SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures which have been finalised before 1 September 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Optison is a diagnostic agent for the enhancement of contrast effect in echocardiography investigations. The active substance is Perflutren (OFP or perfluoropropane) in the form of microspheres stabilised by a thin shell of heat-modified human albumin, suspended in 1% human albumin solution. The microspheres have a mean diameter of 2-4.5 μm and there are approximately 5-8 $x10^8$ microspheres/ml. However, in the context of use it may be more correct to refer to a contrast enhancing system, rather than a substance, since the diagnostic efficiency is related to a number of physical parameters of the system as a whole – number of microspheres/ml, their size, the properties of the Perflutren/albumin interface, etc.

Optison is a development arising from a marketed product based on air as an echogenic contrast-enhancing medium, i.e. air-filled microspheres of heat-modified albumin with diameters from 3.0 to 5.0 μm suspended in a solution of 5% human albumin. The thin shell of heat modified albumin prevents the microspheres from coalescing. However, in the case of air, the shell is permeable to the diffusion of air out of the core and into the surrounding solution whenever the surrounding fluid is not saturated with dissolved air. Because venous blood is not saturated with air, air quickly diffuses out of the microspheres, resulting in a substantial shrinking. The size reduction compromises the echogenicity of the microspheres, and their efficiency and persistence as an ultrasound contrast agent decreases accordingly. In the case of Optison, OFP has such a low aqueous solubility that the driving force for gas exchange into the surrounding blood is reduced. Replacement of air with OFP therefore improves the physical stability of the product, increasing the effective life of the microspheres and increasing the duration of contrast effect from less than 1 minute to more than 4 minutes. This represents a clinically significant advantage.

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

The main ingredients are:

1. Perflutren.
2. Human albumin solution 5% (w/v), PhEur
3. Sodium chloride injection 0.9% (w/v) BP

Other specific ingredients (included in the above) are:

N-acetyltryptophan, Caprylic acid, Sodium hydroxide and Water for Injections.

Container

Optison is filled into 3 ml clear, colourless vials (Ph. Eur. type I borosilicate glass with a 13 mm finish). The vials are closed with Ph. Eur. Type 1 13 mm bromobutyl stoppers and are sealed with aluminium closures.

Development pharmaceutics

Optison is an improved ultrasound contrast agent developed from a marketed product containing air encapsulated as microspheres suspended in a 5% albumin solution. The main difference between the products is that the external albumin shell of Optison encapsulates a core of Perflutren instead of a core of air. The change from air to Perflutren increases the life-time (and hence duration of contrast performance) of the echogenic microspheres *in vivo* because of the lower solubility of Perflutren in human blood (reduced gas exchange) as compared to air.

Development studies focussed on critical aspects of the formulation, e.g. physico-chemical characteristics of the Perflutren-containing microspheres, and the results justified the choice of the final formulation.

Manufacturing process

The manufacturing process is complex. A satisfactory detailed description has been provided.

Process validation

The manufacturing process is performed according to the EEC Guide to Good Manufacturing Practice for Sterile Medical Products. The validation of steam sterilisation (used for the equipment) and dry heat sterilisation (used for the equipment, vials, stoppers) are performed according to Ph Eur. The aseptic processing is mandatory for the sterility assurance of the product, as it can not be sterilised in its final container. The reproducibility of the process has been shown for five consecutive commercial-scale batches. The batch results complied with the specifications for the finished product.

Active substance

In the interests of simplicity, the active substance may be said to be Perflutren (perfluoropropane, OFP); it is not the subject of a pharmacopoeial monograph. However, in the context of use proposed for the product, it may be more correct to refer to the activity of a *contrast-enhancing system*, rather than the activity of a single defined chemical substance, since echogenic contrast enhancement is related to a number of physical parameters of the system as a whole – number of microspheres/ml, their size, the properties of the Perflutren/albumin interface, etc. Similarly, for simplicity, the concentration of the active substance may be defined as 0.22 mg Perflutren gas per ml of product, although it may be more relevant to define the concentration in terms of the number and size of microspheres per ml of product.

Active Substance Specification (OFP)

Information on the manufacture and control of OFP has been provided in a DMF.

The identity, assay and purity of OFP are determined using the same extensively-validated gas chromatographic method. The sample is detected first with a thermal conductivity detector (TCD), and then with a flame ionisation detector (FID), which detects and quantifies the impurities hexafluoropropylene and 2H-heptafluoropropane.

The suitability of the specification has been justified and confirmed by the provision of batch analytical records. Batches were tested for identity, assay and impurities. The presented data confirm the capability to produce a consistent quality at the proposed site.

Other ingredients

Albumin

This is an important ingredient in the formulation and has been subjected to an intensive evaluation, as for OFP. The albumin used is of PhEur quality.

Product specification

Satisfactory specifications have been proposed at release and shelf-life, which should ensure the consistent and reproducible clinical performance of the product.

Batch analysis results included data derived from several batches, used for process validation and stability studies. The presented data meet the proposed product specifications, and show acceptable batch to batch uniformity.

Stability of the active substance

Perflutren is an inert and very stable substance. Satisfactory stability has been shown by means of experimental investigations and bibliographic references.

Stability of the finished product

Data from two separate stability studies were presented. All batches were manufactured by the procedure proposed for the commercial product and all stability studies were conducted in compliance with ICH guidelines on stability testing. On the basis of these results in total, a shelf life of 24 months

when stored at 2-8 °C is justified, and also at room temperature for a limited period of time, and this information appears in the SPC.

