Statistical Analysis Plan

IC-HOT

"Intra-Coronary Hyperoxemic Therapy"

A MULTI-CENTER EVALUATION OF THE DELIVERY OF INTRACORONARY HYPEROXEMIC SUPERSATURATED OXYGEN THERAPY FOR 60 MINUTES IN ANTERIOR ACUTE MYOCARDIAL INFARCTION PATIENTS WITH SUCCESSFUL REPERFUSION (VIA PCI) ≤ SIX HOURS AFTER SYMPTOM ONSET

Device: DownStream System (DS-1) and
DownStream Cartridge (DSC-2)

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IC-HOT Clinical Trial Statistical Analysis Plan

The intention of this Statistical Analysis Plan (SAP) is to detail the planned analysis and reporting for the IC-HOT Clinical Trial.

The undersigned hereby jointly declare that they have reviewed this statistical analysis plan and agree to its content. Furthermore, they confirm that the statistical analysis plan contains the information relevant for the evaluation of study data.

Statistical Analysis Plan Reviewed and Approved By:

Study Statistician:

_____ _____
Name Date

_____
Title

_____
Affiliation

_____
Signature

Sponsor Approval:

_____ _____
Name Date

_____
Title

_____
Affiliation

_____
Signature

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1. STUDY OVERVIEW

1.1. PROTOCOL SUMMARY

Study Description	A Multi-Center, Consecutively Enrolled Single-Arm Study to confirm the safety and effectiveness of the delivery of supersaturated oxygen (SSO_2) Therapy for 60 minutes selectively into the left main coronary artery (LMCA) with a commercially available qualified SSO_2 delivery catheter used with the TherOx® DownStream® System and Cartridge in the treatment of qualified patients presenting with anterior acute myocardial infarction in whom reperfusion with PCI is successful within six hours after symptom onset.
Investigational Product	The TherOx IC-HOT Clinical Study incorporates the use of three primary components. These include a hardware device called the TherOx DownStream System ("system"), a single-use disposable device called the TherOx DownStream Cartridge ("cartridge") and a commercially available, qualified SSO_2 delivery catheter ("delivery catheter"). The cartridge is loaded into and operated by the DownStream System; the cartridge has a tubing set that connects to an arterial sheath on the patient blood draw side and the SSO_2 delivery catheter on the supersaturated infusate patient return side. SSO_2 Therapy is delivered selectively into the LMCA via the SSO_2 delivery catheter, which is positioned at the ostium of the left coronary arterial tree proximal to the bifurcation.
Indications for Use	The TherOx DownStream System, DownStream Cartridge, and SSO_2 delivery catheter are indicated for: the preparation and delivery of SuperSaturated Oxygen Therapy (SSO_2 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.
Objective	To collect confirmatory data supporting the safety and effectiveness of SSO_2 Therapy in the treatment of anterior acute myocardial infarction (AMI) patients who have undergone successful percutaneous coronary intervention (PCI) with stenting within six hours of experiencing AMI symptoms.
Study Design	This is a non-randomized, single-arm study. Subjects who present with anterior STEMI requiring stent placement in the proximal and/or mid LAD who meet all inclusion and exclusion criteria and provide informed

	consent will be treated with primary PCI with stenting, and if successful and uncomplicated then immediately with post-procedure delivery of SSO_2 Therapy for a duration of 60 minutes.
Patient Enrollment	One hundred (100) patients enrolled and treated with SSO_2 Therapy at up to fifteen (15) centers in the US.
Patient Population	Qualifying anterior STEMI patients (1 mm or greater of ST-segment elevation in 2 or more contiguous leads in V1-V4) successfully treated with PCI and stenting within 6 hours from time of symptom onset.
Study Blinding	This study is not blinded.
Inclusion Criteria	Patients must meet **ALL** of the following criteria: **GENERAL INCLUSION CRITERIA:** Candidates for this study must meet **ALL** of the following criteria: **Pre-PCI:** 1. The subject must be ≥18 and ≤80 years of age. 2. AMI must be anterior (ST-segment elevation ≥1 mm in two or more contiguous leads between V1 and V4 or new left bundle branch block). 3. Subject is experiencing clinical symptoms consistent with acute MI of ≤6 hour duration from time of symptom onset until admission to the emergency room. 4. The subject or legally authorized representative has been informed of the nature of the study, agrees to its provisions and has been provided and signed written informed consent, approved by the appropriate Institutional Review Board (IRB). 5. Subject and his/her physician agree to all required follow-up procedures and visits. **ANGIOGRAPHIC INCLUSION CRITERIA:** These are evaluated after the subject has provided signed Informed Consent but prior to enrollment: 6. Based on coronary anatomy, PCI is indicated for revascularization of the culprit lesion(s) with use of a commercially available coronary stent (bare metal or drug-eluting, at operator discretion) in the LAD. 7. The primary stented infarct-related lesion(s) must be in the

	proximal and/or mid-LAD coronary artery (other lesions in the LAD target vessel, including diagonal branches, may be treated if clinically indicated). 8. Baseline (pre-PCI) TIMI flow grade 0, 1, 2, or 3 flow in the LAD. 9. Successful angioplasty is completed ≤6 hrs from symptom onset, as documented by <50% diameter residual angiographic stenosis within all treated culprit lesions with TIMI 2 or 3 flow and no major complications such as perforation or shock. 10. Expected ability to place the SSO_2 delivery catheter in the coronary ostium of the left main coronary system to deliver SSO_2 Therapy with stable, coaxial alignment.
Exclusion Criteria	Patients will be excluded if **ANY** of the following conditions apply: **GENERAL EXCLUSION CRITERIA** **Pre-PCI:** 1. Prior CABG surgery. 2. Prior myocardial infarction, or known prior systolic dysfunction (known ejection fraction <40% by any prior measure or regional wall motion abnormalities; this criterion does not include left ventricular dysfunction induced by the acute MI). 3. Thrombolytic therapy administered for this STEMI. 4. An elective surgical procedure is planned that would necessitate interruption of anti-platelet agents during the first 30 days post-enrollment. 5. Subjects who previously underwent coronary stent implantation and in whom coronary angiography demonstrates stent thrombosis to be the cause of the anterior AMI. 6. Subjects who have previously undergone an angioplasty or stenting procedure in the left anterior descending coronary artery. 7. Subjects with ventricular pseudoaneurysm, VSD, or severe mitral valve regurgitation (with or without papillary muscle rupture). 8. Any contraindication to MRI imaging. This will include any of the following exclusions: a. Cardiac pacemaker or implantable defibrillator; b. Non-MRI compatible aneurysm clip; c. Neural Stimulator (i.e., TENS unit); d. Any implanted or magnetically activated device (insulin

