Diabetic Foot Ulcer (DFU) Biofilm Infection and Recurrence

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1) Background

Diabetic foot ulcers (DFU) are one of the most common reasons for hospitalization of diabetic patients and frequently results in amputation of lower limbs. Of the one million people who undergo non-traumatic leg amputations annually worldwide, 75% are performed on people who have type 2 diabetes (T2DM)[1,2]. The risk of death at 10 years for a diabetic with DFU is twice as high as the risk for a patient without a DFU[3]. The rate of amputation in patients with DFU is 38.4%[4]. Infection is a common (>50%) complication of DFU[1,5-13]. Emerging evidence underscores the significant risk that biofilm infection poses to the non-healing DFU[6,14]. Biofilms are estimated to account for 60% of chronic wound infections[15]. In the biofilm form, bacteria are in a dormant metabolic state. Thus, standard clinical techniques like the colony forming unit (CFU) assay are used to detect infection and may not detect biofilm infection[16]. Thus, biofilm infection may be viewed as a silent maleficent threat in wound care.

In the current standard of care (SoC), a wound closure is defined by the Food and Drug Administration (FDA) by wound area re-epithelialization without drainage[17]. Our pre-clinical large animal work demonstrates that wounds with a history of biofilm infection may meet the above criteria, but the repaired wound-site skin is deficient in barrier function[18,19]. This has led to the concept of functional wound closure wherein the current clinical definition of wound closure is supplemented with a functional parameter – restoration of skin barrier function as measured by low trans-epidermal water loss (TEWL)[18-21].

This study rests on DFU-patient based findings from a NIDDK-DIACOMP funded pilot study (Sen/Gurtner) showing that closed DFU with deficient barrier function are more likely to recur. Biofilm infection as assessed through scanning electron microscopy and wheat germ aggluttin assay performed on debrided tissue causes faulty re-epithelialization, compromising skin barrier function at the closed wound site. Such defects are caused by biofilm-inducible miRs which silence junctional proteins necessary for skin barrier function. The IRB protocol associated with this study, rests on our novel patient-based observation that in wound-edge tissue catenin delta1/p120 catenin (CTNND1) is suppressed as measured through immunohistochemistry. CTNND1 is an essential regulator of E-cadherin stability which is regarded as a master organizer in epithelial phenotype and plays a critical role in maintaining the barrier integrity of skin. Our prior study identified miR-9 is a biofilm induced microRNA that targets adherens junction protein E-cadherin. We propose that miR-9 can target CTNND1 (Aim 1). The validation of miR targeting will be performed quantitative real time PCR, Western blot analyses, Argonaute 2 pull down assay and on bead assay. Thus, this proposal, fully based on the study of DFU patients, seeks to conduct a fully powered clinical study testing whether DFU with a history of biofilm infection closes with deficient barrier function (Aim 2). Aim 3 tests whether such functionally deficient wound closure, manifested as high TEWL, is associated with greater wound recurrence. Per FDA, the significance of association studies is heightened by support of a well-founded biological rationale provided by mechanistic studies[22]. This proposal rests on such mechanisms that have been reported by us in pre-clinical large animal studies[18-20]. The primary parent study will address

molecular mechanisms implicated in biofilm-induced loss of skin epithelial barrier integrity in DFU patients.

2) Objective

The multi-site parent study will address the following three specific aims:

Aim 1: Determine how biofilm induced DFU tissue microRNAs disrupt barrier function of the repairing DFU.

Aim 2: Test the incidence of wound biofilm infection at initial visit and test its association with deficient skin barrier function at the closed DFU-site.

Aim 3: Test whether closed DFU with deficient barrier function is associated with higher rate of recidivism.

Based on the premise, burden and significant gap in knowledge, we propose to perform pilot comparative studies by developing a focus within the parent Diabetic Foot Ulcer (DFU) Biofilm Infection and Recurrence protocol, as announced in NOT-AG-20-034 (funded as a supplement to the parent award NIH/NIDDK-1R01DK125835), by including AD/ADRD populations with the purpose of comparing the outcome and trajectory of wound healing among diabetic vs non-diabetic patients with AD/ADRD.

