

TherOx, Inc. DownStream AO System
PMA P080005 Panel Package
Section 4: Sponsor Executive Summary

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DownStream® AO System
PMA P080005 Panel Package

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1 Introduction

TherOx, Inc. has developed a focal hyperbaric oxygen technology to treat ischemic myocardial tissue in heart attack patients, referred to as "SuperSaturated Oxygen Therapy", or SSO₂ Therapy. This novel focal approach, unlike hyperbaric chambers that rely on full-body exposure to pressurized oxygen gas, creates a highly concentrated aqueous solution of oxygen dissolved in sterile saline called SSO₂ solution, which is then mixed in a low priming volume extracorporeal circuit with a patient's arterial blood. The arterial pO₂ of the blood is elevated to hyperoxemic levels (760-1000 mmHg) and pumped into the coronary arteries after primary percutaneous coronary intervention (PCI) treatment for acute myocardial infarction (AMI) in a single 90-minute infusion.

The TherOx SSO₂ Therapy procedure utilizes three device components: a computerized mobile hardware system, a single-use disposable cartridge, and an infusion catheter. The system is a complex electromechanical hardware device that operates and monitors the extracorporeal circuit throughout the procedure. The cartridge has a three-chambered main body that creates SSO₂ solution from inputs of hospital-supplied oxygen gas and physiologic saline, and mixes the SSO₂ solution with autologous arterial blood to create oxygen-enriched hyperoxemic blood. The cartridge has draw tubing to withdraw the patient's blood and return tubing that attaches to the infusion catheter to return the SSO₂-infused blood back to the patient. Together, the cartridge and catheter comprise the blood-contacting extracorporeal circuit. The aim of the treatment is to resuscitate stunned or damaged myocardium, reducing the size of the infarct and thereby improving cardiac function.

TherOx has conducted thorough device testing and a series of clinical studies to establish the safety and effectiveness of SSO₂ Therapy in treating the intended AMI patient population. This executive summary provides an abbreviated description of the device and its principles of operation, a presentation of the key biocompatibility and non-clinical study results, and the key findings of the clinical trials conducted using SSO₂ Therapy as a PCI-adjuvant procedure to treat AMI patients. More detailed documents within this Panel Package provide an in-depth discussion of these elements, such as the Device Description (Section 6) or the Clinical Summary Report (Section 7). For the benefit of the reviewer, SSO₂ Therapy was referred to previously as "Aqueous Oxygen" or "AO" Therapy, and some documents in the Panel Package use this terminology. In addition, the [REDACTED] catheter is referred to in some documentation as the [REDACTED] catheter; these terms are synonymous.

2 Proposed Indications for Use

The TherOx® DownStream® AO System, DownStream® AO Cartridge, and [REDACTED]™ Infusion Catheter are indicated for: The preparation and delivery of SuperSaturated Oxygen Therapy (SSO₂ Therapy) to targeted ischemic regions of the patient's coronary vasculature immediately following revascularization by means of percutaneous coronary intervention (PCI) with stenting that has been completed within 6 hours after the onset of anterior acute myocardial infarction (AMI) symptoms.

3 Regulatory History

In September 1999, TherOx, Inc. received FDA approval to initiate its Investigational Device Exemption application to conduct a pilot study in acute myocardial infarction patients to evaluate the clinical feasibility of SuperSaturated Oxygen (SSO₂) Therapy. This pilot study was conducted in the

U.S and Italy using prototype equipment. Phase I involved 9 subjects who received SSO₂ Therapy selectively, with the hyperoxemic reperfusion provided through a guiding catheter in the left main coronary ostium at a flow rate of 100 ml/min. In Phase IA involving 20 subjects, the reperfusion was provided sub-selectively in the infarct-related artery through an infusion catheter at a flow rate of 75 ml/min. Results of the Phase I/IA studies showed LV functional improvement in treated subjects.

The AO System, AO Cartridge, and infusion catheter received CE-mark in September 2001 for sale in the European Community. The first commercial AO System placement in Europe was October 20, 2001 in Italy. From that time until present, AO Systems and accessories have been sold in eight hospitals in two countries, including Italy and the Czech Republic.

The Acute Myocardial Infarction with HyperOxemic Therapy, or AMIHOT I trial, initiated in January 2002, was approved under the same IDE number. This study examined the safety and effectiveness of SSO₂ Therapy in both anterior and inferior STEMI patients undergoing successful reperfusion therapy via PCI up to 24 hours from symptom. Results for the Control/SSO₂ Therapy group comparisons for the three co-primary effectiveness endpoints demonstrated a nominal improvement in the test group but did not achieve clinical and statistical significance in the entire study population. However, a post hoc analysis of SSO₂ Therapy patients who were revascularized within 6 hours of AMI symptom onset and who had anterior wall infarction showed a marked improvement in all three co-primary endpoints as compared to Controls.

Working closely with FDA, TherOx, Inc. developed the investigational plan for the AMIHOT II Clinical Trial which served as the pivotal study for PMA application. The promising results observed in this anterior < 6 hr AMI patient population in the AMIHOT I study served as the basis for selecting this cohort in the AMIHOT II trial. The AMIHOT II trial was designed to test the superiority of SSO₂ Therapy in reducing infarct size in anterior AMI patients treated within six hours of symptom onset, with safety evaluation by comparing the incidence of 30-day Major Adverse Cardiac Events (MACE) between the SSO₂ Therapy and Control groups.

The AMIHOT II trial utilized a Bayesian statistical design that allowed for the informed borrowing of data from the previously completed AMIHOT I trial. The Bayesian statistical model was pre-specified; the model required that the posterior probability for success to be greater than 95.0% for both the efficacy and safety endpoints. An unbalanced randomization ratio of 2.8:1 (SSO₂ Therapy: Control) was utilized to satisfy the power requirements of the statistical model. The AMIHOT II study endpoints were met for both effectiveness and safety. The AMIHOT II pivotal study results demonstrated that SSO₂ Therapy is effective in reducing infarct size and is safe when used in accordance with its recommended instructions for use.

TherOx, Inc. utilized a modular PMA approach to submit the Premarket Approval Application to FDA. The PMA was filed as P080005 on March 20, 2008.

As shown in **Figure 1**, the AO System chassis consists of a main enclosure mounted upon a system base. The sheet metal main enclosure contains several electronic subsystems and mounting interfaces for these subsystems. A rear service panel provides access to the internal components and has a power switch. The main enclosure also has two side handles and a front door. A retractable pole for the saline bag is mounted to the main enclosure. The system base supports the main enclosure, contains the power supply and holds the oxygen bottle. The system base has four wheels that fully-articulate for system mobility and lock for stability. The following subsystems are integrated into the AO System chassis:

- The AO Cartridge Subsystem (AOCS) houses and operates the AO Cartridge (the AOCS does not contact saline or blood). The AOCS monitors operating parameters within the AO Cartridge. The AOCS controls the flow of oxygen to the cartridge and controls the flow of saline through the cartridge by actuating moving parts within the cartridge. The AO System operating state is controlled by software within the AOCS.
- The Blood Pump Subsystem (Blood Pump) has a fully occlusive peristaltic (roller) pump that is loaded with the AO Cartridge draw tubing. The blood pump withdraws normoxic arterial blood from the patient's femoral artery and returns hyperoxemic blood via infusion catheter to the coronary arteries.
- The Bubble Detector Subsystem (Bubble Detector) is a custom ultrasound-based device that monitors the return blood flow in the extracorporeal circuit for the presence of microbubbles or air-in-line.
- The Safety Interlock Subsystem (Safety Interlock) stops treatment and isolates the AO Cartridge blood path from the patient if a fault condition is detected. The Safety Interlock continuously monitors signals from other subsystems for fault conditions. The Safety Interlock also has a manually operated Emergency Stop switch to disable SSO₂ Therapy.
- The User Interface Subsystem (User Interface) has a touch-screen display that guides the user through set-up and clinical operation. The User Interface accepts and initiates user commands, and communicates with the AOCS, Blood Pump, and Bubble Detector subsystems.
- The Oxygen Supply Subsystem (Oxygen Supply) uses a hospital-supplied oxygen E-bottle to provide pressurized oxygen to the AO System. A pressure regulator controls the AO Cartridge oxygen supply pressure.
- The Power Supply Subsystem (Power Supply) provides DC power to the electronic subsystems within the AO System. The Power Supply uses either AC Mains or an internal power supply (battery) as the power source.

The AOCS, Blood Pump, Bubble Detector, and User Interface Subsystems contain software to monitor and control subsystem function.

4.2 DownStream AO Cartridge

The AO Cartridge (**Figure 2**) is a sterile, single-use device that is inserted in the AO System during set-up to support SSO₂ Therapy. The cartridge creates SSO₂ solution from hospital-supplied inputs of saline

and oxygen gas. The cartridge provides the blood flow path (tubing) that withdraws arterial blood from the patient and returns hyperoxemic blood to the infusion catheter for delivery to the coronary arteries. As shown in the figure, the cartridge has a three-chamber design; each chamber performs a different step in this process. The cartridge weighs less than one pound and is primarily constructed from injection-molded polycarbonate; the tubing material is polyvinyl chloride (PVC). The cartridge is individually packaged and has a three-year shelf life.

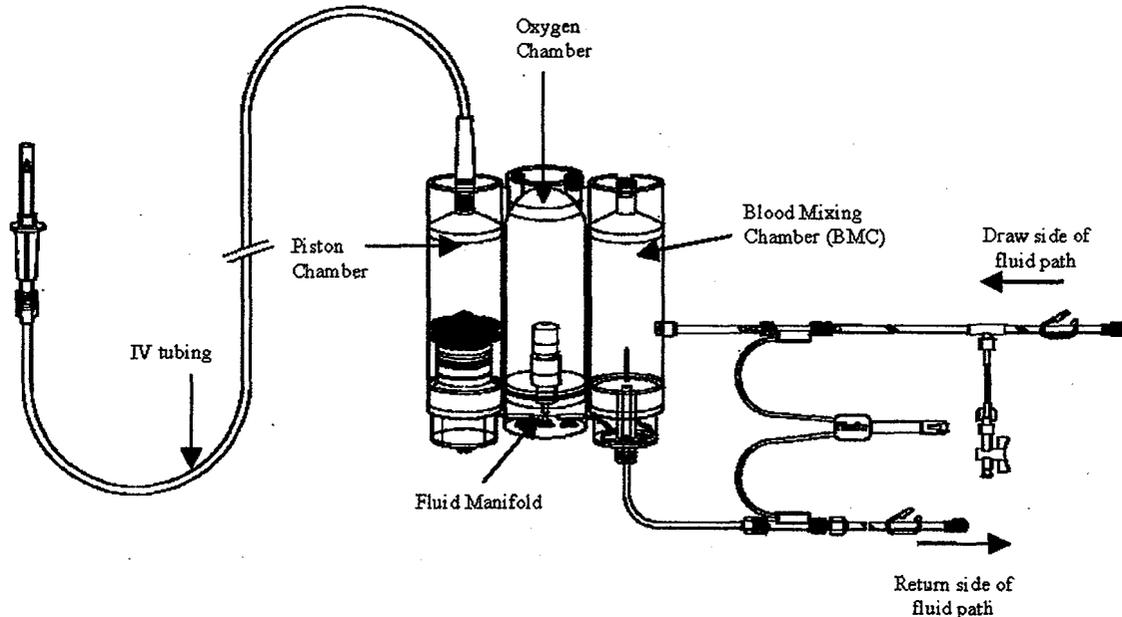


Figure 2. DownStream AO Cartridge

As shown in the figure, the AO Cartridge has three separate chambers and a fluid manifold. The three discrete chambers are the Piston Chamber, Oxygen Chamber, and Blood-Mixing Chamber (BMC). The Piston Chamber utilizes an IV spike to connect to a bag of saline, and operates as a motorized syringe pump to withdraw saline from the bag on the piston downstroke, and to pump saline into the central Oxygen Chamber on the piston upstroke. The Oxygen Chamber is pressurized to (~ 42 atmospheres) of oxygen pressure. Saline is pumped into the Oxygen Chamber through an atomizer nozzle that sprays the saline in a mist of fine droplets, enabling rapid solubilization of oxygen gas. Saline may also be pumped into this chamber through a dilution port rather than the atomizer. A pool of highly oxygenated saline – SSO₂ solution – is maintained at the bottom of the Oxygen Chamber. During therapy, SSO₂ solution flows from the pressurized Oxygen Chamber at a flow rate of 3 ml/min into the Blood Mixing Chamber. This 3 ml/min SSO₂ solution flow is combined with 72 ml/min of inflowing arterial blood in the Blood Mixing Chamber, and 75 ml/min of hyperoxemic blood flow is returned to the patient. The fluid manifold connects the three chambers and meters the flow of saline solution through the cartridge. **Figure 3** depicts the fluid flow paths through the cartridge and the fluid flow control features.

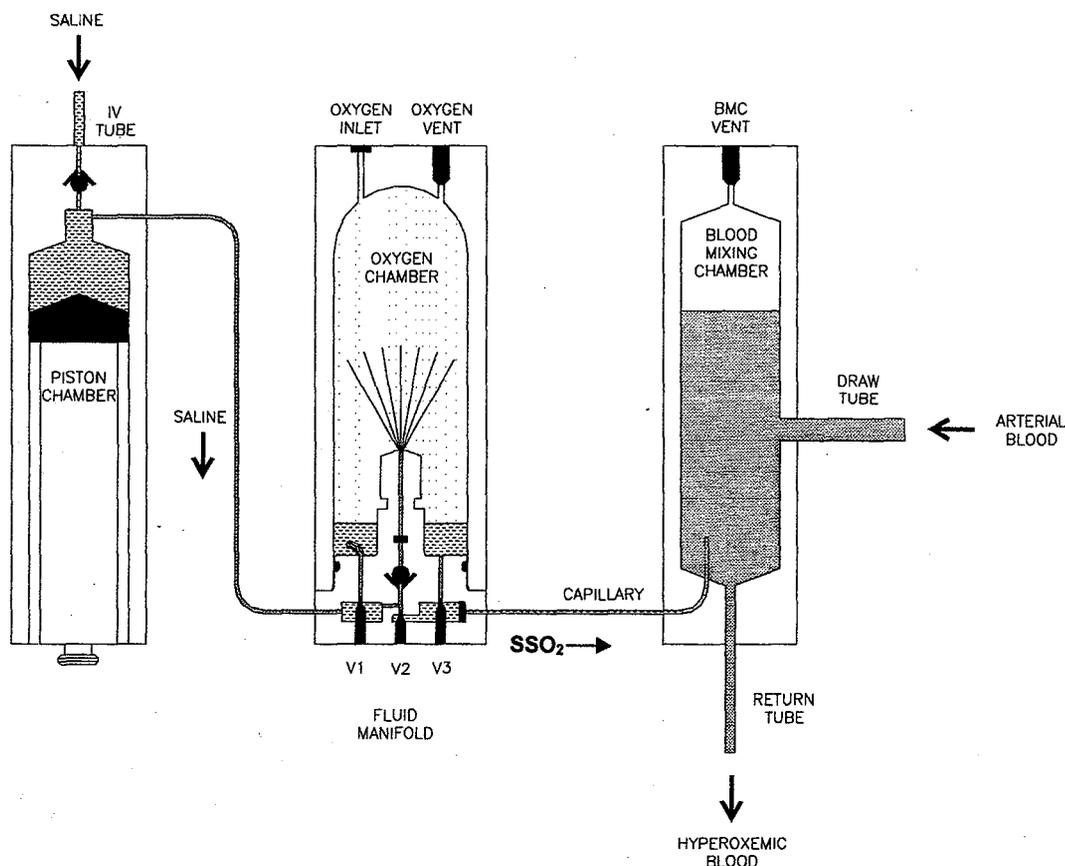


Figure 3. DownStream AO Cartridge Fluid Schematic

4.3 [REDACTED] Infusion Catheter

The [REDACTED] infusion catheter is a sterile, single-use over-the-wire device that may be inserted into patients through commercially available guide catheters 6 F or larger. The catheter's outer diameter (O.D.) is [REDACTED]. The polyethylene catheter body is extruded in a continuous process that transitions from soft tip to the stiffer proximal shaft. The inner lumen is smooth and free of transitions, and the catheter has a single end hole for fluid exit. The usable length is [REDACTED] and the overall length of the catheter is [REDACTED]. The inner diameter (I.D.) of the catheter is nominally [REDACTED] in except at the location of the platinum/iridium radiopaque marker band. The I.D. under the marker band is a minimum [REDACTED]. The catheter is individually packaged and has a three-year shelf life. The [REDACTED] catheter is shown in Figure 4.

Luer Hub: A female luer hub is molded over the proximal O.D. of the shaft. The luer hub enables attachment of the cartridge return tubing to the catheter.

Strain Relief: A polyolefin strain relief is applied over the shaft and luer hub joint with a heat shrinking process.

Proximal Shaft: The catheter has a non-plasticized white high-density polyethylene (HDPE) proximal shaft.

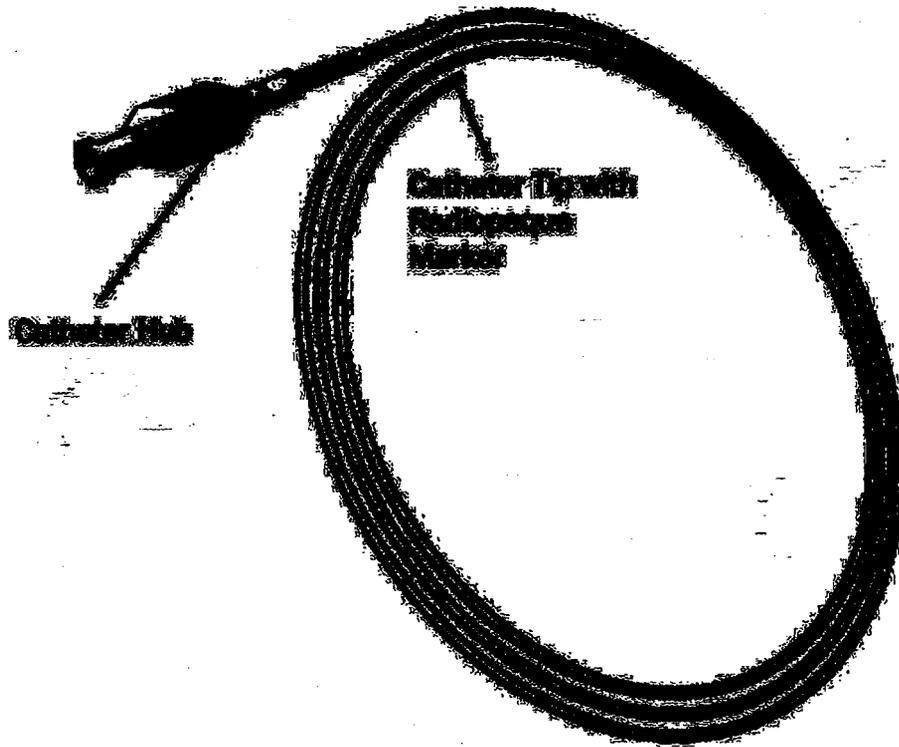


Figure 4. [REDACTED] Infusion Catheter

Distal Tip: From the distal tip termination to a nominal distance of [REDACTED], the distal tip material is a flexible low-density polyethylene (LDPE). The distal tip has a smooth radius to reduce the potential for vascular injury.

Radiopaque Marker Band: The radiopaque platinum/iridium alloy marker band is used to visualize the catheter fluoroscopically during use and is fitted within [REDACTED] of distal tip termination.

