Impact of Endothelin-1 on the Development of Cardiac Allograft Vasculopathy in Heart Transplant Recipients: Endothelin Receptor Antagonism and Vasomotor Function

NCT05373108

July 28, 2021

STUDY PROTOCOL

Sponsor/Institution: UCLA

Drug Support: Actelion Pharmaceuticals US Inc, a Janssen Pharmaceutical company of Johnson and Johnson and represented by Janssen Research and Development, LLC

Funding Support: American Heart Association

Principal Investigator: Rushi Parikh, M.D., FACC, FSCAI

TITLE: Impact of Endothelin-1 on the Development of Cardiac Allograft Vasculopathy in Heart Transplant Recipients

BACKGROUND/SIGNIFICANCE

Dr. Christiaan Barnard performed the first human-to-human heart transplant in South Africa in 1967.(1) Over 50 years later, nearly 5,000 heart transplants are performed annually worldwide, yet the "Achilles' Heel" of long-term survival remains the same—cardiac allograft vasculopathy (CAV).(2,3) CAV is a complex immune and inflammatory-mediated fibroproliferative disease of the graft coronary circulation defined by concentric intimal and negative remodeling (i.e. vessel shrinkage due to medial vasoconstriction and adventitial fibrosis), dual processes that together result in accelerated luminal narrowing.(4) Rapidly progressive disease just 1 year after transplantation has been shown to predict death and/or graft loss at 5 years.(5,6) Despite advances over the past few decades to prevent CAV including the advent of statins, robust immunosuppressive agents to treat acute rejection, and aggressive infectious prophylaxis, recent international registry data demonstrate minimal improvement in the 5-year incidence and survival due to CAV over this period (32% to 29% and 71% to 76%, respectively). Indeed, CAV still accounts for an unacceptably high proportion of long-term graft failure, including 1 in 8 late deaths (beyond 1-year post-transplant).(2) These sobering data highlight that the clinical care of heart transplant recipients in the contemporary era is greatly limited by a lack of effective treatment to prevent and/or slow the progression of CAV. Hence, there is an urgent need to elucidate the mechanisms of CAV in order to target new therapies and impact long-term outcomes.

One compelling mechanistic candidate is endothelin-1 (ET-1), a principal molecular mediator of vascular integrity with powerful vasoconstrictive, mitogenic, and pro-inflammatory effects throughout the vessel wall.(7) Notably, ET-1 also carries immediate therapeutic potential because safe inhibitory drugs—endothelin receptor antagonists (ERAs)—are already clinically well-established (approved for the treatment of pulmonary arterial hypertension [PAH]) and widely available.(8,9) Furthermore, they do not possess and significant drug-drug interactions with the standard post-heart transplant pharmacological regimen.

A series of classic studies using rat models of heart transplantation established the plausibility of ET-1 as a mechanistic driver of CAV. First, Okada et al. observed strong ET-1 immunoreactivity throughout the entire coronary vessel wall post-transplant and found that orally-administered bosentan, a non-selective endothelin receptor antagonist, attenuated these changes and significantly reduced intimal growth and improved the lumen/vessel ratio.(10) Simonson et al. then conducted similar studies using phosphoramidon, an ET-1 converting enzyme inhibitor, and found that it improved allograft survival and decreased CAV. Phosphoramidon-treated rats had less smooth muscle cell-derived intimal thickening, medial tone, adventitial fibrosis, and macrophage infiltration in the coronary vessels.(11,12) Finally, Yamaguchi et al. treated donor hearts with anti-sense oligodeoxynucleotides for ET-1, which markedly

reduced ET-1 mRNA and protein expression and significantly reduced intimal hyperplasia.(13) These studies collectively provide robust causal evidence for ET-1 in the pathogenesis of CAV and confirm that ET-1 exerts effects throughout the vessel wall, contributing to intimal hyperplasia and negative remodeling.

