TITLE PAGE

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability and Systemic Inflammation (HESTIA)

Protocol Number: 01

Short Title: Investigation of Thermal Injury and Intestinal Permeability

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1. SYNOPSIS

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability, and Systemic Inflammation (HESTIA)

Short Title: Investigation of Thermal Injury and Intestinal Permeability

Rationale:

Patients with severe thermal injury [>20% total body surface area (TBSA)] are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

No GSK study treatment will be employed in this study.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients.

Previous literature demonstrates that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study, therefore, is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.

4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

In order to enter this study thermally injured participants will be required to co-enrol in this study and an allied study entitled: A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injury (SIFTI-2) (reference number IRAS ID: 200366). Clinical data, standard of care laboratory data and investigational biomarker data will be shared from the SIFTI-2 study to this study and the combined data from both studies will be used to address exploratory endpoints. Participants of the SIFTI-2 study will be appropriately consented for this data sharing.

Objectives and Endpoints:

Objective	Endpoint
Co-Primary	
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
2. To characterise the effect of thermal injury on small intestinal permeability over time	• Changes in L/M ratio over time

Overall Design:

A prospective, longitudinal study will be conducted in adult (≥18 years old) men and women admitted to a hospital following thermal injury. Measurements of intestinal permeability, inflammation and microbial translocation will be taken over a six month period. A cohort of healthy participants will also be recruited in order to determine the reference against which post-burn permeability measurements and other biomarkers will be compared.

The lactulose-to-mannitol ratio is traditionally used to assess small intestinal permeability and sucralose to assess colonic permeability. Lactulose, mannitol and sucralose [henceforth referred to as sugar test material (STM)] will be intermittently administered enterally for the purpose of intestinal permeability measurement to examine permeability at different points along the GI tract and is described in Section 7. It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

An internal preliminary data review will be conducted. This review is described in Section 10.

Number of Participants:

Table 1 in Section 5.2 described the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Treatment Groups and Duration:

Group 1: Healthy Participants

The total duration of this study for healthy participants will be approximately two weeks, in addition to the screening window:

- Screening: up to 28 days before Day 1
- Day 1: study start and assessments will be performed
- Day 8 and Day 15

Group 2: Thermally injured participants

Thermal injury participants will be asked to participate for a total of 6 months (plus or minus 14 days).

- There will be no screening period. Thermal injury participants will be recruited within 24 hours of their admission to the burns unit at the study site.
- Intense monitoring phase: Assessments will be performed on alternate days for the first 14 days following study enrolment. If the participant is discharged prior to 14 days, the intense monitoring phase will end, but the participant will remain enrolled in the study.
- Convalescent monitoring phase: Assessments at 28 days and 6 months will be made on an outpatient basis if the participant has already been discharged from hospital.

Exception to monitoring periods:

- If a discharged participant attends the centre for routine clinical care on any of days 8-14, then study assessments will be made and samples will be taken

2. SCHEDULE OF ACTIVITIES (SOA)

2.1. Healthy Participants (Group 1)

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Review of inclusion/ exclusion criteria and informed consent	X				
Demography	X				
Medical history (includes substance and alcohol usage at screening) and Medication history	X				
Substance testing (urine)	X				Substances: [Recreational Drugs and Alcohol]
HIV, Hepatitis B and C screening	X				If test otherwise performed within 3 months prior to study entry, testing is not required
Laboratory assessments	X				
Pregnancy test (WOCBP only) (urine)	X	(X)			Only performed again on Day 1 if patient at risk of pregnancy at or since screening

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Blood sampling for biomarkers		X			20ml of blood will be sampled in a single draw on one day, preferably Day 1
Stool sample collection		X			Participants will be given a collection container at screening to bring with them on Day 1.
Brief physical examination including measurements of height and weight Vital Signs (systolic and diastolic blood pressure and heart rate)	X	X	X	X	BMI calculated from height and weight at screening only Examinations should be conducted the day after intestinal permeability measurement.
Medical review (assessment of health status)		X	X	X	Monitor for signs and symptoms of gastro-intestinal infections and other emergent issues
STM Training	X				Refresher training may be provided as needed
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection		X	X	X	Please refer to SRM for full information Intestinal permeability measurement to be performed at home the day before the study visit.