Note: an additional Aim as Aim 4 was added in response to the supplemental funding described above. Due to difficulty in enrolling the AD/ADRD with diabetes and chronic wounds population, only n=2 subjects could be enrolled during the supplemental funding period of 1 year. Now that the supplemental funding has expired, Aim 4 has been removed from this protocol all sections.

3) Study Population

For Aims 1-3: *405 clinically diagnosed Diabetic Foot Ulcer (DFU) patients who are suspected to be infected will be recruited for this larger cohort of patients in the parent study.*

3.1) Inclusion Criteria

1. Male or Female, Age ≥ 18
2. Willing to comply with protocol instructions, including all study visits and study activities.
3. Patient with an open Diabetic Foot Ulcer
4. Adequate arterial blood flow as evidenced by at least one of the following (for wounds below the knee):
    a. TcOM ≥30 mmHg
    b. Ankle-brachial index at least ≥0.7
    c. Toe pressure > 30 mmHg
    d. TBI > 0.6 mmHg

3.2) Exclusion Criteria

1. Individuals who are deemed unable to understand the procedures, risks, and benefits of the study.
2. Wounds closed or to be surgically closed by flap or graft coverage
3. Participants with marked immunodeficiency (HIV/AIDS, or on immunosuppressive medications.
4.
5. Diabetics with a hemoglobin A1c > 15 within 3 months prior to enrollment
6. Participants with autoimmune connective tissue disease
7. Ulcer size and location that does not allow the TEWL measurement per SOP
8. Pregnant women
9. Prisoners
10. Unable to comply with study procedures and/or complete study visits

4) Study Design

Aims 1-3: The parent study is a multi-center trial that initially was conducted among the Indiana University clinical sites, Aiyan Diabetes Center and the University of Arizona. Effective of July 1, 2023, Dr. Sen (PI) and the research team transitioned to the University of Pittsburgh and continued the Multicenter study from this location as a coordinating center site under Dr. Sen as Principal Investigator (PI). The IU clinical locations consisted of Aiyan Diabetes Center and the University of Arizona. Effective July 1, 2023, the IU and Aiyan Diabetes Center were closed and at that time the University of Pittsburgh Medical Center (UPMC) Wound Care Centers and University of Arizona were identified as the current sites moving forward for participant recruitment, enrollment and conduct of study activities. These sites are supported by trained physicians, research nurses, clinical research coordinators and other trained support staff. All staff involved with human subject research have completed requisite human subject training via the Collaborative Investigator Training Initiative (CITI).

Patients enrolled in the study will be followed for 16 weeks for wound closure (*Phase A*) and will then begin *Phase B* (refer to study procedures below). During this time, the wounds will be subjected to Standard of Care (SoC) as per standardized procedures followed by all the sites wound care providers.

Patients enrolled in the study will be initially followed for 16 weeks to monitor for wound closure (Phase A) and will then begin Phase B (refer to study procedures below) if wound closure takes place. In this time, the patient will be subjected to Standard of Care (SoC) as per routine procedures followed by all the sites wound care providers.

5) Study Procedures

5.1) Phase A

5.1a) Study Visit 1 (pre-closure enrollment)

The initial study visit will take place alongside completion of a patient's regularly scheduled wound care clinic visit and the following activities will take place:

- Informed consent will be obtained from the patient
- Baseline demographics, medical history, labs, diagnostic test results, and current medications will be recorded such as:
    - Age, gender, race/ethnicity, smoking status, wound perfusion using one of the following approaches: Transcutaneous oxygen measurement, ABI or TBI measurements.
    - Nutritional status will be recorded as documented by Albumin levels from EMR within the past three months.
- Wound data (etiology, size, location, duration, wound care modality) will be recorded.
- Previous wound treatment for the past 30 days, as well as the total number of debridements since wound onset.
- Wound swab for culture and wound infection history will be obtained, as appropriate.
- Baseline digital imaging of target wound(s).
- Baseline labs: Hemoglobin A1c, Albumin, pre-albumin, GFR, ESR, CRP, urine cotinine, neuropathy/monofilament (10 g) exams. Note: only labs drawn as standard of care since the onset of the current wound will be collected except for Hemoglobin A1c.
- Participants will be asked to complete the SF-12 Health survey, Visual Analogue Pain scale, and Cardiff wound impact questionnaire.
- Wound site evaluation including one of the following: TCOM/TBI/ankle brachial index (ABI) will be completed for participants with wounds below the knee, if not already completed per standard of care (SoC) within the previous 12 months prior to enrollment.
- For a subset (n=40 first consented willing to participate in the sub-study) of subjects a blood perfusion study will be performed by LSI (see below)
- Wound perfusion will also be measured by laser speckle imaging (LSI) using the Pericam PSI-NR instrument (Perimed Inc.). PeriCam PSI is a non-invasive non-contact device providing two-dimensional imaging of peripheral tissue blood perfusion. Reduced blood flow may lead to insufficient tissue oxygenation and, thus, assessment of peripheral vascular function has several clinical applications. The PeriCam PSI System is FDA 510(k) (#K063586) approved instrument imaging of peripheral tissue blood perfusion. The additional Pericam imaging for perfusion will be performed only in a small pilot cohort of n=40 patients at University of Pittsburgh sites to compare this imaging modality with established modalities such as TCOM/TBI/ABI measurements performed within this study.