To date, the published literature in humans has been somewhat inconsistent in establishing a mechanistic role for ET-1 in the pathogenesis of CAV. Ravalli et al. initially compared 13 explanted allografts with severe CAV to 10 explanted native hearts with normal coronary arteries and observed significantly increased ET-1 immunoreactivity throughout the vessel wall in the CAV cohort.(14) Weis et al. next studied 33 heart transplant recipients and showed that increased myocardial ET-1 mRNA expression was associated with coronary endothelial dysfunction (acetylcholine-induced paradoxical vasoconstriction), but that neither ET-1 mRNA expression nor plasma ET-1 were associated with maximal intimal thickness (MIT) on 2D planar intravascular ultrasound (IVUS).(15) Ferri et al. subsequently demonstrated that interstitial ET-1 expression on endomyocardial biopsy at 3-months post-transplant predicted development of angiographic CAV at 2 years in 47 heart transplant patients.(16) Larose et al. then studied 18 heart transplant recipients and measured angiographic epicardial dilation after intracoronary delivery of 1) BQ-123 (selective ETA receptor antagonist), and 2) nitroglycerin (maximal vasodilator). They found that biologically active ET-1 accounted for a significantly larger proportion of vasomotor tone among patients with advanced angiographic CAV, thereby providing the first mechanistic data in humans supporting a causal link between ET-1 and CAV.(17) More recently, Starling et al. measured plasma ET-1 and performed 2D IVUS within 2 months and at 1-year post-transplant in 106 heart transplant patients. They observed that changes in ET-1 but not baseline ET-1 (as a continuous variable) were associated with rapidly progressive CAV (defined as a change in MIT ≥0.5 mm).(18) Taken together, the findings from these studies generally favor ET-1 as a mediator of CAV, but important limitations should be considered. First, the studies were primarily small (<50 heart transplant recipients) and cross-sectional in design. Second, they utilized less sensitive techniques—quantitative coronary angiography (QCA) or 2D IVUS—to evaluate coronary architecture, whereas contemporary 3D volumetric IVUS allows for greater accuracy in detecting smaller changes in the vessel wall.(19) In addition, the IVUS-based analyses focused solely on intimal thickening and failed to assess vessel remodeling, which as described earlier, is an integral component of CAV. Lastly, apart from the small study by Larose et al., functional and/or mechanistic exploration was lacking.

I addressed several of these limitations in a recently published retrospective cohort study examining the association of ET-1 with early CAV in 90 heart transplant patients at Stanford University from 2002-2014.(20) The salient findings of this study were 1) elevated baseline ET-1 was the strongest independent predictor of rapidly progressive CAV at 1 year as measured by volumetric IVUS [odds ratio=4.88 (1.69-14.1), p=0.003] following multivariate adjustment for recipient and donor demographic, cardiovascular, and transplant-related risk factors, and 2) higher baseline ET-1 conferred a significantly lower risk of late death and/or re-transplantation in multivariate Cox regression analysis [hazard ratio=2.94 (1.12-7.72), p=0.02]. These data strongly support the hypothesis that greater ET-1 bioactivity accelerates the development of CAV and suggests that ET-1 blockade may have durable prognostic value for heart transplant recipients.

These published data serve as the foundation for my ongoing AHA-funded Career Development Award entitled “Impact of Endothelin-1 on the Development of Cardiac Allograft Vasculopathy in Heart Transplant Recipients,” which aims to leverage contemporary interventional methods to prospectively define the role of endothelin-1 (ET-1) in the development of CAV in heart transplant recipients through a comprehensive invasive assessment of coronary anatomy, physiology, and vasomotor function. Specific

Aim #1 primarily involves the prospective validation of the ability of baseline ET-1 (measured on day of initial post-transplant coronary angiogram) to predict accelerated CAV at 1 year after transplant (measured using 3D IVUS). Specific Aim #2 primarily involves evaluating the association between baseline ET-1 changes in epicardial coronary physiology (measured by fractional flow reserve as well as other non-hyperemic pressure ratios) and lipid accumulation (measure by near infrared spectroscopy) at 1 year. I began enrolling patients in January 2019, and as of December 17th, 2020 we have enrolled 65 post-heart transplant patients into the study, 30 of whom have undergone their 1-year angiogram and thus completed the study.