	pump); e. Any type of non-MRI compatible ear implant; f. Metal shavings in the orbits; g. Any metallic foreign body, shrapnel, or bullet in a location which the physician feels would present a risk to the subject; h. Any history indicating contraindication to MRI, including claustrophobia or allergy to gadolinium; i. Inability to follow breath hold instructions or to maintain a breath hold for >15 seconds; and j. Known hypersensitivity or contraindication to gadolinium contrast. 9. Known impaired renal function (creatinine clearance <30 ml/min/1.73 m^2 by the MDRD formula) or on dialysis. 10. Known platelet count <100,000 cells/mm^3 or >700,000 cells/mm^3 or a known Hgb <10 g/dL. 11. Subject has active bleeding or a history of bleeding diathesis or coagulopathy (including heparin induced thrombocytopenia), or refusal to receive blood transfusions if necessary. 12. History of intracerebral mass, aneurysm, arteriovenous malformation, or hemorrhagic stroke. 13. Stroke or transient ischemic attack within the past six (6) months, or any permanent neurological defect. 14. Gastrointestinal or genitourinary bleeding within the last two (2) months, or any major surgery (including CABG) within six weeks of enrollment. 15. Subject has received any organ transplant or is on a waiting list for any organ transplant. 16. Subject has other medical illness (e.g., cancer, dementia) or known history of substance abuse (alcohol, cocaine, heroin, etc.) that may cause non-compliance with the protocol, confound the data interpretation, or is associated with limited life expectancy of less than one year. 17. Subject has a known hypersensitivity or contraindication to unfractionated heparin, abciximab, aspirin, bivalirudin, cangrelor, clopidogrel, ticlopidine, prasugrel, eptifibatide, tirofiban or ticagrelor that cannot be adequately premeditated. 18. Current use of warfarin, dabigatran, or factor Xa inhibitors, or known intent to administer these agents after the primary PCI. 19. Subjects presenting with or developing in the cath lab prior to completion of the primary PCI procedure any of the following

	conditions: cardiogenic shock (SBP <80 mmHg for >30 minutes), or requiring IV pressors or emergent placement of an intra-aortic balloon pump (IABP), Impella, or other hemodynamic support for hypotension treatment, or cardiopulmonary resuscitation for >10 minutes, or ventricular fibrillation or tachycardia requiring cardioversion or defibrillation. 20. Severe known cardiac valvular stenosis or insufficiency, pericardial disease, or non-ischemic cardiomyopathy. 21. Any significant medical or social condition which in the investigator's opinion may interfere with the subject's participation in the study or ability to comply with follow-up procedures, including MRI (e.g. alcoholism, dementia, lives far from the research center, etc.). 22. Current participation in other investigational device or drug trials. 23. Previous enrollment in this study. **ANGIOGRAPHIC EXCLUSION CRITERIA:** These are evaluated after the subject has provided signed Informed Consent but prior to enrollment: 24. Anticipated inability to achieve a stable coaxial position in the left main coronary artery with the SSO_2 delivery catheter. 25. Treatment during the index procedure of any lesion in either the left main, LCX (including the ramus), and/or RCA. 26. Post-index procedure planned intervention within 30 days (i.e., PCI of non-target lesions in any vessel, or CABG). Note: Planned revascularization (PCI or bypass) of a non-target lesion >30 days following the index procedure is allowed. 27. Anterior MI is due to thrombosis within or adjacent to a previously implanted stent. 28. Left ventriculography demonstrates severe mitral regurgitation, a ventricular septal defect, or a pseudoaneurysm. 29. Any left main coronary artery stenosis >20%. 30. Any untreated LAD or diagonal branch lesion is present with diameter stenosis $\geq$ 50% in a vessel with reference vessel diameter > 2.0 mm (visually estimated), or for which PCI will be required before the MRI study. 31. Presence of a non-stented coronary dissection with NHLBI grade $\geq$B upon completion of the PCI procedure.
Treatment	Study clinical investigators and research staff will be trained according to

Strategy	the clinical protocol, device Instructions for Use, and study regulatory and reporting requirements. The procedural strategy is as follows: • Subjects presenting with anterior STEMI with symptom to presentation time of less than or equal to 6 hours will be screened for clinical eligibility. • If all clinical eligibility criteria are met, the subject or legal representative must sign an IRB-approved Subject Informed Consent Form. • After Informed Consent is signed, if not already administered, subject will receive a loading dose of aspirin 324 mg chewed or 250-500 mg IV, and at least one ADP antagonist, either clopidogrel 600 mg p.o. or prasugrel 60 mg p.o. or ticagrelor 180 mg p.o. per investigator discretion. These loading doses must be given even if the patient is on home dual anti-platelet therapy. • Subject will undergo coronary arteriography and left ventriculography (required before enrollment). • Subject is screened for angiographic eligibility criteria. Patients who are being considered as potential subjects for enrollment into study may be treated with standard anticoagulation prior to and during the procedure. Procedural anticoagulation must consist of either bivalirudin with or without the addition of cangrelor (in which case GP IIb/IIIa inhibition is not recommended unless required for refractory procedural thrombotic complications) or heparin with either cangrelor or a GP IIb/IIIa inhibitor. If bivalirudin is used, an infusion of 1.75 mg/kg/hr should be continued during the SSO_2 infusion and for 3-4 hours post procedure. Note: if a GP IIb/IIIa inhibitor is used, it should be continued during the SSO_2 infusion and for a total of at least 12 hours post procedure, as per local standard of care. The dose of the GP IIb/IIIa inhibitor and bivalirudin infusions should be down-adjusted for renal insufficiency as per FDA labeled instructions. Intravenous cangrelor may also be used according to label per investigator discretion, administered as a bolus plus infusion, with the infusion continued for 2-4 hours post-PCI. • The patient must receive a commercially available intracoronary stent with post-index procedure angiographic TIMI flow grade 2 or 3. • Post index procedure, if all clinical and angiographic entry