3. Part III: Toxico-pharmacological aspects

In summary, the preclinical documentation was well organised and is adequate for the proposed indication.

Pharmacodynamics

Pharmacodynamic effects relating to proposed indication

Left ventricular opacification (LVO) and endocardial border delineation (EBD) were studied in six anaesthetised mongrel dogs injected i.v. sequentially with either an air-based contrast agent (0.35 to 0.6 ml/kg) or Optison (0.005 to 0.04 ml/kg). Results indicated that intravenous administration of Optison in dogs provided opacification of cardiac chambers and improved the delineation of endocardial borders at a dose 70 times lower than the air-based product. Left ventricular peak intensity appeared to be similar between the air-based product and Optison, however, Optison showed a longer image duration and a greater extent of left ventricular filling.

General pharmacodynamics

Cardiovascular and other physiological parameters were studied in three groups of anaesthetised mongrel dogs treated with 0.25 ml/kg of the 1% human albumin vehicle, 0.25 ml/kg of Optison or dipyridamole (a vasodilator used clinically for myocardial viability evaluation) followed by 0.25 ml/kg Optison.

Generally, the majority of haemodynamic parameters, which were evaluated, increased throughout the monitoring period in animals treated with the albumin vehicle or Optison. This effect was considered to be attributable to the procedure. An initial decrease in the majority of parameters with an apparent rebound increase in pressure parameters was demonstrated in animals pre-treated with dipyridamole. The decreases in pressures were considered to be a delayed effect of the dipyridamole and not due to the administration of Optison. According to the Study Director, there were no consistent changes in the electrocardiograms (electrocardiograms were not included in the report). Clinical pathology, blood gas evaluation and gross necropsy did not reveal any treatment-related changes.

Pharmacokinetics

The pharmacokinetic studies were designed to address the disposition and elimination of the two major components of Optison microspheres, i.e. the human albumin shell and the perfluoropropane gas.

Distribution and elimination of ^{125}I-labelled Optison

Clearance and tissue distribution for ^{125}I-Optison following a single iv bolus dose of 0.25 ml/kg were studied in male Sprague-Dawley rats. Animals selected for the 24-hour sacrifice were placed in metabolism cages. Samples of blood, urine, faeces and selected tissues were collected at pre-determined intervals during the 24 hour observation period. The tissues collected were; heart, kidneys, liver, lungs, thyroid, urinary bladder, bone marrow, brain, and spleen. Prior to tissue collections, animals were perfused with heparinised saline.

Plasma radioactivity levels rapidly declined within the first five minutes following administration of ^{125}I-Optison. Thereafter, plasma ^{125}I levels increased with C_{max} reached at 90 minutes (6.9% of total dose activity) post-treatment, and then the elimination of radioactivity became a dominating process.

The results suggest that most of the intact ^{125}I-Optison microspheres were cleared from the systemic circulation during the first few minutes following treatment. It is likely that only the plasma ^{125}I levels measured at one minute and five minutes post-treatment reflect the plasma kinetics of intact microspheres. The plasma data collected after five minutes post-treatment, and all of the tissue distribution data probably represented radiolabelled albumin fragments and free ^{125}I, and not intact ^{125}I-Optison microspheres. Since the ^{125}I activity recovered in the liver was the highest of all the tissues at all timepoints, it is likely that the liver is the major organ of metabolism and elimination of the radiolabelled albumin shell and its fragments.

Elimination of perfluoropropane gas

The elimination of perfluoropropane (OFP) gas in exhaled air and blood was studied in two pilot studies in rats and more thoroughly investigated in one study in dogs. The conclusion of these elimination studies was that OFP was rapidly cleared through the exhaled air in the anaesthetised dog as shown by an approx. 40 seconds mean residence time. At a comparable dose (1 ml/kg), OFP was still detectable in air samples in several rats 15 min after administration, indicating that the elimination in exhaled air is slower in rats. However, a direct comparison is not possible due to different experimental models.

Toxicology

Single dose toxicity

The safety of Optison following a single administration was evaluated in rats, dogs and Rhesus monkeys. It can be concluded that a single iv injection of Optison 20 ml/kg (x400 the clinical dose) was tolerated in these animals.

Repeated dose toxicity

The results of studies in rats and dogs indicated that intravenous administration of Optison up to 10ml/kg/day for four weeks did not cause treatment-related adverse effects. Pulmonary lesions observed at dosages of 10 & 20 ml/kg in a three-week study in rats were probably related to the high infusion rate of a highly concentrated suspension of microspheres (due to floating in the syringe).

Reproduction studies

No embryonic or teratogenic effects were observed in rats at dosages causing significant maternal toxicity including deaths. In the rabbit study, maternal toxicity increased abortions and premature deliveries, increased embryofoetal deaths, reduced foetal body weight and increased number of foetuses with dilated ventricles in the brain were observed at dosages of 2.5ml/kg. The mechanism for the increased incidence of dilated ventricles in the brain of developing rabbit embryos has to be considered unknown. Since the NOAEL is only x5 the clinical dosage information on this variation is included in the SPC under Sections 4.6 and 5.3.

Genotoxic potential

Optison did not show any genotoxic potential.