- Hemoglobin A1c point of care testing will be drawn for diabetic participants who do not have an A1c available as standard of care (SoC) in their medical record within 3 months prior to enrollment to confirm study eligibility; those with an A1c > 12 will be excluded and no further study activities will be completed. These participants will be recorded as a screen fail and will not be enrolled.
- Wound edge tissue specimen will be collected for laboratory analysis.
    - Wound debridement collection: Debridement of tissue will be conducted as a standard of care (SoC) procedure for these patients. This debrided tissue would normally be disposed of as waste tissue, but for this study we will collect this discarded waste tissue and perform analysis. In brief, waste tissue from debridement of the wound edge will be collected for histology and immunohistochemistry studies. Samples will be used to determine whether the wound is infected using standard lab techniques.
- Swabs will be collected for microbiological and analytical assays.
- The sample will be collected for microscopy, molecular analysis, and histopathology

The following procedures are only applicable to the 40 participants that will be participating in the PSI PeriCam arm.

Note: The participants will not receive results of any of the tests performed; these are for research purposes only.

This specific part of the study is intended to evaluate how fluid passes through the circulatory system. The PeriCam laser speckle perfusion imager allows us to see the perfusion status of a wound.

This is a non-invasive non-contact device providing two-dimensional imaging of peripheral tissue blood perfusion. The PeriCam PSI System is FDA 510(k) (#K120884) approved instrument for imaging of peripheral tissue blood perfusion.

At the time of imaging with PSI, the study coordinator will confirm with participants on their last food/caffeine/smoke intact. Ideally this exposure should not be less than 1h. If within last 1h, then the participant will wait to get the study started at least 1h after their last food/caffeine/smoke. At the start of measurements, they will be asked to lay on their back (supine).

The following activities will take place:

1. Measure blood pressures in ankle and toe (i.e., ABI and TBI), if not already available in the subject's medical records. Measure blood flow pulse in arteries (PVR) to check for any issues that may prevent proper flow and oxygen supply to the foot.
2. Skin temperature of the area around is warmed close to normal body temperature (i.e., 30 deg C) using heating blankets, foot bath or by other means.

3. Measure oxygen supply to the foot using sensors attached to skin until stable reading is obtained.
4. Measure the blood supply to the skin using the imager (Perimed PSI device) until stable reading is obtained.
5. Lift the legs with wedge pillow, measure PSI over wound area until 30 seconds of stable data is obtained.
6. Lower legs back down by removing the pillow and measure with PSI over wound area for 30 seconds
7. Lift legs with wedge pillow again, for 5 minutes, keep measuring PSI over wound area
8. Measure skin temperature with a thermometer when placing tcpO2 electrodes, at first leg elevation, at last leg elevation and at the end of the study.
9. Photos of the wound site will be taken with each leg position.

This imaging will be scheduled the same day with their visit for the DFU Biofilm main study.

Following this visit, the participant's medical records from standard of care (SoC) wound care visits will be reviewed and followed for up to 16 weeks or until their wound has closed, whichever comes first. The research staff will also call the patient and or caregiver, as needed to check for wound closure.

The study team may contact participants or their family members throughout the study, as needed, for any research related communication. These will be brief phone calls in which study team members may inquire about the wound healing status or update the participant or family members on any information relevant to the study that the patient should be informed about.

