Percentage of All Adverse Events Reported During the Post-Marketing Experience (28 Dec 2000 to 27 Dec 2007)

Adverse Events	%
Musculoskeletal and connective tissue disorders	38.7%
Skin and subcutaneous tissue disorders	10.9%
Nervous system disorders	9.7%
General disorders and administration site conditions	9.2%
Respiratory, thoracic and mediastinal disorders	7.2%
Gastrointestinal disorders	5.4%
Vascular disorders	5.2%
Investigations	3.0%
Cardiac disorders	3.0%

Based on this total of 3113 AEs reported during post-marketing experience, the most frequently reported AEs terms within these SOCs are listed in order of descending frequency below.

Table 6.2 Percent Most Frequently Reported Adverse Event Terms within Affected System Organ Classes During the Post-Marketing Experience (28 Dec 2000 to 27 Dec 2007)

Preferred Term or High Level Term (HLT)	Number of Events[a]
Back pain	891 (28.6%)
Flank pain, Muscle spasms, Arthralgia, Neck pain	179 (5.2%)
Urticaria	142 (4.6%)
Chest pain, Chest discomfort	139 (4.5%)
Flushing, Hot flush	114 (3.7%)
Dyspnea	119 (3.8%)
Headache	101 (3.2%)
Pain in extremity	70 (2.2%)
Nausea and vomiting symptoms (HLT)	83 (2.7 %)
Pruritus	66 (2.1%)
Rashes, eruptions and exanthems and Erythemas (HLTs)	38 (1.2%)
Dizziness	59 (1.9%)
Blood pressure decreased, Hypotension	54 (1.7%)
Feeling hot, Feeling abnormal	41 (1.3%)
Heart rate increased, Tachycardia	33 (1.1%)
Blood pressure increased, Hypertension	25 (0.8%)
Cough, Wheezing	21 (0.7%)
Paraesthesias and dysaesthesias (HLT)	19 (0.6%)

[a] The data, including the percentages provided, are based on the number of reports in the safety database received during the post-marketing experience. Determination of the incidence rate for these events cannot be derived from the post-marketing data.

Of note, the majority of these AEs were spontaneous reports from healthcare providers in relevant clinical settings. These settings include large cohorts of both acute and subacute hospitalized patients, as well as outpatients in hospitals and clinics undergoing resting or rest-stress examinations.

Table 6.3 All Serious Adverse Events Reported by System Organ Class During the Post-Marketing Experience (28 Dec 2000 to 27 Dec 2007)

System Organ Class	Frequency counts
Respiratory, thoracic and mediastinal disorders	113 (16.3%)
Skin and subcutaneous tissue disorders	92 (13.2%)
Nervous system disorders	88 (12.7%)
Cardiac disorders	80 (11.5%)
General disorders and administration site conditions	72 (10.4%)
Musculoskeletal and connective tissue disorders	60 (8.6%)
Vascular disorders	47 (6.8%)
Gastrointestinal disorders	41 (5.9%)
Investigations	35 (5.0%)
Immune system disorders	25 (3.6%)
Eye disorders	9 (1.3%)
Infections and infestations	7 (1.0%)
Psychiatric disorders	6 (0.9%)
Renal and urinary disorders	5 (0.7%)
Injury, poisoning and procedural complications	5 (0.7%)
Hepatobiliary disorders	2 (0.3%)
Surgical and medical procedures	3 (0.4%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2 (0.3%)
Reproductive system and breast disorders	1 (0.1%)
Social circumstances	1 (0.1%)
Total	**695**

A total of 695 serious events from 277 cases were reported from 28 Dec 2000 to 27 Dec 2007. The table above displays the most frequently reported AEs within these SOCs listed in order of descending frequency.

6.3.2 Important Identified Risks

Overall we have focused attention on the most common serious reactions, which are clinically either cardiopulmonary, hypersensitivity or CNS reactions. (Note: in Table 6.3 cardiopulmonary reactions are found predominantly within the SOCs cardiac and respiratory, thoracic and mediastinal disorders; hypersensitivity reactions are mostly found in the SOCs

immune system and skin disorders; neurovascular events are predominantly found in the SOC nervous system disorders). However all three clinical categories of events appear to be uncommon to rare events. Our further analyses within each of these categories are described below.

6.3.2.1 Cardiopulmonary Reactions

Serious cardiopulmonary reactions, including cardiac/cardio-respiratory arrest, ventricular fibrillation, and ventricular tachycardia have been reported rarely following DEFINITY® administration. Table 6.4 below lists events of interest by preferred term relating to cardiopulmonary disorders reported during the period 28 December 2000 through 14 April 2008.

There were a total of 91 post-marketing reports of cases with serious cardiopulmonary reactions; seven of these were fatal (refer to Section 6.4). Fourteen of the 91 cases included some symptoms or signs that might also be related to hypersensitivity reactions, though the predominant clinical findings were cardiopulmonary in nature.

Table 6.4 All Cardiopulmonary Events Reported by Preferred Term During the Post-Marketing Experience (28 Dec 2000 to 14 Apr 2008)

Preferred Term	Number
Cardiac arrest	19
Respiratory arrest	13
Ventricular tachycardia	11
Respiratory distress	7
Bradycardia	5
Cardio-respiratory arrest	5
Ventricular fibrillation	5
Atrial fibrillation	3
Acute myocardial infarction	2
Supraventricular tachycardia	3
Cardiac failure	2
Cardiac failure congestive	2
Ventricular extrasystoles	2
Respiratory failure	2
Arrhythmia	1
Atrial flutter	1
Cardiac tamponade	1
Cardiogenic shock	1
Cardiopulmonary failure	1
Cyanosis	1
Extrasystoles	1
Myocardial infarction	1
Pulmonary embolism	1
Shock*	1
Total	**91**

*Not a cardiac or respiratory preferred term

When co-morbid conditions and concomitant medications were reported, it is evident that patients who experienced serious cardiopulmonary reactions typically had one or more acute or chronic conditions that predispose to future clinical cardiopulmonary events regardless of any testing procedures. Reported co-morbidities included: atrial fibrillation, coronary artery disease, abdominal aortic aneurysm, emphysema, congestive heart failure, coronary artery bypass graft, acute heart failure, cardiomyopathy, cardiomegaly, morbid obesity, pulmonary edema, pulmonary emboli, severe biventricular dysfunction, trilobar pneumonia, chronic

obstructive pulmonary disease, ischemic cardiomyopathy, myocardial infarction, pulmonary hypertension, aortic stenosis, critical aortic stenosis, peripheral vascular disease, renal failure, and others. Concomitant medications included: dobutamine, amiodarone, warfarin, losartan, clopidogrel, dopamine, amlodipine, lidocaine, heparin, carvedilol, digoxin, esomeprazole as well as many others typically used for therapies in patients with underlying cardiopulmonary or metabolic diseases.

Because of the well-known phenomenon of "pseudo-complications" (see Main et al, 2008, for a review), it is to be expected that serious clinical events will occur in such high risk populations. At times these events may occur in close proximity to a test, such as DEFINITY®-contrast echocardiography, and therefore form a spurious association with the test that forms no part of the mechanism or cause of the clinical event. Indeed, physicians can be expected to schedule diagnostic tests at the time of major events – for patients undergoing echocardiography such events would include diagnoses such as myocardial infarctions, and acute onset decompensated congestive heart failure. Patients with such diagnoses can be expected to be at significant risk for imminent death. Thus, it would not be surprising that such patients would be scheduled for diagnostic tests at the same time they are at greatest risk for experiencing death.

In addition, we have not been able to clearly associate serious cardiopulmonary reactions with any specific demographic subgroup of patients. Further investigations of large databases of clinical outcome data may be valuable in searching for patient subgroups that may be at risk for reactions after DEFINITY® administration, and form part of our ongoing safety surveillance and commitments (Refer to Section 6.7).

6.3.2.2 Hypersensitivity and Anaphylactic/Anaphylactoid Reactions

Serious hypersensitivity reactions, including anaphylactic and anaphylactoid events have been reported rarely following DEFINITY® administration. Table 6.5 below lists events of interest by preferred term during the period 28 December 2000 through 14 April 2008.

There were a total of 106 post-marketing reports of serious hypersensitivity reactions. Detailed information on potential prior sensitization to perflutren has generally not been available in these reports.

Table 6.5 All Hypersensitivity and Anaphylactic/Anaphylactoid Reactions Reported by Preferred Term During the Post-Marketing Experience (28 Dec 2000 to 14 Apr 2008)

Preferred Term	Number
Uticaria	36
Pruritis	16
Anaphylactic reaction	11
Erythema	7
Lip swelling	6
Pharyngeal oedema	4
Rash	4
Swelling face	3
Anaphylactic shock	2
Laryngeal oedema	2
Angioedema	2
Wheezing	2
Rash generalized	2
Rash macular	1
Rash pruritic	1
Anaphylactoid reaction	1
Palatal oedema	1
Oedema mouth	1
Type IV hypersensitivity reaction	1
Generalized erythema	1
Serum sickness	1
Drug hypersensitivity	1
Total	**106**

Serious hypersensitivity reactions have been reported rarely during post-marketing surveillance. These reactions occurred in both genders, and over a wide range of patient ages. Where information was reported, most cases occurred in patients taking one or more concomitant medications without a discernibly high rate in any class or specific medication. While patients sometimes had prior histories of drug allergies, it is not possible to assess the risk of hypersensitivity reactions in this subgroup as compared with those without prior allergic histories due to inconsistencies in data reporting. Additionally, the reports frequently

identified prior chronic cardiopulmonary or metabolic disorders, as would be expected in a population undergoing contrast echocardiography.