Oncogenic/carcinogenic potential

Not applicable

Local tolerance

No signs of toxicity or irritation, which could be attributable to Optison, were detected in rabbit studies.

Special toxicity studies

No statistical differences in red blood cells haemolysis parameters between Optison-treated rabbits and controls were observed, as well as in *in vitro* compatibility studies using human whole blood.

Ecotoxicity/environmental risk assessment

It was concluded that the manufacture and clinical use of Optison will not cause toxic effects to the environment or alter the physical environment.

GLP status

All toxicity studies (dose range finding studies not included) and the distribution study of ^{125}I-labelled Optison were in compliance with GLP. The general pharmacology study in dogs was not strictly in accordance with GLP, however, the quality of this study was considered to be otherwise acceptable.

Conclusions on the preclinical dossier

Pharmacodynamic studies in dogs have demonstrated opacification of cardiac chambers and improved delineation of endocardial borders. Optison represents improvement in the ultrasound imaging characteristics over air-based products.

Significant findings in the toxicity studies could be attributed to the high sequestration of intact Optison microspheres and/or human albumin fragments in tissues or repeated administration of high volumes of human albumin.

The main preclinical safety problem arising from initial assessment of the preclinical dossier were potential aggregation of microspheres and/or albumin fragments which may contribute to pulmonary congestion and the lack of safety evaluation of OFP gas. It was felt that the risk for potential blockage of pulmonary capillaries in humans should be further evaluated, also taking into account the unintentional administration of a highly concentrated Optison solution (e.g. due to floating or separation in the syringe). In response to this question, data on potential *in vitro* aggregation of Optison microspheres in human blood as well as of stabilised air microspheres was provided, measured before and after ultrasound exposure at several appropriate time points. These data were evaluated and on further analysis the risk of aggregation was considered to be acceptably small, considering the use of the product in accordance with the restrictions imposed in the SPC.

Concerning the OFP gas, the safety of intraocular use (i.e. 0.3ml) in the treatment of retinal detachment in humans has been demonstrated. The OFP gas is considered essentially chemically unreactive, thermally stable and resistant to acid and base hydrolysis. It has an extremely low solubility and it is rapidly and completely eliminated from the lungs. Preclinical studies have not identified any concerns.

4. Part IV: Clinical aspects

Contrast media for various diagnostic ultrasound investigations are thought to help in delineation of cardiac cavities by better definition of the endocardium-blood border and by amplification of Doppler signals. A common feature of these products is the presence of gaseous microbubbles (usually air) with some kind of 'shell' surrounding the gas. These products have been approved for various diagnostic procedures involving the heart, the vessels and the Fallopian tubes. The second generation of contrast media makes use of chemically inert gases instead of air. Optison, when used in conjunction with diagnostic ultrasound, provides opacification of cardiac chambers, improvement in the delineation of cardiac borders and visualisation of wall motion and blood flow within the heart.

Pharmacodynamics

Study FS-1000

The pharmacodynamic action of i.v. injections of Optison have been studied (study FS-1000) in forty healthy volunteers. Subjects randomly received two different injection volumes of Optison as they were assigned to one of four dose groups: Dose Group A (0.5 and 5.0 ml), Dose Group B (1.0 and 10.0 ml), Dose Group C (2.0 and 20.0 ml) and Dose Group D (4.0 and 40.0 ml). Preliminary efficacy of left ventricular opacification and endocardial border delineation were assessed

For left ventricular endocardial delineation, results demonstrated that even the lowest dose of Optison (0.5 ml) was adequate and there was little further improvement at higher doses except that more views could be obtained and contrast effect was longer, exceeding 12 minutes for the highest dose with 4 minutes for the lowest doses. Further, myocardial perfusion in a single view was found at doses as low as 0.5 ml in normal subjects. No statistically significant changes from baseline were observed for vital signs, electrocardiographic measures, pulmonary spirometry or 30 of the 40 monitored clinical laboratory variables after injection of all experimental doses of Optison. There were very few changes in physical or neurological examinations and no statistically significant changes were noted. No immunological response to the administration of Optison was indicated for any of the five immunoglobulins studied in the 16 subjects evaluated. Adverse events were dose-related, being most frequent in the highest dose group (see also the Safety section of this report).

Given the lack of additional efficacy in the high dose groups, it was determined that subsequent clinical trials in patients would be undertaken using a lower dose range.

Pharmacokinetics

The Perflutren component of Optison is rapidly and almost completely eliminated from the body via the lungs in less than 10 minutes, with a pulmonary elimination half-life of 1.3 ± 0.7 minutes. The disposition and elimination of the albumin component of the product has not been studies in humans. Information obtained from a preclinical study with ^{125}I-labelled albumin microspheres indicated that microspheres were rapidly cleared from the circulation and radio-labelled microspheres, albumin shells and ^{125}I was taken up primarily in the liver. The primary route of elimination for radioactivity was the urine. High levels of radioactivity were also retained in lungs for a considerable time, approx. 10% of the total dose 40 minutes after dose administration (compared with 35% in the liver), which may indicate aggregation of albumin shells and/or intact ^{125}I-albumin microspheres.