5.1b) Study Visit 2 (Post-closure, Week 0)

The participant will return to the wound clinic within 10 days of wound closure, or at 16 weeks (+/- 2 weeks) following Study Visit 1 if the wound has not healed, for Study Visit 2. The wound closure evaluation will be per standard of care.

If the participant's wound is noted to be **healed** at or before the end of the 16-week timeline, then the participant will be asked to return to the clinic for Phase B/Study Visit 3.
At Study Visit 2 the following activities will take place:

- Wound data (etiology, size, location, duration, wound care modality, determination of presence of callus) will be recorded.
- A digital photograph of the wound will be taken.
- Medication review and adverse event review will be completed.
- Various labs that have been drawn per Standard of Care since enrollment, medication review, and any adverse events will be recorded.
    - For Trans Epidermal water loss (TEWL) measurements (can be collected using non-invasive devices such as Dermalab or GPSKIN, for

example):The TEWL probe will be placed on the skin.
- The TEWL measurement takes approximately five-ten minutes.
- There will be multiple measurements obtained over the healed wound site.
- A reference (control) TEWL measurement will be taken from intact skin at an anatomically matched site. For example, f the healed wound is on the right foot, we will also measure the same location on the left foot, for comparison.
- Room humidity and temperature readings monitored on the instrument are also recorded.

If the wound has **not healed** by the end of the 16-week timeline, this visit will mark the participant's Final Study Visit and the following information will be collected:

- Wound data (etiology, size, location, duration, wound care modality, determination of presence of callus) will be recorded.
- A digital photograph of the wound will be taken.
- Quality of Life questionnaires such as the SF-12 Health survey, Visual Analogue Pain scale, and Cardiff wound impact questionnaire.

5.2) Phase B

The participant will be seen again following wound closure for follow-up assessment of the healed wound over 12 weeks. There will be visits at 2 and 12 weeks (+/-2 weeks) following Study Visit 2 marking wound closure. These visits will last about 1 hour. Participants will be asked to refrain from using any topical product(s) (lotion, study product, ointments, etc.) the day of the study visits.

5.2a) Study Visit 3 (Post-closure, Week 2 +/- 2 weeks)

At two weeks following Study Visit 2, the patient will be asked to return to the clinic to complete Study Visit 3 and begin Phase B of the study. This visit will last about 1 hour. The participant will be asked to refrain from using any topical product(s) (lotion, ointments, etc.) the day of the study visits. The following study activities will take place during this visit:

- Digital photos of the wound site will be taken.
- Wound site evaluation for signs of recurrence and callus determination, including the date of wound healing will be obtained.
- Medication review and adverse event review will be completed.
- Various labs that have been drawn per Standard of Care since enrollment, medication review, and any adverse events will be recorded.
    - For Trans Epidermal water loss (TEWL) measurements (can be collected using noninvasive devices such as Dermalab or GPSKIN, for example):
        - The TEWL probe will be placed on the skin.
        - The TEWL measurement takes approximately five-ten minutes.

        - There will be multiple measurements obtained over the healed wound site.
        - A reference (control) TEWL measurement will be taken from intact skin at an anatomically matched site. For example, if your healed wound is on your right foot, we will also measure the same location on your left foot, for comparison.
        - Room humidity and temperature readings monitored on the instrument are also recorded.
    - Any relevant lab values collected per standard of care (SoC) since enrollment will be captured and recorded.

In addition to the activities completed during this study visit, research staff will complete weekly phone calls to the participant or caretaker to monitor that the wound remains closed during Phase B of the study.

If the patients wound has **reopened or recured** within the 2 week timeline this visit will mark the participant's Final Study Visit and the following study activities will take place:

- Digital photos of the wound site will be taken.
- Wound site evaluation for signs of recurrence and callus determination, including the date of wound healing will be obtained. You will receive weekly phone calls for wound site evaluation.
- Quality of life questionnaires such as the SF-12 Health survey, Visual Analogue Pain scale, and Cardiff wound impact questionnaire.