Specific Aim #3 of the AHA grant involves defining the contribution of ET-1 bioactivity to coronary vasomotor tone following heart transplantation. This mechanistic aim of the grant requires short-term (1 week) administration of an ERA (Macitentan, which will be provided by Actelion Pharmaceuticals US Inc, a Janssen Pharmaceutical company of Johnson and Johnson and represented by Janssen Research and Development, LLC) in post-heart transplant patients. I anticipate that the findings of this substudy, combined with the data generated from Aims #1 and #2, will provide the necessary groundwork to conduct a pilot randomized, placebo-controlled trial to assess whether or not reducing ET-1 bioactivity via endothelin receptor antagonism using Macitentan slows the development of CAV and improves outcomes in heart transplant recipients. The possible addition of ERAs to the limited CAV armamentarium would be a pivotal shift in the clinical practice paradigm of this complex patient population.

STUDY OBJECTIVES

1. To define the contribution of ET-1 bioactivity to coronary vasomotor tone after heart transplantation*. At 1-year follow-up in a subset of consecutive patients, we will use 3D IVUS to measure vasomotor response (% change in lumen volume) to Macitentan and intracoronary nitroglycerin (the reference standard for maximum dilatory capacity). Of note, patients will have taken a 1-week course of Macitentan 10 mg daily to end on the day of their 1-year angiogram.*
   Hypothesis: ET-1 bioactivity accounts for a greater proportion of vasomotor tone (i.e. higher macitentan/nitroglycerin luminal volume dilation ratio) in accelerated CAV.

2. To establish short-term safety of Macitentan in post-heart transplant patients. *On the day of the 1-year angiogram, potential drug-related side effects will be recorded and laboratory tests including hemoglobin and liver function tests will be collected.*
   Hypothesis: Macitentan is safe in heart transplant patients (no substantial increase in rate of side effects or laboratory abnormalities compared to historically reported rates).

STUDY DESIGN

For the remainder of AHA award period (ends 12/1/21), I will continue to recruit new heart transplant recipients within the first 4 months of their surgery to participate in this prospective cohort study. For those patients already enrolled who have yet to complete their 1-year angiogram (approximately 28 patients as of 11/1/20) and for new enrollees, a consecutive subset of patients will receive a 1-week course of Macitentan and undergo the protocol described below.

The overall study inclusion/exclusion criteria are as follows:

Inclusion Criteria

1) Within 4 months post-transplant
2) ≥18 years old
3) Have a serum creatinine < 2.0 mg/dL (to minimize risk of contrast-induced nephropathy)
4) Able to provide informed written consent.

Exclusion Criteria

1) 1) Multi-organ transplant (i.e. Heart/Lung)
2) 2) Transplant-related complications and comorbidities that preclude the ability to safely perform an invasive coronary evaluation in the cardiac catheterization laboratory
3) Contraindicated to undergo coronary angiography and/or IVUS/physiologic assessment (e.g. pregnant women due to risk of radiation to fetus).

Additional exclusion criteria for the current Macitentan-based study are:

1) 1) Patients who are taking potent CYP3A4 inhibitors (e.g. ketoconazole, ritonavir) as these drugs can expose one to higher levels of macitentan
2) 2) Pregnant women due to possible fetal harm; as above, all women of childbearing potential must have a negative pregnancy test within 1 week of starting Macitentan, and 30 days after completing the one-week course of Macitentan,
3) 3) Cirrhosis or baseline liver function tests (AST/ALT) > 3x the upper limit of normal

There are otherwise no inclusion/exclusion criteria based on gender, race, ethnicity, or language spoken.

Macitentan Protocol

As described above, Macitentan is a nonselective ERA that is approved for use in PAH; the use of Macitentan in post-heart transplant patients is considered investigational. A consecutive subset of eligible patients based on the criteria outlined above will receive a 1-week course of Macitentan 10 mg tablets daily prior to their routine 1-year coronary angiogram (7^{th} and final dose of Macitentan will occur on the day of their 1-year angiogram). On the day of the angiogram, drug-related side effects, adverse reactions, and medication compliance will be recorded. Additionally, laboratory tests including hemoglobin, liver function tests, and creatinine will be obtained.