Clinical diagnostic experience

The clinical program to address the safety and efficacy of Optison was carried out mainly in two phase III studies (FS3000 and FS3500), where Optison was evaluated in comparison with an EU-authorised product containing stabilised microbubbles of air – hereafter referred to as the reference product or air-based product. Bubbles from both agents are theoretically small enough to pass the pulmonary capillary bed in significant amounts to outline the left cardiac cavities. The air-based product has been shown not to alter hemodynamics, coronary blood flow or left ventricular contractility. The more pronounced efficacy of Optison is attributed to the presence of perfluoropropane instead of air. Perfluoropropane is a biologically inert gas and is presently approved in the U.S. as an intraocular device for the treatment of retinal detachment.

Compliance with GCP

All studies regarding Optison presented in the marketing authorisation application (MAA) complied with current European GCP requirements.

Clinical development

The low number (203) of patients in the main phase III studies (FS3000 & FS3500) was justified by the clear diagnostic efficacy of the product. Additional safety studies were also conducted. These studies include two phase I or phaseI/II studies on immunology (FS-1000, FS-6000) with one phase I study on the subject of rechallenge (FS-1250), one phase I study on pharmacokinetics/mass balance (FS-1500), two phase II/phase I studies on safety in specific patient groups with a comparison with the control agent 1% human albumin (FS-6000), pulmonary effects (spirometry, FS-1000; oxygen saturation, all six studies). The six studies mainly oriented towards safety and efficacy of Optison enrolled a total of 308 subjects of whom 279 received Optison.

Two more phase I/II studies were undertaken (FS 2000, FS 5000).

FS 2000 comprised of a clinical evaluation of the safety and efficacy of i.v. Optison for myocardial, kidney and liver perfusion. FS 5000 was done to evaluate safety and efficacy of i.v. Optison for myocardial contrast Echocardiography versus Nuclear imaging. The two last mentioned studies included a total of 59 subjects, all of whom received Optison

An overview of the clinical studies is presented in the table below:

Protocol Number	Dose Range	Type of Study	Number of Patients in Study	Number of Patients Receiving OPTISON
FS-1000	single: 0.5 - 40.0 ml cumulative: 5.5 - 44 ml	Safety, Dose-Ranging, Immunology	40	40
FS-1500	20.0 ml	Mass Balance, Safety	10	10
FS-6000	20.0 ml	Immunology, Safety	50	25
FS-1250	20.0 ml	Immunology, Rechallenge, Safety	5	5*
FS-3000	single: 0.2 - 5.0 ml cumulative: 8.7 ml	Safety, Efficacy for EBD, LVO, Doppler	101	97
FS-3500	single: 0.2 - 5.0 ml cumulative: 8.7 ml	Safety, Efficacy for EBD, LVO Doppler	102	102
FS-5000	single: 2.5 ml cumulative: 30.0 ml	Safety, efficacy for MCE during stress imaging	34	34
FS-2000	heart - 10.0 & 5.0 ml kidney: 15.0 & 5.0 ml liver: 20.0 & 10.0 ml	Safety, efficacy for organ perfusion	25	25
Total Patients			367	338

EBD = Endocardial Border Delineation
LVO = Left Ventricular Opacification
MCE = Myocardial Contrast Echocardiography
*One subject in the FS-1250 study received the 0.2 ml intradermal dose of Optison but not the 20.0 ml intravenous dose.

Diagnostic efficacy

FS-3000 and FS-3500 (Trials performed in the US)

The two open-label, randomised, comparative phase III trials (FS-3000 and FS-3500) were conducted in the USA at fourteen centres recruiting 203 patients, which included a subgroup of 79 patients with chronic pulmonary disease and/or moderately severe cardiac disease. Each patient received intravenous reference product and Optison on separate days, with a minimum of 48 hours to a maximum of 10 days required between the two injection series. Patients were scheduled to receive four intravenous Optison doses: 0.2, 0.5, 3.0 and 5.0 ml; the maximum cumulative Optison volume a patient received was 8.7 ml from the four injections. The maximum cumulative volume of reference product a patient received was 0.30 ml/kg. The objectives of these studies were to determine whether i.v. Optison, when compared with the reference product, provide improved image enhancement of the left ventricle and allowed for a more complete examination.

The primary endpoint was related to endocardial border delineation while the secondary endpoints pertained to left ventricular opacification and Doppler signal enhancement. Efficacy endpoints were assessed by a masked reviewer at an independent core laboratory.

Efficacy criteria

Left ventricular endocardial border delineation was assessed via two methods in the FS-3000 and FS-3500 studies. Left ventricular endocardial length was measured in centimetres by the masked reviewer at the core laboratory who was blinded to contrast agent. The entire well visualised non-contrast apical 4-chamber endocardial length was measured and compared with the well visualised endocardial length subsequent to injections of the two echo-contrast agents. This technique is a quantitative means of describing how well a given contrast agent improves the ability of an unbiased reader to clearly visualise the endocardium, giving the possibility for an accurate assessment of regional and global left ventricular function and size.

The other test of endocardial delineation was performed by dividing the apical 4-chamber view into six segments and grading the ability to see the endocardium of that segment before and after the contrast agent according to a semi-quantitative scale.

Left ventricular opacification was evaluated through two methods, measuring peak contrast intensity and visualising peak left ventricular (LV) filling. The former method consisted of measuring the change from baseline in arbitrary gray scale units after each injection.