5.2 b) Study Visit 4 (Post-closure, Week 12 +/- 2 weeks)

Study Visit 4 will take place at 12 weeks following wound closure (+/- 2 weeks) in which the patient will be required to travel back to clinic to complete the study activities. The following study activities will take place at Study Visit 4:

- Digital photos of the wound site will be taken.
- Wound site evaluation will be completed.
- TEWL measurements will be obtained using a TEWL probe as previously described.
- Medication review and adverse event review will be completed.
- Any lab values collected per standard of care (SoC) since enrollment will be captured and recorded.
- Quality of Life questionnaires such as the SF-12 Health survey, Visual Analogue Pain scale, and Cardiff wound impact questionnaire and diabetic foot scale questionnaire.

If the patient's wound **recurred or reopened** during the 12 week timeline this visit will mark the participant's Final Study Visit and the following study activities will take place

- Wound data (etiology, size, location, duration, wound care modality, determination of presence of callus) will be recorded.
- A digital photograph of the wound will be taken.
- Quality of Life questionnaires such as the SF-12 Health survey, Visual Analogue Pain scale, and Cardiff wound impact questionnaire.

5.3) Potential Risks

All data collected for the proposed study represent parts of the process practiced for standard care of these patients. No specific invasive procedures will be performed on all patients specifically for this study. Debridement is a routine part of wound standard of care (SoC)even if the participant does not participate in the study. No psychological, social, legal or other risks are anticipated from the proposed research. The risk of disclosure of information and linkage of results to PHI by non-project research staff is managed by appropriate institutional security measures that limit access to the research project database. There is NO intervention, or any invasive approaches planned as part of this study. The ABI, PSI PeriCam and TEWL measurements are noninvasive measurements and pose less than minimal risk. HbA1c measurement is a finger stick measurement and is minimal risk. There may be some discomfort with the tissue debridement procedure; however, the wound site will be numbed by applying local anesthesia, as appropriate.

5.3a) Transepidermal Water Loss (TEWL) Measurements

TEWL measurements are non-invasive and have minimal risk. The use of a TEWL probe is a method of measuring water lost from skin due to evaporation. The device in this study protocol is solely meant for research purposes. The device is not invasive and not for diagnosing, curing, mitigating, or treating the wound. The device as used in this research presents non-significant risk and hence is qualified for an abbreviated requirement under 21 CFR 812.2(b). As part of abbreviated requirements IRB approval, informed consent, monitoring, records, and reports will be kept. No progress reports or final reports will be required for FDA compliance.

6) Benefits

There are no direct benefits to individual study participants. Research information produced by the proposed study will help advance our understanding of why chronic wounds do not close. This study is the first to address the significance of TEWL measurements as an invaluable biomarker to predict DFU wound healing outcomes in this subpopulation. The PSI PeriCam studies will address perfusion status in DFU wound healing outcomes.

6.1) Knowledge to be gained

The proposed research aims to uncover novel mechanisms that compromise closure of chronic diabetic wounds in a general population. Knowledge gained from the proposed study has the potential of providing considerable benefits to wound care by identifying a key player that influences the closure of diabetic wounds. Studies in humans in tandem with previous porcine models will help validate the model for additional studies.

7) Payment

The payment schedule for participants will be different depending on what specific cohort of the study they are enrolled in and will vary by the sites and institutional policies. The site consent will have detailed information regarding the payments for each cohort and each study visit.

Compensation will only be given to participants after the completion of all study visit activities. By law, payments to participants over $600 per year are considered taxable income.

8) Statistical Analysis

Statistical power and required sample size are estimated for a multivariable linear regression of a continuous outcome variable (TEWL=Y) with a binary predictor variable (biofilm infection) with an expectation of achieving 80% power assuming conditional (fixed X's) model. TEWL measurement at closure visit 2 is used in the analysis to capture the peak TEWL level, to account for wound closure without complete skin barrier function restoration. The regression model assumes that the conditional expectation of Y is estimated given the realized values of X's. The method of sample size calculation uses the squared multiple correlation coefficient, R^2 as a function of Cohen's effect size. With 0.05 level of significance (Type I error) to detect a small (f-square=0.02) to medium (0.15) and large (0.35) difference in mean TEWL between biofilm infected and non-biofilm infected DFU with 10 additional variables controlled in the model will require an effective sample size of 395 participants. Conservatively, we assumed the effect size to be within small and medium values, equal to 0.03. With these parameters we estimate an effective sample size of 264 participants in. Assuming 20% loss to follow-up, we need at least 330 participants to be enrolled in the study. Because Study Aim 3.0 needs larger sample, we will enroll more than 330 participants (N=405) for Aims 1-3.