4.4 Patient Connections

The AO Cartridge draw tubing connects to the sidearm of the existing femoral arterial sheath that is used for PCI and stenting procedures. Sheath placement may be coaxial (one sheath in one femoral artery) or contralateral (two sheaths in both the right and left femoral arteries), at the physician's discretion. The preferred coaxial configuration, shown in Figure 5, illustrates how arterial blood is withdrawn from the sidearm through the annular space between the guide catheter and sheath; in this configuration, a single 8F introducer sheath can be used. The cartridge draw tubing luer fitting connects to the sidearm. The [REDACTED] catheter is placed through the 6F guide catheter over a guidewire, to the desired target location within a coronary artery. The guidewire is removed prior to initiation of blood flow. When extracorporeal blood flow is initiated, the [REDACTED] catheter and cartridge return tubing are wet-connected to ensure that no gaseous emboli are introduced to the patient during priming. The term 'wet connection' requires that both devices are fully blood-primed and free of trapped air bubbles. The cartridge return tubing luer fitting connects to the luer hub of the [REDACTED] catheter. For the contralateral approach (not shown), a 5F introducer sheath is used on the draw side, while a 6F introducer sheath

provides access for the 6F guide catheter. This alternative approach may be used by physicians who prefer to use two smaller sheaths for arterial access (5F and 6F) instead of a single 8F sheath.

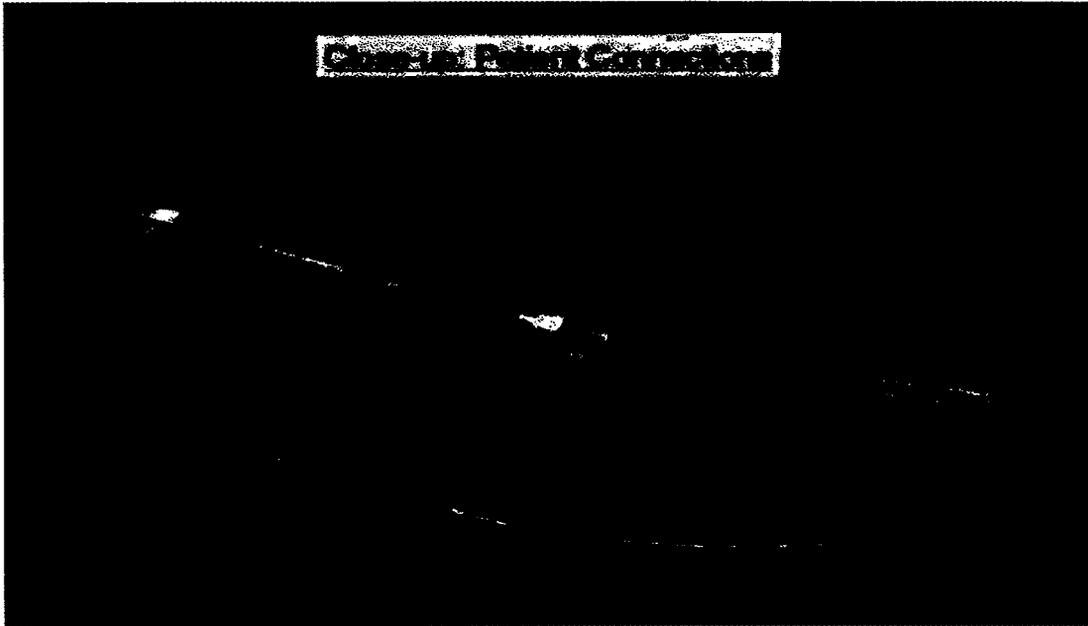


Figure 5. Co-axial Draw/Return Clinical Configuration for SSO₂ Therapy

4.5 Principles of Operation

The DownStream AO System is operated by a trained health care professional (user). Treatment is initiated in the [REDACTED]. The User Interface guides the health care professional (user) through setup and clinical operation. The operating principles for SSO₂ Therapy are provided herein. Two distinct processes are combined in the extracorporeal circuit to perform SSO₂ Therapy. The first process is SSO₂ solution delivery; the second process is extracorporeal blood circulation. The AO System controls and monitors these processes for safety.

SSO₂ Delivery Process

During SSO₂ Therapy, SSO₂ solution is produced in the cartridge and pumped through the capillary into the Blood Mixing Chamber at a rate of 3 ml/min throughout the 90-minute procedure. The additional fluid loading to the patient is therefore $90 \times 3 = 270$ ml. The SSO₂ dissolved oxygen concentration is controlled by the system to achieve hyperoxemic blood pO₂ levels in the range of 760 – 1000 mmHg using patient arterial blood.

Cartridge Preparation (Prep): After the cartridge has been loaded into the system and the user has spiked the IV bag, the user can Prep the cartridge. The purpose of Prep is to saline prime the fluid path in the high-pressure side of the cartridge, establish the minimum liquid level, and pressurize with oxygen. Prep is fully automated after user initiation.

SSO₂ Solution Delivery: The system monitors SSO₂ solution reservoir level within the cartridge Oxygen Chamber. The SSO₂ solution low level sensor detects low level when the reservoir volume

decreases to 5 ml. Detection of low level initiates a fill cycle. The fill cycle starts when the piston actuator drives the piston upward to build pressure in the piston chamber. After the load cell signal reaches a hardware threshold [REDACTED], the piston begins to deliver saline to the Oxygen Chamber. The delivered saline volume is set to 3 ml by commanding the piston stepper motor to turn 50 revolutions after the hardware threshold has been reached. The SSO₂ flow needle valve remains open throughout the fill cycle, maintaining a constant SSO₂ solution flow rate. The fill cycle occurs approximately once every minute during SSO₂ solution delivery.

The system controls the SSO₂ dissolved oxygen concentration by controlling the flow path of saline into the Oxygen Chamber. Saline can enter through the nozzle for atomization and oxygen saturation, or can enter through the dilution port directly into the reservoir (non-oxygenated).

Blood Circulation Process

The process of blood circulation through the DownStream AO Cartridge is similar to other extracorporeal blood circuits, although the blood flow rate and blood contact surface area are significantly lower than other applications (e.g., cardiac bypass). During SSO₂ Therapy administration, arterial blood from the patient circulates through the extracorporeal circuit comprised of the cartridge draw tubing, Blood Mixing Chamber, return tubing, and the [REDACTED] infusion catheter. The circuit priming volume is 60 ml. The system circulates arterial blood using a peristaltic blood pump.

Blood Path Priming and Circulation: Prior to circuit priming, the cartridge has been prepped for SSO₂ delivery, the [REDACTED] catheter has been placed into the target coronary artery, and the cartridge draw tubing has been connected to the sidearm of the arterial draw sheath. Priming the extracorporeal circuit requires two health care professionals; the user operates the system while the physician performs a wet connection between the return tubing and the infusion catheter after both devices are blood-primed. Blood priming is initiated when the user presses and holds the prime switch. This action opens the draw tubing clamp and starts the blood pump.

Prior to wet-to-wet connection with the Infusion Catheter, both the system user and the physician operator confirm that blood priming of the return tubing is complete. Priming is completed after wet-to-wet connection when the blood flow rate measured by the flow probe exceeds 50 ml/min, and the bubble detector has adequate signal strength. After blood priming, the AO System circulates blood at a flow rate of 75 ml/min, controlled with feedback from an ultrasonic flow measurement probe. After SSO₂ solution delivery has been initiated, hyperoxemic blood is returned to the patient at a blood flow rate of 75 ml/min. After 90 minutes of treatment, the flow of SSO₂ solution is discontinued while normoxic blood continues to circulate until the user ends the procedure.

4.6 Device Safety Features

The DownStream AO System monitors operating parameters to detect and respond to unsafe conditions. When an unsafe condition is detected, the system stops treatment and isolates the extracorporeal circuit from the patient. The safety processes described here address potential unsafe conditions that have been identified for the SSO₂ solution delivery and blood circulation processes, as well as general medical electrical equipment operation. These safety processes were designed using a comprehensive risk-based approach.

SSO₂ Solution Delivery safety monitoring: Safety monitoring for SSO₂ solution delivery includes detection of overpressure in the cartridge Piston Chamber and Oxygen Chamber. The cartridge operates with a maximum piston pressure of [REDACTED]. The hardware threshold for piston overpressure is set to [REDACTED] well below the failure limit for the device component. As a redundant protection against piston overpressure, the stepper motor encoder will stop SSO₂ solution production if a motor stall is detected; thus, the potential for motor failure is monitored as well. The operating range for pressure in the Oxygen Chamber is [REDACTED]. The hardware threshold for overpressure of the Oxygen Chamber is set to [REDACTED] well below the failure limit for this device component.

Blood Circulation safety monitoring: Safety monitoring for blood circulation through the AO Cartridge is similar to other extracorporeal blood circuits. The bubble detector continuously monitors the blood path after the system has been primed. The system will not complete the priming sequence if the bubble detector probe is not properly loaded. The bubble detector detects individual microbubbles with diameter $\geq 100 \mu\text{m}$, and quantifies the cumulative bubble volume during SSO₂ Therapy. If the cumulative bubble volume reaches 10 μl during the 90-minute treatment, or signal strength is out of range, the Bubble Detector initiates a system shutdown.

The system monitors the level of blood in the cartridge Blood Mixing Chamber during treatment and initiates a system shutdown if the level is excessively high or low. The chamber vent is always open when minimum blood level is not detected. Thus, any sudden introduction of gas into the chamber will be vented out. The chamber high-level sensor monitors the presence of the gas headspace (gas trap) in the top of the chamber. If this headspace decreases and blood level rises due to excess pressure or a vent valve leak, the chamber high-level sensor detects a fault condition.

Safety systems for SSO₂ Therapy blood circulation include detection of high pressure on the return tubing or low pressure (suction) on the draw tubing. Two disposable pressure transducers are incorporated into the cartridge tubing set. The blood pump head is fully occlusive and can isolate the draw tubing from the BMC at the stop threshold of 2000 mmHg [REDACTED]. The return tubing clamp also isolates the return tubing from the BMC at this level. If the blood pump speed is out of range (high or low), or the motor stalls, a fault condition is generated and therapy is stopped. The Blood Pump is unidirectional, so blood cannot be pumped back to the patient through the draw tubing. The pump head detector also senses if the pump head is opened during operation. Opening the pump head while blood is circulating generates a fault condition.

System Safety Response: If an unsafe (fault) condition is detected, a system shutdown occurs: the blood pump stops and the draw and return tubing lines are isolated from the patient by two automatically-operated tubing clamps that are mounted on the system. In the event of a system shutdown, the cartridge is depressurized and is unloaded manually. A new cartridge must be loaded to continue treatment.

5 Non-Clinical Studies

A summary of non-clinical studies and information for the DownStream AO System, DownStream AO Cartridge, and [REDACTED] Infusion Catheter is presented in this section. Results of biocompatibility

testing, relevant animal studies of SSO₂ Therapy, engineering testing of the device and components, and software testing are included.

5.1 Biocompatibility Testing

The DownStream AO System hardware has no fluid contact or blood contact surfaces. The single use DownStream AO Cartridge, which is loaded into the system, and the intra-coronary Infusion Catheter, have fluid and blood contact.

Biocompatibility tests for the DownStream AO Cartridge and Infusion Catheter were selected in accordance with ISO 10993-1, Biological Evaluation of medical devices – Evaluation and testing, and FDA Blue Book Memorandum #G95-1. Both the AO Cartridge and Infusion Catheter are classified as externally communicating, blood-contacting, short duration devices as defined in ISO 10993-1. All required biocompatibility tests were completed in accordance with the guidelines and were conducted in compliance with Good Laboratory Practices (GLP) as defined in 21CFR§58. The AO Cartridge and Infusion Catheter passed the required biocompatibility testing requirements. Biocompatibility testing results for the cartridge are presented in Table 1; testing results for the catheter are presented in Table 2.

Table 1. Biocompatibility Testing for the DownStream AO Cartridge

Test Performed / Reference Standard	Extract(s)	Extract conditions / Animal model	Test and Control(s)	Results
Cytotoxicity (Ref: ISO 10993-5)	Minimum Essential Medium (MEM)	120 cm ² / 20mL 37°C – 24 hours Animal model: N/A	Negative control: Polyethylene Positive control: Latex	PASS
Sensitization (Ref: ISO 10993-10)	0.9% NaCl Cottonseed oil (CSO)	120 cm ² / 20mL 50°C – 72 hours Animal Model: Guinea pig	0.9% NaCl CSO Freund's Complete Adjuvant (CFA)	PASS
Irritation / Intracutaneous Toxicity (Ref: ISO 10993-10)	0.9% NaCl CSO	120 cm ² / 20mL 50°C – 72 hours Animal Model: Rabbit	0.9% NaCl CSO	PASS
Systemic Toxicity (Ref: ISO 10993-11)	0.9% NaCl CSO	120 cm ² / 20mL 50°C – 72 hours Animal Model: Mouse	0.9% NaCl CSO	PASS
Ames test (Ref: ISO 10993-3)	0.9% NaCl	120 cm ² / 20mL 50°C – 72 hours Animal Model: N/A	Sodium Azide Mitomycin-4 4-nitro-0-phenylene-diamine (NPD) aminofluorene (2AF)	PASS
Hemolysis (Ref: BTC protocol No. P0204010*)	0.9% NaCl	120 cm ² / 20mL 50°C – 72 hours Animal Model: N/A	Negative control: 0.9% NaCl Positive control: Sodium carbonate solution (0.1%)	PASS

* The Biological Test Center (BTC) performs hemolysis testing on device extracts per internal procedures.

Table 2. Biocompatibility Testing for the MI-Cath Infusion Catheter

Test Performed / Reference Standard	Extract(s)	Extract conditions / Animal model	Test and Control(s)	Results
Cytotoxicity (Ref: ISO 10993-5)	Minimum Essential Medium (MEM)	4 grams / 20mL 37°C – 24 hours Animal model: N/A	Negative control: Polyethylene Positive control: Latex	PASS
Sensitization (Ref: ISO 10993-10)	0.9% NaCl Cottonseed oil (CSO)	120 cm ² / 20mL 50°C – 72 hours Animal Model: Guinea pig	0.9% NaCl CSO Freund's Complete Adjuvant (CFA)	PASS
Irritation / Intracutaneous Toxicity (Ref: ISO10993-10)	0.9% NaCl CSO	4 grams / 20mL 50°C – 72 hours Animal Model: Rabbit	0.9% NaCl CSO	PASS
Systemic toxicity (Ref: ISO10993-11)	0.9% NaCl CSO	4 grams / 20mL 50°C – 72 hours Animal Model: Mouse	0.9% NaCl CSO	PASS
Hemolysis (Ref: BTC protocol no. P0105005*)	0.9% NaCl	120 cm ² / 20mL 50°C – 72 hours Animal Model: N/A	Negative control: 0.9% NaCl Positive control: Sodium carbonate solution (0.1%)	PASS
Thrombogenicity (Ref: ISO 10993-4)	N/A	6 hour intravascular implant Animal model: Canine	Control article: [REDACTED] Infusion Catheter	PASS
Complement Activation (Ref: ISO 10993-4)	Normal Human Serum	6 cm ² / 1.0mL 37°C – 60 minutes Animal Model: N/A	Positive Control: Cobra Venom Factor Negative Control: High Density Polyethylene Comparison Article: [REDACTED] Infusion Catheter	PASS
Ames test (Ref: ISO 10993-3)	0.9% NaCl Dimethyl Sulfoxide (DMSO)	118 cm ² / 22.6mL 37°C – 72 hours Animal Model: N/A	Sodium Azide Mitomycin-4 4-nitro-0-phenylene-diamine (NPD) 2 aminofluorene (2AF)	PASS

* The Biological Test Center (BTC) performs hemolysis testing on device extracts per their internal procedures.

5.2 In Vitro Engineering Testing

Verification and Validation (V&V) testing was performed on the component subsystems of the AO System and on the fully integrated device to ensure conformance with requirements. AO System software was developed, documented, and validated in compliance with IEC 60601-1-4 and FDA guidance.

5.2.1 DownStream AO System Bench Testing

AO System V&V testing was performed on the integrated system and at the subsystem level. The following is a summary of the key test data:

- **General Requirements**

General requirements for medical electrical equipment include safety testing to standards (IEC 60601-1 and IEC 60601-1-2) and confirmation of design control through a review of applicable documentation. System software was developed and controlled using procedures that comply with IEC 60601-1.4.

- **AO Cartridge Subsystem (AOCS) Testing**

The AOCS interfaces with the AO Cartridge within the AO System housing. AOCS testing was conducted on a stand-alone configuration in a diagnostic (service) mode. This stand-alone subsystem configuration facilitated calibrated measurements of power supply voltages, oxygen supply pressure and temperature through a series of test conditions. When operating the AO Cartridge, the average volume of each piston stroke was 3.05 ml, within specification (2.5 – 3.5 ml). The ability of the AOCS to support SSO₂ delivery above the operational pressure limit of [REDACTED] was verified. The needle valve actuators were hydrostatically proof-tested at pressures up to [REDACTED]. The cartridge housing was proof-tested to 1600 psig. V&V testing demonstrated that the AOCS met all design requirements.

- **Blood Pump Subsystem Testing**

The system is equipped with a peristaltic blood pump, ultrasonic flow probe, and flow-feedback controller; together these elements comprise the Blood Pump Subsystem (Blood Pump). The Blood Pump was tested with an AO System configured in a diagnostic (service) mode. Testing was conducted with 5% (w/v) saline solution; this test fluid is a blood analog with respect to the ultrasonic flow probe response. The Blood Pump sustained flow of 75 ml/min at pressures up to the system operating limits. In addition, the Blood Pump controlled flow within 75 ml/min ± 10% over the typical range of return pressure 800 – 1600 mmHg [REDACTED]. V&V testing demonstrated that the Blood Pump met all design requirements.

- **Oxygen Supply Subsystem Testing**

The AO System has an Oxygen Supply Subsystem that regulates the pressure and flow of oxygen gas from the oxygen bottle into the system. The conformance of the required components of this subsystem for oxygen gas handling was verified through testing. The ability of the pressure regulator to control oxygen supply pressure within the specified range [REDACTED] was verified. The set point [REDACTED] and service life of the relief valve (100,000 cycles) was verified. V&V testing demonstrated that the Oxygen Supply met all design requirements.

- **Power Supply Subsystem Testing**

V&V testing was conducted on the Power Supply Subsystem of an AO System configured in a diagnostic (service) mode. The conformance of the Power Supply components to system requirements was validated. The AO System power demand was 219 W maximum when charging and 198 W maximum when charged, meeting the requirements. Testing demonstrated that the Power Supply capacity exceeded the 250 W continuous output requirement. The Power Supply batteries provided 106 minutes-of operation after being charged for 10 hours (from low battery condition), meeting the requirement of one-hour minimum operation. V&V testing demonstrated that the Power Supply met all design requirements.

- **Integrated AO System Safety Testing**

The AO System is equipped with a hardware Safety Interlock that shuts down system operation safely in the event of a fault condition. Safety testing was conducted on three AO Systems; each system was tested with an AO Cartridge and a simulated extracorporeal circuit. Twenty-seven safety events were generated according to test protocol. Twenty-four of these events were intended to generate shutdowns, while three events were intended to generate warnings. Examples of these events include out-of-range flow rates, pressures, and presence of air-in-line. For each of the twenty-four shutdown events, the AO System detected the condition and stopped treatment, meeting the requirements. For the three warning events, the AO System generated the appropriate warning as intended. V&V testing demonstrated that the AO System generated the correct safety response as per requirements.