6.3.2.3 Possible Mechanisms and Risk of Hypersensitivity Reactions

Serious hypersensitivity reactions were not observed within the pre-approval clinical studies performed with DEFINITY®. In the post-marketing experience since 2001, uncommon or rare serious hypersensitivity reactions have been reported. Detailed information on potential prior sensitization to perflutren or other microbubble contrast agents has generally not been available in these reports. Also, these reports do not include any information on immunologic tests that may have been performed to assess the underlying mechanisms. It is therefore not presently possible to determine with certainty if these reactions represent classic Type 1 hypersensitivity reactions or results from another mechanism, such as complement activation-related pseudoallergy (CARPA) or are due to other undefined mechanisms. Refer to Safety and Pharmacology Section 4.2 for additional detail.

The mechanism leading to hypersensitivity reactions following DEFINITY® administration remains unclear. We cannot exclude the possibility that some of these reactions represent CARPA. Both type 1 hypersensitivity reactions and CARPA-like reactions occur with all other radiocontrast agents, such as those used for X-ray angiography, CT or MRI scanning. The rates of hypersensitivity reactions appear lower than among these other classes of contrast agents. Other than those patients with known prior hypersensitivity to perflutren, it is not yet possible to predict the risk of hypersensitivity reactions in the overall population typically receiving DEFINITY®.

6.3.2.4 Seizures and Seizure Like-Reactions

Serious neurological AEs such as loss of consciousness and seizures following DEFINITY® exposure have been reported in the post-market period. The mechanism of these events is

unclear but theoretically could involve an allergic-type reaction or vasovagal response. Some events have occurred within 30 minutes of DEFINITY® administration and a causal relationship cannot be excluded. However, these events appear uncommon or rare, and were not seen during pre-market clinical trials.

All post-marketing reports of seizures and seizure-like reactions, including syncope, loss of consciousness and coma associated with DEFINITY® use were reviewed. Table 6.6 lists the events related to seizures.

A cumulative compilation and assessment of possible seizures and seizure-like reactions to DEFINITY® was performed from 28 December 2000 to 27 December 2007 using the database. Table 6.6 displays the number of events reported in the post market period.

Table 6.6 Seizures and Seizure-Like Adverse Events Reported in Association With DEFINITY® During the Post-Marketing Experience (28 Dec 2000 to 27 Dec 2007)

Preferred Term(s)	Number of Events
Convulsion	18
Loss of consciousness	15
Syncope and syncope vasovagal	13
Dizziness	10
Coma	9
Grand mal convulsion	2
Tremor	2

In the overall post-market experience there were 65 case reports of possible seizures and seizure-like reactions. Of the 65 reports, 63 were spontaneously reported from worldwide sources and 2 were received from a clinical trial. Forty-eight (48) of the 65 reports qualified for classification as serious: 46 spontaneous reports and 2 clinical trial reports, and 12 were considered life-threatening. None were fatal.

The 65 case reports, included 31 females and 28 males (6 unknown gender), ranging in age from 26 to 86 years with a mean age of 56 years. Thirty-seven (37) of the 65 reports were included only neurological AE terms and 28 were reports which included at least one coded term that might be related to a hypersensitivity reaction as well as a neurological event. However, the predominant clinical findings were neurologic and not anaphylactoid in nature.

Of the 65 reports, 28 were effectively treated with medication and/or medical intervention (i.e., IV fluids, oxygen, diphenhydramine, steroids), 29 did not require any treatment and treatment was unknown in 8 reports. Of the 12 life-threatening reports, 1 was not considered related to DEFINITY® by the reporter and the remaining 11 were not assessed.

6.4 Events with a Fatal Outcome

There were 14 reports of fatal outcomes following DEFINITY® administration. These 14 reports describe 9 males and 4 females (1 unknown) ranging in age from 34 to89 years.

Table 6.7summarizes the 14 fatal outcomes during the post-marketing period to the date of this document. Ten of the 14 deaths occurred in patients with significant co-morbidities and high acuity including underlying CAD or CHF. The remaining four case reports contained limited or no information on medical history, patient characteristics or other aspects of care.

A recently published review of the October 2007 changes to the US PI, including discussion of the four of the seven cases of fatal cardiac arrest that occurred during or within 30 minutes of DEFINITY® administration, has suggested the possible role of "pseudo-complication" in these cases. They point out that complications occurring after any medical procedure may be attributable to progression of the underlying disease state rather than the procedure itself.

Table 6.7 Fatalities Following DEFINITY® Exposure

CIOMS	Reporting Method	Age/Gender	Dosing	Adverse Event	Timing Relative to DEFINITY® Exposure
12755260	Spontaneou	34/female	dose and method of administration unknown	cardiac arrest.	immediately after dosing
12986592	Spontaneous	mid-70-80/male	2mL of DEFINITY®; unknown method of adminsitration	cardiac arrest.	2 minutes after dosing
1373470	Spontaneous	67/male	dilute bolus: 1mL/4mL NS	cardiac arrest	30 minutes after dosing
13816079	Spontaneous	70/male	dilute bolus: 1.5mL/8.5mL NS (4.5mL administered)	cardiac arrest	within 30 minutes
14046221	Spontaneous	73/male	bolus dose: 0.5mL (5uL/kg)	cardiac arrest	within 30 minutes
14086243	Spontaneous	late 70s/male	dilute bolus: 10cc incrementally administered	cardiac arrest	within 30 minutes
14142442	Spontaneous	63/male	4cc of 1 vial, reconsitituted per insert administered over 30 minutes	"infusion reaction" and cardiac arrest	15 minutes after dosing
13983879	Spontaneous	unk/female	unknown dose and method of administration	death following transesopheagal echo, no specifics	greater than 30 minutes of administration
14021828	Spontaneous	54/female	unknown dose and method of administration	cardiac arrest	1 hour post-dose
11841004	Spontaneous	unk/unk	unknown dose and method of administration	death, no specifics	60-90 minutes of dosing
12410643	Investigator sponsored clinical trial	63/male	dilute bolus: 1.3ml/20mL NS (10-14mLs administered as a continuous hand injection)	cardiac arrest likely secondary to arrhythmia	several hours post-dose
14116255	Spontaneous	71/male	unknown dose and method of administration	cardiac arrest	approximately 8 hours post-dose
1270724	Spontaneous	57/male	dilute bolus: 5mL administered	SOB, agitation, found dead	approximately 12 hours post-dose
13664537	Spontaneous	89/female	dilute bolus: 1.3mL/8mL NS (2mL administered)	back pain, SOB, dizziness hypotension	not reported

The following narratives represent the seven cases with a fatal outcome occurring within 30 minutes of DEFINITY® administration. It should be noted that all except for Case 6, involved patients with severe preexisting life-threatening conditions. For Case 6 there was minimal medical history provided.

Case 1: (13734702) This was a 67 year old male whose medical history included coronary artery disease, atrial fibrillation, hypertension, ischemic cardiomyopathy, dyslipidemia, hypothyroidism and Type 2 diabetes. His concomitant medications included: furosemide, aspirin, digoxin, carvedilol, lisinopril, lovastatin/extended-release nicotinic acid, levothyroxine, rosiglitazone, glipizide, and metformin. In addition, Tc-99m sestamibi was also administered on the same day he received DEFINITY®.

Prior to a resting echocardiogram in March 2007, the patient received a dilute bolus of DEFINITY® (1ml in 4mLs of normal saline) administered as an IV push over 20 seconds. No adverse reactions were noted during the resting echo. Thirty (30) minutes following DEFINITY® dosing and approximately 1 minute after beginning a treadmill exercise stress test, he experienced a cardiac arrest. Basic and advanced life support was administered and he was transported to a medical center where he died 5 days later. The cause of death was reported as anoxic encephalopathy, cardiopulmonary failure and acute myocardial infarction. No relationship to DEFINITY® was provided by the reporter.

Case 2: (12986592) This was a male, reported as "mid-70's" to 80 years old, with a recent myocardial infarction, a very low ejection fraction and progressive worsening of his clinical condition. He was admitted to a Coronary Care Unit and, approximately 4 hours later, underwent an echocardiogram and received 2mL of DEFINITY®. Prior to the echocardiogram, he had received an unknown sedative for agitation. Approximately two minutes after completing the echocardiogram, the patient had a fatal cardiac arrest. The report was received in 2005 and the reporter considered the death unrelated to DEFINITY®.