In accordance with the Risk Evaluation and Mitigation Strategy (REMS) program for Macitentan, women of childbearing potential (i.e. pre-menopause) will be required to enroll in REMS and 1) have a confirmed negative pregnancy test within 1 week of starting Macitentan, and 2) take an additional pregnancy test 30 days after completing the 1-week course of Macitentan. The results of these pregnancy tests will be recorded in the participant's case report form.

Per the overall study protocol, a 10-cc blood arterial blood sample will be drawn into an EDTA tube to measure plasma ET-1 levels after arterial access is obtained. Next, coronary angiography will be performed, after which an initial IVUS run of the left anterior descending coronary artery protocol will be done without administering nitroglycerin (Macitentan run). Afterwards, 200 mcg of intracoronary nitroglycerin (reference standard for maximum dilatory capacity) will be given and a second IVUS run (Nitroglycerin run) will be performed in order to calculate the Macitentan:Nitroglycerin luminal volume dilation ratio. Of note, 3D volumetric IVUS analyses will be performed by a blinded, independent IVUS core lab at Stanford University. Finally, coronary physiology indices including fractional flow reserve will be measured.

Of note, blood samples will be centrifuged at 4° C within 30 minutes of being drawn and subsequently stored in cryovials at -80° C. Remaining blood specimens after ET-1 analysis will remain banked and available for future research purposes.

Primary Endpoint
ET-1 bioactivity as measured by contribution to vasomotor tone via the Macitentan:Nitroglycerin luminal volume dilation ratio

Secondary Endpoint
Frequency of drug-related side effects

STATISTICAL PLAN
First, I will evaluate the correlation between 1-year ET-1 levels and the Macitentan:Nitrogylcerin luminal volume dilation ratio; we hypothesize that higher ET-1 levels will be positively correlated with higher Macitentan/Nitroglycerin luminal volume dilation ratios.

Next, in order to test the hypothesis that ET-1 bioactivity accounts for a greater proportion of vasomotor tone (i.e. higher Macitentan/Nitroglycerin luminal volume dilation ratio) in patients with accelerated CAV at 1 year, the Macitentan subset will be divided into 2 groups—those with and without accelerated CAV at 1-year post-transplant—and the Macitentan/Nitroglycerin luminal volume dilation ratio will be compared between these 2 groups. Accelerated CAV will prospectively be defined as > 75th percentile of lumen volume loss measured by 3D IVUS because prior studies using 2D IVUS reported 20-30% rates of accelerated disease.(2,15,16) Of note, I will also run a separate sensitivity analysis using the cutoff identified in our recently published retrospective analyses.

Power analysis and sample size estimation
Although Larose et al. used QCA (as opposed to IVUS) to define accelerated CAV and evaluate vasomotor response to endothelin receptor antagonism, I used their data to estimate the required sample size.(17) Assuming 80% power, a 2-tailed alpha of 0.05, a 25% prevalence rate of accelerated CAV, and a 15% drop-out rate, a sample size of approximately 25 patients is needed to detect their previously reported standardized effect sizes for the outcomes of interest (Larose and colleagues reported an ERA/Nitroglycerin ratio of 12.9% ± 26.3 in patients without advanced CAV versus 53.2% ± 28.6% in patients with advanced CAV.(12) To date, I have enrolled 65 patients into the parent AHA study, 30 of whom have undergone their 1-year angiogram and thus completed the study (i.e. 35 patients have yet to undergo their 1 year angiogram and eligible to receive Macitentan). I anticipate enrolling at least 40 additional patients into the study (end date for enrollment 11/30/21). Thus, between these new study participants and the 35 awaiting 1-year angiograms, I will easily be able dose Macitentan in > 25 patients, with a goal of 40-50 patients total. However, this assumes that we are able to begin able to dispense Macitentan in the coming months. This expected Macitentan cohort size will provide adequate power to test the aforementioned hypothesis. Finally, we will compare the rates of drug-related side effects with historically reported rates.