• Integrated AO System Performance Testing

Performance V&V testing was performed by conducting simulated SSO₂ Therapy in bench testing. Testing was conducted on three AO Systems configured in normal (clinical) mode consistent with the system's intended use. This testing utilized an *in vitro* clinical simulation of SSO₂ Therapy using slaughterhouse bovine blood (SBB) as the recirculation fluid. Testing required that each AO System control an AO Cartridge to produce hyperoxemic output pO₂ levels between 760 – 1000 mmHg with input SBB conditioned to different pO₂ levels. SBB blood gas samples were taken to establish the three input blood pO₂ ranges: 80 – 90 mmHg, 150 – 200 mmHg, and 250 – 350 mmHg. Test results demonstrated that for all three arterial blood concentration ranges, each of the three AO Systems tested produced a return blood concentration between 760 – 1000 mmHg as required. In addition, the blood flow rate was externally verified during each SSO₂ Therapy simulation. The average blood flow rate for the nine tests was 73.0 ml/min (range 70.0 – 76.5 ml/min). The average SSO₂ solution flow rate was 2.9 ml/min (range 2.8 – 3.0 ml/min). V&V testing demonstrated that the AO System satisfies its essential performance requirements.

5.2.2 DownStream AO Cartridge Bench Testing

The AO Cartridge successfully passed V&V testing for all requirements. Key bench test data are presented in **Table 3** and include results for therapy simulation, blood path (tubing) integrity, proof-pressure testing, hemolysis, and packaging.

Table 3. AO Cartridge Test Results

Test Description	Sample Size	Acceptance Criteria	Data	Result
Performance simulation	N=10	Saline Prep AO Cartridge in < 5 min Deliver pO ₂ >760 mmHg within 10 min Complete 3 hours of circulation	Prepped in 203 sec 224 sec max 864 mmHg at 10 min 811 mmHg minimum Completed 3 hours (treatment + 90 min)	PASS
Blood Path integrity	N=10	No leakage or failures from testing Priming volume less than 100 ml	No leakage at 60 psig No failures at 3.3 lb Prime volume 60.9 ml (61.6 ml max)	PASS
Test 100 cycles at design pressure	N=10	Perform 100 cycles (deliver 300 ml) at design pressure	All AO Cartridges delivered >300 ml at above	PASS
Proof test at 1.5X design pressure	N=10	Five minute proof test, at 1200 psi; no damage to cartridge	All AO Cartridges completed proof testing at 1200 psig	PASS
Blood Hemolysis testing	N=10	3-hour blood test; blood hemolysis less than 20 mg/dL/hr	Hemolysis index 10 mg/dL/hr	PASS
Shelf life testing	N=29	Meets non-destructive and destructive performance tests	3-year real-time aged product satisfied test requirements	PASS
Packaging testing	N=29	Test per ISTA 1A. Peel test (1 lb min). Leak test per ASTM F2096-01.	All samples passed shipping, peel test and submersion leak test requirements	PASS

5.2.3 Infusion Catheter Bench Testing

The Infusion Catheter successfully passed V&V testing for all requirements. Key bench test data are presented in **Table 4** and include results for therapy simulation, catheter handling properties, leak, proof, tensile, and burst testing, and packaging.

Table 4. Infusion Catheter Test Results

Test Description	Sample Size	Acceptance Criteria	Data	Result
Physical testing	N=30	Measure priming volume Flow test per ISO 10555-3 Annex A	Prime volume 1.60 ml (1.51 – 1.65 ml range); Flow rate per standard is 19.73 ml/min	PASS
Non-destructive performance	N=30	Catheter pushability, stiffness, leak test, proof test at [REDACTED]	Catheters completed all non-destructive tests without failure	PASS
Destructive performance	N=30	Catheter tensile failure above 2.25 lb Catheter burst failure above 200 psig	Four sections tested per catheter; failure at 3.275 lb minimum Burst test [REDACTED]	PASS
Shelf life testing	N=30	Meets non-destructive and destructive performance tests	3-year accelerated aged product satisfied test requirements	PASS
Packaging testing	N=30	Test per ISTA 1A. Peel test (1 lb min). Leak test per ASTM F2096-01	All samples passed shipping, peel test and submersion leak test requirements	PASS
Pressure drop and clinical simulation	N=5	Circuit pressure less than 26.9 psig Supports hyperoxemic blood delivery.	Circuit pressure drop 23.4 psig (24.5 psig max) at 75 ml/min; Catheters supported hyperoxemic blood delivery	PASS

5.2.4 Sterilization

The [REDACTED] Infusion Catheter and the DownStream AO Cartridge are ethylene oxide sterilized at a contracted sterilization facility. The sterilization cycle uses an Ethylene Oxide/ Carbon Dioxide gas mixture (8.5% and 91.5% respectively) with a minimum EO gas concentration of 255 mg/l. The nominal gas dwell time is 14.5 hours.

TherOx has validated the sterilization cycle using the “overkill” method outlined in ANSI/AAMI/ISO 11135. The validation consisted of three half-cycles, one fraction cycle, and three full cycles. The validation demonstrated that a sterility assurance level (SAL) of at least 10^{-6} is achieved. The sterilization cycle is re-validated annually.

5.2.5 Packaging

The [REDACTED] Infusion Catheter is inserted into a coiled polypropylene protective tube and placed in a Tyvek/poly pouch. The pouch is heat sealed, labeled, and placed in a unit box. The unit box is labeled and placed in an outer case. The cases are palletized and terminally sterilized with an ethylene oxide sterilant and carbon dioxide gas mixture. The packaged catheter has a three-year shelf life.

The DownStream AO Cartridge is placed in a custom thermoformed tray made of high impact styrene. A clear PETG lid snaps into the tray, keeping the AO Cartridge securely in place. The lid is well vented and does not interfere with the EO sterilization process. The tray is then placed in a Tyvek/poly pouch. The pouch is heat sealed, labeled, and placed in a unit box. The unit box is labeled and placed in an outer case. The cases are palletized and terminally sterilized with an ethylene oxide sterilant and carbon dioxide gas mixture. The packaged AO Cartridge has a three-year shelf life.

5.3 *In Vivo* Pre-Clinical Studies

Controlled studies were performed in both porcine and canine AMI models to investigate the safety, effectiveness, and mechanism of action of SSO₂ Therapy^{1,2,3,4}. These studies reported improved LV function, infarct size reduction, and a mechanism of action that acts to counter reperfusion injury. The key summary points from animal studies are:

- SSO₂ Therapy administration post- acute AMI suggested improved heart function as measured by left ventricular ejection fraction (LVEF) and regional wall motion score index (RWMSI) as compared with non-treated controls. These animal studies suggested improved LVEF and RWMSI at certain time points.
- SSO₂ Therapy administration post-AMI results in tissue salvage, as determined by post-sacrifice histological measurements of infarct size. Control animals exhibited larger infarcts than SSO₂-treated animals.
- The potential for optimum tissue salvage occurred when 90 minutes of therapy was provided. There was no significant difference when greater than 90 minutes of therapy was given in this animal model, possibly associated with an O₂ toxic effect in animals receiving 180-minute infusions.
- Based on the study protocols utilized, there is limited information available concerning deleterious effects such as end-organ thromboembolism, hemolysis, etc. SSO₂ Therapy was well-tolerated in the AMI models under study.
- SSO₂ Therapy administration post-AMI has exhibited regional myocardial blood flow improvement in treated animals as compared to controls.
- A significant reduction in myeloperoxidase (MPO) levels was observed in SSO₂ -treated animals versus controls; reduced MPO levels indicate improvement in underlying myocardial hypoxia.
- Transmission electron microscopy (TEM) photographs obtained in swine infarct studies suggest amelioration of endothelial cell edema and restoration of capillary patency in ischemic zone cross-sectional histological examination of SSO₂-treated animals as compared to non-treated control animals⁵.

The pre-clinical animal studies conducted with SSO₂ Therapy application in AMI models reported acute improvements in cardiac function and metabolic indicators of myocardial health, as well as infarct size reduction, in comparison with non-treated controls. The study limitations include the use of prototype equipment to deliver therapy rather than the final device configuration, the lack of concurrent pathology/histopathology to assess organ-specific effects, and the limited sample sizes employed in several of the studies.

6 Summary of Clinical Studies

¹ Spears JR *et al.* Hyperoxemic Reperfusion with Aqueous Oxygen Improves Left Ventricular Function and Microvascular Flow in the Postischemic Canine Myocardium. *J Am Coll Cardiol* 1998; 31(2):Abstr Suppl A:449A (1185 - 127).

² Spears JR *et al.* Reperfusion Microvascular Ischemia: Attenuation with Aqueous Oxygen. *Circulation* 2000; 102(18): Abstr Suppl II:646(3132).

³ Spears JR *et al.* Aqueous Oxygen Hyperbaric Reperfusion in a Porcine Model of Myocardial Infarction. *J Invasive Cardiol* 2002; 14(4):160 - 6.

⁴ Spears JR *et al.* Aqueous Oxygen Attenuation of Reperfusion Microvascular Ischemia in a Canine Model of Myocardial Infarction. *ASAIO J* 2003; 49(6):716 - 20.

⁵ Bartorelli AL. Hyperoxemic Perfusion for Treatment of Reperfusion Microvascular Ischemia in Patients with Myocardial Infarction. *Am J Cardiovasc Drugs* 2003; 3(4):253 -63.

6.1 Early Feasibility Studies

Phase I/IA

The first human study of SSO₂ Therapy in AMI was an IDE-sanctioned Phase I pilot study conducted in the U.S. and Italy beginning in [REDACTED] involving 29 anterior AMI subjects⁶. This study was conducted in two separate phases: Phase I involved 9 subjects who received SSO₂ Therapy selectively, with the hyperoxemic reperfusion provided through a guiding catheter in the left main coronary ostium at a flow rate of 100 ml/min. In Phase IA involving 20 subjects, the reperfusion was provided sub-selectively in the infarct-related artery through an infusion catheter at a flow rate of 75 ml/min. To assess ventricular function, left ventriculography was performed pre- and post-SSO₂ infusion, and regional wall motion was assessed pre-SSO₂ Therapy, during SSO₂ infusion, and at 24-hours, 1 and 3-months post-SSO₂ Therapy. SSO₂ was infused successfully in all cases. No adverse events (AEs) were documented that were related to the DownStream AO System or required accessories, or to SSO₂ Therapy administration.

Results of the Phase I/IA studies showed LV functional improvements post-AMI; mean ejection fraction (EF) improved from 48.6% to 56.0% over three months ($p < 0.001$). In addition, wall motion score index, a measure of ventricular contractility, improved significantly over three months as well. The analysis showed that these improvements in global LV functional measures were due to recovery of ventricular function in the infarct zone; regional WMSI assessments showed no change in the non-infarct zone^{7,8}. These results pointed to strong functional improvements in the heart after SSO₂ Therapy administration, with no documented adverse events that were related to the DownStream System or accessories. Results from the Phase I/IA study fixed the infusion flow rate and sub-selective delivery and served as a basis for additional clinical trials.

OYSTER-AMI Study

The clinical use of the current-generation DownStream AO System for administration of SSO₂ Therapy was examined first in the Supersaturated Oxygen in ST-Elevation Reperfused-AMI, or OYSTER-AMI study conducted on anterior STEMI patients by [REDACTED]. OYSTER-AMI examined the safety and effectiveness of SSO₂ Therapy in a 41-patient study (21 SSO₂ Therapy subjects, 20 matched-control subjects).

The non-randomized study evaluated both SSO₂ Therapy patients and a control population of case-matched subjects. Ninety (90) minutes of SSO₂ infusion was performed with hyperoxemic blood reperfusion provided via sub-selective infusion catheter into the infarct-related artery post-PCI with stent placement. Cardiac enzyme data were evaluated as an indirect measure of the extent of infarction, and LV functional recovery was evaluated by serial 2-D contrast echocardiography at six time points: immediately post-PCI (baseline), and at 24 hrs, 7 days, 1 month, 3 months, and 6 months post-procedure in both groups. LVEF and wall motion score index (16-segment model) were evaluated at each of these time points. A total of 41 subjects were examined in the OYSTER-AMI study, including 21 SSO₂ Therapy patients and 20 Control patients.

⁶ Dixon SR *et al.* Initial Experience with Hyperoxemic Reperfusion after Primary Angioplasty for Acute Myocardial Infarction. *J Am Coll Cardiol* 2002; 39(3):387-92.

⁷ Dixon SR *et al.* Early recovery of infarct zone function with hyperoxemic reperfusion after primary percutaneous transluminal coronary angioplasty. *Am J Cardiol* 2000; 86 (Suppl. 8A): 8i.

⁸ Dixon SR *et al.* Global and regional left ventricular function after intracoronary hyperoxemic perfusion with the Aqueous Oxygen system in acute myocardial infarction: TherOx study. *Circulation* 2000; 102 (Suppl.): II-386.

Results for Cardiac enzyme data showed a significant decrease in the time to peak creatine kinase (CK), measured from time from symptom onset (SSO₂ Therapy = 9.4 ± 3.4 hrs vs. Control = 14.2 ± 5.3 hrs; $p < 0.001$). The difference in CK half-life was significant as well: SSO₂ Therapy = 23.4 ± 8.9 hrs vs. Control = 30.5 ± 5.8 hrs ($p < 0.01$). These decreases observed in the SSO₂ Therapy group translate into less total CK enzyme release. These results were accompanied by functional recovery, as seen in LVEF and wall motion score index measurements. For SSO₂ subjects, the six-month relative improvement in LVEF was 28%; in contrast, the Control group only improved by 2.5% ($p < 0.01$). Similarly, relative improvements in wall motion score index from baseline to six months were 26% in the SSO₂ Therapy group as compared to 2.4% in the Control group.

The improvements in LV functional recovery, ST elevation recovery, and reduction in cardiac enzyme release observed in OYSTER-AMI suggested that SSO₂ Therapy potentially could be used effectively in a STEMI population.

6.2 AMIHOT I Clinical Trial

Background

The AMIHOT I study examined the safety and effectiveness of SSO₂ Therapy in both anterior and inferior STEMI patients undergoing successful reperfusion therapy via PCI up to 24 hours from symptom onset; 269 randomized patients were enrolled in 23 investigational sites. This Phase II study, termed the Acute Myocardial Infarction with HyperOxemic Therapy, or AMIHOT I trial, was conducted from [REDACTED]

Study Design

The AMIHOT I study objective was to determine whether the adjunctive administration of SSO₂ Therapy after PCI and stenting in a group of patients presenting less than or equal to (\leq) 24 hours from AMI symptom onset improved left ventricular function and reduced the area of infarction with no increased incidence of 30-day Major Adverse Cardiac Events (MACE) when compared to a Control group receiving PCI with stenting alone. The AMIHOT I clinical trial was prospectively designed as a randomized (1:1), controlled, multicenter trial.

The AMIHOT I study design included three co-primary effectiveness endpoints to be evaluated by standard statistical tests of superiority with the following goals:

- 5% reduction in infarct size as measured by the percent of left ventricular volume, assessed by [REDACTED] SPECT imaging at $14 (\pm 7)$ days post PTCA/stent placement.
- 0.2 unit increase in regional wall motion score index (WMSI) in the infarct zone over three months (90 ± 7 days) as evidence of left ventricular function recovery.
- ST-segment recovery as evidenced by a 50% lower ST-deviation vs. time trend curve area in the SSO₂ treatment group during the first three hours of continuous monitoring as an indicator of myocardial ischemia reversal.

The primary safety endpoint was based on the number of patients experiencing Major Adverse Cardiac Events (MACE), comprising the total incidences of death, reinfarction, target vessel revascularization, and stroke within one month (30 days) of enrollment.

Methods

Key AMIHOT I selection criteria considered patients who were diagnosed with acute myocardial infarction (AMI) and admitted to the hospital within 24 hours of symptom onset. Qualifying AMIs met specific electrocardiographic and angiographic criteria prior to randomization, including a > 1 mm ST-segment elevation as measured by ECG, and a pre-PCI/stenting angiographic TIMI score of 0, I, or II in the cardiac catheterization laboratory. Successful revascularization with PCI was required for AMIHOT I subjects, as measured by a post-procedure TIMI score \geq II. SSO₂ Therapy patients received an intracoronary 90-minute infusion of hyperoxemic blood post-PCI via infusion catheter.

Results and Conclusions

Two hundred sixty-nine (269) patients were randomized into the AMIHOT I trial, including 135 Control subjects and 134 SSO₂ Therapy subjects. A comparison of baseline demographic and clinical patient characteristics between the two randomized groups revealed no statistically significant differences that might suggest a potential bias in the clinical outcome for these patients.

Results for the Control/SSO₂ Therapy group comparisons for the three co-primary effectiveness endpoints demonstrated a nominal improvement in the test group; this nominal improvement did not achieve clinical and statistical significance when evaluating the entire study sample. However, a post hoc analysis of SSO₂ Therapy patients who were revascularized within 6 hours of AMI symptom onset and who had anterior wall infarction showed a marked improvement in all three co-primary endpoints as compared to Controls.

Infarct size results are expressed as a percentage of the left ventricle. This endpoint was established on the basis of numerous peer-reviewed publications of this biomarker that demonstrate a 5% median infarct size reduction is clinically meaningful. The use of the median in the evaluation of this endpoint is predicated on the obvious skewness (particularly due to the number of zeroes) of the data distribution typically seen in infarct size studies. The all-patient < 24-hour group showed a 2% absolute reduction in median infarct size, from 13% for Control subjects to 11% in the SSO₂ Therapy group, although this difference was not statistically significant (Wilcoxon rank-sum test; one-sided p-value = 0.3). This result did not achieve statistical significance. The infarct size reduction for anterior STEMI subjects treated within 6 hours of symptom onset showed that SSO₂ Therapy subjects exhibited a 14% absolute reduction in median infarct size from 23% in the Control group to 9% in the SSO₂ Therapy group (Wilcoxon rank-sum test; one-sided p-value = 0.04). Expressed in terms of mean data, infarct size was reduced from 17.4% in the Control group to 16.9% in the SSO₂ Therapy group for the entire study cohort, a mean reduction of 0.5%, but this statistic is less meaningful in light of the right-skewed distribution involved. In the anterior < 6 hr cohort, mean infarct size was reduced in this patient subgroup from 23.0% in the Control group to 17.3% in the SSO₂ Therapy group, a mean reduction of 5.7%. Due to the skewness of the infarct size dataset, characterized by a right-tailed distribution rather than a normal distribution, the use of the mean as a measure of central tendency for infarct size studies is uncommon.

Effectiveness results for the other two co-primary endpoints (regional wall motion score index improvement, ST area reduction) in the AMIHOT I trial were consistent with the infarct size data. The results for regional wall motion score index improvement (decrease) at 3 months (90 days), as compared to baseline, demonstrated a nominal improvement in all patients in the SSO₂ Therapy group as compared to Controls (-0.62 vs. -0.57, respectively, ANCOVA one-sided p-value = 0.24); the results were

statistically significant only when the anterior ≤ 6 hr population was examined (-0.75 vs. -0.54 for SSO₂ Therapy and Controls, ANCOVA one-sided p-value = 0.03). As seen in the infarct size measurements, the observed improvement in SSO₂ Therapy subjects versus Controls is greatest in the ≤ 6 hr anterior patient population.

ST-segment area reduction is believed to represent the continuing ischemic burden on the heart in the post-acute phase for AMI patients. Results for this biomarker obtained at 3 hours post-PCI were consistent with both infarct size and wall motion data; results were comparable for all patients (median ST area = 0 μ V-min for both groups, Wilcoxon rank-sum test one-sided p-value = 0.5) and significantly better in the anterior ≤ 6 hr patient group (median areas = 0 vs. 311 μ V-min for SSO₂ Therapy and Controls, Wilcoxon rank-sum test one-sided p-value = 0.01).