Case 3: (13816070) This was a 70 year old male with a medical history that included myocardial infarction, triple coronary artery bypass surgery, atrial fibrillation, congestive heart failure (CHF), peripheral edema, deep vein thrombosis, previous smoker, shortness of breath and an allergy to lisinopril. Concomitant medications included albuterol inhaler, aspirin, carvedilol, losartan, furosemide, potassium supplement, varenicline, simvastatin, warfarin, fluoxetine, and sildenafil.

In June 2007, the patient received 4.5mL of a dilute bolus of DEFINITY® (1.5mL in 8.5mL of normal saline) during an echocardiogram to evaluate worsening CHF. Approximately five minutes following completion of the echocardiogram, he complained of dizziness. His systolic blood pressure was 50mmHg; he became cyanotic and went into cardiac arrest. He was treated with epinephrine in case it was an allergic reaction. Cardiopulmonary resuscitation was instituted but he was pronounced dead on arrival at the emergency room. Suspected massive pulmonary embolism was reported as the cause of death. No relationship to DEFINITY® was provided by the reporter.

Case 4: (12755260) This case was a 34 year old, morbidly obese female (>350 lbs) who was admitted to an intensive care unit in October 2004 with severe tri-lobar pneumonia and hypoxia. She was being treated for numerous concurrent medical conditions including postpartum cardiomyopathy and pulmonary embolism. Her respiratory state required mechanical ventilation and her hypotension was treated with two pressor agents. Concomitant medications included dopamine, norepinephrine, vecuronium bromide, famotidine, ferrous sulfate, ibuprofen, heparin, azithromycin, ceftriaxone, vancomycin, pipercillian/tazabactam, lidocaine and epinephrine. Echocardiography, including DEFINITY® administration (dose unknown), revealed severe bi-ventricular dysfunction and a large mass in the right ventricle, thought to be thrombus. Immediately after DEFINITY® administration, the patient developed bradycardia leading to asystole. Resuscitation was performed for 30 minutes without success. An autopsy revealed pulmonary edema, multiple

pulmonary emboli, large right ventricular thrombus, acute heart failure with moderate dilation of the right ventricle and left ventricle, and cardiomegaly. The treating physicians and the pathologist considered the death unrelated to DEFINITY®.

Case 5: (14046221) This case was a 73 year old Canadian male with three-vessel coronary artery disease and left ventricular (LV) dysfunction, LV ejection fraction: 25-30% who had undergone coronary bypass graft surgery in December 2007, 10 days prior to a DEFINITY®-enhanced echocardiogram (bolus dose 0.5mL (5uL/kg). This was performed to assess LV function as, on post-operative day 8-9, he was noted to be short of breath and to have elevated white blood cell count and plasma creatinine. The contrast echo was well tolerated and the LVEF was noted to be 12%. Approximately 5 minutes after the completion of the study and as the patient was leaving the examination room, he collapsed and was found to have asystolic cardiac arrest. Resuscitation led to ventricular fibrillation but was ultimately not successful. Preliminary information from an autopsy revealed a severely dilated LV with no thrombus and left lung pneumonia.

Case 6: (14086243) A health professional reported that a male patient in his late seventies became unresponsive and died following DEFINITY® administration. The patient received DEFINITY® as a part of resting echo study as an in-patient. The duration of the hospitalization and the admitting diagnosis was not available. DEFINITY® 10cc diluted in normal saline was incrementally administered during the test. The test took place sometime between July 2007 and September 2007. The patient became unresponsive and slumped over in his wheelchair 10 minutes following the test. Consequently the patient underwent resuscitation procedure, but within 30 minutes of the completion of the test, the patient died. The cause of death was not available. Medical assessment notes: this patient became unresponsive and subsequently died 30 minutes after administration of injection DEFINITY®.

Case 7: (14142442) A 63 year old male with a history of CAD, CABG, diabetes, hypertension, hyperlipidemia, hyperuricemia, and a drug allergy to carvedilol (reaction unknown). Concomitant medications included allopurinol, amiodarone, aspirin, clopidogrel bisulfate, esomeprazole, exetimibe, fosphenytoin sodium, glipizide, insulin, metformin, nicotinic acid, simvastatin, valsartan, and pipercillin/tazobactam. On [redacted] the patient received 4cc of 1 vial of DEFINITY®, reconstituted as per insert and administered over 30 minutes. Approximately 15 minutes following DEFINITY® administration patient developed "infusion reaction" (undefined) and cardiac arrest. He was transported to an emergency room and subsequently admitted to the ICU and placed on ventilator. The family chose to withdraw life support on [redacted].

6.4.1 Summary and Conclusion

The adverse events reported during post-marketing use of DEFINITY® occurred in patients with complex medical conditions, multiple co-morbidities and concomitant medications. The majority of patients had clinically significant underlying cardiac conditions and were undergoing assessment and evaluation, including contrast echocardiography. Because these cases are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. As indicated by the reporters, most of these cases were probably related to the patients' serious underlying disorders. However, the temporal association of DEFINITY® administration with some of these cardiopulmonary events means that a contributing effect of DEFINITY® cannot be excluded.

6.4.2 Review of Recent Retrospective Literature

Two important recent publications also address the risks of serious adverse events and fatalities following perflutren administration (Kusnetzky et al, 2008; Herzog, 2008). These studies both represent retrospective review of large single center echocardiography databases

that contain demographics, imaging and safety data gathered during the routine use of DEFINITY® in clinical practice since it became available.

Kusnetzky et al reported results from 18,671 consecutive echocardiography cases among inpatients at a large tertiary care facility in Kansas. Cases included 12,475 non-contrast and 6,195 DEFINITY®-contrast echocardiograms. Vital status up to 24 hours after echo was available for all patients. Incidence of death between the two groups was examined with a chi-square test. No patients died within 1 hour of echocardiography. In the non-contrast group, 46 patients (0.37%) died within 24 hours, and 25 (0.42%) died in the DEFINITY® group. There was no statistical difference between the two groups (p=0.60). The DEFINITY® group had significantly longer ICU stays and included a greater proportion of serious comorbid conditions including diabetes mellitus, hypertension, COPD, CAD and CHF. The authors concluded that there was no evidence for an increased risk of mortality (power 80% to detect a relative risk of 1.84) despite a greater level of clinical acuity and more co-morbid conditions.

Herzog reported data from a large echocardiography database and associated records of adverse events from a large country medical center in Minnesota. Data were abstracted for all 12,974 cases receiving DEFINITY® over the past 5 years. Twenty adverse events were reported, of which 4 were serious, and none were fatal. The serious reactions were cardiopulmonary or hypersensitivity type reactions and all resolved. The overall adverse event rate was 0.12% and the serious event rate was 0.031%. This case series did not report control data from non-contrast echocardiography exams. However, these reported rates of adverse events and serious adverse events are similar to the rates we would estimate from our post-market surveillance program.

These large single site experiences provide important additional evidence that the risk of serious or fatal reactions following DEFINITY® administration is low. Lantheus Medical

Imaging further explored the risk of fatalities in a retrospective study of the Premier Perspective™ Database as described below.

6.5 Premier Database Study

6.5.1 Background and Methods

To further evaluate the risk of fatalities associated with contrast echocardiography, we have conducted a retrospective, observational study to evaluate short term mortality in a population undergoing an echocardiogram with DEFINITY® versus those undergoing a non-contrast echocardiogram. We sought to examine the temporal association between DEFINITY® use and in-hospital deaths and compare it with a cohort of inpatients also undergoing similar echocardiography examinations but without contrast exposure.

Patients were selected from United States hospitals participating in the Premier Perspective™ Database, which contains information from approximately 5.5 million patient discharges per year from not-for-profit, non-governmental, community and teaching hospitals, and health systems representing data from 602 US hospitals. Premier's database is the largest hospital-based, service-level comparative database in the USA and provides detailed resource utilization data along with patients' primary and secondary diagnosis and procedure codes. Service level information is available for each hospital day and includes medication information (i.e., drug name and strength, quantity dispensed and unit cost). This database contains inpatient information, including diagnoses based on the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9M).

The patient information collected includes patient demographics (age, gender, race/ethnicity), principal and secondary diagnoses, principal and secondary procedures, length of stay, drug utilization. All patient records used in this study were de-identified in compliance with the Health Insurance Portability and Accountability Act of 1996. This study met the criteria for

exemption of Institutional Review Board oversight under the Common Rule §45 CRF 46.101(b) (4).

The results presented below represent data from inpatients who underwent a transthoracic echocardiogram either with or without DEFINITY® contrast administration during January 1, 2002 through October 31, 2007. The primary outcome was 24-hour mortality. Twenty-four hour mortality was defined as a discharge code of deceased on same day as the echocardiogram or the following day. This approach permitted the capture of deaths that occurred on the day following the echocardiogram to accommodate for patients that may have died very late in the day and could not be immediately coded into the system and to attain 24 hour follow-up for cases where the echocardiogram was performed late in the day. The degree of severity of illness and risk of mortality variables were based on an algorithm developed by 3M (3M™ APR-DRG Software) and was constructed by considering (1) the primary admitting diagnosis, (2) the secondary diagnoses, (3) the age of the patient, and (4) the presence of certain procedures. The following cardiopulmonary comorbidities: congestive heart failure, myocardial infarction, acute coronary syndrome, pulmonary hypertension, pulmonary emboli, respiratory failure including hypoxemia, emphysema, ventricular arrhythmias and QT interval prolongation and all associated clinical conditions were mapped to correlating ICD-9 codes using the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9M).