Visual assessment of peak LV end-systolic and end-diastolic filling was completed using a qualitative scale (i.e., 0 = none/ 0% LV filling, 1 = faint contrast/ 33% LV filling, 2 = intermediate contrast/ 67% LV filling and 3 = full LV chamber opacification / 100 % LV filling). Efficacy was measured for those patients achieving > 2+ LV filling post-injection of the contrast agent. All efficacy assessments were made by a core laboratory.

Enhancement of the spectral Doppler signal was assessed subsequent to each injection of Optison in these studies. Each investigator, as well as the core laboratory, made assessments using the following scale: 1 = optimal, 2 = good, 3=adequate, 4 = poor, 5 = absent, and X = not clinically measurable.

Additionally, wall motion was graded by the core laboratory as another efficacy variable.

The proposed indication for Optison is improved assessment of cardiac function and verification of anatomical structures. Thus, the intent was to show both statistically significant and clinically relevant improvement of echocardiographic imaging with Optison, as compared with another agent, or, as in the case of Doppler, with no contrast agent at all.

The efficacy variables were also used in one preclinical phase 1 study (FS1000) and Optison was found to be efficacious for left ventricular opacification and border delineation.

Results

There were 199 evaluable patients. Left ventricular border length increased from non-contrast to contrast significantly more with Optison doses than with the reference product ($p = 0.0001$, for both FS-3000 and FS-3500). In the FS-3000 study, the increase from non-contrast to contrast following an injection of 3.0 ml of Optison was 7.8 cm at end-diastole, compared with 3.7 cm following 0.22 ml/kg of reference product. Likewise, in the FS-3500 trial, the increases were 7.1 cm and 3.1cm, respectively, for Optison and reference product.

Administration of 3.0 ml of Optison resulted in 96% of the patients having an improvement in one or more border segments (from inadequate to any other grade) compared with 65% improvement for the reference product, at 0.22 ml/kg in the FS-3000 trial. Similarly, improvement for Optison and reference product in the FS-3500 study were 91% and 63%, respectively. When analysing the results from the impaired function subgroups (those patients with pulmonary or cardiac disease in whom the reference product is thought to be less effective), improvements of 100% for Optison and 60% for reference product were noted in the FS-3000 trial, while improvements of 89% for Optison and 67% for the reference were noted in the FS-3500 trial.

Visual assessment differences of left ventricular filling measured at end-diastole were statistically significant. In the FS-3000 study, administration of 3.0 ml Optison resulted in 86% of the patients achieving 100% filling compared to 44% for the reference product at 0.22 ml/kg, while 92% of the patients achieved greater than 67% filling for Optison and 60% achieved a similar age for the reference. In the FS-3500 trial, the of patients achieving 100% and greater than 67% filling were 89% and 97%, respectively, for Optison (3.0 ml) and 29% and 52%, respectively, for the reference product (0.22 ml/kg).

Optison improved Doppler signals of the right and left pulmonary veins. At any given dose in the FS-3000 study, Optison converted 42% to 77% of inadequate non-contrast right pulmonary vein Doppler signals and 76% to 93% of inadequate left pulmonary vein Doppler signals to a clinically useful level. Similarly, in the FS-3500 study, improvements in the right and left pulmonary veins were 47% to 69%, and 74% to 91%, respectively. No substantial improvement in Doppler signal across the aortic and mitral valves was seen since virtually all non-contrast signals were already adequate at baseline.

The percent of patients with improvement in wall motion visibility from non-contrast to contrast increased significantly with Optison compared with the reference product in apical 2- and 4- chamber views (Chi-Square tests, $p<0.001$ for FS-3000 and FS-3500). In the FS-3000 study, rates of improvement for patients were 47% for the reference product at 0.22 ml/kg compared to 84% for Optison at 3.0 ml in the apical 2-chamber view, and 58% for the reference product compared to 82% for Optison in the apical 4-chamber view. Similarly, in the FS-3500 study, the apical 2-chamber views

were improved in 24% and 59% for the reference product and Optison, respectively, while the apical 4-chamber view improvements were 62% and 73% for the respective agents.

The study centre investigators indicated a duration of contrast enhancement of two dimensional images for 3.0 ml of Optison at 4 minutes compared to less than one minute for 0.22 ml/kg of the reference product for patients enrolled in FS-3000. In the FS-3500 clinical trial, duration of contrast enhancement for 3.0 ml of Optison was noted, on average, at 4.6 minutes. The investigators stated that they were able to obtain more echocardiographic views, evaluate wall motion in more patients and were able to obtain a diagnostic study more often with Optison.

After all images were read by the masked reviewer, those patients with at least four of six (>66%) endocardial segments read as "not adequately visualised" at baseline were sub-analysed. The data from these 36 and 49 patients from FS-3000 and FS-3500, respectively, were analysed to determine how many converted to what was defined as a "diagnostic study", i.e., at least five of six (83%) adequately (or better) delineated segments. It was determined that seeing at least 83% of the endocardium adequately would constitute a "diagnostic quality" study. From this group receiving 3.0 ml Optison and 0.22 ml/kg reference product, there was a conversion rate from "non-diagnostic" to "diagnostic quality" of 26 (72%) and 37 (76%) in the FS-3000 and FS-3500, respectively, after Optison compared with only 8 (22%) and 14 (29%) in the FS-3000 and FS-3500, respectively, after the reference product.