Key safety data revealed no statistically significant differences in the composite primary endpoint of one-month (30 days) Major Adverse Cardiac Event (MACE) rates between the SSO₂ Therapy and Control groups. The 30-day MACE results are presented in Table 5. MACE includes the combined incidence of death, reinfarction, target vessel revascularization, and stroke. MACE rates were quite similar in the two arms; in total, 9/134 (6.7%) subjects in the SSO₂ Therapy group and 7/135 (5.2%) subjects in the Control group experienced 30-day MACE. A formal test of non-inferiority yields a p-value = 0.02 when evaluating the null hypothesis that the SSO₂ Therapy group MACE rate is at least 8% [absolute] higher than Control group MACE rate).

Table 5. AMIHOT I Primary Safety Endpoint Evaluation (30-Day MACE)

Adverse Event	Randomization Group			
	Control (N=135)		SSO ₂ Therapy (N=134)	
	Events ¹ (n)	Pts with Events (n/N; %)	Events ¹ (n)	Pts with Events (n/N; %)
Composite 30-Day MACE ³	10	7/135 (5.2%)	11	9/134 (6.7%)
Death		2/135 (1.5%)		4/134 (3.0%)
Target Vessel Revascularization ²		3/135 (2.2%)		3/134 (2.2%)
Reinfarction		3/135 (2.2%)		3/134 (2.2%)
Stroke		2/135 (1.5%)		1/134 (0.7%)

¹Event count: count of number of unique event types experienced per patient

²AMIHOT main vessel or branches

³p-value = 0.02 (Unconditional Test of Non-Inferiority using difference of two Binomial Proportions)

In summary, although a slight trend was observed for SSO₂ Therapy patients as compared to Control subjects, the results for the entire AMIHOT I population did not achieve the requisite level of statistical significance for the three co-primary effectiveness endpoints. However, post-hoc analysis of the anterior AMI ≤ 6 hr patient cohort suggested the potential for therapeutic effectiveness in this subgroup, with improvements noted for all three effectiveness endpoints. These effectiveness results, coupled with a comparable 30-day MACE safety profile between the SSO₂ Therapy and Control groups, formed the basis of the pivotal AMIHOT II trial to confirm the validity of the AMIHOT I findings for anterior AMI ≤ 6 hr patients.

6.3 AMIHOT II Clinical Trial

Results from the AMIHOT I study demonstrated an improvement in left ventricular function and infarct size reduction in high-risk anterior AMI patients treated with PCI within six hours of symptom onset. These consistent effectiveness results, coupled with a comparable 30-day MACE safety profile between the SSO₂ Therapy and Control groups, formed the basis of a new trial (AMIHOT II) to build upon the AMIHOT I experience. The need for a validating study for these findings and the framework of the pivotal AMIHOT II trial was discussed in a meeting conducted on [REDACTED] between FDA and TherOx. Working collaboratively with FDA, TherOx proposed a randomized trial focused upon this vulnerable < 6 hr anterior AMI patient cohort, devising a Bayesian statistical trial design that enabled some degree of borrowing of primary endpoint data from the AMIHOT I trial for the analysis of the AMIHOT II trial. The AMIHOT II trial was approved under IDE number [REDACTED] in a letter from FDA dated [REDACTED].

6.3.1 Study Design and Endpoints

Study Objective

To determine whether intracoronary perfusion of hyperoxemic blood in the SSO₂ Therapy group immediately after successful PCI/stenting within 6 hours of symptom onset for the treatment of anterior acute myocardial infarction reduces the area of infarction (% left ventricle) as measured by [REDACTED] SPECT imaging at 14 days post-PCI, with no worse than a 6% (absolute) increase in the incidence of Major Adverse Cardiac Events (MACE), comprising the combined incidence of death, reinfarction, target vessel revascularization, and stroke at the latter of either 30 days post-PCI or hospital discharge, when compared to a Control group receiving PCI/stenting alone.

Primary Effectiveness Endpoint

Reduction in infarct size as measured by percent of left ventricular volume, assessed by [REDACTED] SPECT imaging at 14 days post PCI/stenting, as an indicator of treatment effectiveness by test of superiority.

Primary Safety Endpoint

A composite safety endpoint based on the incidence of death, reinfarction, target vessel revascularization, and stroke occurring less than or equal to one month (30 days) after enrollment or until hospital discharge, whichever is later. The composite safety endpoint was evaluated by a test of non-inferiority within a safety delta less than or equal to 6.0%. In addition, all Serious Adverse Events whether they are determined to be device-related or not were investigated and reported as part of the overall evaluation of device safety.

Endpoint Evaluation

Infarct size was measured by [REDACTED] SPECT imaging at 14 (\pm 7) days by the independent [REDACTED] at the [REDACTED]. In addition, SSO₂ Therapy was required to show non-inferiority in the incidence of 30-day Major Adverse Cardiac Events (MACE) within a 6.0% safety delta. MACE is a composite endpoint that includes the combined incidence of death, reinfarction, target vessel revascularization, and stroke. Primary safety endpoint adjudication was performed by the independent Clinical Events Committee (CEC).

The AMIHOT II trial had a Bayesian statistical design that allows for a flexible degree of borrowing of data from the previously completed AMIHOT I trial, where greater similarity of the studies would allow for a higher degree of borrowing of information. The Bayesian statistical model was pre-specified; the model required that the posterior probability for success to be greater than 95.0% for both the efficacy and safety endpoints. An unbalanced randomization ration of 2.8:1 (SSO₂ Therapy: Control) was utilized to satisfy acceptable power requirements.

6.3.2 Study Management

Study Committees

The AMIHOT II study utilized four study committees. The Executive and Steering Committees reviewed and approved the study design, and were responsible for general study oversight. Two additional oversight groups were employed in the AMIHOT II clinical trial, the Data and Safety Monitoring Board (DSMB) and the Clinical Events Committee (CEC). The DSMB was responsible for reviewing the aggregate study results to ensure that patient welfare and safety were being maintained. The DSMB made recommendations on study continuation at each of their meetings. The Clinical Events Committee reviewed and adjudicated all adverse events. In addition, the CEC adjudicated all Primary Safety Endpoints, non-MACE adverse events and Steering Committee Success Endpoints. An independent third party [REDACTED] managed both the CEC and DSMB. The committees were directed to submit all correspondence through [REDACTED]. Moreover, all Sponsor correspondence to the CEC or DSMB was directed through [REDACTED] to maintain a level of independence between these oversight committees and the sponsor.

Independent Core Laboratories

The AMIHOT II clinical trial utilized three independent core laboratories for data analysis and interpretation. The core laboratories were blinded to the subjects' randomization assignment and clinical outcome. These three core laboratories were:

- [REDACTED] provided infrastructure, expertise, and independent analysis of index procedure angiograms for the AMIHOT II clinical trial. The [REDACTED] developed the rationale, measurement, and data assessment for AMIHOT II cath lab angiographic information.
- [REDACTED] provided an independent interpretation of AMIHOT II ST-Segment monitoring data obtained with the NorthEast 12-lead integrated circuit ST monitors. The AMIHOT II clinical protocol required that all study subjects receive continuous 24 hours ST-Segment monitoring.
- [REDACTED] conducted independent analysis of the infarct size primary endpoint that was measured using [REDACTED] SPECT nuclear imaging. SPECT imaging data interpretation and calculations were used to evaluate infarct size reduction as a percentage of left ventricular volume in both groups. The nuclear scan was performed fourteen (14) days (± 7 days) post-index procedure.

6.3.3 Patient Selection Criteria and Randomization

Study Inclusion Criteria

Pre-PCI:

1. Patient must be ≥ 18 years of age
2. AMI must be anterior
3. Patient is experiencing clinical symptoms consistent with anterior AMI of < 6 hour duration from time of symptom onset until admission to the emergency room
4. Complete medical history, history of AMI, previous coronary interventions, list of medications given within last 24 hours
5. 12-lead qualifying ECG criteria: Anterior infarction (ST-segment elevation ≥ 1 mm in two or more contiguous leads between V1 and V4 or new left bundle branch block (LBBB) with documentation of LAD system culprit lesion)
6. Patient provides written, Informed Consent
7. Patient and his/her physician agree to all required follow-up procedures and visits
8. Women of childbearing potential who have a negative pregnancy test (applies to female patients only)

Angiographic Inclusion Criteria: Evaluated after the subject provided signed Informed Consent but prior to randomization:

9. Based on coronary anatomy, PCI is indicated for culprit lesion with anticipated use of an Intra-Coronary Stent
10. TIMI 0, I, or II flow is present on the initial angiographic injection of the infarct-related artery
11. Successful angioplasty as documented by $< 50\%$ diameter residual angiographic stenosis within and associated with the culprit lesion and \geq TIMI II flow and no major complications such as perforation or shock
12. Documented time of reperfusion is ≤ 6 hours from the documented time of symptom onset

Exclusion Criteria

Pre-PCI:

13. Patients with ventricular pseudoaneurysm, VSD, or papillary muscle rupture.
14. Absolute contraindications to anticoagulant therapy, including hemorrhagic diathesis or thrombocytopenia
15. Systemic Arterial pO_2 is < 80 mmHg with supplemental oxygen
16. Placement of an intra-aortic balloon pump (IABP)
17. Patient has had coronary bypass surgery during the 30 day period preceding PCI
18. Severe known cardiac valvular stenosis or insufficiency, pericardial disease, or non-ischemic cardiomyopathy
19. Patients requiring cardiopulmonary resuscitation for > 10 minutes
20. Cardiogenic shock (SBP < 80 mm Hg for more than 30 minutes unresponsive to fluids or requiring intravenous pressors or placement of an IABP)
21. Expected survival of less than 6 months due to non-cardiac condition

22. Current participation in other investigational device or drug trials that have not finished the primary efficacy endpoint follow-up parameters
23. Patient has had a hemorrhagic stroke during the 6 month period preceding PCI
24. Physician discretion regarding unacceptability for enrollment

Angiographic Exclusion Criteria: Evaluated after the subject provided signed Informed Consent but prior to randomization:

25. Any proximal coronary diameter stenosis > 40 % that would restrict native flow with the infusion catheter in place
26. Infarct-related vessels that are either saphenous vein grafts and/or small second order coronary vessels that do not supply significant areas of myocardium
27. Presence of a non-stented coronary dissection upon completion of the PCI procedure
28. Unprotected left main diameter stenosis > 60%
29. Severe target vessel calcification or tortuosity
30. Multi – vessel disease that in the judgment of the investigator is best treated with emergent or urgent CABG or additional PCI within 30 days
31. In the investigator's opinion, the target vessel is unsuitable for either placing the infusion catheter or treatment with PCI

Randomization

Patients who met the study's selection criteria were randomized into the AMIHOT II clinical trial after successful enrollment screening and providing Informed Consent. Patients were randomly assigned, on a 2.8:1 basis, to either the SSO₂ Therapy group or the Control group (PCI/stenting). The unbalanced randomization split was derived from the requirements of the Statistical Analysis Plan. Patients were stratified on the basis of time to reperfusion (0-3 hours or >3-6 hours) and lesion location (proximal or non-proximal); therefore, the randomization was performed separately for each of the 4 combinations of these two variables. The TherOx AMIHOT II clinical trial utilized an automated randomization service, termed the [REDACTED] operated by the independent [REDACTED] for all participating investigational sites. The randomization procedure was designed and validated in accordance with the randomization plan outlined in the Statistical Analysis Plan.

6.3.4 Study Procedures

Baseline

The following baseline examinations and tests were performed on patients who agreed to participate in the study by signing the approved Informed Consent:

- Medical History and physical examination
- 12 Lead ECG and application of 24-Hour Holter Monitor
- Administration of study medication
- Cardiac Enzymes
- Clinical Chemistry, Hematology, and Liver Panel

Procedure Medications

All enrolled patients were to receive the following protocol required medications:

- Aspirin – 325 mg soluble aspirin given in E.R. or prior to catheterization
- Clopidogrel 300 – 600 mg p.o., then 75 mg p.o.q. daily for a minimum of 1 month in all patients undergoing PCI/stent procedure. (May give up to four hours post procedure.)
- Intravenous Heparin
- Low-flow nasal oxygen (3-5 l/min.) or oxygen mask (5-10 l/min.) to maintain systemic arterial $pO_2 > 80$ mmHg

Glycoprotein IIb/IIIa Inhibitors

Use of platelet IIb/IIIa reception inhibitors during the AMIHOT II study was allowed at the physician's discretion, consistent with observing the standard of care in AMI treatment with PCI. The use (or non-use) of these agents, along with the categorical time of administration, was documented for each subject.

Heparin Management

The AMIHOT II study utilized heparin-only per protocol. The target Activated Clotting Time (ACT) was ≥ 250 seconds for all patients receiving SSO₂ Therapy. ACT was measured and recorded as a baseline reading prior to SSO₂ Infusion and subsequently every 30 minutes during SSO₂ Therapy administration.

Coronary Angiography

After arriving at the catheterization laboratory, patients were prepared for PCI according to standard hospital procedures. When performing coronary angiography, the entire distal target vessel with capillary and collateral flow was to be shown to allow for assessment of pre-procedure TIMI blush score. Left ventriculography was to be performed in the right anterior oblique view and two consecutive sinus beats were to be available for analysis. Pre and post-PCI angiographic data were sent to the angiographic core laboratory for analysis.

PCI/Stenting Procedure

Only commercially available bare metal or drug-eluting stents were used in AMIHOT II patients. Following successful PCI/stenting, with all angiographic inclusion criteria satisfied and in the absence of any angiographic exclusion criteria, patients were randomized.

SSO₂ Therapy Procedure

AMIHOT II investigators had the option of utilizing either one or two arterial access sheaths for SSO₂ Therapy. When the protocol-recommended coaxial approach was employed, blood was withdrawn from the femoral artery via the annular space between the arterial sheath and the guiding catheter. This configuration required a 2-French (F) size difference between the sheath and guiding catheter to enable blood withdrawal at the 72 ml/min flow rate.

The protocol allowed for an alternative to the coaxial approach, termed the contralateral approach. With contralateral access, a second arterial access site was utilized in the other femoral artery. This configuration allowed the investigator to utilize a smaller sheath on the guiding catheter side and a 5F or 6F introducer sheath in the contralateral femoral artery.

As part of approved IDE Supplement [REDACTED], the [REDACTED] infusion catheter was qualified as an alternative to the [REDACTED] for SSO₂ Therapy delivery. The guide catheter and arterial sheath size requirements are different for these two catheters. The [REDACTED] infusion catheter requires a 7F guiding catheter, and thus a 9F arterial sheath for coaxial setup. The [REDACTED] catheter has a slightly smaller outer diameter (O.D.) than the [REDACTED], requiring a minimum 6F guiding catheter and 8F arterial sheath for coaxial setup.

The following steps were performed during SSO₂ Therapy administration, after completion of successful PCI:

Catheter Placement and Patient Set-Up

The guiding catheter was placed at the ostium of the infarct-related artery (IRA). After positioning of the guiding catheter, the infusion catheter was advanced over the guidewire into the IRA. Positioning of the infusion catheter within the IRA was at the discretion of the investigator for optimum infusion, but not distal to the stent. Prior to initiation of SSO₂ Therapy, the investigator removed the guidewire and rechecked the infusion catheter position under fluoroscopy. SSO₂ Therapy was initiated in the cardiac catheterization laboratory. Per the device IFU, the recommended procedure was to complete SSO₂ Therapy administration in the cath lab, but investigators did have the option of transferring the patient to an appropriate holding area, or the Coronary Care Unit (CCU), after initiating the infusion.

DownStream AO System and AO Cartridge Use

The AO System and AO Cartridge are set up per the Instructions for Use and Operators Manual. Prior to SSO₂ Therapy initiation, baseline systemic arterial pO₂ and blood pressure data were recorded. The trained system operator manually adjusted the AO System pO₂ range via the touch-screen display after the baseline systemic arterial pO₂ value was available. During the 90-min infusion time, physiological parameters such as blood pressure, systemic arterial pO₂, and heart rate/rhythm were recorded at 30-minute intervals. The pO₂ range on the AO System was updated as required based on changes in the patient's systemic arterial pO₂ level.

SSO₂ infusion was discontinued after 90 minutes, and normoxic blood continued to circulate through the circuit until the user manually shut down the system, typically within 1-2 minutes after hyperoxemic infusion had stopped.

SSO₂ Therapy Device Performance

Device performance information, including date and time of SSO₂ Infusion, the number of cartridges used, cartridge tracking information, SSO₂ Infusion time, and system operating parameters were recorded for every AMIHOT II clinical case. These data were recorded on discrete log files maintained in the AO System memory. Log files were analyzed by TherOx Engineering per established procedures and protocols for each cartridge utilized during the AMIHOT II study.

In-Hospital Procedures

eECG Monitoring

A continuous 24-hour 12-lead ECG Holter Monitor was placed on patients in the immediately after obtaining Informed Consent. The monitor was removed after 24 hours and recorded data were sent to

the eECG Core Laboratory for analysis. Other ECGs were performed at any time during the index hospitalization as warranted for clinical management.

Cardiac Enzymes, Clinical Chemistry, and Hematology

Cardiac enzymes (CK, CK-MB and troponins) were drawn at baseline, 8, 16 and 24 hours post-PCI. Clinical chemistry and hematology and liver panel results were obtained at baseline and 24-hours post-PCI.

Patient Management and Hospital Discharge

No restrictions were placed upon the standard-of-care procedures administered by participating investigational sites in the post-PCI in-hospital period. After leaving the cath lab, patients were sent to the CCU, Step-Down Unit, or Coronary Care Floor at the investigator's discretion; their transfer location was recorded. All medications administered to patients during their hospital stay were recorded. Timing of hospital discharge was at the investigator's discretion for each individual patient.

SPECT Imaging

A resting scan was performed at 14 days (± 7 days) post-PCI and results sent to the core lab. A phone call was encouraged from the site Clinical Coordinator prior to the scheduled scan date to ensure patient compliance.

30-Day Follow Up

A clinical follow-up visit was required on or after day 30 to be completed no later than day 45 post-PCI per protocol requirements. The purpose of this follow up visit was to assess the patient's health status and to assess definitively whether the patient experienced a MACE event within the 30-day primary endpoint window. If the patient was hospitalized longer than 45 days, the follow-up was to be completed at the time of hospital discharge.

6- and 12-Month Patient Surveys

A telephone survey was obtained at the 6-month and 12-month intervals post-PCI (± 30 days) to ascertain patient status.

Table 6 summarizes the required schedule for AMIHOT II study assessments.

Table 6. AMIHOT II Study Assessments

	H&P/Consent	24-Hour ECG	Angiogram	Blood Labs	Blood Pressure	Arterial Blood Gas (ABG)	Heart rate/rhythm	ACT	Sestamibi Imaging	Follow-up Visit	Telephone Survey
Enrollment Screening/ Baseline	♥	♥				♥					
Pre-PCI/Stent		C O N T I N U O U S	♥	♥				♥			
Post PCI/Stent			♥	♥							
30 min. SSO ₂ Infusion											
60 min. SSO ₂ Infusion											
90 min. SSO ₂ Infusion											
8 hours ± 2 hours					♥						
16 hours ± 2 hours					♥						
24 hours ± 2 hours					♥						
14 days ± 7 days									♥		
30 days +15 days										♥	
6 months ± 30 days										♥	
12 months ± 30 days										♥	

6.3.5 Study Enrollment

Figure 6 depicts a patient accountability flow chart for the AMIHOT II study.