Descriptive statistics were summarized using counts and percentages for categorical variables and means and standard deviations for continuous variable. For statistical comparisons, chi-square tests were used to analyze categorical variables and t-tests were used to analyze continuous variables. We used the SAS System for Windows, Version 9.1.

6.5.2 Results

6.5.2.1 Demographics

The table below displays the summary demographic data from the cohort. A total of 4,300,966 patients were identified from the database; 58,254 were DEFINITY® enhanced echocardiograms and the remaining 4,242,712 were non-contrast echocardiograms.

The DEFINITY® group had more males, more Caucasians and had significantly older patients than the non-contrast group (Table 6.8).

Table 6.9, Table 6.10 and Table 6.11 further characterize the patient populations. The DEFINITY® population had significantly greater illness severity, risk of mortality (Table 6.9). The frequencies of unstable cardiopulmonary conditions in each group are shown in Table 6.10 and Table 6.11, and indicate significantly greater proportions in the DEFINITY® group for 5/6 conditions. Hospital length of stay was longer in the DEFINITY® group while ICU length of stay was the same for both groups (Table 6.12).

Table 6.12 shows the 24 hour mortality was approximately 1% in each group (p=0.613), in spite of the greater severity of illness and greater risk of mortality in the DEFINITY® group. Multivariate logistic regression analysis was used to compare 24 hour mortality, while controlling for case mix and the following covariates: race, gender, age, hospital, admission type, contraindications, comorbidities, and severity of illness. The results showed that 24 hour mortality, after adjusting for all covariates, was significantly lower for patients in the DEFINITY® group (Adjusted Odds Ratio 0.76 (CI = 0.70, 0.82)).

Table 6.8 Demographics

Inpatient Rest Echocardiogram Discharges	Non Contrast Echocardiograms 4,242,712		DEFINITY®-enhanced Echocardiograms 58,254		P-value
	Discharges	Percentage	Discharges	Percentage	
Age					
0-17	172,347	4.06%	17	0.03%	
18-44	388,568	9.16%	4,588	7.88%	
45-64	1,158,840	27.31%	21,322	36.60%	
65-74	864,825	20.38%	14,448	24.80%	
75-79	547,784	12.91%	7,304	12.54%	
80 +	1,110,348	26.17%	10,575	18.15%	
Mean (Std Dev)	65.05 (20.4997)		65.67 (14.2798)		<.0001
Median	70		67		
Gender					
Female	2,257,118	53.20%	22,683	38.94%	<.0001
Male	1,985,496	46.80%	35,570	61.06%	
Unknown	98	0.00%	1	0.00%	
Race					
Caucasian	2,704,364	63.74%	43,609	74.86%	<.0001
African American	687,859	16.21%	7,302	12.53%	
Hispanic	227,896	5.37%	882	1.51%	
Other/Unknown	622,593	14.67%	6,461	11.09%	

Table 6.9 Patient Characteristics

	Non Contrast Echocardiograms		DEFINITY®-enhanced Echocardiograms		P-value
Inpatient Rest Echocardiogram Discharges	**4,242,712**		**58,254**		
	Discharges	**Percentage**	**Discharges**	**Percentage**	
Severity of Illness*					
1 = Minor	724,482	17.08%	6,564	11.27%	
2 = Moderate	1,572,921	37.07%	19,166	32.90%	<.0001
3 = Major	1,319,188	31.09%	20,318	34.88%	
4 = Extreme	622,195	14.67%	12,127	20.82%	
Unknown	3,926	0.09%	79	0.14%	
Risk of Mortality*					
1 = Minor	1,333,117	31.42%	13,884	23.83%	
2 = Moderate	1,575,570	37.14%	20,810	35.72%	
3 = Major	874,792	20.62%	13,984	24.01%	<.0001
4 = Extreme	455,307	10.73%	9,497	16.30%	
Unknown	3,926	0.09%	79	0.14%	

*APR-DRG (all patient refined, diagnosis related groups)

Table 6.10 Frequency of Unstable Cardiopulmonary Conditions I

	Non Contrast Echocardiograms		DEFINITY®-enhanced Echocardiograms		P-value
Inpatient Rest Echocardiogram Discharges	**4,242,712**		**58,254**		
	Discharges	**Percentage**	**Discharges**	**Percentage**	
1. Worsening or clinically unstable congestive heart failure	1,341,263	31.61%	25,040	42.98%	<.0001
2. Acute myocardial infarction or acute coronary syndrome	592,128	13.96%	13,871	23.81%	<.0001
3. Serious ventricular arrhythmias or high risk for arrhythmia due to prolongation of the QT interval	200,923	4.74%	4,742	8.14%	<.0001
4. Respiratory failure, as manifest by signs and symptoms of hypoxemia	606,615	14.30%	12,480	21.42%	<.0001
5. Severe emphysema, pulmonary emboli or other conditions that may cause pulmonary hypertension	54,803	1.29%	765	1.31%	0.6478
6. Pulmonary hypertension	244,475	5.76%	4,380	7.52%	<.0001

Table 6.11 Frequency of Unstable Cardiopulmonary Conditions II

	Non Contrast Echocardiograms		DEFINITY®-enhanced Echocardiograms		P-value
Inpatient Rest Echocardiogram Discharges	**4,242,712**		**58,254**		
	Discharges	**Percentage**	**Discharges**	**Percentage**	
Unstable CP ConditionUnstable CP Conditions per Discharge					
# Unstable CP Condition: 1	1,402,865	33.07%	22,756	39.06%	<.0001
# Unstable CP Condition: 2	544,235	12.83%	11,367	19.51%	<.0001
# Unstable CP Conditions: 3	148,794	3.51%	4,097	7.03%	<.0001
# Unstable CP Condition: 4	23,765	0.56%	808	1.39%	<.0001
# Unstable CP Conditions: 5	1,450	0.03%	53	0.09%	<.0001
# Unstable CP Condition: 6	30	0.00%	0	0.00%	<.0001
Discharges with 1 or more Unstable CP Condition	2,121,139	49.99%	39,081	67.09%	<.0001

Unstable CP Condition defined by ICD-9 codes:
Unstable CP Condition #1: icd_code encompassing 428
Unstable CP Condition #2: icd_code encompassing 410 or icd_code encompassing 411
Unstable CP Condition #3: icd_code in (426.82, 427.1 ,427.4, 427.5)
Unstable CP Condition #4: icd_code in (415.1, 518.0, 518.81, 518.83, 518.84, 518.4, 518.5, 518.82, 799.02, 799.1)
Unstable CP Condition #5: icd_code in (492, 492.0, 492.8, 518.1)
Unstable CP Condition #6: icd_code in (415.0, 416.0, 416.8, 416.9)

Table 6.12 **24-Hour Mortality and Length of Hospital Stay**

	Non Contrast Echocardiograms		DEFINITY®-contrast Echocardiograms		P-value
Rest Echocardiogram Discharges	**4,242,712**		**58,254**		
	Discharges	**Percentage**	**Discharges**	**Percentage**	
24-Hour Mortality	45,789	1.08%	616	1.06%	0.613
Mean/Median Length of Stay (Days)	7.85	5	8.40	5	<.0001
Mean/Median ICU Length of Stay (Days)	6.37	3	6.31	3	0.4124

6.5.3 Discussion

This large hospital based cohort using billing claims data revealed similar 24 hour mortality either with or without the use of DEFINITY® for resting echocardiography within a hospitalized patient population. The age spectrum was similar for both DEFINITY® contrast and non-contrast echo populations. More males and Caucasians received DEFINITY® than those receiving only non-contrast echo. DEFINITY® patients had significantly greater underlying illness severity and a higher risk of acute mortality. Although the mortality rate were similar, patients receiving DEFINITY® had significantly more unstable or severe cardiopulmonary conditions in 5/6 of the clinical categories (Table 6.10, Table 6.11). Multivariate logistic regression analysis of the 24 hour mortality data showed a statistically significant 24% risk reduction (adjusted odds ration 0.756) in DEFINITY® patients after adjustment for severity of illness and risk of mortality, and other co-morbidities as described above. Since DEFINITY® is not intended as a therapeutic agent, the risk reduction may result from improved diagnosis and associated improvements in patient management decisions. *To our knowledge, this is the first evidence that use of* DEFINITY® *in patients with significant cardiovascular co-morbidities may improve survival among hospitalized patients.*

6.6 Overall Post-Marketing Safety Summary and Conclusions

Small absolute numbers (<300) of serious adverse events including very small numbers fatalities have occurred following the use of DEFINITY® in routine clinical practice since its introduction in 2001. Most of these events have been identified through spontaneous post-marketing AEs reports. Over the same period, approximately two million doses of DEFINITY® have been administered to patients. We recognize that the rates of serious adverse events are difficult to estimate based upon post-market reports. However, for use of DEFINITY®, it may be that reporting of serious reactions may be improved since

DEFINITY® is always administered in a clinically monitored environment and delivered in the presence of healthcare providers. Accordingly it appears that these serious events are rare or very rare.