	criteria are met, the patient is formally enrolled into the study and may receive SSO_2 Therapy. The introduction of the infusion catheter is the point of intent-to-treat enrollment, whether SSO_2 Therapy is successfully administered or not. • The patient will remain in the cardiac catheterization laboratory for the entire SSO_2 Therapy procedure. Cine angiography w/o contrast will be performed at the beginning of and 30 minutes into the SSO_2 infusion procedure to document stable position of the delivery catheter. • Post SSO_2 Therapy control angiography will be obtained in each enrolled patient to document position of the delivery catheter, TIMI flow, and to assess for intravascular complications.
Patient Follow-Up	Baseline, procedural, post-procedure, in-hospital, and clinical follow-up at 30 days. Cardiac MRI will be performed at 4 days (acceptable range 3-5 days) and at 30 days (±7 days; range 23-37 days) to evaluate device effectiveness. Primary data collection including adverse event reporting is through 30 days; patient safety will be tracked and reported through one year.
Safety Endpoint	The primary endpoint of the study is the rate of Net Adverse Clinical Events (NACE), comprised of the composite incidence of death, reinfarction, clinically-driven target vessel revascularization, stent thrombosis (ARC definite or probable), new onset heart failure or readmission for heart failure, and TIMI major or minor bleeding.
Additional Study Endpoints	Effectiveness is a secondary endpoint for this study. Effectiveness data from dual time point cardiac MRI scans at day 4 and day 30 will be collected and reported, using 30-day median infarct size (% left ventricle (LV) necrosis) as the primary effectiveness outcome measure. Infarct size data in this study will be compared to a matched control population from the INFUSE-AMI clinical trial, and to the AMIHOT II study. In addition, myocardial structural and functional data, including microvascular obstruction at 4 days, and global and regional LV volumes and function at both time periods will be evaluated. Data will only be analyzed in subjects completing the cardiac MRI study in which the imaging data is received and analyzable by the core laboratory.

Additional Safety Data	In addition to 30-day NACE, the following information will be reported and summarized: • Additional bleeding events through 30 days, categorized by the BARC criteria • All other 30-day adverse events • Individual NACE component event rates through 1 year
Event Assessment	An independent Clinical Events Committee (CEC) will review and adjudicate all significant study adverse events, including any potential NACE events or cardiac-related events. Adjudication will be performed in accordance with a prospective CEC Charter including clinical event definitions.
Safety Oversight	An independent Data Safety Monitoring Board (DSMB) will review and assess overall study safety both during and at the conclusion of the study.
Study Sponsor	TherOx, Inc. 17500 Cartwright Road, Suite 100 Irvine, CA 92614 USA TEL: (949) 757-1999 FAX: (949) 757-1989

1.2. Study Assessment Time Points

The study assessment time points will consist of assessments at enrollment/baseline, pre-PCI, post-PCI, during SSO_2 infusion, post-60 min SSO_2 infusion, 12 and 24 hours (± 2 hours) post-PCI, 4 (±1) days, 30 days (±7) and 6/12 months. These time points are outlined in **Table 1.** The time point for primary analysis of safety and effectiveness data is 30 days.

Table 1. Data Collection Procedures

PROCEDURE / TEST	Pre-PCI - Stent	PCI / Stent Procedure	Post- PCI / Stent	Baseline SSO_2	30 min SSO_2	60 min SSO_2	60 (±30) min post-SSO_2	12 (±2) hrs	24 (±2) hrs	Cardiac MRI (4 ± 1 days)	30 (±7) days	6 and 12 mos (±30 days)
Subject Medical / Clinical History / Physical Exam	√											
Subject Informed Consent	√											
General Inclusion / Exclusion Criteria	√											
Angiographic Inclusion / Exclusion Criteria			√									
Cardiac Biomarkers: CK, CK-MB, and Troponin	√							√	√	√[1]		
Arterial blood gas	√		√	√								
WBC, CBC, creatinine, platelet count	√[8]								√[2]			
Cardiac MRI										√[3]	√[4]	
HR, BP	√	√	√	√	√	√						
ECG	√						√[5]			√	√	
Anticoagulation (per protocol)	√											
Antiplatelet loading dose	√											
Cardiac cath lab procedures and information		√										
Cine angiogram w/o contrast of angiographic delivery catheter				√	√	√						
Coronary angiogram with TIMI flow grade assessment	√	√[6]	√			√						
ACT (per protocol)		√		√	√[9]	√						
SSO_2 Therapy Procedure			√	√	√	√						
Per Protocol Medications	√	√	√	√	√	√		√			√	√
Dual Antiplatelet Medication	√	√	√					√[7]			√[7]	√
Concomitant Cardiac Medications	√	√	√	√	√	√		√			√	√
Adverse Events				√	√	√	√	√	√	√	√	√

[1]Required only if cardiac events.
[2] Measured the day after the index procedure and then daily until the nadir value for Hgb, Hct, platelet count and the peak value for serum creatinine is reached or the patient is discharged. Baseline and nadir information will be collected.
[3]All subjects to have in hospital cardiac MRI.
[4]A protocol deviation will be recorded if the MRI is performed between 38 and 90 days but those images will be evaluated by the core laboratory and the results will be analyzed.
[5] ECG to be performed 60±30 minutes after the last angiogram after the SSO_2 procedure.
[6] Left ventriculography required prior to enrollment.
[7] Post procedure DAPT is according to current recommended standards as published by the AHA/ACC/ESC, which recommends a minimum of twelve (12) months DAPT for subjects receiving a stent.
[8] Must have screening HCT, HGB, platelet count and creatinine measurement per inclusion/exclusion criteria; however, lab results do not need to be available prior to enrollment.
[9] The mid-procedure 30-min ACT sample is required if patients receive heparin for procedural anticoagulation.

2. SAMPLE SIZE

The intent of this study is to collect confirmatory patient safety and effectiveness data on the intracoronary perfusion of hyperoxemic blood administered to qualifying anterior acute myocardial infarction patients who have undergone successful PCI with stenting within 6 hours of symptom onset. A total of 100 qualifying patients will be enrolled in this trial and treated with SSO_2 Therapy. Safety and effectiveness data will be analyzed in accordance with this plan.