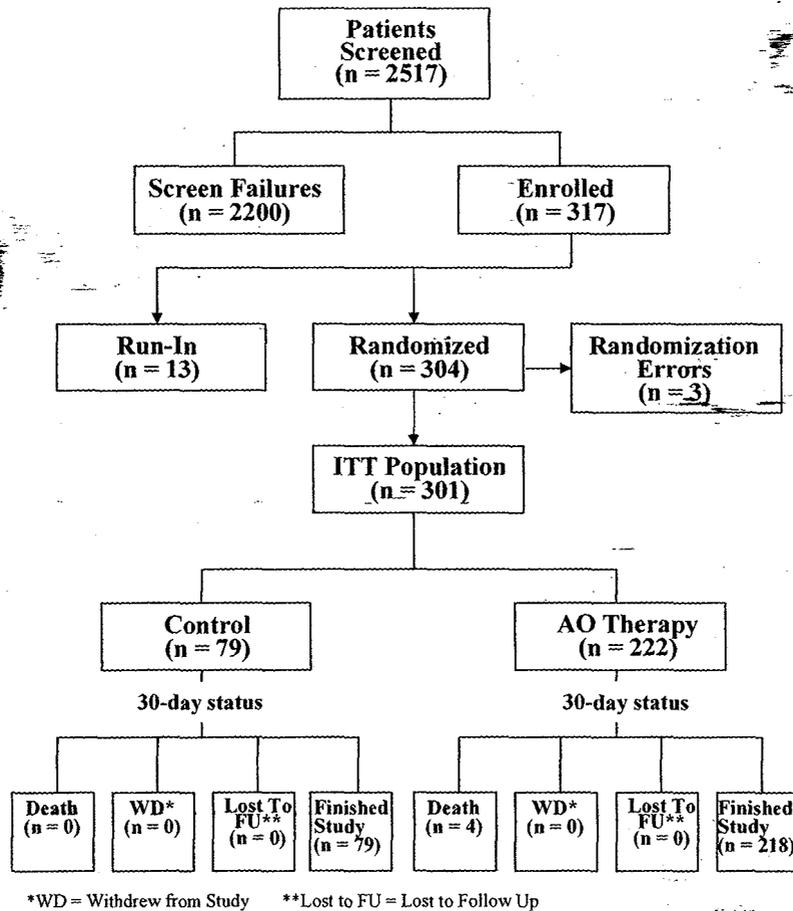


Figure 6. AMIHOT II Patient Accountability Flow Chart

As shown in the figure, a total of 2,517 subjects were screened for potential enrollment in the AMIHOT II trial. Of this total, 2,200 subjects were classified as screen failures, failing to meet the eligibility criteria for the study. A total of 317 subjects were enrolled, including 301 randomized ITT subjects, 13 non-randomized run-in cases, and three subjects randomized in error. The ITT analysis sample consists of 79 Control subjects and 222 SSO₂ Therapy subjects. Unless otherwise specified, the data tables presented in this discussion of AMIHOT II trial results are based upon the ITT sample.

The study's endpoint assessment was performed at 30 days post-procedure. At this time point, the status of all 79 Control and 222 SSO₂ Therapy subjects was known. The Control group had zero (0) deaths, zero (0) patients lost to follow up, and zero (0) patient withdrawals, for a total of 79 subjects who finished the study. The SSO₂ Therapy group had four (4) deaths, zero (0) patients lost to follow up, and zero (0) patient withdrawals, for a total of 218 subjects who finished the study.

A total of 21 participating investigational sites enrolled patients into the study, including [REDACTED] US and OUS sites. A total of 123/301 (40.9%) subjects were enrolled at US sites, as compared to 178/301 (59.1%) enrolled at OUS sites.

6.3.6 Pre-Randomization (Baseline) Data

A review of baseline patient information demonstrates comparability between the SSO₂ and Control study groups. **Tables 7 and 8**, shown below, display group data for baseline patient characteristics and cath lab procedural results. Continuous data are expressed in terms of the median values. Angiographic data were evaluated by an independent core laboratory, the [REDACTED]

Table 7. AMIHOT II Baseline Patient Characteristics

	Control Group (N=79)	SSO ₂ Therapy Group (N=222)	p-value ¹
Age (years)	59	60	0.28
Male	87.3%	77.9%	0.07
Diabetes	13.9%	16.2%	0.63
Hypertension	45.6%	46.9%	0.85
Hyperlipidemia	43.0%	45.1%	0.76
Current Smoking	43.0%	38.3%	0.46
Prior Myocardial Infarction	8.9%	9.0%	0.97
Prior PCI of target vessel	10.1%	5.9%	0.20

¹Mann-Whitney test for continuous data or Chi Square for frequencies

Table 8. AMIHOT II Cardiac Catheterization Laboratory Procedural Results (pre-randomization)

	Control Group (N=79)	SSO ₂ Therapy Group (N=222)	p-value*
Time intervals (min):			
Symptom Onset to ER arrival	90	110	0.39
Door to Balloon	75	77	0.63
Symptom Onset to reperfusion	171	195	0.42
Infarct lesion location:			0.64**
Proximal LAD ¹	46.8%	47.7%	
Mid LAD	51.9%	49.1%	
Distal LAD	0%	2.3%	
Diagonal branch of LAD	1.3%	0.9%	
LVEF %	40	40	0.53
Stent implanted	97.5%	99.1%	0.28**
Glycoprotein IIb/IIIa inhibitor use	64.6%	68.0%	0.57
Rescue PCI (failed thrombolytics)	8.9%	5.0%	0.27**
TIMI flow pre-PCI:²			0.07
0/1	69.9%	75.5%	
2	13.7%	17.1%	
3	16.4%	7.4%	
TIMI flow post-PCI:²			0.23**
0/1	2.8%	1.4%	
2	4.2%	10.2%	
3	93.0%	88.4%	

¹LAD = left anterior descending coronary artery

²as determined by independent angiographic core laboratory

*Mann-Whitney test for continuous data or Chi Square for frequencies unless otherwise noted

**Exact Chi-Square test

Table 8 shows that the study groups were well matched in terms of cath lab procedural characteristics as well. As shown in the table, median time interval data for door-to-balloon, symptom onset to emergency room arrival, and symptom onset to reperfusion times are nominally longer for the SSO₂ Therapy group. Other procedural characteristics, including infarct lesion location, baseline left ventricular ejection fraction (LVEF), and stent implantation showed good comparability with no statistical differences between the study groups. The incidence of rescue PCI cases was nominally higher in the Control group.

An analysis of pre-PCI TIMI flow in the infarct vessel showed slightly better flow in the Control group, as measured by the angiographic core laboratory. Post-PCI TIMI flow numbers were nominally better in the Control group, but this finding was not statistically significant.

6.3.7 Laboratory Data

Baseline and 24-hour laboratory data were collected for the study groups. These data included Complete Blood Count (CBC) without differential, clinical chemistry, hemodynamic information, liver panel enzymes, and cardiac enzymes. With respect to these many parameters, the groups were comparable at baseline and 24 hours. No clinical laboratory data were collected to suggest a deleterious impact from the SSO₂ Therapy infusion upon these parameters. A larger drop in hematocrit was observed in the SSO₂ Therapy group than the Control group at 24 hours compared to baseline, a change

attributed to greater fluid loading, extra blood loss from discarding the AO Cartridge (and multiple cartridge use), and a potentially higher rate of minor bleeding. Bleeding will be discussed in **Section 6.3.10, Safety Data.**

6.3.8 SSO₂ Therapy Procedural Data

Data and discussion are presented for SSO₂ Therapy arterial access information, device usage parameters, including intra-procedural time intervals, hemodynamic and laboratory data during SSO₂ Therapy administration, and device failures.

SSO₂ Therapy Arterial Access Data

During the AMHOT II study, two catheters were used to perform the SSO₂ infusion. The [REDACTED] infusion catheter was the only qualified infusion catheter for SSO₂ Therapy at the beginning of the study. When enrollment was approximately two-thirds completed, an alternative to the [REDACTED] was introduced, the TherOx [REDACTED] infusion catheter. The [REDACTED] catheter has a slightly smaller OD than the [REDACTED], enabling the use of a smaller 6F guide catheter as compared to the 7F guide necessary for the [REDACTED]. With the coaxial access configuration, the [REDACTED] therefore enabled the use of an 8F sheath as compared to a 9F sheath for the [REDACTED]. As a result, physicians chose the coaxial access option more often after the introduction of the [REDACTED] totaling 72/75 (96.0%) cases as compared to only 88/147 (59.9%) for the [REDACTED] (see **Table 9**). These data are significant because SSO₂ Therapy group access site bleeding events track with the choice of infusion catheter (due to the choice of two arterial access sites vs. one), as discussed in **Section 6.3.10, Safety Data.**

Table 9. SSO₂ Therapy Procedure: Arterial Access Data

	SSO ₂ Therapy Group (n=222) (n/N; %)
Guide Catheter Introducer Sheath	
Sheath Size:	
7F	55/222 (24.8%)
8F	48/222 (21.6%)
9F	118/222 (53.2%)
10F	1/222 (0.5%)
Infusion Catheter	
[REDACTED]	147/222 (66.2%)
TherOx [REDACTED]	75/222 (33.8%)
Draw Sheath Approach:	
Coaxial	160/222 (72.1%)
w/ [REDACTED]	88/147 (59.9%)
w/ TherOx [REDACTED]	72/75 (96.0%)
Contralateral femoral artery	62/222 (27.9%)
w/ [REDACTED]	59/147 (40.1%)
w/ TherOx [REDACTED]	3/75 (4.0%)

Cartridge Usage and SSO₂ Infusion Time

Table 10 shows SSO₂ Therapy procedural data and DownStream AO Cartridge device usage information.

Table 10. SSO₂ Therapy Procedure: Device Usage Information

	SSO ₂ Therapy Group (n=222)
Time Intervals	
Reperfusion to initiation of SSO₂ Therapy (min)	
(mean ± SD) (n)	57 ± 28.9 (n=214)
(median ± IQR) (range)	52 ± 28 18 – 240
Symptom Onset to initiation of SSO₂ Therapy (min)	
(mean ± SD) (n)	266 ± 77.5 (n=214)
(median ± IQR) (range)	250 ± 106 101 – 540
SSO₂ Infusion Time (min)	
0-59 min infusion time (n/N; %)	25/222 (11.3%)
60-89 min infusion time (n/N; %)	9/222 (4.1%)
90 min infusion time (n/N; %)	165/222 (74.3%)
> 90 min infusion time (n/N; %)	23/222 (10.4%)
Number of Cartridges Used¹ (n/N; %)	
0	1/222 (0.5%)
1	150/222 (67.6%)
2	48/222 (21.6%)
3	20/222 (9.0%)
4	3/222 (1.4%)
SSO₂ Therapy Delivery Location² (n/N; %)	
CCL	152/216 (70.4%)
Holding area	5/216 (2.3%)
CCU	53/216 (24.5%)
Other	6/216 (2.8%)
Cartridge-Level Data	
AO Cartridge Usage Outcome	
Number of cartridges exposed to patient contact ¹ (N)	318
Completed 90-min treatment (n/N; %)	181/318 (56.9%)
Shutdown prior to 90-min completion (n/N; %)	137/318 (43.1%)
Reasons for shutdown: (n/N; %)	
Cartridge loading	42/137 (30.7%)
Infusion catheter set-up	39/137 (28.5%)
Priming sequence	11/137 (8.0%)
Tube set handling	19/137 (13.9%)
Device Failure (cartridge or system)	3/137 (2.2%)
User initiated shutdown pre-90 min	23/137 (16.8%)

¹Defined as number of blood-wetted cartridges

²All SSO₂ Therapy procedures were initiated in the CCL; CCL = Cardiac catheterization laboratory

As seen in **Table 10**, the median time delay from reperfusion of the target vessel to initiation of SSO₂ Therapy was 52 minutes. **Table 10** also displays SSO₂ infusion time data. The protocol-prescribed infusion time was 90 minutes. The AO System is programmed to deliver this time duration of infusion with a single cartridge. However, during the AMIHOT II study, a total of 34/222 (15.3%) SSO₂ Therapy patients received less than 90 minutes infusion time. 25/222 (11.3%) SSO₂ Therapy patients received less than 60 minutes of infusion time. The majority of the 23 patients receiving more than 90 minutes infusion time received 91-95 minutes total, using multiple cartridges.

Causes of Premature DownStream AO System Shutdown

The system is equipped with an integrated hardware Safety Interlock that stops therapy if operating parameters such as blood flow rate or perfusion pressure go out of range. If the system shuts down, the tube set is isolated from the patient by automated clamps, and a new cartridge must be installed to re-start the procedure. A total of 71/222 (32.0%) SSO₂ Therapy subjects had multiple cartridge use (see **Table 10**). In total, 318 AO Cartridges were exposed to blood contact during the study. 181/318 (56.9%) were used to complete the 90-min SSO₂ infusion. A total of 137/318 (43.1%) experienced a shutdown prior to the 90-min infusion completion.

The categorical reasons for system shutdowns are shown in **Table 10**. These reasons were determined by analysis of system log file data by TherOx Engineering, and are overwhelmingly due to use errors. The most common reasons for premature system shutdown were related to cartridge loading, totaling 42/137 (30.7%) shutdowns. Infusion catheter set-up issues, typically involving the over-tightening of a [REDACTED] adapter onto the infusion catheter, were the second-most-common shutdown cause (39/137; 28.5%). Priming sequence errors accounted for 11/137 (8.0%) premature shutdowns. The net consequences of these three common types of use errors were three-fold. First, these errors necessitated the use of multiple cartridges. Second, time delays were introduced that postponed the delivery of SSO₂ Therapy in the earliest possible timeframe post-AMI. Lastly, the priming volume of blood in each cartridge is approximately 60 ml; because this volume was discarded as per the system IFU, additional blood loss was incurred with each cartridge use.

The second general category of AO System shutdowns occurs during the course of the hyperoxemic infusion. As shown in **Table 10**, the most common shutdown cause of this type is user-initiated shutdown. The next most common shutdown cause after SSO₂ Therapy has been initiated successfully is tube set handling (e.g. inadvertent kinking/constriction of the tube set), accounting for 19/137 (13.9%) premature shutdowns. Lastly 3/137 (2.2%) shutdowns were caused by device failures during clinical cases. Two system failures and one cartridge failure occurred during clinical cases; none of these device failures either caused or had the potential to cause patient injury. The result of these failures was a failure-to-treat.

SSO₂ Therapy Intra-Procedural Laboratory Assessments

Table 11 displays hemodynamic and laboratory data obtained at 30-min intervals during SSO₂ Therapy administration, including blood pressure measurement, heart rate and rhythm, arterial pO₂ information, and activated clotting time (ACT). The results displayed in **Table 11** demonstrate that patient vital signs and systemic arterial pO₂ were stable from the pre-infusion time point through the completion of SSO₂ Therapy administration.

Table 11. Hemodynamic and Laboratory Data During SSO₂ Therapy¹

	Post-Stent (pre-infusion)	30 min	60 min	90 min
Systolic BP (mmHg)				
(mean ± SD) (n)	124.9 ± 17.4 (n=221)	125.2 ± 17.3 (n=201)	128.0 ± 19.8 (n=196)	126.4 ± 17.6 (n=192)
(median ± IQR)	125 ± 23	124 ± 23	125 ± 25	123 ± 20
(range)	76 – 180	76 – 175	70 – 186	90 – 182
Diastolic BP (mmHg)				
(mean ± SD) (n)	78.5 ± 12.9 (n=221)	80.7 ± 13.7 (n=201)	82.1 ± 13.8 (n=196)	80.4 ± 13.9 (n=192)
(median ± IQR)	80 ± 15	80 ± 17	80 ± 18.5	80 ± 19
(range)	41 – 120	38 – 119	50 – 124	41 – 119
Heart Rate (bpm)				
(mean ± SD) (n)	76.5 ± 15.7 (n=221)	72.9 ± 12.9 (n=201)	72.5 ± 12.5 (n=196)	72.6 ± 11.8 (n=192)
(median ± IQR)	75 ± 17	72 ± 15	71 ± 15	71 ± 14
(range)	34 – 187	46 – 110	40 – 124	41 – 109
ABG data				
Systemic arterial pO₂ (mmHg)				
(mean ± SD) (n)	140.5 ± 72.4 (n=221)	140.7 ± 61.1 (n=200)	144.0 ± 57.8 (n=196)	139.9 ± 51.3 (n=187)
(median ± IQR)	114.7 ± 74.7	125.1 ± 64.6	133.3 ± 69.2	127.5 ± 62.0
(range)	34.5 – 460.0	53.0 – 452.0	51.0 – 403.0	63.0 – 378.0

¹If multiple assessments were made at a given time point, the last assessment was used for data analysis. If pre-infusion data were missing, screening data were used to replace the missing values (if available).

6.3.9 Effectiveness Endpoint Results

6.3.9.1 Primary Endpoint Results, Statistical and Clinical Significance

The primary effectiveness endpoint of the study required the demonstration of superiority of infarct size reduction with SSO₂ Therapy, as compared to patients receiving PCI with stenting alone. The surrogate endpoint established for this study was infarct size measurement by XXXXXXXXXX SPECT imaging at 14 (±7) days post-procedure. This endpoint was established on the basis of numerous peer-reviewed publications of this biomarker that demonstrate a 5% median infarct size reduction is clinically meaningful. The use of the median in the evaluation of this endpoint is predicated on the obvious skewness (particularly due to the number of zeros) of the data distribution typically seen in infarct size studies. The study population included qualified subjects who experienced anterior acute myocardial infarction revascularized by means of PCI with stenting within 6 hours of symptom onset. Results showed that median infarct size was reduced from 26.5% of the left ventricle in the Control group to 20.0% in the SSO₂ Therapy group in the AMIHOT II study, an absolute median reduction of 6.5%.

At first glance, this effect size appears smaller than the median reduction observed in the AMIHOT I study anterior < 6 hr AMI cohort, exhibiting a median reduction of 23% to 9% between the Control and SSO₂ Therapy groups. However, due to the smaller sample sizes involved (~ 50 per group) and gaps in the center of the distribution, smoothed median scores of 24% and 17.5% are a better representation of these AMIHOT I data, and reflect a 6.5% absolute median reduction. This reduction is consistent with the effect observed in the AMIHOT II trial.

Alternatively, mean infarct size was reduced in the AMIHOT II study from 27.1% in the Control group to 23.2% in the SSO₂ Therapy group, a mean reduction of 3.9%, but this statistic is less meaningful in light of the right-skewed frequency distribution involved.

A pre-specified hierarchical Bayesian model was used to evaluate the infarct size data for statistical significance, and incorporated results from the AMIHOT I study as well. To account for the skewness of the data, a log-transformed scale was used. Differences of means on the log-transformed scale correspond roughly to differences in median on the original scale; this correspondence is exact if the log-transformed data are truly normally distributed. The model considered all AMIHOT I infarct size data, modeled in four categories: anterior/non-anterior wall infarction and time to reperfusion less than or equal to, or greater than, 6 hours. The model allowed for a flexible amount of pooling of the AMIHOT I infarct size data, with a greater degree of pooling if the AMIHOT II study results were consistent with those observed in AMIHOT I. Using this model, the Bayesian posterior probability of superiority is 96.9%, thus satisfying the pre-established efficacy endpoint.

The AMIHOT II study was neither designed nor powered to detect differences in mortality. However, the medical literature indicates that infarct size reduction is correlated with clinical endpoints. Published clinical evidence supports a median infarct size difference of 5.0% as clinically meaningful, in terms of correlation with ultimate reduction in late (> 30 days) mortality. These studies are the basis for utilizing  SPECT measurement of infarct size as a valid surrogate endpoint in clinical trials. Most of the evidence supporting the validity of this endpoint is summarized in two reviews^{9,10}; a few key details are presented here for emphasis.