Analytic Strategy
For continuous variables, we will express normally distributed data as mean±standard deviation and non-normally distributed data as median (interquartile range). We will express categorical variables as frequency (percentage). Given our relatively small sample size, we will use non-parametric Wilcoxon Rank-Sum and Rank-Sign tests to assess for differences between groups of variables, as appropriate.

Statistical analyses will be carried out with the SPSS system, version 26 (SPSS Inc., Chicago, IL). A p-value <0.05 will be deemed statistically significant.

SAFETY

Potential Risks

As outlined in our IRB-approved informed consent form, there are greater than minimal risks associated with Macitentan. Although Macitentan is safe and well-tolerated, after 1 week of therapy, there is a possibility for side effects including but not limited to headaches, fluid retention and swelling, nasopharyngitis, bronchitis, anemia, and elevation of liver function tests. The details of these potential risks will be outlined in detail with the potential participants during the informed consent process. In addition, laboratory tests including hemoglobin and liver function tests will be checked the day of the patient's 1-year angiogram (i.e. Day 7 of Macitentan). Finally, Macitentan is contraindicated in pregnancy. As outlined above, women of childbearing potential will be required to enroll in REMS and have a confirmed negative pregnancy test within 1 week of starting Macitentan as well as take an additional pregnancy test 30 days after completing Macitentan.

Protection Against Risk

Importantly, the protocol has been designed to perform vasomotor function testing in response to Macitentan at a time point coincident with routine 1-year clinical surveillance at UCLA. The additional risk of performing a second IVUS run is negligible. Additional procedures to minimize risk will include: 1) conducting the invasive studies outlined above in a cardiac catheterization laboratory that is fully equipped with state-of-the-art technology for visualization of catheter and wire placements and intracoronary imaging as well as cardiac monitoring and facilities for resuscitation, 2) employing procedures for ensuring aseptic and sterile environment, 3) having highly trained personnel conduct the procedures, 4) having a formal data and safety monitoring plan (refer to "Data and Safety Monitoring Plan" section below), and 5) detailed informed consent.

Potential Benefits of the Proposed Research

There are no direct/immediate benefits to the study participants. However, data obtained will comprehensively define the role of ET-1 in the development of early CAV. These findings will potentially lead to a study of whether endothelin receptor antagonists attenuate CAV in heart transplant recipients, the results of which could have major clinical implications for all heart transplant recipients.

Data Security

Clinical and procedural data will be stored in a secure-web-based database (UCLA REDcap). The database will be password protected, and only members of the research team will have access to the database password. The database will not be coded, but will have study participant ID numbers so that when the data is extracted for analysis, the name, MRN, and DOB can be excluded. Blood specimens will similarly be labeled using participant ID numbers. The participant ID numbers will only be available to the research team. This REDcap set-up is a standard infrastructure for clinical research studies like this one and very much minimizes the risk of patient health information being disclosed.

Data and Safety Monitoring Plan

The proposed research involves greater than minimal risk, though this risk is predominantly encountered as part of the subjects' routine post-transplant clinical care at UCLA (i.e. risks associated with performing coronary angiography, IVUS, etc.). The protocol-specific risk that is greater than minimal in nature is the potential for an adverse reaction to Macitentan, though this is very unlikely given the short duration of therapy (1 week), established safety and tolerability of Macitentan, and lack

of drug-drug interactions with standard post-heart transplant pharmacologic regimens. Given these greater than minimal risks, I have created the following formal Data and Safety Monitoring Plan:

1. Safety data will be captured at the time of the 1-year angiogram (Day 7 of Macitentan) and at any time point when an adverse event (AE) occurs during the study period (1-week course of Macitentan)
2. As Principal Investigator, I will monitor data and safety internally. I will assess each AE for designation as serious AE (SAE) or AE
3. All study-related AEs will be reported to the UCLA IRB and Janssen per protocol below
4. Specific safety data collection and reporting will be conducted in accordance with the Janssen protocol below (pages 7-13)

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SAFETY EVENT AND PRODUCT QUALITY COMPLAINTS REPORTING

1 Sponsor/Institution Responsibilities

1.1 Health Authority

As the sponsor/institution of the Study and to the extent required by any applicable laws or regulations in each country/territory, UCLA shall be solely responsible for complying, within the required timelines,

with any safety reporting obligation towards the competent health authorities, the Ethics Committees (EC) or Independent Review Board (IRB) and the participating (co- or sub-) investigators.