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
AE/SAE and Concomitant medication reviews	(X)	←==========→			Day 1 will include concomitant medication review only

2.2. Thermal Injury Participants (Group 2)

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)													6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
Review of inclusion/ exclusion criteria and informed consent	X																Participants will be co-consented to the SIFTI-2 study
Medical history (includes substance and alcohol usage) and Medication history	X																
Substance testing (urine)	X																Substances: [Recreational Drugs, Alcohol]
Pregnancy test (WOCBP only) (urine)	X															X	To be repeated at 6 months prior to final STM administration
HIV, Hepatitis B and C screening	X																If test otherwise performed within 3 months prior to study entry, testing is not required

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)														6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
Initial assessment of Burns	X																Calculation of %TBSA Location of thermal injury and depth
Fluid balance (total input/output)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X*		Daily (over 24 hr) *only if participant still admitted
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection	X		X		X		X		X		X		X		X	X	Measurements every 48 hours from first measure. Preference: D1, 3, 5, 7, 9, 11, 13. Otherwise: D2, 4, 6, 8, 10, 12, 14. Please refer to SRM for full method.
Brief Physical Examination															X	X	Following final intestinal permeability measurement. Can be omitted if patient is still admitted to hospital.
Stool sample collection	←==========→ (A sample of the first stool produced following admission)													X	X	X	Time to first stool collection; preferably on Day 1. Then on Days 14 and 28 and 6 months
Wound Healing Assessments														X	X	X	This is to assess time to 95% wound healing

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)														6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
AE/SAE and Concomitant medication reviews	X	←==========→														X	AE/SAE monitoring will begin after the first administration of STM

- The timing and number of planned study assessments may be altered during the course of the study based on newly available data or factors outside of the study such as priority medical care to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol deviation or require a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

3. INTRODUCTION

3.1. Study Rationale

The purpose of this study is to describe the kinetics and magnitude of increases in intestinal permeability which are observed as a result of thermal injury. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

3.2. Background

3.2.1. Thermal Injury, Intestinal Permeability and Multi-Organ Dysfunction Syndrome

Patients with severe thermal injury >20% TBSA are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients (Figure 1).

Figure 1 Changes in Intestinal Permeability

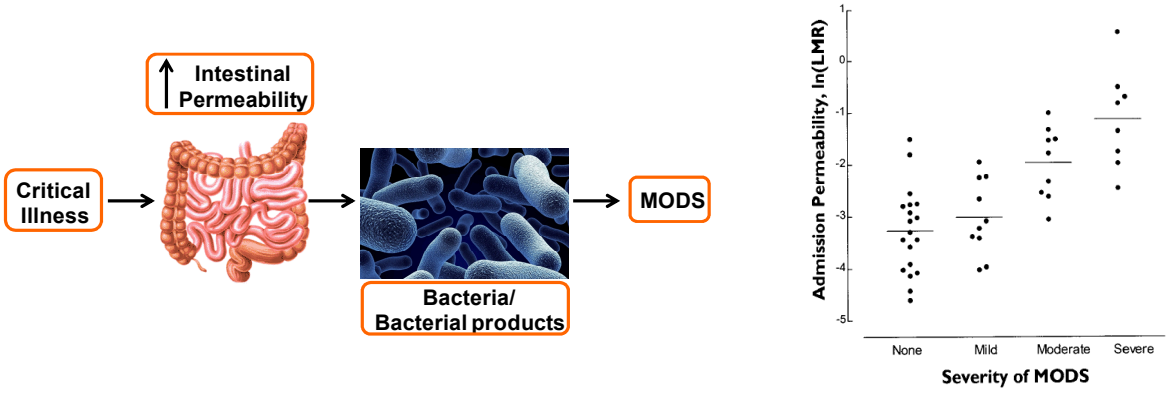


Figure 1 (Left panel) Hypothesis: The gut is the major driver of MODS in critical illness (Right panel) Correlation between intestinal permeability as measured by lactulose/mannitol ratio and the severity of MODS in critically ill patients. Intestinal permeability was determined by measuring the differential absorption of lactulose (increased in the damaged gut) and mannitol (freely absorbed in the normal and damaged