Three published studies have shown that sestamibi infarct size is associated with a difference in late patient mortality. The first of these¹¹ studies reported two-year follow-up in 274 patients at the . The measured discharge infarct size was quite small with a median of 12% to the left ventricle. Despite a low two-year mortality rate of 3%, sestamibi infarct size was highly associated with both overall mortality (Chi-squared = 8.66, p=0.003) and cardiac mortality (Chi-squared = 11.89, p<0.001). A separate multicenter study of 249 patients¹² also showed a similar significant association between sestamibi infarct size at discharge and one-year mortality. In addition, in a larger population of 1,164 patients in the CORE trial¹³ infarct size substudy, six-month mortality was significantly related to infarct size (Chi-squared = 9.1, p=0.03). In the "overlap group" of 753 patients who also underwent ejection fraction measurements, the odds ratio for infarct size for six-month mortality was 1.033, i.e., for each one percent increase in infarct size, the odds of mortality in the next six months were 1.033 times higher. A 5% increase in median infarct size would therefore mean that the odds of six-month mortality were $(1.033)^5 = 1.176$ times higher. A patient with an infarct size that was greater by 5% of the left ventricle would therefore have a 17.6% greater odds of dying in the next six months.

⁹ Gibbons, RJ *et al.* Myocardium at risk and infarct size after thrombolytic therapy for acute myocardial infarction: implications for the design of randomized trials of acute intervention. *J Am Coll Cardiol* 1994; 24:616-23.

¹⁰ Gibbons RJ *et al.* The quantification of infarct size. *J Am Coll Cardiol* 2004; 44:1533-42.

¹¹ Miller TD *et al.* Infarct size after acute myocardial infarction measured quantitative tomographic ^{99m}Tc sestamibi imaging predicts subsequent mortality. *Circulation* 1995; 92:334-41.

¹² Miller TD *et al.* Technetium-99m sestamibi infarct size predicts mortality. *Am J Cardiol* 1998; 81:1491-3.

¹³ Burns RJ *et al.* for the CORE study investigators: The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. *J Am Coll Cardiol* 2002; 39:30-6.

The sensitivity of the study results to the choice of the pre-specified Bayesian model and other parameters was evaluated using a series of alternative Bayesian models. The results of these analyses showed that the posterior probability of superiority was $> 95.0\%$ for every case tested, and that the primary conclusion that SSO₂ Therapy reduces infarct size in the AMI population under study is robust. The magnitude of the treatment effect also exceeds the benchmark for clinical significance of 5% median infarct size reduction that has been established in the clinical literature.

Additional information is provided below for a more detailed discussion of the AMIHOT II infarct size results, the Bayesian models used to evaluate infarct size reduction, and the magnitude of the treatment effect.

6.3.9.2 AMIHOT II Infarct Size Results

Results are shown in **Table 12** for AMIHOT II infarct size results. This table represents a secondary analysis of data based upon a frequentist paradigm. Because the AMIHOT II study was designed and powered within the context of Bayesian hierarchical modeling of both AMIHOT I and II data, evidence of superiority was neither required nor expected from AMIHOT II data alone. In all, 72/79 (91.1%) Control subjects and 209/222 (94.1%) SSO₂ Therapy subjects had SPECT scans that were submitted to the core laboratory and were of sufficient quality to permit infarct size measurement. The results shown in **Table 12** are based on these evaluable data.

Infarct size results are displayed in **Table 12** as median \pm IQR for all subjects, and for key patient subsets of interest. Patients were categorized for the purposes of exploring the effects of time to reperfusion, infarct location within the target LAD vessel, age, gender, prior MI, diabetes, pre- and post-PCI TIMI flow grade, baseline LVEF, stent type (BMS or DES), and geographic location (US/OUS). Two-sided p-values were calculated for individual comparisons using the Mann-Whitney test; interaction p values for patient subsets were calculated using two-way ANOVA with infarct size results expressed on the log-transformed scale ($\ln(\text{infarct size} + 10)$). These p-values should be interpreted cautiously because these comparisons were not corrected for multiple comparisons.

Table 12 shows that median infarct size was reduced from 26.5% in AMIHOT II Control patients to 20% in SSO₂ Therapy patients ($p=0.10$), representing a 6.5% median reduction. **Table 12** shows that patients with shorter time to reperfusion (0-3 hrs vs. $>3 - 6$ hrs) exhibited a larger infarct size reduction in SSO₂ Therapy subjects as well. This result is directionally consistent with the previously discussed AMIHOT I results, for which shorter reperfusion times on a longer time scale (0-6 hrs. vs. > 6 hrs) favored SSO₂ Therapy subjects as well. Subjects with distal target vessel lesions appeared to show a stronger treatment effect as well; Control subjects with non-proximal LAD lesions had larger infarct size ($21.5 \pm 30\%$) as compared to SSO₂ Therapy subjects ($14 \pm 26\%$; $p = 0.017$). Results for patients older than the median age of 60 years also favored SSO₂ Therapy; Control subjects older than 60 had greater infarct size ($29.5 \pm 36\%$) compared to SSO₂ Therapy subjects ($19 \pm 32\%$; $p = 0.031$). Small sample size caveats apply to these qualitative comparisons of patient subsets, but the results are supportive of a treatment effect from the adjunctive use of SSO₂ Therapy that is more effective in patients with shorter time to primary intervention, with more available circulation for the convective transfer of hyperoxic blood flow.

Table 12. Infarct Size at 14 Days (% of Left Ventricle), Evaluation of Infarct Size for AMIHOT II ITT Analysis¹ (infarct size values presented as median data with interquartile range (IQR))

	Control Group (n=79) (median ± IQR) (n)	SSO ₂ Therapy Group (n=222) (median ± IQR) (n)	p value*
All Patients	26.5 ± 35.5 (n=72)	20 ± 31 (n=209)	0.10
Time strata (actual)			0.026
0 – 3 hrs to reperfusion	32 ± 35 (n=41)	14 ± 27 (n=88)	0.004
> 3 hrs to reperfusion	21 ± 30 (n=31)	26 ± 32 (n=121)	0.6
Infarct location (actual)			0.11
Proximal LAD	29.5 ± 36 (n=34)	30 ± 33.5 (n=100)	0.9
Non-proximal LAD	21.5 ± 30 (n=38)	14 ± 26 (n=109)	0.017
Age			0.11
Age < 60 (median)	20 ± 35 (n=42)	21 ± 30 (n=101)	0.9
Age ≥ 60 (median)	29.5 ± 36 (n=30)	19 ± 32 (n=108)	0.031
Gender			0.2
Male	24 ± 36 (n=65)	20 ± 31 (n=167)	0.3
Female	38 ± 27 (n=7)	20.5 ± 23 (n=42)	0.044
Prior Myocardial Infarction			0.9
Prior MI	37 ± 39 (n=6)	32 ± 32 (n=19)	0.7
No Prior MI	24 ± 36 (n=65)	19 ± 29 (n=189)	0.13
Diabetes			0.7
Diabetic (Type I or II)	20 ± 39 (n=10)	21 ± 26.5 (n=32)	0.8
Non-diabetic	28 ± 35 (n=61)	19.5 ± 31 (n=172)	0.09
Pre-PCI TIMI flow grade²			0.10
0/I	31.5 ± 34 (n=46)	26.5 ± 30 (n=154)	0.5
II	24.5 ± 27 (n=10)	6 ± 19 (n=34)	0.033
III	8 ± 30 (n=10)	2 ± 17 (n=15)	0.3
II/III	19 ± 28.5 (n=20)	4 ± 19 (n=49)	0.030
Post-PCI TIMI flow grade²			0.9
0-II	43 ± 36 (n=5)	26 ± 18 (n=21)	0.7
III	24 ± 37 (n=59)	20 ± 31 (n=181)	0.3
Baseline LVEF³			0.8
LVEF < 40%	33 ± 23 (n=29)	29 ± 28 (n=69)	0.2
LVEF ≥ 40%	18.5 ± 29.5 (n=36)	14 ± 30 (n=116)	0.15
Stent Type			0.2
DES ⁴	31 ± 27 (n=35)	21 ± 31 (n=117)	0.09
BMS ⁴	18 ± 34 (n=35)	19 ± 27 (n=90)	0.9
Site Location			0.5
US	29.5 ± 36 (n=30)	26 ± 31 (n=85)	0.5
OUS	23.5 ± 34 (n=42)	17 ± 26.5 (n=124)	0.10

¹Available data for ITT patients analyzed as per Statistical Analysis Plan

*two-sided p value calculated using Mann-Whitney test; interaction p values shown in bold calculated using 2-way ANOVA on the log-transformed scale

²Angiographic core laboratory assessment

³Investigator assessment

⁴DES = drug-eluting stent; BMS = Bare metal stent

6.3.9.3 Imputation of Missing Infarct Size Data

Reasonable efforts were made to obtain complete data for all patients; however, missing observations did occur due to patients lost to follow-up or noncompliance with required assessments. In order to account for this, and to be able to perform the ITT analysis on the primary outcomes, the missing

outcomes were imputed in accordance with the study Statistical Analysis Plan. Missing infarct size values were imputed by randomly drawing from non-missing values within well-defined strata. The first-order imputation used strata defined by study (AMIHOT I or II), subgroup (Ant/NonAnt by LT6/GT6), and treatment arm (SSO₂ Therapy or Control). The second-order imputation further divided on gender (Male or Female), and age (less than 60, greater than equal to 60). Scientifically, it is plausible that data within one of these strata could be considered missing at random¹⁴, at least to first order. Multiple imputations of each missing value were drawn, and inference for each of the completed data sets was generated.

In addition, to evaluate the impact of data imputation on the outcomes, a secondary analysis ignoring missing responses also was conducted, as shown in the previous section. Results showed that the overall impact of the additional imputed data was minor. The AMIHOT II study results using only available data show an absolute median infarct size reduction of 6.5% in the SSO₂ Therapy group. Using the 1st and 2nd order imputation methods described above, the associated median infarct size reductions are 6.0% and 6.7%, respectively, in good agreement with the non-imputed results.

6.3.9.4 Evaluation of Infarct Size Results Using Simple Pooling

Additional exploratory analyses were performed for the combined AMIHOT II infarct size data and AMIHOT I anterior < 6 hr patient infarct size data, referred to here as 'simple pooling'. This model provides an idealized scenario for combining the AMIHOT I and II infarct size data for the subgroup of interest (anterior < 6 hr infarcts only). The Bayesian hierarchical model conservatively considers other AMIHOT I patient subgroups (*i.e.*, non-anterior infarcts and > 6 – 24 hr infarcts), so this pooled model can be thought of as a simplified construct, but one that is instructive with respect to understanding the magnitude of the treatment effect in the target population. A correction for study level was incorporated to account for the overall baseline differences in the AMIHOT I and II infarct size results. This pooled analysis exhibited an absolute median infarct size reduction of 6.5%, from 25% in the Control group to 18.5% in the SSO₂ Therapy group. This result is consistent with the overall treatment effect calculated from each study separately, as presented previously.

6.3.9.5 Results of Primary Analysis Using Pre-Specified Bayesian Model

The results for the Bayesian analysis of infarct size reduction are presented herein using the pre-specified hierarchical model described in the Statistical Analysis Plan. The data were log-transformed in order to remove some degree of skewness. **Figure 7** displays histograms that display the AMIHOT II infarct size distributions for the two study groups; the skewed right-tailed distributions are typical and expected for these data. **Table 13** shows the Control and SSO₂ Therapy group infarct size results as (mean ± SE) on the logarithmic scale. Sample sizes are provided for both the AMIHOT I and II studies. For discussion purposes, this model is referred to as M1.

The important output of the Bayesian hierarchical model is the posterior probability of superiority, which indicates the likelihood that the SSO₂ Therapy group infarct size is smaller than that of the Control group. The study endpoint required that the posterior probability of superiority be greater than 95.0%, taking into account data from all ITT subjects, and providing for imputation of data for subjects without a readable SPECT scan. The 2nd order imputation analysis factors in the most background

¹⁴ R.J.A. Little DBR. Statistical Analysis with Missing Data. 2nd ed. New York: John Wiley & Sons, Inc.; 2002.

information about the patients missing data and is considered the primary analysis result, in accordance with the Statistical Analysis Plan.

The results of the infarct size analyses in **Table 13** demonstrate that the study endpoint was met successfully for the ITT analysis, with or without imputation; the posterior probability of superiority = 95.1% using available data without imputation, 95.5% using 1st order imputation, and 96.9% using 2nd order imputation. Sufficient Monte Carlo simulations were performed to ensure one-tenth-of-one-percent precision for these calculated posterior probabilities.

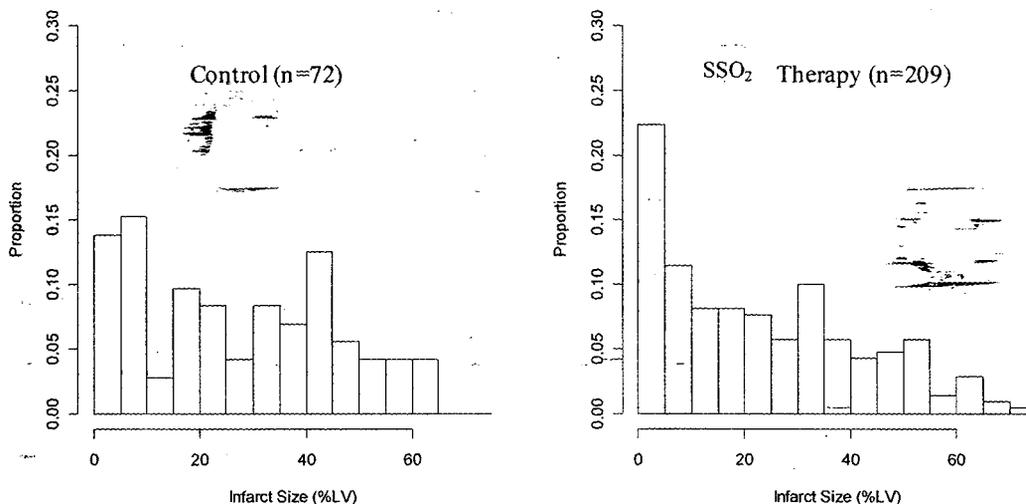


Figure 7. AMIHOT II Infarct Size Distributions

Table 13. Infarct Size at 14 Days (% of Left Ventricle), Bayesian Evaluation of Primary Endpoint, Sensitivity of Imputation Methods¹: Model M1 (Pre-specified Model)
(infarct size values presented on log-transformed scale with mean and standard error (SE))

	Control Group (mean ± SE) (n ²)	SSO ₂ Therapy Group (mean ± SE) (n ²)	Difference (± SE)	Posterior Probability of Superiority ⁴
ITT Analysis				
No imputation (available data)	3.42 ± 0.06 (n=52/68; 72)	3.30 ± 0.04 (n=49/71; 209)	-0.12 ± 0.07	95.1%
1 st Order Imputation	3.42 ± 0.06 (n=53/79; 79)	3.30 ± 0.04 (n=52/81; 222)	-0.12 ± 0.07	95.5%
2 nd Order Imputation	3.43 ± 0.06 (n=53/79; 79)	3.30 ± 0.04 (n=52/81; 222)	-0.13 ± 0.07	96.9%

¹Analysis performed three ways: No imputation, 1st order imputation, 2nd order imputation methods

²Sample size for Bayesian Evaluation given as (x/y; z) where x = number of Anterior ≤ 6 hours in AMIHOT I, y = number of other subjects in AMIHOT I and z = number of subjects in AMIHOT II (all Anterior ≤ 6 hours).

³Posterior mean difference between SSO₂ and Control groups incorporating data from AMIHOT I study into the hierarchical model.

⁴Posterior probability that the average SSO₂ Therapy Group infarct size is smaller than the Control Group infarct size.

6.3.9.6 Alternative Bayesian Models to Examine Robustness of Results

A series of additional Bayesian hierarchical models were developed to examine the robustness of the results to different choices of model parameters, prior distributions, and the degree of borrowing from AMIHOT I data. These models were not part of the pre-specified Statistical Analysis Plan but were developed in concert with FDA's request to examine the sensitivity of the study results to a number of factors. For simplicity, these models are referred to in the text as "M2", "OL", "H1", etc. (recalling that the pre-specified model is "M1") with the following brief descriptions of the purpose of each of the models:

- Model OL: Analyzes the data through a non-parametric ordinal logistic model that also allows for the straightforward calculation of the magnitude of the median infarct size reduction.
- Model M2: A variant of pre-specified model M1 that allows for greater variability in Control group infarct size level between the AMIHOT I and II studies.
- Model H1: An alternative Bayesian hierarchical model similar to our pre-specified model M1, but has 6 additional random effects terms, leading to higher degrees of borrowing from AMIHOT I where this is warranted.
- Model H2: A variant of model H1 to incorporate the two-way ANOVA mean model for the subgroup effect, to account for the potential interaction between infarct location and time to reperfusion in the AMIHOT I data.
- Model H3: A variant of model H1 to incorporate a term for possible center effects from the AMIHOT I and II investigational sites.
- Model H4: A variant of model H1 that includes the time to reperfusion as a continuous variable and treatment by time to reperfusion interaction effects.

The results of Model OL are calculated on actual (non-transformed) scale, and are presented in **Table 14**. Results of the other models are calculated on the log-transformed scale and are shown in comparison to model M1 in **Table 15**. Results are shown with respect to available data and using multiple imputation for missing data. As shown in the tables, every alternative Bayesian model formulated to analyze the data produced a higher posterior probability of superiority that infarct size is reduced with adjunctive SSO₂ Therapy than the pre-specified Model M1. Notably, the non-parametric ordinal logistic model OL, which analyzes the data on actual rather than a transformed scale, calculates posterior probabilities of superiority of 99.0% using available data. Because the data are on actual scale, this model is well-suited to estimate the magnitude of the treatment effect. The calculated median infarct sizes are 25.5% and 18.8% for the Control and SSO₂ Therapy groups, an absolute reduction of 6.7%, in close agreement with the non-Bayesian calculated effect sizes presented previously.

These alternative analyses demonstrate that the study conclusions are robust with respect to the superiority of SSO₂ Therapy in reducing infarct size for this study population.

Table 14. Infarct Size at 14 Days (% of Left Ventricle), Bayesian Evaluation of Primary Endpoint Using Ordinal Logistic Regression Model (Model OL)

	Control Group (Median ± SE)	SSO ₂ Therapy Group (Median ± SE)	Odds Ratio (95% CI) ¹	Posterior Probability of Superiority
No imputation (available data)				
Model OL	25.5 ± 2.9	18.8 ± 1.5	1.61 (1.07, 2.43)	99.0%
Multiple Imputation				
Model OL	26.1 ± 2.9	18.7 ± 1.5	1.69 (1.12, 2.54)	99.4%

¹The OL model is applied to 5 categories (approx. quintiles) of infarct size; the odds ratio is interpreted as an increased odds for the SSO₂ Therapy group to have a lower category of infarct size than the Control group

Table 15. Infarct Size at 14 Days (% of Left Ventricle), Bayesian Evaluation of Primary Endpoint Using Alternative Bayesian Models (results shown on log-transformed scale)

	Control Group (mean ± SE)	SSO ₂ Therapy Group (mean ± SE)	Difference ¹ (± SE)	Posterior Probability of Superiority ²
No Imputation (available data)				
Pre-specified Model M1	3.42 ± 0.06	3.30 ± 0.04	-0.12 ± 0.07	95.1%
Model M2	3.44 ± 0.06	3.30 ± 0.04	-0.13 ± 0.07	96.6%
Model H1	3.45 ± 0.06	3.31 ± 0.04	-0.15 ± 0.07	97.7%
Model H2	3.45 ± 0.06	3.31 ± 0.04	-0.14 ± 0.07	97.3%
Model H3	3.46 ± 0.07	3.32 ± 0.06	-0.14 ± 0.08	96.6%
Model H4	3.45 ± 0.06	3.31 ± 0.04	-0.15 ± 0.07	97.9%
Multiple Imputation³				
Pre-specified Model M1	3.43 ± 0.06	3.30 ± 0.04	-0.13 ± 0.07	96.9%
Model M2	3.45 ± 0.06	3.31 ± 0.04	-0.14 ± 0.07	97.9%
Model H1	3.46 ± 0.06	3.31 ± 0.04	-0.16 ± 0.07	98.4%
Model H2	3.46 ± 0.06	3.31 ± 0.04	-0.15 ± 0.07	98.4%
Model H3	3.47 ± 0.07	3.31 ± 0.05	-0.15 ± 0.08	97.7%

¹Posterior mean difference between SSO₂ Therapy and Control groups incorporating AMIHOT I study data into the hierarchical model.