1.2 General

The Institution shall notify (Actelion Pharmaceuticals US Inc, a Janssen Pharmaceutical company of Johnson and Johnson and represented by Janssen Research and *Development, LLC* (Company) immediately in case of a suspension of recruitment or premature stop of the concerned clinical study because of a safety concern; preferably by means of a telephone contact with the Company Representative, alternatively by email within twenty-four (24) hours of the decision.

Institution shall provide to the Company copies of any and all relevant extraordinary* (not including routine initial or follow-up individual case safety report (ICSR submission) correspondences with health authorities and ethics committees regarding any and all serious adverse events (irrespective of the association with the Janssen Product(s) Under Study). Copies of such correspondence shall be provided within 24 hours of such report or correspondence being sent to applicable health authority.
*See definitions section 4.6

Training: Institution shall be responsible for training the Study personnel (including the Investigator) on managing safety information arising from the Study according to agreed procedures and the requirements of this Agreement.

Management of Safety Data: The Institution and Investigator will provide safety information arising from the Study to the Company on adverse events, special situations including pregnancies and product quality complaints as defined within this exhibit. This safety information will be documented by the Investigator and reported as described in this exhibit from the time a subject has signed and dated an Informed Consent Form (ICF) until 30 days after the last dose of the Janssen Product(s) Under Study. All subsequent AEs and SAEs beyond 30 days after the last dose of the Janssen Product(s) under study shall be collected/reported if the investigator considers the AE/SAE to be causally related to the use of the Janssen Product(s) Under Study.

For the purposes of this Study, the Janssen Product(s) Under Study is (are): macitentan (Opsumit®)

Maintenance of Safety Information: All safety data arising from the Study shall be maintained in a clinical database in a retrievable format. The Institution and Investigator shall provide a summary of all non-serious adverse events (NSAES) annually, and both serious and non-serious adverse events will be summarized in the final Study report (excluding those from subjects not exposed to a Janssen product). The summary shall include a listing of: Patient ID, adverse event term (uncoded), severity, relationship to Janssen Product(s) Under Study, and action taken with Janssen Product(s) Under Study. However, in certain circumstances more frequent provision of safety data may be necessary, e.g. to fulfill a regulatory request, and as such the safety data shall be made available within a reasonable timeframe at the Company's request.

Follow-up: All (serious and non-serious) adverse events reported for a Janssen Product(s)Under Study shall be followed up in accordance with good clinical practice (GCP).

1.3 Reporting of Safety data and Product Quality Complaints (PQCs) to Company

All adverse events and special situations, whether serious or non-serious, related or not related, collected as per protocol design, following exposure to a Janssen Product(s) Under Study are to be documented by the investigator and recorded in the CRF and in the subject's source records. Investigators must record in the CRF their evaluation concerning the relationship of the adverse event to a Janssen Product(s) Under Study.

The Institution will submit (and procure that the Investigator submits), to the identified Company Representative in each Country/Territory the following safety information:

Type of report	**Timelines**	**How to report**
Serious Adverse Event	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Adverse Events of Special Interest (AESI)	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Reports of drug exposure during pregnancy (maternal and paternal), with or without SAE.	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Reports of Suspected transmission of any infectious agent via administration of a Janssen Product, with or without an SAE	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Abnormal Pregnancy Outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomaly, ectopic pregnancy)	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Special Situation (SS), if associated with an SAE	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties, on a Serious Adverse Event Form.
Special Situation (not associated with an SAE)	Annually and in final study report.	Recorded in CRF and reported annually and in final study report.
Product Quality Complaints	Within 3 business days of becoming aware of the event(s)	By a secure means, as agreed by the Parties.
Non-Serious Adverse Event* (*Not meeting other listed 3 business day reporting criteria e.g. AESI or SS)	Annually and in final study report.	Recorded in CRF and reported annually and in final study report.
Follow-up information	Transmitted within the same timeframes as above i.e. if initial report was required to be submitted within 3 business days, follow-up information shall	Transmitted via same method as above i.e. if initial method of transmission was on an SAE form, follow-up information

	be submitted within the same timeframe.	shall be transmitted in the same manner.