2.1. Primary Safety Endpoint

The primary endpoint of the IC-HOT trial to achieve study success is the 30-day rate of Net Adverse Clinical Events (NACE), compared against an objective performance goal (OPG) based from the rate of an appropriate historical control population from the INFUSE-AMI trial. For safety, the composite NACE endpoint includes a hierarchical total of the following events:

- Death (all-cause)
- Reinfarction
- Target Vessel Revascularization (clinically driven)
- TIMI major or minor bleeding
- New onset severe heart failure or rehospitalization for heart failure
- Stent thrombosis (ARC definite or probable)

This composite endpoint includes safety categories that are of significance in contemporary AMI studies and includes the specific risks that were identified as areas of concern by FDA with regards to SSO_2 Therapy. It should be noted that an observed event of myocardial rupture would be included in the Death category. The IC-HOT study will use well-established clinical event definitions[1] for the component NACE event categories:

DEATH
(Per ARC Circulation 2007:115: 2344-2351)

[1] Gibson, CM, Maehara A, Stone GW, et al. Rationale and design of the INFUSE-AMI study: A 2 × 2 factorial, randomized, multicenter, single-blind evaluation of intracoronary abciximab infusion and aspiration thrombectomy in patients undergoing percutaneous coronary intervention for anterior ST-segment elevation myocardial infarction. *Am Heart J* 161(3):478-86.

All deaths are considered cardiac unless an unequivocal non-cardiac cause can be established. Specifically, any unexpected death even in patients with coexisting potentially fatal non-cardiac disease (e.g. cancer, infection) should be classified as cardiac.

Cardiac death:
Any death due to proximate cardiac cause (e.g. MI, low-output failure, fatal arrhythmia), unwitnessed death and death of unknown cause, all procedure related deaths including those related to concomitant treatment.

Vascular death:
Death due to non-coronary vascular causes such as cerebrovascular disease, pulmonary embolism, ruptured aortic aneurysm, dissecting aneurysm, or other vascular cause.

Non-cardiovascular death:
Any death not covered by the above definitions such as death caused by infection, malignancy, sepsis, pulmonary causes, accident, suicide or trauma.

MYOCARDIAL INFARCTION (MI)
(Per ARC Circulation 2007:115: 2344-2351)

Myocardial Infarction Classification and Criteria for Diagnosis

Classification	Biomarker Criteria*	Additional Criteria
Periprocedural PCI	Troponin >3 x URL or CK-MB >3 x URL	Baseline value <URL
Periprocedural CABG	Troponin >5 x URL or CK-MB >5 x URL	Baseline value <URL and any of the following: new pathologic Q waves or LBBB, new native or graft vessel occlusion, imaging evidence of loss of viable myocardium
Spontaneous	Troponin >URL or CK-MB >URL	
Sudden death	Death before biomarkers obtained or before expected to be elevated	Symptoms suggestive of ischemia and any of the following: new ST elevation or LBBB, documented thrombus by angiography or autopsy
Reinfarction	Stable or decreasing values on 2 samples and 20% increase 3 to 6	If biomarkers increasing or peak not reached then insufficient

	hours after second sample diagnose recurrent MI.	data to diagnose recurrent MI.

URL=Upper Reference Limit (defined 99th percentile of normal reference range);
LBBB=Left Bundle-branch Block
* Baseline biomarker value requiring before study procedure and presumes a typical rise and fall

– **Periprocedural MI After PCI**
The periprocedural period includes the first 48 hours after PCI.

– **Periprocedural MI After CABG**
The periprocedural period includes the first 72 hours after coronary artery bypass grafting (CABG).

– **Spontaneous MI**
MI after the periprocedural period may be secondary to late stent complications or progression of native disease. Performance of ECG and angiography supports adjudication to either a target or non-target vessel in most cases.

With the unique issues and pathophysiological mechanisms associated with these later events as well as the documented adverse impact on short-and long-term prognosis, a more sensitive definition than for periprocedural MI of any elevation of troponin above the upper range limit is used. All late events that are not associated with a revascularization procedure simply as spontaneous.

– **Electrocardiographic Classification**
Within this category it is distinguished:

– **Q-wave MI (QMI)**
Development of new pathological Q waves in 2 or more contiguous leads (according to the Minnesota code as assessed by the ECG core lab) with or without post- procedure CK or CK-MB levels elevated above normal.

– **Non Q-wave MI (NQMI)**
All MIs not classified as Q waves.

– **Relation to the Target Vessel**
All infarcts that cannot be clearly attributed to a vessel other than the target vessel will be considered related to the target vessel.

Clinically-driven Target Vessel Revascularization (TVR)

TVR is defined as any repeat percutaneous intervention of the target vessel or bypass surgery of the target vessel. The target vessel consists of the target lesions + any additional lesions in the main epicardial coronary artery or branches containing the target lesion (the left anterior descending artery, left circumflex coronary artery or right coronary artery). TVR will be considered clinically driven if there is clinical or electrocardiographic evidence of ischemia with an angiographic ≥50% diameter stenosis (core laboratory measured), or an angiographic ≥70% diameter stenosis (core laboratory measured) in the absence of ischemia.

TVR will be sub-divided into target lesion revascularization (TLR) and non-TLR. The target lesion is defined as the treated segment from 5 mm proximal to the stent and to 5 mm distal to the stent.

STENT THROMBOSIS

(Per ARC Circulation 2007:115:2344-2351), with modification for SSO_2 delivery:

Stent Thrombosis should be reported as a cumulative value at the different time points and with the different separate time points.

1) Intra-procedural stent thrombosis during the PCI procedure (before SSO_2) - will be adjudicated by the angiographic core laboratory, but is not considered an endpoint event.

2) Stent thrombosis during SSO_2 delivery - may or may not be considered an endpoint event (see below)

3) ARC stent thrombosis period: Time 0 is defined as the time point after the guiding catheter and diagnostic catheter for SSO_2 Therapy delivery has been removed and the subject has left the cardiac catheterization lab.

Sub-categorization of ARC Stent Thrombosis Timing

Acute stent thrombosis*:	0 - 24 hours post stent implantation
Subacute stent thrombosis*:	>24 hours – 30 days post stent implantation
Late stent thrombosis†:	30 days - 1 year post stent implantation
Very late stent thrombosis†:	>1 year post stent implantation

* Acute + subacute = early stent thrombosis.