We are not aware, beyond those already examined, of specific animal models that would easily predict such rare clinical events or allow patient subgroups that are predisposed to be prospectively identified. We do, however, intend to use model systems to further investigate possible effects if they are identified. The low frequency of events makes it difficult to identify such reactions in the pre-market clinical studies as well. Eight fatal adverse events did occur in our pre-market studies, though all fatal events occurred more than a day after DEFINITY® administration (most several days later), and were considered related to underlying medical conditions rather than to DEFINITY® administration. Given the spectrum of underlying cardiovascular disease prevalent at high rates in the intended target population for use of DEFINITY®, it is not surprising to see an association of serious cardiopulmonary reactions, including rare fatalities, in the post market experience. Main et al (2007) reviewed the issue and the authors argue that many such serious adverse cardiopulmonary reactions are likely "pseudo-complications", that is, events resulting from underlying disease regardless of any temporal association with DEFINITY® exposure. Following this argument, Kuznetsky et al (2008) reviewed short term in hospital mortality in over 18,000 patients in a single hospital experience, including those who had echocardiography performed with or without contrast administration. They concluded that there was no excess risk of fatality associated with DEFINITY®, while those exposed to DEFINITY® were actually sicker at baseline prior to exposure. Kuznetsky's results prompted our own large scale retrospective review of in-hospital mortality within 24 hours of DEFINITY®. In a cohort of over 50,000 patients that received DEFINITY® and over 4 million receiving echocardiography without DEFINITY®, the data indicate that use of DEFINITY®, rather than increase the risk of fatality, may have sufficient benefit on diagnosis and patient management to improve survival.

Since the trigger mechanism underlying these adverse reactions is not clear, some reactions may be pseudocomplications and therefore unrelated to DEFINITY® use, and since the patients who often stand to benefit the most from diagnostic information produced by contrast echocardiography (Main et al, 2007), we conclude that specific contraindications for certain patient populations is not warranted at this time (except for those with known prior hypersensitivity reactions to perflutren-containing microbubble contrast agents). Lantheus Medical Imaging does agree that appropriate warnings about the potential for serious hypersensitivity, cardiopulmonary and or central nervous system reactions are made, as well as directing the responsible physician to make a careful risk-benefit assessment in unstable patients.

6.7 Safety Surveillance and Safety Commitments

Our continuing commitment to gathering and understanding the safety profile of DEFINITY® as used in clinical practice is described below. As referenced in Section 6.2 (Safety Surveillance Methods and Process) of the document a Medical Safety Team (MST) was initially constituted by DuPont Pharmaceuticals prior to DEFINITY®'s entry into Phase 3 trials, then transitioned to BMS (upon sale of the Company in 2001) to enable members to develop a deep understanding of the compound's safety profile over a period of years. Since the sale of BMS Medical Imaging to Lantheus Medical Imaging, Inc. (LMI) in January 2008, pharmacovigilance activities have been performed by LMI in conjunction with Bristol-Myers Squibb (BMS). A multidisciplinary team ensures continuous proactive assessment of product safety throughout its life cycle. In addition to individual case and periodic aggregate spontaneous adverse event (AE) data review and reporting, we conduct review of adverse events reported in clinical trials sponsored by LMI, global literature review, and other signal detection activities as part of internal company procedures, utilizing close physician review and descriptive assessments to further facilitate early identification and characterization of emerging safety issues of specific importance, ongoing benefit/risk evaluation and timely updating of DEFINITY® labeling.

In summary, safety signal detection includes the integration of all reasonably available sources of safety information to identify and characterize unrecognized safety risks or changes in those which are currently expected adverse drug reactions. As needed, changes in the safety profile of DEFINITY® are addressed by appropriate adjustments to risk communication and risk minimization strategies, such as changes in Company Core Safety Information and Product Labels. Such changes in safety information are included with the aggregate reports and ad hoc communications with Health Authorities. The ultimate objective of signal detection is the early identification, characterization, and communication of clinically important adverse events to achieve patient benefit at the lowest risk.

This process describes a comprehensive, integrated risk management plan to ensure the maintenance of a favorable benefit-risk balance for DEFINITY®. The RMP has been designed to continually assess known or potential risks. The risk management plan also gathers further information on risks among patients with pulmonary hypertension and other subgroups of patients with serious underlying cardiopulmonary disorders. The safety surveillance process has been designed to continually assess known or potential risks. As new safety data emerges, the information will be communicated to Health Authorities in scheduled aggregate reports or on an ad hoc basis as warranted.

6.7.1 Additional Ongoing and Planned Post Market Surveillance Studies

Lantheus Medical Imaging has committed to perform several post-market safety assessment studies in addition to routine pharmacovigilance activities to ensure appropriate use of DEFINITY® in clinical practice. These commitments are described below in addition to voluntary additional investigations and assessments that are underway. In general, the results of these investigations, individually or collectively, will be reviewed periodically for any new adverse safety trends and recommendations for additional clinical or pre-clinical investigations or adjustments in prescribing information made accordingly.

6.7.1.1 CaRES Safety Study

We have implemented the CaRES registry (**C**ontrast echocardiography **RE**gistry for **S**afety Surveillance) as of February 2008. To our knowledge this is the first large, multicenter, real-world, prospective registry established to gather and evaluate safety information about the use of DEFINITY® during routine clinical practice in the USA. The specific purposes of the registry are to:

1. Gather adverse and serious adverse event data prospectively on consecutively enrolled patients with a clinical need for contrast echocardiography;

2. Adjudicate any serious events independently to better assess the mechanism and any potential causal link to DEFINITY®;

3. Gather information on the results of monitoring vital signs, electrocardiograms and oxygen saturation and its impact on managing any reactions to DEFINITY®.

At least 1600 patients with a clinical need for contrast echocardiography will be enrolled from approximately10 registry sites. The registry, which was initiated in February 2008, will gather information on patient demographics, characteristics, reason for study, as well as all adverse events and serious adverse events. All patients will have vital signs, ECG monitoring and cutaneous oxygen saturations assessments during and for 30 minutes following DEFINITY® administration to evaluate the value of these monitoring tools.

We expect to analyze available data from the registry on a quarterly basis beginning no later than September 2008. Serious adverse events will have detailed follow-up information gathered and will be reviewed by a separate Clinical Event Committee. We expect to publish the results of the registry on a periodic basis determined by the overall enrollment rate, and to have completed the study no later than January 2010.

At the present time, the registry has 5 enrolling centers and has enrolled 107 cases.

6.7.1.2 Pulmonary Artery Pressure Study

In order to assess regulatory concerns that patients with pulmonary hypertension are at increased risk of serious adverse reactions following DEFINITY® administration, we will perform a randomized, placebo-controlled investigation of pulmonary and systemic hemodynamics using invasive monitoring. The study will compare changes in pulmonary and systemic pressures at various time points following administration of DEFINITY® in patients with and without chronic pulmonary hypertension. This study will be performed in approximately 30 patients undergoing clinical cardiac catheterization procedures. At least 15 of these patients will have pulmonary artery hypertension documented on baseline pulmonary artery pressure assessment, and at least 15 will have normal baseline pulmonary artery pressures. The study will be completed no later than November 2009.

6.7.1.3 Retrospective Database Study

To assess the mortality risk associated with both non-contrast and DEFINITY® contrast echocardiography, and following our initial work with data from the Premier Perspective™ Database, we are designing an observational clinical study using an existing outcomes database to compare in-hospital mortality in critically ill patients undergoing echocardiography with and without DEFINITY®. The analysis plan will be pre-specified and be provided to the FDA prior to commencement of the analysis. It will address, using appropriate statistical techniques, mortality risk in a fashion that adjusts for major patient demographics, characteristics and other parameters that may reasonably affect outcomes. The results of the study may confirm our findings, and those of independent researchers (Kusnetzky et al 2008), that there is no increased overall risk of mortality following DEFINITY® administration.

This new database study will also be completed no later than November 2009.

6.7.1.4 Healthcare Provider Education and Training

Lantheus Medical Imaging is committed to the appropriate and timely education of the physician community regarding the potential risks associated with the use of DEFINITY®. The recent changes to the DEFINITY® US PI have been communicated to healthcare providers widely across the US, including an updated healthcare provider alert and the updated US PI. All medical information, presentations of DEFINITY® safety, efficacy and risk benefit analyses, have or will be updated before they are used further. All Lantheus Medical Imaging field staff and other staff that provide information to healthcare providers have been re-trained with the new US label, and will disseminate only revised materials to the healthcare community. We have planned local, regional and national level educational programs over the next several months regarding the benefit and risks associated with the use of DEFINITY® to be delivered by our staff and by members of the healthcare community who are experienced practitioners of echocardiography.