The Institution and/or Investigator is responsible for ensuring that these cases are complete and if not, are promptly followed-up. A safety report is not considered complete until all clinical details needed to interpret the case are received.

Note: In the event the Study is blinded, the Investigator will submit an unblinded SAE or pregnancy exposure report to Company.

If the Investigator/agent of the Investigator receives safety information and is aware it involves a non-study J&J product, it will be reported to Company, indicating it is an incidental spontaneous report discovered during the course of the study.

Pregnancy:
If a subject becomes pregnant during the study, in addition to reporting the pregnancy within 3 business days, a determination regarding Janssen Product(s) Under Study discontinuation and subject discontinuation must be made by the investigator in alignment with the protocol inclusion/exclusion criteria and in consultation with the reference safety information.
Because the Janssen Product(s) Under Study may have an effect on sperm, pregnancies in partners of male subjects exposed to a Janssen Product(s) Under Study will be reported by the Investigator within 3 business days of their knowledge of the event using the Serious Adverse Event Form. Depending on local legislation this may require prior consent of the partner.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

1.4 Sponsor confirmation/review of SAEs

Company Representative or designee will provide the Institution and Investigator with a list of the Study's SAEs (previously received by the Company from the Institution and the Investigator) on a semi-annual basis. Investigator will confirm to Company that all applicable SAEs related to Janssen Product(s) Under Study have been reported to Company.

2 Company Responsibilities

Company Representative or designee will provide the Institution and Investigator with the following safety information:

- Local Package Insert for the determination of expectedness https://www.opsumit.com/
- ICF risk wording
- any new information, which becomes available during the course of the Study, which may affect the overall safety profile of the Janssen Product(s) Under Study.

3 Company Safety Contact Information

Contact details for the Company representative for submission of information required under this exhibit.

SAE/Pregnancy Notifications: IIS-BIO-VIRO-GCO@its.jnj.com
PQC: IIS-BIO-VIRO-GCO@its.jnj.com
Phone: contact the study assigned Janssen Trial Manager
Fax: 1-866-451-0371

4 Definitions

4.1 Adverse Event (AE)

Any untoward medical event in a patient administered a pharmaceutical product which does not necessarily have to have a causal relationship with the treatment. An adverse event can be any unfavourable and unintended sign (including abnormal finding or lack of expected pharmacological action), symptom, or disease temporarily associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that (investigational or non-investigational) product. This includes any occurrence that is new in onset or aggravated in severity from the baseline condition, or abnormal results of any diagnostic procedures, including laboratory test abnormalities.

4.2 Adverse Event of Special interest

Adverse events of special interest are events that the Company is actively monitoring as a result of a previously identified signal (even if non-serious). Adverse events of special interest of macitentan (Opsumit®) will be reported to the Company as defined in the protocol. In case of any amendments to the AESI list, Company will notify the Institution/Investigator and the Institution/Investigator will inform sites. Institution/Investigator will ensure that these changes are updated in the protocol as soon as practical.

4.3 Serious Adverse Event (SAE)

Any adverse event occurring that:

- Results in death;
- Is life-threatening;
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Any suspected transmission of any infectious agent via administration of a medicinal product
- Is considered medically significant*

*Any untoward medical occurrence that is considered medically significant. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not result in death, be life-threatening or require hospitalization but may be considered a serious adverse drug experience when, based on appropriate medical judgement, that may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the other outcomes listed in the bulleted list above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse or malignancy.