† Includes "primary" as well as "secondary" late stent thrombosis; "secondary" late stent thrombosis is a stent thrombosis after a target segment revascularization (e.g., for restenosis).

Categories (ARC [Definite, Probable, Possible] and Non-ARC [SSO_2-Therapy related])

a. Definite stent thrombosis

Definite stent thrombosis is considered to have occurred by either angiographic or pathologic confirmation.

Angiographic confirmation of stent thrombosis*

The presence of an intracoronary thrombus that originates in the stent or in the segment 5 mm proximal or distal to the stent and presence of at least 1 of the following criteria within a 48-hour time window:

- Acute onset of ischemic symptoms at rest
- New ischemic ECG changes that suggest acute ischemia
- Typical rise and fall in cardiac biomarkers (refer to definition of spontaneous MI)
- Non-occlusive thrombus
    - Defined as a (spheric, ovoid, or irregular) noncalcified filling defect or lucency surrounded by contrast material (on 3 sides or within a coronary stenosis) seen in multiple projections, or persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream.
- Occlusive thrombus
    - TIMI 0 or TIMI 1 intrastent or proximal to a stent up to the most adjacent proximal side branch or main branch (if originates from the side branch).

Pathological confirmation of stent thrombosis

Evidence of recent thrombus within the stent determined at autopsy or via examination of tissue retrieved following thrombectomy.

*The incidental angiographic documentation of stent occlusion in the absence of clinical signs or symptoms is not considered a confirmed stent thrombosis (silent occlusion).

b. Probable stent thrombosis

Clinical definition of probable stent thrombosis is considered to have occurred after intracoronary stenting in the following cases:

- Any unexplained death within the first 30 days‡

- Irrespective of the time after the index procedure, any MI that is related to documented acute ischemia in the territory of the implanted stent without angiographic confirmation of stent thrombosis and in the absence of any other obvious cause

‡ For studies with ST-elevation MI population, one may consider the exclusion of unexplained death within 30 days as evidence of probable stent thrombosis.

c. Possible stent thrombosis

Clinical definition of possible stent thrombosis is considered to have occurred with any unexplained death from 30 days after intracoronary stenting until end of study follow-up.

Protocol definition of stent thrombosis

For this protocol, the primary definition of stent thrombosis is ARC definite or probable stent thrombosis, or SSO_2 Therapy-related stent thrombosis associated with new onset chest pain and either new or worsening ischemia and/or reduced TIMI flow.

Note: ARC possible stent thrombosis and SSO_2 Therapy-related stent thrombosis not associated with new onset chest pain and either new or worsening ischemia and/or reduced TIMI flow will not be considered primary endpoint events.

TIMI Bleeding:

TIMI Major Bleeding is defined as intracranial hemorrhage or overt bleeding associated with a decrease in Hgb >5 g/dL (or >15% of hematocrit), or use of any blood product transfusion. TIMI Minor Bleeding is defined as observed blood loss (e.g., gross hematuria or hematemesis, heme-positive coffee ground emesis, heme-positive melena, hematoma or retroperitoneal bleeding) associated with a hemoglobin decrease of >3 g/dL (or a hematocrit decrease of >9%), or a hemoglobin decrease >4 g/dL (or >12% of hematocrit) with no bleeding site identified.

Severe Heart Failure:

Severe heart failure is defined as heart failure requiring intubation, intra-aortic balloon pump (IABP) insertion, 100% oxygen by face mask, documented PaO_2 <60 mmHg or radiographic evidence of pulmonary edema, or heart failure necessitating re-hospitalization.

Safety Endpoint: Objective Performance Goal

As a benchmark with a similar anterior wall AMI population, the INFUSE-AMI study yielded a 10.7% 30 day NACE event rate for control subjects receiving no aspiration and no intracoronary abciximab (control subject data analysis performed for TherOx by the INFUSE-AMI Principal Investigator Gregg Stone, MD, and the Biostatistics Department at the Cardiovascular Research Foundation). In INFUSE-AMI all of the components of the NACE endpoint were adjudicated using specific definitions, except TIMI bleeding. The same definitions will be used for the present study (except that TIMI bleeding will be adjudicated). The INFUSE-AMI study reflects current cath lab practice, and therefore the use of a composite endpoint benchmark from the control group's observed event rates is an appropriate comparator for the present prospective study.

The proposed threshold for success of the prospective trial is that the observed 30-day NACE rate in the SSO_2 group will be no greater than the 30-day NACE rate observed in the INFUSE-AMI control group, 10.7%.

Furthermore, the specific 30-day NACE event categories of death, stent thrombosis, myocardial rupture, and SAE bleeding will be examined in terms of individual events. The numbers provided in **Table 4** below represent the FDA-recommended 30-day event rate guidelines for these individual safety endpoints. FDA will conduct clinical review of event counts, severity, and patient circumstances. Events will be reviewed and evaluated in the context of individual patient circumstances, relationship to the investigational device, and overall patient status in an overall determination of device safety.

Table 4. FDA Guidelines for Acceptable Adverse Event Rates

30-Day NACE Event	**IC-HOT Trial FDA Guideline**
Death	≤3.0%
Stent Occlusion	≤3.0%
Myocardial Rupture	≤1.0%
TIMI Major and Minor Bleeding	≤3.0%

Weighing the frequency, severity, and causal factors of the observed adverse events in this study, the safety profile of SSO_2 Therapy will be measured against the observed effectiveness results in an overall evaluation of the risk-benefit profile.

To further evaluate the safety profile of Optimized SSO_2 Therapy, the treatment cohort will be followed throughout the post-approval data collection period (12 months), however the primary endpoint will be at 30 days.

For the purpose of comparing the new, modified NACE endpoint for this confirmatory study with previous studies of SSO_2 Therapy, the data from the AMIHOT II study were re-examined using the new, modified NACE endpoint definitions. **Table 5** shows summary data for AMIHOT II. In the total patient sample, 13.1% (29/222) of SSO_2 patients experienced 30-day NACE. Therefore, the IC-HOT clinical trial NACE endpoint OPG of 10.7% is more conservative than the AMIHOT II results, and is anticipated to exhibit lower event rates than AMIHOT II due to safety improvements with both the SSO_2 delivery catheter placement and a more refined patient anticoagulation regimen.