6.7.1.5 Conclusions

The ultimate objective of signal detection is the early identification, characterization, and communication of clinically important adverse events to achieve patient benefit at the lowest risk. The Lantheus Medical Imaging Safety Surveillance plan described above provides a robust process for continuing evaluation of the safety of DEFINITY® as used in clinical practice, and in certain special populations that may be at increased risk of serious adverse reactions.

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Current U.S. Prescribing Information

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515838-0408 April 2008

DEFINITY®

VIAL FOR (Perflutren Lipid Microsphere)

INJECTABLE SUSPENSION

For Intravenous Use

WARNING: Serious Cardiopulmonary Reactions

Serious cardiopulmonary reactions, including fatalities, have occurred during or following perflutren-containing microsphere administration.

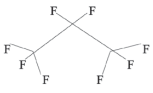
- Assess all patients for the presence of any condition that precludes DEFINITY® administration (see CONTRAINDICATIONS).
- In patients with pulmonary hypertension or unstable cardiopulmonary conditions, monitor vital sign measurements, electrocardiography and cutaneous oxygen saturation during and for at least 30 minutes after DEFINITY® administration (see WARNINGS).
- Always have resuscitation equipment and trained personnel readily available.

DESCRIPTION

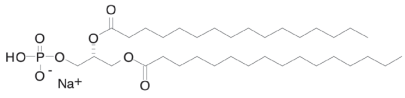
The DEFINITY® vial contains components that upon activation yield perflutren lipid microspheres, a diagnostic drug that is intended to be used for contrast enhancement during the indicated echocardiographic procedures. The vial contains a clear, colorless, sterile, non-pyrogenic, hypertonic liquid, which upon activation with the aid of a Vialmix®, provides a homogeneous, opaque, milky white injectable suspension of perflutren lipid microspheres. The suspension of activated DEFINITY® is administered by intravenous injection.

The perflutren lipid microspheres are composed of octafluoropropane encapsulated in an outer lipid shell consisting of (R) – hexadecanoic acid, 1-[(phosphonoxy)methyl]-1,2-ethanediyl ester, monosodium salt (abbreviated DPPA); (R) - 4-hydroxy-N,N,N-trimethyl-10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium, 4-oxide, inner salt (abbreviated DPPC); and (R)-∝-[6-hydroxy-6-oxido-9-[(1-oxohexadecyl)oxy]-5,7,11-trioxa-2-aza-6-phosphahexacos-1-yl]- ω-methoxypoly(ox-1,2-ethanediyl), monosodium salt (abbreviated MPEG5000 DPPE).

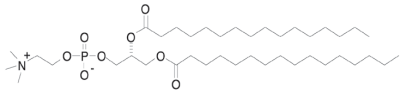
Octafluoropropane is chemically characterized as 1,1,1,2,2,3,3,3-octafluoropropane. It has a molecular weight of 188, empirical formula of C_3F_8 and has the following structural formula:



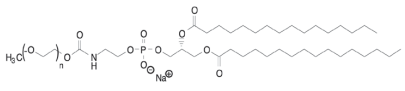
DPPA has a molecular weight of 670, empirical formula of $C_{35}H_{68}O_8PNa$, and following structural formula:



DPPC has a molecular weight of 734, empirical formula of $C_{40}H_{80}NO_8P$, and following structural formula:



MPEG5000 DPPE has an approximate molecular weight of 5750 represented by empirical formula $C_{265}H_{527}NO_{123}PNa$, and the following structural formula:



Prior to Vialmix® activation, the DEFINITY® vial contains 6.52 mg/mL octafluoropropane in the headspace. Each mL of the clear liquid contains 0.75 mg lipid blend (consisting of 0.045 mg DPPA, 0.401 mg DPPC, and 0.304 mg MPEG5000 DPPE), 103.5 mg propylene glycol, 126.2 mg glycerin, 2.34 mg sodium phosphate monobasic monohydrate, 2.16 mg sodium phosphate dibasic heptahydrate, and 4.87 mg sodium chloride in Water for Injection. The pH is 6.2-6.8.

After activating the contents of the vial in a Vialmix®, each mL of the milky white suspension contains a maximum of 1.2 X 10^{10} perflutren lipid microspheres, and about 150 µL/mL (1.1 mg/mL) octafluoropropane. The microsphere particle size parameters are listed in Table 1 below:

Table 1: Microsphere Size Distribution

	Microsphere particle size parameters
Mean diameter range	1.1 µm – 3.3 µm
Percent less than 10 µm	98%
Maximum diameter	20 µm

See DEFINITY® Activation, Preparation, and Handling Instructions.

CLINICAL PHARMACOLOGY

PHARMACODYNAMICS

After activation of DEFINITY® and intravenous injection, the physical acoustic properties of activated DEFINITY® (see DESCRIPTION) provide contrast enhancement of the endocardial borders during echocardiography. The perflutren lipid microspheres exhibit lower acoustic impedance than blood and enhance the intrinsic backscatter of blood.

In animal models the acoustic properties of activated DEFINITY® were established at or below a mechanical index of 0.7 (1.8 MHz frequency). In clinical trials, the majority of the patients were imaged at or below a mechanical index of 0.8.

In a crossover trial of 64 patients randomized to both bolus and infusion, the duration of clinically useful contrast enhancement for fundamental imaging was approximately 3.4 minutes after a 10 µL/kg bolus and was approximately 7.1 minutes during the continuous infusion of 1.3 mL activated DEFINITY® in 50 mL saline at a rate of 4 mL/min.

PHARMACOKINETICS

Human pharmacokinetics information is not available for the intact or degassed lipid microspheres. The pharmacokinetics of octafluoropropane gas (OFP) were evaluated in healthy subjects (n=8) after the IV administration of activated DEFINITY® at a 50 µL/kg dose.

Octafluoropropane (OFP) Protein Binding

OFP gas binding to plasma proteins or partitioning into blood cells has not been studied. However, OFP protein binding is expected to be minimal due to its low partition coefficient into whole blood.

Metabolism

OFP is a stable gas that is not metabolized. The phospholipid components of the microspheres are thought to be metabolized to free fatty acids.

Elimination

OFP was not detectable after 10 minutes in most subjects either in the blood or in expired air. OFP concentrations in blood were shown to decline in a mono-exponential fashion with a mean half-life of 1.3 minutes in healthy subjects.

SPECIAL POPULATIONS

The pharmacokinetics of octafluoropropane gas (OFP) were evaluated in subjects (n=11) with chronic obstructive pulmonary disease (COPD). The mean half-life of OFP in blood was 1.9 minutes. The total lung clearance of OFP was similar to that in healthy subjects.

Microspheres may obstruct the vasculature of some patients. See WARNINGS for use in subjects with cardiac shunts and pulmonary hypertension.

The pharmacokinetics of activated DEFINITY® has not been studied in subjects with hepatic diseases or congestive heart failure.

Gender:

The effects of activated DEFINITY® appeared to be similar in men and women.

Age/Race:

The effects of age and race on the pharmacokinetics of activated DEFINITY® have not been studied.

Pediatrics:

The pharmacokinetics of activated DEFINITY® in pediatric subjects has not been studied. The safety of injecting activated DEFINITY® in neonates and infants with immature pulmonary vasculature has not been studied (see WARNINGS).

Elderly:

The pharmacokinetics of activated DEFINITY® in the elderly has not been studied.

DRUG-DRUG INTERACTIONS

Drug-drug interactions for activated DEFINITY® have not been studied.

CLINICAL TRIALS

A total of 249 subjects were evaluated in clinical trials (208 received activated DEFINITY® and 41 placebo). In this group, 154 (61.8%) were male and 95 (38.2%) were female; 183 (73.5%) were White, 38 (15.3%) were Black, 21 (8.4%) were Hispanic, and 7 (2.8%) were classified as other racial or ethnic groups. The mean age was 53.9 years (range 18 to 87).

Activated DEFINITY® was evaluated in four controlled clinical trials: Two open-label baseline controlled, unpaired blinded image evaluation studies and two identical placebo-controlled, unpaired blinded image evaluation studies. Subjects were eligible for these studies if they had two or more (of six) non-evaluable segments in either the apical 2- or 4-chamber view in non-contrast fundamental echocardiography.

In the baseline controlled studies, a total of 126 (67 in study A and 59 in study B) subjects received a bolus dose of 10 µL/kg activated DEFINITY®. The outcome measures in these studies included the blinded assessment of ejection fraction (EF), endocardial border length (EBL) obtained by direct measurement, and qualitative assessment of wall motion.

In the two placebo-controlled studies a total of 123 subjects were randomized in 1:2 ratio to receive two I.V. bolus doses of either saline (placebo) or activated DEFINITY® 10 µL/kg (17 placebo vs. 33 activated DEFINITY® patients and 24 placebo vs. 49 activated DEFINITY® patients, respectively). The outcome measure for assessing the effectiveness of activated DEFINITY® was the blinded assessment of improvement in ventricular chamber enhancement (measured by videodensitometry at end-diastole and end-systole).