gut) following oral administration and expressed as the ratio of lactulose to mannitol (L/M) [Olquin; 2005].

There are some data showing that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.
4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

3.2.2. Intestinal Barrier Function and Its Measurement

The intestinal barrier combines a physical and immunological barrier. Epithelial cells are connected by tight junctions and prevent the passage of bacteria, toxins and antigens into the systemic circulation [Bjarnason, 1995]. Paneth cells, located in crypts of the small intestine, produce anti-microbial substances (e.g. lysozyme and defensins) and other immune cells patrol the lamina propria [Ayabe, 2000]. Barrier function can be disturbed by diseases such as inflammatory bowel disease; by drugs such as aspirin and alcohol; by ischaemia and has been observed following a number of acute injuries such as burns, trauma and radiation injury [Bjarnason, 1995; Derikx, 2006]. This disturbance results in the translocation of the intestinal flora (pathogenic or commensal) to the systemic circulation. Microbes are accompanied by proteins which normally form part of the tight intracellular junctions of the intestinal epithelium, such as claudins, and other enterocyte-derived proteins [Grootjans, 2010]. In this study, intestinal permeability will be measured directly using oligosaccharide absorption and indirectly by looking for micro-organisms and soluble markers of intestinal barrier dysfunction in the systemic circulation.

Since the 1970s, oligosaccharides have been used as test probes to measure the function of the intestinal barrier [Menzies, 1972]. Lactulose, a large polysaccharide, does not normally cross the intestinal barrier, but following damage can cross the epithelium and enter the systemic circulation. It is not metabolised, so is filtered in the kidney and excreted in the urine. The fractional excretion (amount administered / amount recovered in urine) of lactulose is therefore a measure of intestinal permeability. The amount of lactulose entering the urine is dependent on a number of factors including renal function,

gastric emptying, and degradation in the large bowel by commensal bacteria; thus, a monosaccharide such as mannitol, which passes freely across the healthy intestinal barrier, is often co-administered to 'normalise' lactulose measurements. Following administration of both lactulose and mannitol the fractional excretion of the sugars is expressed as a ratio where mannitol is the denominator. Lactulose and mannitol absorption occurs mainly in the proximal small intestine and is complete within approximately 5 hours of oral administration. This approach has been previously used successfully in patients with severe burn injury and intestinal permeability was found to correlate with episodes of sepsis [Doig, 1998].

In order to assess the permeability of the large bowel a third oligosaccharide, sucralose, will also form part of the sugar absorption test. This synthetic sweetener (marketed by Tate and Lyle as 'Splenda') is not subject to the same degradation by large bowel commensal flora as lactulose and is therefore a better measure of large bowel permeability than lactulose. Again, this has formed part of previously described studies aiming to measure intestinal permeability [Del Valle-Pinero, 2013].

Lactulose, mannitol and sucralose will be co-administered to both healthy participants and participants following thermal injury. In order to document accurately the time course of change in permeability, thermally injured participants will be asked to undergo the test on alternate days for 14 days (the intense monitoring phase) followed by two convalescent samples at day 28 and month 6 (thermal injury participants). In order to produce an accurate baseline measurement, healthy participants will be asked to undergo three measurements of intestinal permeability over approximately a two week period.