Table 5. AMIHOT II 30-day NACE Summary Data

	Death	**Re-MI**	**TVR**	**Bleeding**	**CHF**	**Stent thrombosis**	**Total NACE***
SSO_2 group (n=222)	1.8% (n=4)	1.8% (n=4)	3.6% (n=8)	3.6% (n=8)	3.6% (n=8)	4.0% (n=9)	13.1% (n=29)

*Total NACE = the number of unique patients experiencing one or more NACE events

In addition to 30-day NACE, the following safety data will also be reported and summarized:

- Additional bleeding events through 30 days, categorized by the BARC criteria
- All other 30-day adverse events
- Individual NACE component event rates through 1 year

2.2. Additional Study Endpoints

Effectiveness

Effectiveness is a secondary endpoint for this study. Effectiveness data from dual time point cardiac MRI scans at day 4 and day 30 will be collected and reported, using 30-day median infarct size (% left ventricle (LV) necrosis) as the primary effectiveness outcome measure. Current practice uses 30-day cardiac MRI infarct size for final infarct size measurement in clinical studies[2,3]. Final infarct size at days 4 and 30 will be analyzed and compared to the a matched set of patients from the INFUSE-AMI trial, a study of very similar entry criteria (STEMI with proximal or mid LAD treatment) in which infarct size was assessed by cardiac MRI at the same core laboratory at the same time frame (both 4 and 30 days). Matching (1:1) will be performed on the following parameters which are known affect infarct size: Time from symptom onset to first device; baseline and post PCI TIMI flow (core laboratory); proximal vs. only mid LAD treatment; and angiographic collateral flow (if possible). In addition, since intracoronary abciximab was associated with reduced infarct size in INFUSE-AMI, these patients will be excluded from the match if possible. Attempts will be made to match all 100 SSO_2 patients. However, as the 4-day MRI was pre-specified in INFUSE-AMI in only half the patients, this may not be possible for the early time point. In this case a 2:1 match will be performed.

As an additional effectiveness analysis, the final infarct size at 30 days from the present study will be compared to the final infarct size from the SSO_2 Therapy group of the AMIHOT II study. The AMIHOT II study utilized 14-day Tc-99m sestamibi SPECT imaging for final infarct size measurement, which was the gold standard at that time. SPECT has been replaced in favor of cardiac MRI due to several reasons, including the greater ease of use and convenient collection of myocardial functional and structural data from the scans. Both techniques measure final infarct size and are validated against histological measurements[4,5,6].

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[2] Stone GW, Maehara A, Witzenbichler B, et al. Intracoronary abciximab and aspiration thrombectomy in patients with large anterior myocardial infarction: the INFUSE-AMI randomized trial. *JAMA* 2012 May 2;307(17):1817-26.

[3] Carlsson M, Arheden H, Higgins CB, et al. Magnetic resoncance imaging as a potential gold standard for infarct quantification. *J Electrocardiol* 2008;41(6):614-20.

[4] Medrano R, Lowry RW, Young JB, et al. Assessment of myocardial viability with 99mTc sestamibi in patients undergoing cardiac transplantation. A scintigraphic/pathological study. *Circulation* 1996;94:1010-17.

[5] Kristensen J, Mortensen UM, Nielsen SS, et al. Myocardial perfusion imaging with TC-99m sestamibi early after reperfusion reliably reflects infarct size reduction by ischaemic preconditioning in an experimental porcine model. *Nucl Med Com* 2004;25:495-500.

[6] Hsu LY, Natanzon A, Kellman P, et al. Quantitative myocardial infarction on delayed enhancement MRI. Part I: Animal validation of an automated feature analysis and combined thresholding infarct sizing algorithm. J Magn Reson Imaging 2006;23(3):298-308.

The effectiveness comparisons are not powered, and while statistical comparisons will be reported (as described below); they are for descriptive purposes only.

3. STUDY POPULATION FOR ANALYSIS

Patients who meet the study's selection criteria and provide informed consent will be consecutively enrolled into this multi-center clinical trial. An all treated subjects population will be used as the primary analysis population for safety data. The all treated subjects population is defined as all patients enrolled into the trial. Study enrollment occurs when the study device is introduced into the patient (*i.e.*, the delivery catheter). The all treated subjects analyses will include patients who die, are lost to follow up, and patients with protocol deviations. All available data, regardless of whether data are derived within specified time windows, will be included in the analysis. For the all treated subjects analysis, patients who do not complete the entire course of treatment with SSO_2 Therapy will be included.

4. INDEPENDENT REVIEW COMMITTEES

4.1. Clinical Events Committee

During the course of the study, an independent Clinical Events Committee (CEC) will be responsible for the adjudication of clinical events.

The CEC is responsible for adjudicating protocol-defined adverse events. The committee determines whether the reported events meet the established definitions for the adverse events that will be tracked in this study. This process includes a thorough review of the source documents for the event and an independent adjudication of the event according to protocol definitions.

The CEC may or may not agree with conclusions drawn by the investigator from a specific investigational site; in this case, the CEC's conclusion will serve as the final decision for submission to regulatory authorities and for reporting and publication. Furthermore, should the committee members become aware of an event through the review of source documents from the research subjects, they will be able to adjudicate those events even if the site investigator has not reported them.

In the course of reviewing an event, the CEC will determine the following:

- Agreement or disagreement with event as reported by the study investigator

- Adverse event classification
- Relation of adverse event to investigational device
- Need for additional data

Events to be adjudicated for this study include:

- **Disease specific**:
  - Death (cardiac death, vascular death, non-cardiac death; if the cause of death is unknown it will be adjudicated as cardiac death)
  - Revascularization (PCI or CABG): clinically driven, non-clinically driven
  - Recurrent angina or reinfarction (Q-wave MI, Non-Q-wave MI, target vessel related, non-target vessel related)
  - Arrhythmias (2^{nd} and third degree heart block, atrial fibrillation, flutter or supraventricular tachyarrhythmias, non-sustained or sustained ventricular tachycardia or fibrillation)
  - Cerebrovascular accident (including stroke and TIA)
  - Stent thrombosis (ARC definite or probable)
  - New onset heart failure
  - Hospital readmissions (cardiac heart failure related, cardiac non-heart failure related, non-cardiac)
  - Embolism (including air emboli and thromboemboli)
  - All bleeding (TIMI scale and BARC scale) and blood produce transfusions
- **Procedure related:**
  - Vascular complications: access site hematoma, pseudoaneurysm, arteriovenous fistula peripheral ischemia or nerve injury
  - Bleeding complications, including Intracerebral Hemorrhage (ICH), and Hemolysis
  - Need for surgical intervention at the access site
  - Transfusion
  - Infection
- **Device specific:**
  - Dislodgement of the device requiring repositioning
  - Dissection of the LMCA or its branches
  - Thrombosis (including catheter thrombus)
  - Intra-procedural ischemia related to catheter position in LMCA
  - Any clinically-driven discontinuation of SSO_2 Therapy before 60 minutes

4.2. Additional Event Definitions:

Death, myocardial infarction, and stent thrombosis will be adjudicated according to the definitions provided in **Section 2.1**. The definition for myocardial rupture, which will be used as an individual sub-component of NACE for specific review, is provided herein.