Interim Analysis: A blinded interim analysis will be done at 25% and 50% of enrollment. The PIs will be blinded from the interim analysis to maintain the validity and integrity of the study. The purpose of the interim analysis is to inform us the effectiveness of program-related resources and activities that will help the study team get informed decisions regarding modifications, if any, in logistical, monitoring and recruitment procedures.

9.1) Data Collection, Analysis and Management

Clinical data will be recorded in **REDCap (Research Electronic Data Capture** (*HIPAA compliant*)**)** as clinical research database at IU. Effective Nov 01, 2023, the Redcap database will be transferred to the University of Pittsburgh under Dr. Sen's oversight. All data collected from all sites within this project will continue to be housed within this central database. Prior to being given access to the data, HIPAA and human subjects certified study staff will be trained in the collection of patient-reported outcomes. Study data will be collected using Standardized **Case Report Forms (CRFs**), with clear, uniform instructions for study staff. Researcher's access to files will be governed by institutional secure data access policies. Effective November 01, 2023, the REDCap platform will be transferred and operated by the University of Pittsburgh Clinical Research team.

9.2) Data Quality and Security

The study team will review all CRFs. All incoming data will be monitored, with particular attention to: ***1)*** enrollment and follow-up reports as entered into the database, ***2)*** potential adverse events, and ***3)*** missing data or extreme values. Regular reporting to the study team will work to reconcile or report issues as needed and to monitor participant retention. To ensure quality, 10% of the study medical records will be re-abstracted. If the error rate is found to be >5%, staff will be retrained and more extensive data quality checks will take place, with full on-site audit. Study data integrity, adverse events, and design considerations will be reported at all scheduled OSMB. The clinical database administrator will perform quality control assessments for data completeness (missing data) and accuracy (variable range checks, and within and between data field checks) to identify data that appear inconsistent, incomplete, or inaccurate.

When identified, potentially erroneous data will be communicated to the on-site research coordinators to address quality control queries. Corrections and changes to the data will be reviewed by data management staff. Queries will be considered resolved when corrections to the database are made, or when an explanation is provided that allows data management staff to address the query. An ongoing data cleaning effort will progress continuously throughout data collection.

9.3) Adverse Event/Serious Adverse Event Collection and Reporting

- An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participants' involvement in the research, whether or not considered related to participation in the research.

- A serious adverse event (SAE) is any adverse event that results in death; is life threatening or places the participant at immediate risk of death from the event as it occurred; requires or prolongs hospitalization; causes persistent or significant disability or incapacity; results in

congenital anomalies or birth defects; is another condition which investigators judge to represent significant hazards.

- An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the following criteria:
    1. unexpected, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the study population.
    2. related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
    3. suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

- Monitoring of AEs, SAEs or UPs is defined in conjunction with existing SOPs at study sites and as defined by the study protocol.

9.4) Classifying Adverse Events

Adequate review, assessment, and monitoring of adverse events requires that they be classified as to severity, expectedness, and potential relatedness to the study intervention.

Severity

1. Mild: Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.

2. Moderate: Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.

3. Severe: Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating. Severity is not synonymous with seriousness. A severe rash is not likely to be an SAE. Likewise, a severe headache is not necessarily an SAE. However, mild chest pain may result in a day's hospitalization and thus is an SAE.

Expectedness

AEs will be assessed as to whether they were expected to occur or unexpected, meaning not anticipated based on current knowledge. Categories are:

1. Unexpected: nature or severity of the event is not consistent with information about the condition under study or intervention in the protocol, consent form, product brochure, or investigator brochure.
2. Expected: event is known to be associated with the intervention or condition under study.

Relatedness

The potential event relationship to the study intervention and/or participation is assessed by the site investigator. The following scale will be use to categorize an event:

3. Definitely Related: The adverse event is clearly related to the investigational agent/procedure – i.e. an event that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that could not be reasonably explained by the known characteristics of the participant's clinical state.

4. Possibly Related: An adverse event that follows a reasonable temporal sequence from administration of the study intervention follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by a number of other factors.

5. Not Related: The adverse event is clearly not related to the investigational agent/procedure. - i.e. another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

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