Endocardial Border Length: As shown in Table 2, compared to baseline, a single bolus dose of 10 µL/kg activated DEFINITY® increased the length of endocardial border that could be measured at both end-systole and end-diastole. The mean change in border length from baseline at end-diastole was statistically significant for all readers in the apical 4-chamber view and for 3 out of 4 readers for the apical 2-chamber view. The mean change in border length from baseline at end-systole was statistically significant for 3 out of 4 readers for the apical 4-chamber view and for 2 out 4 readers for the apical 2-chamber view.

Ventricular Chamber Enhancement: Left ventricular chamber enhancement after an activated DEFINITY® dose of 10 µL/kg was significantly increased from baseline compared to placebo in both views at the mid-ventricular and apical levels at end-diastole. Similar results were noted at end-systole, with the exception of the 4-chamber view.

Wall Motion: In a retrospective analysis, in a subset of subjects (n=12 to 47, depending on reader) having at least 2 adjacent segments non-evaluable on non-contrast imaging, activated DEFINITY® converted a baseline non-evaluable image to an evaluable image in 58 to 91% of the patients, depending on the reader. In the converted images, the accuracy of wall motion (i.e., normal versus abnormal) improved in 42-71% of the patients, depending on the reader, however, improvement in the specific diagnostic accuracy (e.g., hypokinetic, akinetic etc.) was not established. Also, in 13 to 37% of the patients, depending on the reader, activated DEFINITY® was found to obscure the wall motion rendering the image non-evaluable.

Ejection Fraction: In the 2 baseline controlled studies, ejection fraction results were evaluated in comparison to MRI. The results were evaluated by 3 blinded, independent radiologists. **In these studies, although there was a statistically significant increase in ventricular chamber enhancement, activated DEFINITY® did not significantly improve the assessment of ejection fraction compared to the baseline images.**

Table 2
MEAN (SD) ENDOCARDIAL BORDER LENGTH (CM) BY BOTH APICAL 2- AND 4-CHAMBER VIEWS AT END-SYSTOLE AND END-DIASTOLE BY STUDY, EVALUABLE SUBJECTS

Study/View	Endocardial Border Length – Blinded Read			
	Mean(SD) at End-Diastole		Mean(SD) at End-Systole	
	Reader 1	Reader 2	Reader 1	Reader 2
Study A: (N = 67)				
Apical 2-chamber				
Baseline	8.0(3.4)	4.7(2.8)	7.1(3.3)	4.3(2.6)
Post-DEFINITY®	12.8(5.2)*	5.8(2.6)*	10.6(5.0)*	4.4(2.3)
Apical 4-chamber				
Baseline	8.1(3.3)	4.5(2.6)	7.6(3.2)	4.5(2.7)
Post-DEFINITY®	13.5(5.2)*	6.8(3.3)*	11.5(4.4)*	5.3(3.1)
Study B: (N = 59)				
Apical 2-chamber				
Baseline	4.3(2.6)	7.8(5.3)	4.1(2.4)	6.5(5.1)
Post-DEFINITY®	5.7(4.7)*	8.2(6.5)	5.5(4.4)*	6.9(6.3)
Apical 4-chamber				
Baseline	4.0(2.7)	9.2(5.9)	3.8(2.6)	7.3(5.6)
Post-DEFINITY®	7.1(5.5)*	11.5(7.5)*	5.9(5.3)*	8.7(6.3)*

Activated DEFINITY® Bolus Dose = 10 µL/kg
*Significant change from baseline (paired t-test, $p<0.05$)

In an open administration, crossover trial, 64 patients were randomized to receive both bolus (10 µL/kg) and infusion (1.3 mL activated DEFINITY® in 50 mL saline at the rate of 4 mL/min) dosing of activated DEFINITY®. Outcome measures for this study included clinically useful ventricular cavity enhancement and endocardial border length. Similar results were seen as described above.

Optimal activated DEFINITY® doses and device settings for harmonic imaging have not been established.

INDICATIONS AND USAGE

Activated DEFINITY® (Perflutren Lipid Microsphere) Injectable Suspension is indicated for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border.

The safety and efficacy of DEFINITY® with exercise stress or pharmacologic stress testing have not been established.

CONTRAINDICATIONS

Do not administer DEFINITY® to patients with known or suspected:

- Right-to-left, bi-directional, or transient right-to-left cardiac shunts,
- Hypersensitivity to perflutren (see WARNINGS).

Do not administer DEFINITY® by intra-arterial injection.

WARNINGS

Serious Cardiopulmonary Reactions:
Serious cardiopulmonary reactions, including fatalities, have occurred during or following perflutren-containing microsphere administration. The risk for these reactions may be increased among patients with pulmonary hypertension or unstable cardiopulmonary conditions (acute myocardial infarction, acute coronary artery syndromes, worsening or unstable congestive heart failure, serious ventricular arrhythmias or respiratory failure, including patients receiving mechanical ventilation). In these patients, monitor vital signs, electrocardiography, and cutaneous oxygen saturation during and for at least 30 minutes after DEFINITY® administration. In the absence of these underlying conditions, observe patients closely during and following DEFINITY® administration.

In postmarketing use, uncommon but serious reactions observed during or shortly following perflutren-containing microsphere administration included fatal cardiac or respiratory arrest, loss of consciousness, convulsions, symptomatic arrhythmias (atrial fibrillation, supraventricular tachycardia, ventricular tachycardia or fibrillation), hypotension, respiratory distress or cardiac ischemia (see ADVERSE REACTIONS).

Always have cardiopulmonary resuscitation personnel and equipment readily available prior to DEFINITY® administration and monitor all patients for acute reactions.

Anaphylactoid Reactions:
Post-marketing reports of acute anaphylactoid reactions including shock, bronchospasm, upper airway swelling, loss of consciousness, urticaria and pruritus, have occurred in patients with no prior exposure to perflutren-containing microsphere products. Monitor all patients for signs and symptoms of anaphylactoid reactions (see ADVERSE REACTIONS).

Systemic Embolization of DEFINITY® in Patients with Cardiac Shunts:
In patients with right-to-left, bi-directional, or transient right-to-left cardiac shunts phospholipid-encapsulated microspheres can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation resulting in microvascular occlusion and ischemia. In an animal study utilizing intra-arterial administration of activated DEFINITY®, microsphere trapping was seen in small arterioles <15 µm, especially at branch points and in capillaries at all doses tested, including doses directly applicable to those used in humans. An animal study utilizing intravenous administration did not result in arterial microvascular obstruction presumably because of filtering by the lungs. Do not administer DEFINITY® by intra-arterial injection (see CONTRAINDICATIONS).

High Ultrasound Mechanical Index:
High ultrasound mechanical index values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. Additionally, end-systolic triggering with high mechanical indices has been reported to cause ventricular arrhythmias. The safety of activated DEFINITY® at mechanical indices greater than 0.8 has not been evaluated. The safety of activated DEFINITY® with the use of end-systolic triggering has not been evaluated.

QTc Prolongation:
ECG parameters for doses up to 10 µL/kg were monitored in 221 subjects at multiple time points from 1 hour to 72 hours after the first bolus injection. In the 221 subjects, QTc prolongations of >30 msec were noted in 64 (29%) subjects. Forty-six out of 64 subjects with QTc prolongations were further evaluated and 39% (18/46) showed associated cardiac rhythm changes. The effects of concomitant drugs were not studied.

PRECAUTIONS

Information For Patients

Patients receiving activated DEFINITY® should be instructed to inform their healthcare provider if they:

1. have a congenital heart defect, or recent worsening of heart or lung conditions,
2. have had prior reactions to DEFINITY® (see CONTRAINDICATIONS and WARNINGS),
3. may be pregnant, are trying to become pregnant, or are nursing.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies with activated DEFINITY® have not been performed to evaluate carcinogenic potential. Evidence of genotoxicity was not found in the following studies with activated DEFINITY®:
1) bacterial mutagenesis assay (Ames assay), 2) *in vitro* mammalian mutagenesis assay, 3) *in vitro* human lymphocyte chromosome aberration assay, and 4) *in vivo* rat micronucleus assay.

Impairment of male or female fertility was not observed in rats and rabbits treated with activated DEFINITY® at up to 1 mL/kg (24x and 15x maximal human dose based on body surface area, respectively).

Pregnancy Category B

Reproduction toxicity studies have been performed in rats and rabbits at up to 3 mL/kg and, 1 mL/kg (24x and 15x maximal human dose based on body surface area for rats and rabbits, respectively). The studies revealed no evidence of an effect of activated DEFINITY® treatment on the developing fetus. Adequate and well-controlled studies in pregnant women have not been conducted. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers

Studies to detect if activated DEFINITY® is excreted in human milk have not been conducted. Because many drugs are excreted in human milk, caution should be exercised when activated DEFINITY® is administered to a nursing woman.

Pediatric Use

The safety and effectiveness of activated DEFINITY® have not been established in the pediatric population (see WARNINGS).