MYOCARDIAL RUPTURE: Myocardial rupture is typically characterized by a sudden demise without prior hemodynamic instability and no severe arrhythmia. Myocardial rupture can be defined as "hemodynamic deterioration with new onset of either a ventricular septal defect, papillary muscle rupture, or myocardial free wall rupture as evidenced on angiography, echocardiography or other imaging study, or autopsy".

Without confirmatory evidence of rupture, myocardial rupture may be a clinical diagnosis if accompanying adverse events occur such as sudden death, severe hemodynamic instability accompanied by a new or enlarging pericardial effusion or other events that would strongly suggest that a rupture has occurred.

5. PLANNED STUDY ANALYSES

5.1. Interim Analyses

No interim analyses for this study are planned.

5.2. Primary Analysis and Reporting

The primary analysis for this study will be performed after all patients have completed the 30-day follow-up assessment.

6. STATISTICAL METHODS

6.1. General

All statistical analyses will be performed using the all treated subjects patient sample as described above. Samples representing subsets will be evaluated in the same manner as the analysis of the all treated subjects samples. Statistical analysis will be performed and reported when all patients have completed the 30-day follow-up assessment.

Data from this study will be tabulated using descriptive statistics. Continuous variables will be presented as mean and standard deviation, as well as median, range and interquartile range. For categorical variables, relative frequencies will be provided and 95% confidence intervals using the Jeffrey's procedure will be computed[7].

6.2. Methods for Handling Missing Data

Reasonable efforts will be made to obtain complete data for all patients; however, missing observations will occur due to patients lost to follow-up or noncompliance with required assessments. The reasons for missing data will be evaluated (*e.g.*, patient is deceased, lost to follow up, missed appointment, etc.). The distribution of prognostic factors between patients with data and those without data will be assessed to evaluate any potential sources of bias.

Adjustments for missing NACE component safety data or missing 30-day infarct size data will be performed only if deemed necessary and will be described completely. In the case of evidence showing systematic patterns of missing data ("informative" missing data), alternative strategies for analyzing such data, depending on the pattern, will be investigated. In addition to an analysis of all available data, sensitivity analyses will be considered, including an analysis using multiple imputation methods for missing observations. Denominators for adverse event incidence rates will be based on the all treated subjects population sample.

6.3. Evaluation of Site Heterogeneity

To evaluate differences among sites in the trial, summary tables by site will be presented for baseline variables, including demographics and medical history, baseline clinical variables, and PCI procedure variables, as well as for the NACE assessment variables. Sites with fewer than five patients will be pooled. For continuous normally distributed variables, the mean, standard deviation, range and two-sided 95% confidence interval around the mean, will be presented by site. For categorical parameters, data will be summarized by site by their respective proportions and two-sided 95% confidence intervals.

[7] Brown LD, Cai TT, and DasGupta A. Interval estimation for a binomial proportion. Statist Sci 2001;16:101-33.

Analyses will be performed to evaluate any association between site and each of the variables of interest. For continuous variables statistical tests for differences in means among study sites will be performed using a one-way analysis of variance (ANOVA) model with a term for site. For categorical variables, tests for differences among sites will be performed using Fisher's Exact test. The critical safety assessment variables will be evaluated similarly for site effect. If variables are found to differ by study site, then the variable and/or study site may be identified for special consideration in subsequent analyses. The site effect will be further examined in a multivariate analysis to determine if site is independently associated with outcome.

6.4. Additional Analyses

Exploratory analyses not identified in this SAP may be performed. Any post-hoc, or unplanned analyses performed which are not identified in this SAP will be identified in the Clinical Study Report.

For further scientific interest, in order to look for explanatory variables related to the occurrence of endpoint events or variance in effectiveness outcomes, multiple logistic regression techniques may be used to create models predicting study outcomes. These analyses are intended to be exploratory in nature. Candidate variables for inclusion in the model will be chosen from among the baseline and demographic variables with input from clinicians.

The following covariates are pre-specified and may be included in secondary analyses as covariates or evaluated with exploratory/subgroup analyses to assess their influence on study outcomes:

age
sex
BMI
time to reperfusion (continuous)
lesion location (proximal LAD, mid LAD,)
TIMI flow (pre and post PCI)
use of IIb/IIIa inhibitors
hypertension history
diabetes history

smoking history
impaired renal function
multi-vessel disease
current Killip class
previous cardiovascular disease history
bleeding requiring transfusion
study center
time to reperfusion (0-3 hrs and 3-6 hrs)
lesion location (proximal, non-proximal)

Any such analyses are intended to be exploratory in nature and secondary with respect to device approval.

6.5. Evaluation of Study Data

6.5.1. Study Subjects

The number of subjects enrolled by site will be provided as a table.

Disposition of study subjects will be described as a table. Categorical reasons for non-enrollment of all screened subjects will be listed, and all enrolled subjects will be accounted for. The frequency and percent of subjects by site and overall receiving either 0, partial or complete SSO_2 Therapy, completing the MRI at 3-5 and 30 days, completing the 30 day follow-up and health status, and died or were lost to follow-up will be presented. Adherence to study inclusion/exclusion criteria and other protocol requirements will be descriptively tabulated.

A by-subject listing will include the reference data for these tables.

Tabular visit data collection information for the 30-day follow-up, 6 month follow-up, and 1 year follow-up will be provided as well as the reference by-subject listing.