²Posterior probability that the average SSO₂ Therapy Group infarct size is smaller than the Control Group infarct size.

³Multiple imputation computations not performed for Model H4.

6.3.9.7 Secondary Effectiveness Endpoint

The secondary effectiveness marker of the study was ST-segment elevation measured in the first 3 hours post-PCI by area under the curve, using continuous ECG recording. At 3 hours post-PCI, a total of

48/75 (64.0%) Control subjects and 121/202 (59.9%) SSO₂ Therapy subjects had ST area = 0, which can be interpreted as no continuing ischemia.

6.3.10 Safety Data

6.3.10.1 Overview

Section 6.3.10 presents a summary of safety data for all Intent-to-Treat (ITT) patients treated in the randomized phase of the AMIHOT II clinical trial. This safety summary addresses primary safety endpoint data for 30-day Major Adverse Cardiac Events (MACE), as well as other serious adverse events (SAEs) and non-serious adverse events (AEs) through 30 days as well. Additional discussion is presented for both bleeding events and early (< 30 days) stent occlusions. Results for late MACE events (at one year) are also presented. Summary tables are presented for adverse events, including the adjudicated event classification, group totals, and event relationship. All adverse events and safety endpoint events were reviewed and adjudicated by the independent Clinical Events Committee (CEC). The types and numbers of these events are summarized in the following tables as adjudicated by the CEC.

An independent Data and Safety Monitoring Board (DSMB) was utilized for safety review throughout the study. The independent DSMB conducted periodic reviews of the composite safety endpoint and reviewed the cumulative safety data at scheduled intervals to make recommendations regarding continuation of the study. At each DSMB meeting there was unanimous agreement by the board that the study should continue and that there were no safety concerns with the AMIHOT II trial that would require permanent or temporary trial stopping or change to the study design.

6.3.10.2 AMIHOT II 30-Day MACE Results

MACE Definitions

The composite safety endpoint for the AMIHOT II clinical trial is the incidence of Major Adverse Cardiac Events (MACE) including death, reinfarction, target vessel revascularization, and stroke within 30 days. The components of this composite safety endpoint are defined as follows:

30-Day Stroke: Neurological deficit lasting 24 hours or longer, or lasting less than 24 hours with a brain imaging study showing infarction. Stroke events occurring within the 30-day (or hospital discharge) MACE window will be considered primary safety endpoint events.

30-Day Reinfarction: Presence of recurrent ischemic symptoms thought to be of cardiac origin of at least 20 minutes duration and redevelopment of ST-Segment elevation in two (2) or more contiguous precordial leads and/or worsening of existing Q waves or development of new pathologic Q waves in the precordial leads. For defining reinfarctions occurring 96 hours or more after the index event, re-elevation of CK-MB isoenzyme may be utilized as a substitute for ST segment changes. Note: degree of ST change cannot be stipulated during periprocedural phase because it may be related to underlying persistent ST segment changes related to presentation event. Reinfarction events occurring within the 30-day (or hospital discharge) MACE window will be considered primary safety endpoint events if they occur in the region of the originally treated infarct location.

30-Day TVR (Target Vessel Revascularization): Revascularization of AMIHOT II study-related vessel by means of PCI or CABG. Target Vessel Revascularization events occurring within the 30-day (or hospital discharge) MACE window will be considered primary safety endpoint events. Any intervention performed in the cath lab at the time of treatment will not be considered a TVR.

30-Day Death: Including all deaths occurring from time of randomization through day 30 or until hospital discharge, whichever is later.

All reported study primary safety endpoints were reviewed by the CEC and classified according to the definitions outlined above into the appropriate MACE event category. De-identified source documentation and clinical imaging were provided to the CEC for review and adjudication of the primary safety endpoints. The primary endpoint of 30-Day MACE includes all MACE events occurring through the 30th day post procedure.

The primary safety endpoint for the AMIHOT II trial required a determination of non-inferiority in the 30-day MACE rate, comparing the SSO₂ Therapy group with the Control group, within a safety delta of 6.0%. Endpoint evaluation was performed using a Bayesian hierarchical model that factored in the 30-day MACE data from both the AMIHOT I and II studies. The evaluation of the endpoint for both the ITT and Per Protocol (PP) samples was considered co-primary. The observed 30-day MACE rates were 3.8% in the Control group and 5.4% in the SSO₂ Therapy group in the AMIHOT II trial for the ITT analysis, and 3.8% in each arm for the PP analysis. **Table 16** displays the 30-day MACE individual component rate data for the AMIHOT II study (ITT sample). As shown in the table, a trend in slightly higher 30-day mortality and MACE was noted in the SSO₂ Therapy group as compared to Controls.

The MACE rates overall were low given the study patient population which consists of individuals suffering from anterior acute myocardial infarction. Anterior AMI patients have a higher incidence of significant morbidity and mortality than those with non-anterior MI. This patient population has been reported elsewhere to have 30-day MACE rates in the range of 4.4% to 10.5%.^{15,16} In the AMIHOT II study, patients met strict inclusion/exclusion criteria prior to enrollment, including recent onset of symptoms (within 6 hours), successful and uncomplicated PCI procedure with the intention of intra-coronary stent placement, no significant concurrent cardiac morbidity or need for further intervention within 30 days, and no cardiogenic shock or periods of cardiopulmonary resuscitation (CPR) for > 10 minutes. The low overall MACE rate in both groups likely reflects the selective nature of the study population.

¹⁵ Stone GW, Grines CL, Cox DA, et al. Comparison of angioplasty with stenting, with or without abciximab, in acute myocardial infarction. *N Engl J Med* 2002;346:957-66.

¹⁶ Antoniucci D, Rodriguez A, Hempel A, et al. A randomized trial comparing primary infarct artery stenting with or without abciximab in acute myocardial infarction. *J Am Coll Cardiol* 2003;42:1879-85.

Table 16. AMIHOT II 30-Day MACE Rates

Adverse Event	Randomization Group			
	Control (N=79)		SSO ₂ Therapy (N=222)	
	Events (n)	Pts with Events n (%)	Events (n)	Pts with Events n (%)
30-day MACE assessment available		79/79 (100.0%)		222/222 (100.0%)
Composite 30-Day MACE	5	3/79 (3.8%)	19	12/222 (5.4%)
Death	0	0/79 (0.0%)	4	4/222 (1.8%)
Target Vessel Revascularization ¹	3	3/79 (3.8%)	9	8/222 (3.6%)
Reinfarction	2	2/79 (2.5%)	6	4/222 (1.8%)
Stroke	0	0/79 (0.0%)	0	0/222 (0.0%)

¹LAD target vessel or branches

6.3.10.3 Evaluation of Primary Endpoint Using Pre-Specified Bayesian Model

The primary safety endpoint was based on the non-inferiority of the cumulative incidence of 30-day MACE within a safety delta of 6.0%, assessed through Bayesian hierarchical modeling. In concept, study endpoint success was evaluated for the AMIHOT II study contingent upon the results of the AMIHOT I trial. The model was structured so that the AMIHOT I results were sub-divided into four patient subgroups of interest: anterior vs. non-anterior AMI, treated ≤ 6 or > 6 hours from symptom onset. More specifically, the safety endpoint was satisfied if there was a high posterior probability of non-inferiority ($> 95\%$) in the AMIHOT II trial conditional on the safety data from both trials. For the safety endpoint analysis, the ITT and per protocol (PP) analyses were considered co-primary. The ITT results are shown here and are essentially unchanged using the PP analysis.

The degree of borrowing from the results of the AMIHOT I study in analyzing the results of the AMIHOT II study was contingent upon the similarity of data in the two trials. A high degree of similarity would have resulted in a large amount of pooling, but dissimilarity of the trials (either in SSO₂ Therapy versus Control differences or in overall average efficacy measure or mean MACE rate) would have resulted in a limited amount of pooling. It was thus mathematically impossible for the AMIHOT II trial to trend towards a neutral or negative outcome and still meet the statistical criteria for study success, and the design preserved (frequentist) type I error rates of no more than 5% under the null hypothesis of inferior safety (*i.e.*, the SSO₂ Therapy group MACE rate exceeding the Control group rate by at least the safety delta of 6%).

The study was neither designed nor powered to achieve the study endpoints as a stand-alone trial outside of this Bayesian framework. Thus, the study relied upon a reasonable degree of similarity between AMIHOT I and AMIHOT II data in order to be able to pool some of the positive evidence from the AMIHOT I trial in analyzing the AMIHOT II endpoints. Table 17 shows the results of the Bayesian analysis of 30-day MACE rates for the AMIHOT I and II studies within the framework of the model. MACE rate data are displayed as (mean \pm SE) for the composite safety endpoint. The difference in MACE rates is also displayed in addition to the calculated posterior probability of non-inferiority. As seen in the table, the 30-day MACE rates for both the Control and SSO₂ Therapy groups are very similar for the ITT sample, $5.0 \pm 1.4\%$ and $5.9 \pm 1.4\%$, respectively. The posterior probability of an increase of

less than 6% in 30-day MACE rate is 99.5%, successfully achieving the study endpoint. Results are similar in the PP sample, with a posterior probability of 99.9%. Sufficient Monte Carlo simulations were performed to ensure precision to one-tenth-of-one-percent.

Table 17. Primary Safety Endpoint Evaluation (30-Day MACE): AMIHOT I & II ITT and PP Bayesian Analysis

	Control Group (mean ± SE) (%) (n ¹)	SSO₂ Therapy Group (mean ± SE) (%) (n ¹)	Difference² (± SE) (%)	Posterior Probability of Non-Inferiority³
ITT Analysis	5.0 ± 1.4 (n=53/79; 79)	5.9 ± 1.4 (n=52/81; 222)	0.9 ± 2.0	99.5%
PP Analysis	5.1 ± 1.5 (n=51/73; 78)	4.7 ± 1.5 (n=47/72; 186)	-0.4 ± 2.0	99.9%

¹Sample size for Bayesian Evaluation given as (x/y; z) where x = number of Anterior ≤ 6 hours in AMIHOT I, y = number of other subjects in AMIHOT I and z = number of subjects in AMIHOT II (all Anterior ≤ 6 hours).

²Posterior mean difference between MACE rate in SSO₂ and Control groups incorporating AMIHOT I data into hierarchical model.

³Posterior probability that the SSO₂ Therapy group MACE rate is not more than 6 percentage points larger than the Control group rate.

A series of alternative Bayesian hierarchical models were developed to examine the robustness of the results to different choices of model parameters, prior distributions, and the degree of borrowing from AMIHOT I data. These models were not part of the pre-specified Statistical Analysis Plan but were developed in concert with FDA's request to examine the sensitivity of the study results to a number of factors. The results of these models showed that the AMIHOT II safety endpoint was robust to changes in the Bayesian model, and that the posterior probability of non-inferiority was > 95.0% for every model-variant tested.

6.3.10.4 30-Day Mortality

As shown in **Table 16**, a total of four (4) deaths occurred within 30 days post-procedure, all in the SSO₂ Therapy group (1.8%). All four (4) deaths were determined to be cardiac-related deaths. Three (3) deaths were adjudicated by the Clinical Events Committee to be related to the patient's primary disease state (coronary artery disease). Of these three (3) deaths, two (2) patients expired from myocardial rupture within the territory of the LAD at 4 and 9 days, respectively. One patient passed from hypoxic encephalopathy resulting from a cardiac arrest that occurred prior to enrollment. This patient was a protocol deviation for the AMIHOT II trial and should have been excluded per protocol, as she had presented with a loss of consciousness for over five minutes prior to trial enrollment.

The fourth death was determined by the CEC to be related to the SSO₂ Therapy procedure. [REDACTED] initially underwent a difficult and complicated index PCI procedure associated with prolonged periods of no reflow. A TIMI flow of only Grade 2 was able to be established post-PCI. At four minutes into the SSO₂ Therapy procedure, the patient experienced an arrhythmia consisting of ventricular tachycardia and ventricular fibrillation. These events culminated in cardiac arrest. This adjudication by the CEC was a conservative judgment based primarily on event timing, as the patient experienced cardiac arrest four (4) minutes post-initiation of SSO₂ Therapy.

6.3.10.5 Additional 30-Day Serious Adverse Events

During the 30-day follow-up period, 57/222 (25.7%) patients in the SSO₂ Therapy group and 15/79 (19.0%) patients in the Control group presented with one or more serious adverse events (SAEs). **Table 18** displays the summary of all observed SAEs during the 30-day follow-up period for both groups. SAEs are displayed both by System Organ Class (e.g., Cardiac Disorders, Gastrointestinal Disorders) and for individual event codes. 30-day SAE rates are nominally higher in the SSO₂ Therapy group as compared to Controls (25.7% vs. 19.0%), and the majority of events in both groups were classified as Cardiac Disorders, which also trended higher in the SSO₂ Therapy group (14.9% vs. 10.1%).

Table 18. Summary of All Serious Adverse Events within 30 Days

Category	Adverse Event	Randomization Group			
		Control (N=79)		SSO ₂ Therapy (N=222)	
System Organ Class	MedDRA PT	Events (n)	Pts with Events (n/N; %)	Events (n)	Pts with Events (n/N; %)
Assessment Complete			79/79 (100.0%)		222/222 (100.0%)
Assessment Available ¹			79/79 (100.0%)		222/222 (100.0%)
All serious adverse events		19	15/79 (19.0%)	89	57/222 (25.7%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		0	0/79 (0.0%)	1	1/222 (0.5%)
	ANEMIA	0	0/79 (0.0%)	1	1/222 (0.5%)
CARDIAC DISORDERS		9	8/79 (10.1%)	41	33/222 (14.9%)
	ANGINA PECTORIS	1	1/79 (1.3%)	4	4/222 (1.8%)
	AORTIC VALVE STENOSIS	1	1/79 (1.3%)	0	0/222 (0.0%)
	ATRIAL FIBRILLATION	0	0/79 (0.0%)	1	1/222 (0.5%)
	CARDIAC ARREST	0	0/79 (0.0%)	1	1/222 (0.5%)
	CARDIAC FAILURE CONGESTIVE	4	4/79 (5.1%)	8	8/222 (3.6%)
	CARDIAC TAMPONADE	0	0/79 (0.0%)	1	1/222 (0.5%)
	CARDIOGENIC SHOCK	1	1/79 (1.3%)	5	5/222 (2.3%)
	CORONARY ARTERY DISEASE	1	1/79 (1.3%)	0	0/222 (0.0%)
	CORONARY ARTERY OCCLUSION	0	0/79 (0.0%)	1	1/222 (0.5%)
	CORONARY ARTERY STENOSIS	0	0/79 (0.0%)	1	1/222 (0.5%)
	MYOCARDIAL ISCHEMIA	0	0/79 (0.0%)	4	4/222 (1.8%)
	MYOCARDIAL RUPTURE	0	0/79 (0.0%)	2	2/222 (0.9%)
	PERICARDITIS	1	1/79 (1.3%)	2	2/222 (0.9%)
	PULMONARY EDEMA	0	0/79 (0.0%)	4	4/222 (1.8%)
	SICK SINUS SYNDROME	0	0/79 (0.0%)	1	1/222 (0.5%)

¹Complete or event reported in the interval. Event rate denominators utilize number of subjects with assessment available

Table 18 (continued). Summary of All Serious Adverse Events within 30 Days

		Randomization Group			
Category	Adverse Event	Control (N=79)		SSO ₂ Therapy (N=222)	
System Organ Class	MedDRA PT	Events (n)	Pts with Events (n/N; %)	Events (n)	Pts with Events (n/N; %)
	VENTRICULAR FIBRILLATION	0	0/79 (0.0%)	3	3/222 (1.4%)
	VENTRICULAR TACHYCARDIA	0	0/79 (0.0%)	3	3/222 (1.4%)
GASTROINTESTINAL DISORDERS		1	1/79 (1.3%)	0	0/222 (0.0%)
	GASTROESOPHOGEAL REFLUX DISEASE	1	1/79 (1.3%)	0	0/222 (0.0%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		2	2/79 (2.5%)	6	5/222 (2.3%)
	ADVERSE DRUG REACTION	0	0/79 (0.0%)	1	1/222 (0.5%)
	CHEST PAIN	2	2/79 (2.5%)	2	2/222 (0.9%)
	HYPOTHERMIA	0	0/79 (0.0%)	1	1/222 (0.5%)
	PYREXIA	0	0/79 (0.0%)	2	2/222 (0.9%)
INFECTIONS AND INFESTATIONS		0	0/79 (0.0%)	3	3/222 (1.4%)
	BACTEREMIA	0	0/79 (0.0%)	1	1/222 (0.5%)
	PNEUMONIA	0	0/79 (0.0%)	1	1/222 (0.5%)
	URINARY TRACT INFECTION	0	0/79 (0.0%)	1	1/222 (0.5%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS		2	2/79 (2.5%)	15	11/222 (5.0%)
	CORONARY ARTERY DISSECTION	0	0/79 (0.0%)	1	1/222 (0.5%)
	DRUG TOXICITY	0	0/79 (0.0%)	1	1/222 (0.5%)
	STENT OCCLUSION	2	2/79 (2.5%)	12	9/222 (4.1%)
	TRAUMATIC HEMATOMA	0	0/79 (0.0%)	1	1/222 (0.5%)
NERVOUS SYSTEM DISORDERS		0	0/79 (0.0%)	2	2/222 (0.9%)
	HYPOXIC ENCEPHALOPATHY	0	0/79 (0.0%)	1	1/222 (0.5%)
	TRANSIENT ISCHEMIC ATTACK	0	0/79 (0.0%)	1	1/222 (0.5%)
PSYCHIATRIC DISORDERS		1	1/79 (1.3%)	1	1/222 (0.5%)
	ANXIETY	1	1/79 (1.3%)	1	1/222 (0.5%)

Table 18 (continued). Summary of All Serious Adverse Events within 30 Days

Category	Adverse Event	Randomization Group			
		Control (N=79)		SSO ₂ Therapy (N=222)	
		Events (n)	Pts with Events (n/N; %)	Events (n)	Pts with Events (n/N; %)
RENAL AND URINARY DISORDERS	MedDRA PT	1	1/79 (1.3%)	4	4/222 (1.8%)
	HEMATURIA	0	0/79 (0.0%)	1	1/222 (0.5%)
	RENAL FAILURE ACUTE	1	1/79 (1.3%)	3	3/222 (1.4%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		0	0/79 (0.0%)	2	2/222 (0.9%)
	PNEUMONIA ASPIRATION	0	0/79 (0.0%)	1	1/222 (0.5%)
	RESPIRATORY DISORDER	0	0/79 (0.0%)	1	1/222 (0.5%)
VASCULAR DISORDERS		3	2/79 (2.5%)	14	12/222 (5.4%)
	CAROTID ARTERY STENOSIS	1	1/79 (1.3%)	0	0/222 (0.0%)
	CATHETER SITE HEMATOMA	0	0/79 (0.0%)	3	3/222 (1.4%)
	CATHETER SITE HEMORRHAGE	1	1/79 (1.3%)	1	1/222 (0.5%)
	HEMORRHAGE	0	0/79 (0.0%)	1	1/222 (0.5%)
	HYPOTENSION	0	0/79 (0.0%)	4	3/222 (1.4%)
	RETROPERITONEAL HEMORRHAGE	0	0/79 (0.0%)	1	1/222 (0.5%)
	VASCULAR PSEUDOANEURYSM	1	1/79 (1.3%)	4	4/222 (1.8%)

6.3.10.6 30-Day Adverse Events

An overall summary of 30-day adverse events (AEs) is presented in **Table 19**. During the 30-day follow-up period, 119/222 (53.6%) patients in the SSO₂ Therapy group and 37/79 (46.8%) patients in the Control group presented with one or more AEs during study follow-up. SSO₂ Therapy group subjects exhibited a slightly higher incidence of AEs (53.6%) compared to patients randomized to the Control group (46.8%), possibly due to the fact that SSO₂ Therapy subjects received an additional 90 minutes of catheterization time, were administered increased anticoagulation therapy (heparin), and required either a larger single arterial access sheath or a second femoral arterial access site. These factors would be expected to subsequently increase the rates of adverse events.