ADVERSE REACTIONS

Clinical Trials Experience

A total of 1716 subjects were evaluated in pre-market clinical trials of activated DEFINITY®. In this group, 1063 (61.9%) were male and 653 (38.1%) were female, 1328 (77.4%) were White, 258 (15.0%) were Black, 74 (4.3%) were Hispanic, and 56 (3.3%) were classified as other racial or ethnic groups. The mean age was 56.1 years (range 18 to 93). Of these, 144 (8.4%) had at least one treatment-related adverse reaction (Table 3). There were 26 serious adverse events and 15 (0.9%) subjects discontinued because of an adverse event.

Deaths and serious adverse events: Among the 1716 activated DEFINITY® patients, 19 (1.1%) suffered serious cardiopulmonary adverse events including eight deaths. The deaths occurred several days after activated DEFINITY® administration and appear to be related to the course of underlying disease. Of the 11 other serious adverse events, which appeared within days of the drug administration (2-15 days), all appeared to be a progression of underlying cardiac and non-cardiac disease. However, a role for DEFINITY® in the initiation or course of these adverse events can not be ruled out.

Discontinuations: There were 15 discontinuations reported with a mean age of 41.5 years. Nine of these patients were discontinued after the first injection. One patient experienced a hypersensitivity reaction with urticaria and pruritus and all the other patients experienced dizziness, chest pain, dyspnea or back pain. Adverse events appeared within minutes (1 – 15 min) of the drug administration and were of moderate intensity resolving usually without treatment within minutes or hours after onset.

For all adverse events, the overall incidence of adverse experiences was similar for the <65 year age group and the ≥65 year age group, similar in males and in females, similar among all racial or ethnic groups and similar for bolus and infusion dosing. As shown in Table 3, the most common adverse events were reported in the Central and peripheral nervous system (3.1%), Body as a Whole (2.4%) and Gastrointestinal system (1.8%). The most common events were headache (2.3%), back and renal pain (1.2%), flushing (1.1%) and nausea (1.0%).

Table 3
Treatment-Related, New-Onset Adverse Experiences Occurring in ≥0.5% of All Activated DEFINITY®-Treated Subjects

	All activated DEFINITY® (N=1716)	
Total Number of Treatment-Related A.E.'s	269	(8.4%)
Total Number of Subjects with a Treatment-Related A.E.	144	
WHOART body system WHOART preferred term	n	(%)
Application Site Disorders	11	(0.6)
Injection Site Reactions	11	(0.6)
Body as a Whole	41	(2.4)
Back/renal pain	20	(1.2)
Chest pain	13	(0.8)
Central and peripheral nervous system disorder	54	(3.1)
Headache	40	(2.3)
Dizziness	11	(0.6)
Gastrointestinal system	31	(1.8)
Nausea	17	(1.0)
Vascular (extracardiac) disorders	19	(1.1)
Flushing	19	(1.1)

N=Sample size 1716 subjects who received activated DEFINITY®
A.E.=Adverse Experience
n=Number of subjects reporting at least one A.E.

Other treatment-related adverse experiences that occurred in <0.5% of the activated DEFINITY®-dosed subjects were:

Body as a Whole: Fatigue, fever, hot flushes, pain, rigors, and syncope
Cardiovascular: Abnormal ECGs, bradycardia, tachycardia, palpitation, hypertension and hypotension
Digestive: Dyspepsia, dry mouth, tongue disorder, toothache, abdominal pain, diarrhea and vomiting
Hematology: Granulocytosis, leukocytosis, leukopenia, monocytosis and eosinophilia
Musculoskeletal: Arthralgia
Nervous System: Leg cramps, hypertonia, vertigo and paresthesia
Platelet, Bleeding, and Clotting: Hematoma
Respiratory: Coughing, hypoxia, pharyngitis, rhinitis and dyspnea
Special Senses: Decreased hearing, conjunctivitis, abnormal vision and taste perversion
Skin: Pruritus, rash, erythematous rash, urticaria, increased sweating, and dry skin
Urinary: Albuminuria and abnormal urine
Miscellaneous: Lymphadenopathy

Post-Marketing Experience

The following adverse reactions have been identified during the post-marketing use of perflutren-containing microsphere products. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Fatal cardiac arrests and other serious but non-fatal adverse reactions were uncommonly reported. Most of these uncommon reactions included cardiopulmonary symptoms and signs such as cardiac or respiratory arrest, hypotension, supraventricular and ventricular arrhythmias, respiratory distress or decreased oxygenation. Reports also identified neurologic reactions (loss of consciousness or convulsions) as well as anaphylactoid reactions (see WARNINGS).

OVERDOSAGE

The clinical consequences of overdosing with activated DEFINITY® are not known. Treatment of an overdose should be directed toward the support of all vital functions and prompt institution of symptomatic therapy (see CONTRAINDICATIONS, WARNINGS, and PRECAUTIONS).

DOSAGE AND ADMINISTRATION

DEFINITY® IS INTENDED FOR ADMINISTRATION ONLY AFTER ACTIVATION IN THE VIALMIX® APPARATUS. Before injection, this product must be activated and prepared according to the instructions outlined below. The Vialmix® apparatus should be ordered from Lantheus Medical Imaging, Inc., 331 Treble Cove Road, North Billerica, MA 01862. For customer orders call 1-800-299-3431.

DEFINITY® may be injected by either an intravenous bolus or infusion.

Bolus: The recommended dose for activated DEFINITY® is 10 microliters (µL)/kg of the activated product by intravenous bolus injection within 30-60 seconds, followed by a 10 mL saline flush. If necessary, a second 10 microliters (µL)/kg dose followed by a second 10 mL saline flush may be administered 30 minutes after the first injection to prolong contrast enhancement.

Infusion: The recommended dose for activated DEFINITY® is via an IV infusion of 1.3 mL added to 50 mL of preservative-free saline. The rate of infusion should be initiated at 4.0 mL/minute, but titrated as necessary to achieve optimal image enhancement, not to exceed 10 mL/minute.

The maximum dose is either two bolus doses or one single intravenous infusion. The safety of bolus and infusion dosing in combination or in sequence, has not been studied.

Imaging: After baseline non-contrast echocardiography is completed, the mechanical index for the ultrasound device should be set at 0.8 or below (see WARNINGS). Then inject activated DEFINITY® (as described above) and begin ultrasound imaging immediately. The activated DEFINITY® echocardiogram images should be evaluated in combination with the non-contrast echocardiogram images.

DEFINITY® ACTIVATION, PREPARATION AND HANDLING INSTRUCTIONS:

1. Allow the vial to warm to room temperature before starting the activation procedure.
2. Activate DEFINITY® by shaking the vial for 45 seconds using a Vialmix®.

   Note: illustrations of this procedure are contained in the Vialmix® Users Guide.

   WARNING: DO NOT USE THIS DRUG UNLESS IT HAS COMPLETED A FULL 45 SECOND ACTIVATION CYCLE IN THE VIALMIX®. DEFINITY® WILL NOT BE PROPERLY ACTIVATED UNLESS THE FULL 45 SECOND ACTIVATION CYCLE IS COMPLETED. DO NOT REACTIVATE the vial if Vialmix® did not complete a full 45 second cycle. DO NOT REACTIVATE a successfully activated DEFINITY® vial (see step 3). DO NOT USE a Vialmix® that is not functioning properly. Refer to the "VIALMIX® User's Guide" for the "VIALMIX® CALIBRATION AND REPLACEMENT PROCEDURES" to ensure that a properly functioning Vialmix® is used.
3. Immediately after activation in the Vialmix®, activated DEFINITY® appears as a milky white suspension and may be used immediately after activation. If the product is not used within 5 minutes of Vialmix® activation, the microspheres should be resuspended by 10 seconds of hand agitation by inverting the vial before the product is withdrawn in a syringe. The activated DEFINITY® may be used for up to 12 hours from the time of Vialmix®, but only after the microspheres are resuspended by hand agitation. Store the activated DEFINITY® at room temperature in the original product vial.
4. Invert the vial and withdraw the activated milky white suspension using the Intellipin™ (Dispensing Pin) or 18 to 20 gauge syringe needle. Withdraw the material from the middle of the liquid in the inverted vial. DO NOT INJECT AIR INTO THE DEFINITY® VIAL.
5. Use the product immediately after its withdrawal from the vial; do not allow the product to stand in the syringe.

FOR SINGLE USE ONLY: DEFINITY® does not contain bacterial preservative. Bacterial contamination with the risk of post-administration septicemia can occur following the puncture of the elastomeric septum. It is essential to follow directions for activation of DEFINITY® carefully and to adhere to strict aseptic procedures during preparation.

HOW SUPPLIED

DEFINITY® is supplied as a single use 2 mL clear glass vial containing clear liquid. Each package (clear plastic clamshell) contains four (4) single-use vials.

STORAGE

Store between 2-8°C (36°-46°F) in a refrigerator.

CAUTION: Federal law prohibits dispensing without prescription.

Distributed By

Lantheus Medical Imaging
331 Treble Cove Road
N. Billerica, Massachusetts 01862 USA

For ordering, tel. toll free: 800-299-3431
All Other Business: 800-362-2668
(For Massachusetts and International, call 978-667-9531)

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