6.5.2. Background Demographic and Medical History

Baseline patient characteristics will be presented descriptively. Patient baseline variables will include age, gender, ethnicity, race, height, weight, BMI, and significant medical

history, door to balloon time, time from symptom onset to balloon, Killip class, thrombolytic treatment, adherence to study inclusion/exclusion criteria, and baseline laboratory values

By-subject listings will include the reference data for these tables.

6.5.3. Index PCI, SSO_2 Therapy, Core Lab Variables, and MRI

Tabulations for the index PCI procedure, hemodynamics, and pre-screening ABG and ACT, and procedural medications will be provided. PCI procedure variables will include the guide catheter and femoral arterial sheath sizes, presence of multi-vessel disease, target lesion characteristics, pre-and post-procedure TIMI flow and % stenosis, presence of dissection, number of stents, total stented length, stent type and complications.

SSO_2 Therapy procedure data, including arterial access variables, device usage information, intra-procedural data, including HR, BP, arterial blood gas measurements and ACT values, and post-therapy data will be presented descriptively.

By-subject listings will include the reference data for these tables.

The Angiographic Core Lab values tabulations include pre-PCI angiographic data, post thrombectomy prior to stent angiographic data, pre-stent and post-stent worst angiographic data, post-PCI prior to SSO_2 angiographic data, angiographic quantitative measurements, post-SSO_2 angiographic data, and vessel level characteristics.

Tabulations for thromboembolic complications will be provided.

By-subject listings will include the reference data for these Core Lab tables.

Tabulations for compliance to the scheduled cardiac MRI scans for all patients and for only in-time window patients will be provided. Cardiac MRI Core Laboratory data will be tabulated for all patients for only in-time window patients and for all patients with paired MRI scan data (i.e., both 3-5 day and 30 day MRI data).

6.5.4. Hospital Discharge Variables

The percentage of patients with AICD placement during hospitalization, and the discharge location or disposition will be tabulated. Days in hospital, ICU/CCU and step-down unit will be presented descriptively.

6.5.5. Laboratory Variables

Baseline, 12-hour, and 24-hour labs will be presented descriptively by time period (pre versus post treatment). Percentages of patients with values outside pre-specified ranges may be tabulated as well. Laboratory variables include cardiac enzymes, hematology, and clinical chemistry values. In addition, peak biomarker results will also be tabulated.

A by-subject listing will include the reference data for these tables.

6.5.6. Safety Assessment

All adverse events will be recorded and tabulated and grouped by event type overall, and by visit; occurring in-hospital, at the 30-day visit, and post-30 days through 1 year.

An independent Clinical Events Committee will adjudicate any suspected NACE events, cardiac adverse events, vascular events, bleeding, and other events that may have a device relationship. Adverse events will further be categorized as serious or not serious and as primary events or associative symptoms/events.

An adverse event is defined as serious whenever the adverse event is fatal, life-threatening, disabling, results in patient hospitalization or prolongation of hospitalization, or requires medical intervention to prevent permanent impairment of a body structure or function.

Events will be further grouped according to diagnosis when the diagnosis is identified by the reporter. The diagnosis will be categorized as the Primary Event. Associative events are defined as reported/coded events (*e.g.*, signs/symptoms) associated with a precipitating primary event/diagnosis (*e.g.*, a primary event/diagnosis of GI bleed with associative events of nausea, hematemisis, and anemia).

Incidence of adverse events for each reporting period will be tabulated (number and percentage) overall and by categorization up to the 1 month (30 day) follow up visit. Further, adverse events will be summarized by relationship to the investigational device, interventional procedure, and disease.

The in-hospital and 30-day clinical outcomes, including the composite Net Adverse Cardiac Events (NACE) rate, the composite Target Vessel Failure (TVF) rate, the individual NACE and TVF component event rates, as well as the rate of other protocol-specified events requiring adjudication will be similarly tabulated for all CEC adjudicated events as well as all site-reported events.

The critical safety data to be evaluated in this trial, including death, myocardial rupture, stent occlusion and SAE bleeding through 30 day follow-up, will be tabulated overall and by onset period (intraprocedure, prior to discharge, discharge to 30 days). The observed event rates for each component event type will be assessed in comparison to the FDA guideline for acceptable critical safety event rates, and with respect to individual patient circumstances for each event. Relative frequency and 95% confidence interval will be computed for each event type.

A by-subject listing will include the reference data for these tables.

6.5.7. Concomitant Medications

Concomitant medications will be tabulated by study visit time, and a by-subject listing will be provided.

6.6. Programming Considerations

6.6.1. Statistical Software

Statistical analyses will be generated using SAS® Software version 9.1 or later.

6.6.2. Analysis Data Sets

Analysis data sets will be prepared by the study Data Coordinating Center and provided to TherOx Inc. and the Biostats group at the Cardiovascular Research Foundation, who

will perform the data analysis in accordance with this plan. Analysis data sets will be validated prior to distribution of data. The organization and contents of the data sets will follow agreed upon specifications.

6.6.3. Analysis Display Formatting

Table, listing, and figure outputs will, where possible, stand on their own. That is, each table, listing, and figure output will be appropriately titled, footnoted, and labeled such that the contents of the output are clear without reference to other study documents.

Mean, standard deviation, median and range (min, max) will be presented for continuous data. Other summary statistics (*e.g.*, median, quartiles, 5%, 95% intervals) may be used as appropriate. Categorical data will be presented as % (n/N) with 95% confidence intervals. Percentages will be rounded and reported to a single decimal point (xx.x%). Percentages corresponding to null categories may be suppressed. P-values, when included, will be reported to three decimal places with a leading zero (0.001). All p-values reported on default output from statistical software may be reported at the default level of precision. P-values <0.001 will be reported as <0.001 not 0.000.

Population(s) represented on the tables or data listings will be clearly identified in the title of the Table. Sub-populations will provide sufficient detail to ensure comprehension of the population used for analysis in a table or figure. Population sizes may be presented as totals in the column header as (N=xx), where appropriate. When presenting summary statistics (*e.g.*, n/N), N represents the number of patients with non-missing values.

6.6.4. Rules and Definitions

Derived or computed variables will be documented in an analysis dataset specification that will be prepared prior to generation of analysis datasets. Derived data will be identified and documented in the SAS programs that create the analysis files.