Table 19. Overall Summary of Adjudicated Adverse Events within 30 Days

Adverse Event (AE)	Randomization Group			
	Control (N=79)		SSO ₂ Therapy (N=222)	
	# of Events	# (%) of Pts with Events	# of Events	# (%) of Pts with Events
Any Adverse Event	58	37 (46.8%)	256	119 (53.6%)
SSO ₂ device related AE			0	0 (0.0%)
SSO ₂ procedure related AE			46	40 (18.0%)
Index PCI procedure related AE	16	14 (17.7%)	43	34 (15.3%)
Coronary Artery Disease related AE	23	17 (21.5%)	94	64 (28.8%)
Study Medication related AE	0	0 (0.0%)	2	2 (0.9%)
Other relationship ¹	18	15 (19.0%)	62	46 (20.7%)
Unknown relationship	1	1 (1.3%)	9	9 (4.1%)
Serious Adverse Event (SAE)	19	15 (19.0%)	89	57 (25.7%)
SSO ₂ device related SAE			0	0 (0.0%)
SSO ₂ procedure related SAE			17	14 (6.3%)
Index PCI procedure related SAE	4	4 (5.1%)	17	14 (6.3%)
Coronary Artery Disease related SAE	9	8 (10.1%)	42	32 (14.4%)
Study Medication related SAE	0	0 (0.0%)	0	0 (0.0%)
Other relationship ¹	6	5 (6.3%)	10	10 (4.5%)
Unknown relationship	0	0 (0.0%)	3	3 (1.4%)
Adverse Event related to AMIHOT II Vessel	8	5 (6.3%)	26	20 (9.0%)

¹Including pre-existing condition, concurrent condition, concurrent intervention and other relationships

6.3.10.7 30-Day Stent Occlusion Events

Stent occlusion continues to be a serious potential issue in PCI. A detailed discussion of the AMIHOT II 30-day stent occlusion events is provided here, referencing the patient-level data provided in **Table 20**. A total of 9/222 (4.1%) SSO₂ Therapy patients and 2/79 (2.5%) Control patients experienced twelve (12) and two (2) stent occlusion events respectively within this time window. The table provides additional information for these events, including the number of days post-procedure on which the event occurred, the stent type, whether glycoprotein IIb/IIIa inhibitors were utilized in the cardiac catheterization laboratory, the total infusion time for SSO₂ Therapy patients, which infusion catheter was used for SSO₂ Therapy subjects, and whether the adverse event was CEC-adjudicated to be related to the SSO₂ Therapy procedure, the index PCI procedure, or to native coronary artery disease.

Of the nine (9) SSO₂ Therapy patients who experienced stent occlusion within 30 days, six (6) of these subjects experienced events that were adjudicated to be related to the index PCI procedure, while three

(3) of these subjects experienced events that were adjudicated to be related to the SSO₂ Therapy procedure. These three (3) events that were related to the SSO₂ Therapy procedure occurred immediately during the infusion; one event [REDACTED] occurred while the cartridge was primed and before the infusion was started. Similarly, the events observed in [REDACTED] occurred at 4 minutes and 1 minute into the infusion, respectively. Each of these three (3) events were determined to be related to the SSO₂ Therapy procedure due to the temporal relationship of having a sub-selective catheter present in the coronary artery; significantly, none of these three (3) events were determined to be related to the device itself by the CEC (event relationship to the SSO₂ Therapy procedure and the device were distinct categories of determination by the CEC).

Of the six (6) SSO₂ Therapy subjects who experienced stent occlusion within thirty (30) days and whose events were adjudicated to be related to the index PCI procedure, additional information is available for three (3) of these subjects to help delineate the cause of these events. [REDACTED] admitted to cocaine and heroin abuse post-randomization, and reported using heroin prior to experiencing stent occlusion on day 4 post-procedure. Compliance with dual antiplatelet medication was questionable. In the narrative for [REDACTED], who experienced stent occlusion on day 13 post-procedure, the site reported that this subject was non-compliant with dual antiplatelet medications (aspirin and clopidogrel) because of an inability to take oral medications. Finally, [REDACTED], who experienced stent occlusion after only three minutes of SSO₂ Therapy, was noted by the CEC to have a filling defect visible on the final angiogram prior to initiating the SSO₂ Therapy procedure that should have been treated during the index PCI procedure. Taken together, this supporting information provides further assurance that these events had a causal relationship to factors other than the SSO₂ Therapy procedure.

Table 20. AMIHOT II 30-Day Stent Occlusion Adverse Events

Patient ID	Patient Assignment	Days to Event	Stent Type (BMS/DES ¹)	Iib/IIIa Use	Infusion Time (mins)	Infusion Catheter Type	Relationship ²
[REDACTED]	SSO ₂ Therapy	4	DES	Yes	90	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	8	DES	Yes	90	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	13	DES	Yes	1	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	0	DES	Yes	3	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	0	BMS	Yes	21	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	0	DES	Yes	0	[REDACTED]	SSO ₂ Procedure
[REDACTED]	SSO ₂ Therapy	19	DES	Yes	0	[REDACTED]	CAD
[REDACTED]	SSO ₂ Therapy	4	BMS	Yes	91	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	6	BMS	Yes	91	[REDACTED]	PCI
[REDACTED]	SSO ₂ Therapy	0	BMS	No	4	[REDACTED]	SSO ₂ Procedure
[REDACTED]	SSO ₂ Therapy	7	BMS	Yes	90	[REDACTED]	PCI
[REDACTED]	Control	4	BMS	No	NA	NA	PCI
[REDACTED]	Control	0	BMS	No	NA	NA	PCI
[REDACTED]	SSO ₂ Therapy	0	BMS	Yes	1	[REDACTED]	SSO ₂ Procedure

¹BMS = Bare Metal Stent, DES = Drug Eluting Stent

²PCI = Percutaneous Coronary Intervention, CAD = Coronary Artery Disease, SSO₂ Procedure = TherOx SSO₂ Therapy Procedure

6.3.10.8 30-Day Bleeding Events

Due to the SSO₂ Therapy procedure that involved the use of a larger arterial access sheath for blood withdrawal, the possible use of a second introducer, additional anticoagulation therapy, and an extended procedural time to administer the infusion, bleeding was an expected adverse event in this study. **Table 21** summarizes bleeding events within 30 days by comparing the two study groups. These events were adjudicated by the Clinical Events Committee and categorized into mild, moderate and severe categories. These categories are defined as follows:

- **Mild:** Bleeding that does not require transfusion or result in hemodynamic compromise
- **Moderate:** Bleeding requiring transfusion that is defined as any blood loss requiring transfusion of blood products
- **Severe:** Intracranial bleeding or bleeding that results in substantial hemodynamic compromise requiring treatment

As shown in **Table 21**, an increase in all bleeding events was observed in the SSO₂ Therapy group as compared to the Control group (24.8% vs. 12.7%). An increase in bleeding events was observed in mild, moderate, and severe bleeding for both access site and non-access site bleeding events.

Table 21. 30-day Bleeding Events by Severity

			Randomization Group			
			Control (N=79)		SSO ₂ Therapy (N=222)	
Location	Bleeding Category	Adverse Event	# of Events	n (%) of Pts with Events	# of Events	n (%) of Pts with Events
All Bleeding Events			10	10 (12.7%)	58	55 (24.8%)
All Severe/Life Threatening Bleeding Events			1	1 (1.3%)	3	3 (1.4%)
Access Site			9	9 (11.4%)	41	41 (18.5%)
	Mild	Catheter site hematoma	8	8 (10.1%)	34	34 (15.3%)
	Moderate	Catheter site hematoma	0	0 (0.0%)	5	5 (2.3%)
		Catheter site hemorrhage	0	0 (0.0%)	1	1 (0.5%)
	Severe	Catheter site hemorrhage	1	1 (1.3%)	0	0 (0.0%)
		Retroperitoneal hemorrhage	0	0 (0.0%)	1	1 (0.5%)
Non-Access Site			1	1 (1.3%)	17	16 (7.2%)
	Mild	Anemia	0	0 (0.0%)	6	6 (2.7%)
		Hematuria	0	0 (0.0%)	1	1 (0.5%)
		Implant site hematoma	0	0 (0.0%)	1	1 (0.5%)
		Urogenital hemorrhage	0	0 (0.0%)	1	1 (0.5%)
	Moderate	Anemia	1	1 (1.3%)	1	1 (0.5%)
		Hemorrhage	0	0 (0.0%)	4	4 (1.8%)
		Traumatic hematoma	0	0 (0.0%)	1	1 (0.5%)
	Severe	Cardiac tamponade	0	0 (0.0%)	1	1 (0.5%)
		Hematuria	0	0 (0.0%)	1	1 (0.5%)
Events Requiring Transfusion			1	1 (1.3%)	14	14 (6.3%)

The majority of the bleeding events which occurred in the trial for both study groups were access site related and were primarily mild access site hematomas. Additional data indicates that the use of the [redacted] catheter may decrease the rate of access site events associated with the SSO₂ Therapy procedure. As discussed in Section 6.3.8, the introduction of the [redacted] catheter during the study led to the almost exclusive use of the coaxial access approach with one arterial access puncture. Table 22 displays access site complications for both the [redacted] and [redacted] catheters. As seen in the table, in comparison to SSO₂ patients receiving therapy with the [redacted] catheter, patients who received treatment with the [redacted] catheter exhibited a decreased event rate from 27.2% to 13.3%. Furthermore, the 13.3% [redacted] catheter access site complication rate is comparable to rates presented by the Control group (12.7%).

Table 22. Access Site Events within First 30 Days by Infusion Catheter Type

	SSO ₂ Therapy Group			
	[REDACTED] (N=147)		[REDACTED] (N=75)	
Location	Events (n)	Pts with Events (n/N; %)	Events (n)	Pts with Events (n/N; %)
Any Access Site Complication	44	40/147 (27.2%)	10	10/75 (13.3%)
ARTERIOVENOUS FISTULA	1	1/147 (0.7%)	0	0/75 (0.0%)
BACTEREMIA	2	2/147 (1.4%)	0	0/75 (0.0%)
CATHETER SITE HEMATOMA	31	31/147 (21.1%)	8	8/75 (10.7%)
CATHETER SITE HEMORRHAGE	1	1/147 (0.7%)	0	0/75 (0.0%)
CATHETER SITE INFECTION	0	0/147 (0.0%)	1	1/75 (1.3%)
ECCHYMOSIS	1	1/147 (0.7%)	0	0/75 (0.0%)
HYPOTENSION	1	1/147 (0.7%)	0	0/75 (0.0%)
PYREXIA	1	1/147 (0.7%)	0	0/75 (0.0%)
RETROPERITONEAL HEMORRHAGE	1	1/147 (0.7%)	0	0/75 (0.0%)
SYNCOPE VASOVAGAL	2	2/147 (1.4%)	0	0/75 (0.0%)
VASCULAR PSEUDOANEURYSM	3	3/147 (2.0%)	1	1/75 (1.3%)

Although SSO₂ Therapy patients had a higher incidence of bleeding events than Control patients, many of these events were primarily mild hematomas that resolved without transfusion or interventional procedures. Furthermore, delivery of SSO₂ Therapy with the [REDACTED] catheter resulted in an access site event rate directly in line with the access site event rate in the Control group, due to the use of a single arterial access puncture rather than two. Because the [REDACTED] catheter is no longer commercially available, the TherOx [REDACTED] catheter is the exclusive infusion catheter for future use, and the reduced access site bleeding rates observed with its adoption are indicative of expectations in commercial practice.

6.3.10.9 Late Safety Profile (one year)

Table 23 presents a cumulative accounting of MACE events through the 1-year assessment.

Table 23. Cumulative MACE to 1 Year

Adverse Event	Control (n=79)		SSO ₂ Therapy (n=222)	
	Events (n)	Pts with Events (n/N; %)	Events (n)	Pts with Events (n/N; %)
MACE Assessment complete through 1 year		75/79 (94.9%)		220/222 (99.1%)
MACE Assessment available ¹ through 1 year		77/79 (97.5%)		220/222 (99.1%)
Composite Cumulative MACE ²	11	8/77 (10.4%)	47	28/220 (12.7%)
Death	1	1/77 (1.3%)	8	8/220 (3.6%)
Target Vessel Revascularization ³	7	7/77 (9.1%)	25	19/220 (8.6%)
TVR: Clinically Driven		5/77 (6.5%)		18/220 (8.2%)
TVR: Objective evidence of ischemia in AMIHOT II lesion	5	5/77 (6.5%)	16	11/220 (5.0%)
TVR: Target Lesion Revascularization	7	7/77 (9.1%)	23	18/220 (8.2%)
TVR: Urgent or Emergent		4/77 (5.2%)		13/220 (5.9%)
Reinfarction	3	3/77 (3.9%)	13	9/220 (4.1%)
Stroke	0	0/77 (0.0%)	1	1/220 (0.5%)

¹Complete or event reported in the interval. Event rate denominators utilize number of subjects with assessment available.

²For Cumulative summaries, subjects who died prior to 1 year are considered to have assessment complete through 1 year.

³AMIHOT main vessel or branches

At 1-year follow up, 28/220 (12.7%) SSO₂ Therapy patients exhibited a total of forty-seven (47) MACE events. In comparison, 8/77 (10.4%) Control patients presented a total of eleven (11) events. Both populations exhibited similar rates of death, TVR, reinfarction and stroke through 1 year. Within the SSO₂ Therapy group, the overall MACE rate throughout 1-year follow-up is entirely reflective of patients with a history of coronary artery disease and previous myocardial infarction. Based upon these data, SSO₂ Therapy does not increase risk for late MACE events.

No SSO₂ Therapy patients demonstrated a serious adverse event determined to be related to the SSO₂ Device or SSO₂ Therapy procedure after day 30. Additionally, there were no non-serious adverse events in the SSO₂ Therapy group after day 30 that were determined to be related to the SSO₂ device or SSO₂ Therapy procedure. Because SSO₂ Therapy is an infusion with no implantable component, and no mechanical manipulation of the heart, the absence of late device/procedure-related adverse events of any kind is a logical and expected outcome. Overall, the AMIHOT II late safety data revealed no demonstrable differences between the study groups in terms of reported events.

6.3.10.10 Summary of AMIHOT II Safety Data

The AMIHOT II clinical trial was undertaken to assess the safety and effectiveness of the SSO₂ Therapy procedure in patients presenting with anterior acute myocardial infarction treated with PCI within six

hours of symptom onset. The randomized, controlled study enrolled subjects from the specified target population who were treated according to the protocol. The resulting scientific evidence supports the following conclusions regarding the safety of SSO₂ Therapy.

Patients assigned to receive adjunctive SSO₂ Therapy following PCI experienced a similar incidence of MACE events assessed at 30 days when compared to patients treated with PCI alone. The primary study endpoint hypothesis of non-inferiority in 30-day MACE rates within a safety delta of 6% was achieved. Late MACE rates at 6 months and up to one year showed that MACE events remain consistent with rates expected in this patient population.

SSO₂ Therapy patients demonstrated a similar incidence, type, and relationship of Serious Adverse Events assessed at 30 days, 6 months, and one year, when compared to Control patients. A general review of Serious Adverse Events also reveals that the types and frequencies of adverse events observed in this clinical study were entirely consistent with this patient population based upon published medical literature.

SSO₂ Therapy patients presented with a higher incidence of vascular disorder events and a higher incidence of bleeding events. SSO₂ Therapy patients received an additional 90 minutes of catheterization time and extended anticoagulation therapy during the 90-minute infusion period. More significantly, SSO₂ Therapy patients enrolled prior to the introduction of the [REDACTED] catheter required a 9F arterial sheath or a second contralateral arterial sheath in order to accommodate [REDACTED] infusion catheter. Increased procedure time and anticoagulation in conjunction with the increased needs for vascular access would be expected to subsequently increase the rates of vascular and bleeding adverse events. The majority of these observed events consisted of mild bleeding events associated with the access site. These events resolved quickly without any long-term residual effects. Importantly, the incidence of access site events decreased with the use of the [REDACTED] catheter, thus enabling smaller sheath sizes (8F) used for coaxial delivery of SSO₂ Therapy with only a single arterial puncture.

The types and frequencies of other adverse events observed in this clinical study were similar across groups and entirely consistent with this patient population.

In summation, the 30-day MACE rate in the AMIHOT II trial was very low overall (5.4%) considering the patient population under study. SSO₂ Therapy patients had a similar 30-day MACE rate to that observed in Control patients. The type and frequency of other adverse events which occurred in this trial suggest that SSO₂ Therapy is associated with a safety profile which is largely similar to that seen with current percutaneous treatment modalities, including PCI, balloon angioplasty, and stent placement.

7 Conclusions

The AMIHOT II clinical trial was a prospective, multicenter, randomized evaluation of the safety and effectiveness of Supersaturated Oxygen (SSO₂) Therapy administered adjunctively in a population of anterior AMI patients revascularized by PCI with stenting within six hours of symptom onset. The primary effectiveness endpoint, superiority in infarct size reduction measured by SPECT imaging at 14 days, was achieved. The absolute median infarct size reduction was 6.5% in SSO₂ Therapy patients as compared to the Control group, translating into an approximate 25% relative reduction. This level of infarct size reduction has been demonstrated to improve clinical outcomes in over twenty years of

controlled studies of this surrogate endpoint. Bayesian hierarchical modeling was used to evaluate the study endpoint hypothesis; infarct size results from the AMIHOT I and II studies yielded a statistically significant outcome, with a calculated posterior probability of superiority of 96.9% that infarct size is smaller in SSO₂ Therapy patients. The study's primary safety endpoint was non-inferiority in the occurrence of 30-day MACE, evaluated within a safety margin of 6.0%. In the AMIHOT II study, 30-day MACE rates were comparable between the study groups (Control 3.8%; SSO₂ Therapy 5.4%). Bayesian hierarchical modeling demonstrated a posterior probability of non-inferiority of 99.5% in consideration of AMIHOT I and II combined 30-day MACE data. Analysis of other non-endpoint safety data showed that SSO₂ Therapy may be associated with an increase in minor bleeding; however, this increase was partially mitigated during the trial with the introduction of a smaller infusion catheter. In conclusion, the clinical results demonstrate that SSO₂ Therapy is safe and effective as an adjunctive therapy in anterior AMI patients treated with PCI within six hours of symptom onset when used in accordance with its labeling. The results of this study indicate that SSO₂ Therapy is effective in reducing infarct size and is safe when used in accordance with its recommended instructions for use.