FS-2000, FS-5000 (Trials performed in Europe)

Two single centre trials (i.e., one Phase I/II and one Phase II) were conducted in the UK by Molecular Biosystems, Inc. (MBI). Neither of these trials was performed in order to support the indication sought in the current application and will not be commented on further

Influence of administration procedure on efficacy evaluation

To adequately maintain the stability of the product and compare safety and efficacy results within and between studies, study site personnel were instructed to withdraw and inject Optison at a rate no faster than 1 ml/second. They were also instructed not to take longer than one minute from suspension to injection time. When injecting Optison, they were advised to use either an intravenous line or heparin lock. If using an intravenous line, they were instructed to hang a bag of normal saline and run it at a “to keep open” rate (TKO). If they were using a heparin lock, they were told to fill one syringe with normal saline or dextrose in water and flush to assure patency.

Summary on diagnostic efficacy

At proposed normal dosages of 0.5 to 3.0 ml (safely allowable up to 40.0 ml) Optison injections provided a ≥ 2+ left ventricular opacification in 86-92% of the patients investigated. Improved border delineation was observed in 89-96% of the phase III trial patients. These results are superior to those of the approved reference contrast agent used for comparison in the same patients (34-60% and 63-65%, respectively). Furthermore Optison demonstrated conversion of about 75% of patients with non-diagnostic studies to diagnostic levels. The comparable figure of the reference product was 25%.

In the FS-3000 study Optison converted 42-77% of inadequate non-contrast right pulmonary vein Doppler signals and 76-93% of inadequate left pulmonary vein Doppler signals to a clinically useful level. In the FS-3500 study, similar improvements in the right and left pulmonary veins were 47-69% and 74-91%, respectively.

Safety

Ultrasound contrast agents are intended as adjuncts to a non-invasive diagnostic technique and should not, therefore, add significantly to the risk of the test they assist.

However, the theoretical safety problems concerning the proposed use of Optison focus on certain high-risk organs such as the lungs and the brain, and certain risk populations of patients e.g. patients with pulmonary diseases or acute vascular lesions.

Safety parameters were collected and analysed for the assessment of both clinical and statistical significance. The table below lists all the clinical studies performed and the safety evaluations by protocol and the time of the evaluations relative to administration of the test agent. Individual reports

detail these safety evaluations and specific protocol deviations. All adverse events were monitored from the time a patient was on study until the end of the study-specific post-injection follow-up period

Table 2. Summary of Safety Evaluations by Protocol

	FS-1000* (n = 40)	**FS-1500* (n = 10)**	**FS-6000** (n = 50)**	**FS-1250* (n = 5)**	**FS-3000/ 3500*** (n = 203)**	**FS-5000 (n = 34)**	**FS-2000 (n = 254)**
12-lead ECG	X	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X	X
Neurological Examination	X						
Spirometry	X		X				
Vital Signs	X	X	X	X	X	X	X
O_2 Saturation	X	X	X	X	X		
Chemistry Panel	X	X	X	X	X	X	X
Haematology	X	X	X	X	X	X	X
CPK Isoenzymes	X	X	X	X	X	X	X
Urinalysis			X		X		X
PT	X	X	X	X	X	X	
PTT	X	X	X	X	X	X	
Non-contrast imaging (baseline)						X++	X++
Resting OPTISON Contrast ECG						X++	
Stress OPTISON ECG						X++	
AEs	X	X	X	X	X	X	X

* All evaluations performed at baseline, and 2 and 24 hours post-study.
** All evaluations performed at baseline, and 2 and 48 hours post-study.
*** All evaluations performed at baseline, 30 minutes and 48 hours (up to 10 days) post-study for each test agent.
O_2 Saturation monitored every 2 minutes on study. Vital signs monitored at 5 to 20 minute intervals on study. A minimum of 48 hours and a maximum of 10 days were required between each test agent.
++ On-study-only.

FS1000

No statistically significant effects were found in the vital signs, ECG variables, spirometry measurements, physical or neurological examinations or 30 of the forty clinical laboratory variables evaluated. Of the 10 clinical variables that attained statistical significance no clinically meaningful changes were observed.

The statistically significant changes in oxygen saturation noted were related to the rate of change of oxygen saturation between different dose groups. These rates of change were clinically irrelevant and the statistical changes were due to the large number of time points measured and the extremely small variability in the measurement. Mean oxygen saturation varied between 97 and 99% for the various dose groups which is within the normal range.

No immunological response to the administration of Optison was found for the 5 immunoglobulins studied (IgA, IgD, IgE, IgG and IgM).

None of the adverse events that occurred in 15 subjects were definitely attributed by the investigator to the administration of Optison. Although almost half of the events occurred in the high dose group, all events were transient and required no medical attention. The most frequent adverse events were headache, nausea, a warm sensation/flushing, and light-headedness, some of which are consistent with the injection of albumin. The study was conducted in an environment where subjects were housed together for several days before and after the study and it became clear from the comments made to investigators before receiving the agent that the subjects were aware of adverse events experienced by some of those preceding subjects.

Based on the results, it was concluded that Optison injections are safe in single doses up to 40 ml and cumulative volumes of 44 ml.

FS1250

No clinically significant changes for the majority of safety parameters, including vital signs, ECG measurements, spirometry readings, oxygen saturation and physical examination variables, were noted for any of the patients. Only one clinically significant laboratory result was observed for an individual patient (i.e., an increase in lactic dehydrogenase from 119 to 313 U/l). No other liver function tests were abnormal and no obvious cause for this increase was determined.