4.3.1 Hospitalization

For reports of hospitalization, it is the sign, symptom or diagnosis which led to hospitalization that is the serious event for which details must be provided. Any event requiring hospitalization or prolongation of hospitalization that occurs during a study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into a study. [Note: Hospitalizations that were planned before the signing of ICF and where the underlying condition for which the hospitalization was planned has not worsened will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.]

4.3.2 Life-threatening Conditions

As it relates to life-threatening conditions,
The cause of death of a subject in a study within 30 days of the last dose of Janssen Product(s) Under Study, whether or not the event is expected or associated with the study drug, is considered a serious adverse event.

Disease progression should not be recorded as an adverse event or serious adverse event term; instead, signs and symptoms of clinical sequelae resulting from disease progression/lack of efficacy will be reported if they fulfil the serious adverse event definition.

4.4 Special Situations

The following special situations must be reported to Company with or without an associated serious adverse event (SAE):

- Drug exposure during pregnancy (paternal, maternal)
- Suspected transmission of any infectious agent via administration of a Janssen Product(s) under study.

The following special situations must be reported to the Company when associated with a serious adverse event (SAE):

- Overdose of Janssen Product(s) under study
- Exposure to Janssen Product(s) under study from breastfeeding
- Suspected abuse/misuse of Janssen product(s) under study
- Inadvertent or accidental exposure to Janssen Product(s) under study
- Any failure of expected pharmacological action (i.e., lack of effect) of Janssen Product(s) under study
- Medication error (includes potential, intercepted or actual) involving a Janssen product (with or without patient exposure to the Janssen Product(s) under study, e.g., name confusion)
- Unexpected therapeutic or clinical benefit from use of Janssen Product(s) under study

If no SAE is associated with the special situation, the special situation should be recorded in the CRF and sent annually to the Company.
These safety events may not meet the definition of an adverse event; however, the Parties agree that for reporting purposes, they are deemed to be adverse events.

4.5 Product Quality Complaints

A PQC may have an impact on the safety and efficacy of a Company product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of patients, investigators, and the Company, and are mandated by regulatory agencies worldwide. The Company has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information. The Institution agrees that Lot and/or Batch #s shall be collected, when available, for all PQC reports, including reports of failure of expected pharmacological action (i.e., lack of effect) of a Janssen Medicinal Product. A sample of the suspected product shall be maintained for further investigation if requested by the Company.

Any complaint that indicates a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product or delivery system is considered a PQC. Not all PQCs involve a patient.

Examples of PQC include but are not limited to:

- Mislabelling or misbranding
- Information concerning microbial contamination, including a suspected transmission of any infectious agent by a product
- Any significant chemical, physical, or other changes that indicate deterioration in the distributed product
- Any foreign matter reported to be in the product
- Mixed product, e.g., two drugs are mixed-up in the packaging process
- Incorrect tablet sequence (e.g., oral contraceptive tablets)
- Insecure closure with serious medical consequences, e.g., cytotoxics, child-resistant containers, potent drugs
- Suspected counterfeit or tampered product
- Adverse Device Effects including any adverse event resulting from insufficiencies or inadequacies in the instructions for use, the deployment, implantation, installation, operation, or any malfunction of a medical device or combination product. This also includes any event that is a result of a use error or intentional misuse and dosing device malfunctions (e.g. auto-injector button not working, needle detaching from syringe, etc.)
- Physical defect (e.g. abnormal product odour, broken or crushed tablets, etc.)

4.6 “Extraordinary” correspondence

Correspondence with a regulatory authority or ethics committee regarding a safety issue that may impact the safety or benefit-risk balance of the Janssen Product(s) Under Study, and/or may impact patients or public health. Examples include:

- Safety issues relating to a quality defect
- Major safety issues identified with changes in the nature, severity or frequency of known serious adverse reactions which are medically significant or the detection of new risk factors for the development of a known adverse reaction or a new serious adverse reaction.
- Major safety issues identified in the context of ongoing or newly completed post-marketing studies e.g. an unexpected increased rate of fatal or life-threatening adverse events.