Although 6 adverse events were reported, all events were classified as mild in severity and not related to the product. One subject, however, experienced urticaria and pruritus as a result of the intradermal injection with 0.02 ml Optison (i.e., skin test); this subject subsequently did not receive the 20 ml rechallenge intravenous dose of Optison. This individual was tested for any evidence of an immunological response to Optison and all tests proved negative. The cause of the positive skin test was not determined but was possibly due to dermographism.

FS-1500

Approximately 93.4% of the OFP component of Optison was eliminated within ten minutes of dosing with a pulmonary half-life of 1.3 minutes. When one patient with a malfunctioning expired air collection bag (i.e., a leaky sample container bag) was excluded from analysis, the recovery rate improved to 98.5%.

Several clinically significant laboratory changes were observed (i.e., one patient demonstrated a significant increase in total bilirubin levels while a second patient experienced a slightly elevated total CPK at two hours which was resolved at 24 hours post-injection). This may be considered normal for an invasive procedure.

Changes involving ECG measurements and vital signs were not clinically significant, while oxygen saturation and expired air changes were neither statistically nor clinically significant.

Five of the enrolled subjects reported adverse events (AEs); no AEs were definitely attributed to Optison while one event was attributable to the procedure of expired air collection.

FS-6000

Optison did not produce immunological responses in the 50 participants enrolled in this study. Comparable observations were noted for patients injected with either Optison or 1% human albumin. No statistically significant effects and few clinically relevant changes were observed for the ECG assessments, physical examinations, vital signs, oxygen saturation measurements and clinical laboratory variables. Clinically significant changes were comparable between Optison and 1% human albumin. There were no statistically significant differences in oxygen percent saturation between patients who received Optison compared with those who received 1% human albumin.

No statistically significant differences in ECG assessment between participants injected with Optison or 1% human albumin were noted at either two or forty-eight hours post-injection.

The statistical tests indicated no statistically significant differences between 1% human albumin and Optison for all vital signs or clinical laboratory measurements.

No statistically significant changes in physical examination assessments were noted between participants injected with Optison and 1% human albumin.

A total of nineteen participants experienced a total of 32 AEs. Eight participants had one or more AEs after injection with 1% human albumin while eleven other participants had one or more AEs after Optison, with incidence rates of 32% and 44%, respectively. There were no statistically significant differences between the two rates. All participants recovered without treatment except for one hepatic disease patient who received 1% human albumin and experienced mild (without intervention) chest pain that was classified as not related to the study by the investigator. The most commonly reported AEs were headache (6), cold feeling or chills (4) and light-headedness (3). AE rates were comparable for patients who were injected with either Optison or 1% human albumin; most of the events were minor, did not last long and resolved without medical attention.

FS-3000 and FS-3500 (Studies performed in the U.S.)

No statistically significant effects and few clinically relevant changes were observed in ECG assessments, physical examinations, vital signs and oxygen saturation measurements for either of these Phase III studies. A few clinically significant ECG changes were noted, such as ST-T changes, Q-wave changes possibly diagnostic of myocardial infarction, ventricular arrhythmias and other rhythm disturbances. Changes such as these over a five to ten day period in a patient with ischaemic heart disease are expected.

Clinically relevant vital sign incidence rate changes were only 1.1% for FS-3000 and only 0.2% for FS-3500; heart rate, respiration rate and blood pressure changes were noted in both cardiac and hypertensive patients. There were no significant differences between the reference product and Optison with regard to these changes in vital signs.

At various study timepoints, only 0.2% changes in all laboratory parameters were clinically important; most observed increases and decreases were judged to be related to concurrent illnesses and/or diseases.

Clinically relevant oxygen percent saturation changes (defined as greater than 7.5% difference from baseline) were noted at a rate of 0.4% in the FS-3000 study and 0.2% in the FS-3500 trial. This low rate of change is consistent with no significant effect of Optison and can be assumed to reflect concurrent cardiovascular diseases.

The incidence of physical examination parameter changes were between 2-3% for both trials, were similar for both agents, and probably could be attributed to the concurrent diseases of these patients.

Altogether 31/203 patients enrolled in the two trials experienced one or more AEs. Of the 49 AEs reported (i.e., 33 for FS-3000 and 16 for FS-3500), the investigators characterised twelve as not related, eight as uncertain, twenty-six as related to the injection and three as related to the procedure. Most of the events were minor, transient and did not require further medical attention to resolve. The most frequently reported adverse effects associated with the administration of Optison were transient altered taste (2.5%), headache (2.0%) and warm sensation/flushing (2.0%). The percent of patients reporting one or more adverse events after Optison was 6.5% compared with 9.0% for the reference product.

Assessment of specific safety issues

Use in special patient groups: Elderly, patients with hepatic, renal or pulmonary impairment; patients with concomitant acute (cerebro) vascular disease

Hepatic and renal disease, although present in a fairly small number of patients, can be regarded as having been adequately reviewed through the available data provided by the company. These types of patients seem to run a very low risk by using Optison.

Acute cardiovascular states, e.g. acute myocardial infarctions or acute cerebrovascular lesions, are not included in the company database. However, a number of patients undergoing coronary angioplasty are included and argued to characterise an acute vascular state through the effect of the balloon or the stent used in the procedure. This is true and in view of the fact that intravenous delivery of Optison constitutes the only way contained in the application, there should be only a minor risk in patients with

acute cardiovascular states, even if there are a few serious adverse events reported with the use of Optison in intracoronary injections, i.e. in much larger concentrations of the contrast medium.

The use of Optison in acute febrile states of infection or systemic inflammation has not been elucidated by the patient inclusions in the various studies. One cardiac disease of this kind, where echocardiography is common and where knowledge of the left ventricular function is often mandatory for the successful care of the patient, is endocarditis with or without its complications. Even if the use of Optison in such patients seem to constitute very small risks, the use of Optison in such patients should be preceded by a warning in the SPC, that patients of this kind has not been subjected to Optison so far.

Finally, as for respiratory and pulmonary diseases, there should be only minor risks in conjunction with intravenous injections of Optison, considering the gas in the bubbles is shown to be biologically inert and the shell is shown to present no serious challenge as to biological activities. Only one disease entity seems to constitute a concern, still. Since only a few cases of moderate pulmonary hypertension are included in the studies, it is suggested to issue a contra-indication in the SPC, when considering the use of Optison in cases of severe pulmonary hypertension, e.g. systolic pulmonary pressure above the range of 90 mm Hg.

No serious adverse events have been reported in the included patients. A large amount of data speak in favour of Optison being generally of low risk due to the biologically inert gas and the shell of human albumin, where both constituents have been thoroughly investigated as to potential hazards and come out clean out of this scrutiny. However, a few disease states, of interest in the context of intravenous contrast bubbles, have not been represented in the studies and warnings should be issued in the SPC till further information is gathered during postmarketing experiences.

The Optison application for marketing authorisation included safety data on 367 patients. Since the time of the filing, an additional 170 patients have been completed in controlled clinical trials involving cardiac and hepatic patients. This brings the total number of patients studied with intravenous Optison to 537. None of these 537 patients have experienced a serious or unexpected adverse event. The overall safety claim of Optison has leaned on data from clinical trials using a related product based on stabilised microbubbles of air, also authorised to the applicant in this case. The two products are identical, except that the gas used for Optison is perfluoropropane, whereas air has been used for the reference product. Optison has identical physical and immunological properties as the reference product. Thus, the safety data base for the reference represents valid evidence to further support the safety profile presented with intravenous Optison. An updated safety report for the reference product was specifically requested by the CPMP in the list of questions, and provided by the applicant.

Over 3,400 patients have been injected world-wide with the reference product in 70 controlled clinical trials. No serious adverse events have been observed in any of the patients enrolled in these trials which were definitely attributable to the administration of the reference product. Summaries of these 70 trials are presented. The most common adverse events were abnormal taste, flush and dizziness. In 80 patients undergoing intra-coronary and intra-aortic injections of the reference product (Protocol nr 13491-A2 and 13192-A1) one case of hypotension, one case of dyspnea and chest pain, one case of borderline myocardial infarction and one case of ST-T-changes did occur. In one Doppler carotid study of i.v. reference product (Protocol nr 461A/461B) on 14 patients one case of neuropathy, one case of stupor and one case of vertigo occurred. In another study of intra-coronary injections of reference product in 229 patients (Protocol nr E1607) 5/6 adverse events in 3 patients could be related to myocardial cell damage. These adverse events could be of a potentially dangerous nature, however, they were all observed with a totally different way of drug administration.

Post-marketing experience is not applicable for the reference product as it has not yet been marketed in the European countries where authorisation has been obtained.

However, the reference product is approved by the U.S. Food and Drug Administration for the same indications contained within this application. Over 17,000 patients have received intravenous reference product in the United States with no serious adverse events reported.

It can therefore be accepted that the overall safety of Optison is adequately covered by the vast experience of the reference product throughout the world.

5. Overall conclusions and benefit/risk assessment

The quality and stability of the product are considered to be satisfactory. The physicochemical parameters essential for the uniform clinical performance of this product have been adequately defined, and are controlled in a satisfactory way.

Concerning diagnostic efficacy, Optison injections are advantageous in achieving left ventricular cavity opacification with concomitant increased accuracy for cardiac function assessment. The overall incidence of adverse events are relatively low and of mild severity. The intravenous use of Optison permits improved visualisation of endocardial borders in the majority of patients in whom endocardial borders are not visible during the echocardiography examination without contrast-improving measures. The use of Optison can salvage poor quality echocardiograms and obviate the need for more invasive, costly testing for assessment of cardiac function.

However, to balance these conclusions:

Optison should only be used in those patients where studies without contrast enhancement have been shown to be inconclusive.

Clinical experience in certain patient groups is limited and this is reflected in the SPC, especially Section 4.4, Special warnings and special precautions for use.

The CPMP on the basis of the quality, efficacy and safety data submitted, recommended that a Marketing Authorisation should be granted for this product, when used according to the conditions defined in the SPC.

6. Post-Authorisation

Allergic type symptoms (e.g. anaphylactic reaction, face oedema, urticaria) and transient nervous system disorders (e.g. dizziness, paraesthesia, tinnitus, or visual disorders), have been rarely reported post-marketing.

Several research groups have reported that echo-contrast agents in combination with ultrasound caused more severe damage in various animal and *in vitro* models than ultrasound alone. Typically, ruptures of small capillaries were produced, leading to bleeding and other secondary effects. Although these biological side effects have not been reported in humans, a recommendation to use a *low mechanical index* has been included in section 4.4 of the SPC.