3.2.3. The Intestinal Microbiome and Thermal Injury

During the intense and convalescent monitoring phases of this study, samples of stool will be collected. These samples will undergo gene sequence analysis in order to determine the composition of the intestinal microbiota. These results will be compared with culture results from peripheral whole blood samples. The hypothesis is that raised intestinal permeability will correlate with an increased frequency of bacteraemia and that the particular bacteria detected in blood will correlate with the composition of the intestinal microbiota.

Additionally, it has been demonstrated that thermal injury alters the composition of the intestinal microbiome [Hammer, 2015]. The ultimate aim is to be able to block changes in intestinal permeability which might affect this change in composition and are therefore interested, in the current study, to assess the impact of thermal injury on the microbiome.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Non-investigational Medicinal Product Use		
Oral administration of sucralose in solution is unlikely to be palatable.	The dose of sucralose to be administered is 2g per test. A tolerable sweetness score (e.g. 11.3 (diet Pepsi)) would require the sucralose to be administered in 4 litres of water.	Sucralose is to be administered in capsules when given by mouth. If being administered by nasogastric or nasojejunal tube the capsules are to be emptied into the lactulose and mannitol solution
Depending on the volume of administration, the final STM solution may be hyper-osmolar. Administration directly into the jejunum via a nasojejunal tube may then result in osmotic movement of water into the intestine causing distention and discomfort.	This is based on the physiological principle that the stomach normally regulates the osmolality of its contents passing into the small intestine. Administration via nasojejunal tube (but not nasogastric tube) bypasses this process.	The lactulose, mannitol and sucralose will be delivered nasojejunally in 50ml of water and followed by a 50ml water flush. This makes the solution iso-osmolar (300 mosmol/Kg in situ). following nasojejunal administration
Lactulose and mannitol can produce an osmotic laxative effect following enteral administration.	Both lactulose and mannitol are used clinically as osmotic laxatives. The typical dose of lactulose (for the treatment of constipation) would be approximately 21g daily.	The amount of lactulose being used in this study is 5g, 75% below the standard laxative dose. The dose of mannitol is equally low compared to that contained in laxatives.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
STM administration for the measurement of intestinal permeability requires a hold in feeding which may be longer than a standard feeding hold.	Intestinal permeability assessment requires the administration of STM (see Section 7) and the collection of urine samples at defined time points (see the SRM). This procedure has previously been used in patients with severe thermal injury [Doig, 1998] but has not been studied extensively; participant fasting is required pre and post administration of these sugars; and repeated administration as per the SoA tables is also unexplored.	Where possible, tube feeding targets will be volume and not time-based to reduce the amount of feed missed on test days. Moreover, fasts for the tests will, where possible, be aligned with clinically indicated feeding holds (such as fasts required before theatre, or scheduled overnight feed holds).
Other		
The degree of injury sustained by some participants may be severe.	Participants with severe thermal injury experience significant morbidity and high levels of mortality. Therefore it is anticipated that these participants will be subject to multiple medical complications which may impact the study assessments and period (See SoA tables)	Ensure that routine care in the burns unit is not compromised by study participation. Prompt reporting of any adverse events which are related to study procedures and may affect study safety.
Omission of lactulose and sennosides which are frequently administered as a part of routine burn management.	Lactulose is a part of the STM and its administration for clinical reasons would complicate intestinal permeability measurement significantly. Equally, the mechanism of actions of sennosides is to cause irritation of the GI tract	Polyethylene glycol (e.g. Movicol) will be used as an alternative osmotic laxative. If senna is required for clinical purposes, then its use must be documented in the CRF.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	and may result in increased permeability.	
The STM will be prepared for administration on the assessment day and will not be sterile.	There is a risk that the sugar test material formulation may be contaminated with yeast or bacteria. The presence of contamination within the STM formulation delivered via the feeding tube in thermal injury participants could present an infection risk.	Microbiology release testing will be conducted by Tayside pharmaceuticals. The STM will be prepared on the assessment day to minimise this risk. Lactulose/Mannitol will be refrigerated from the point of manufacture and until use. The site investigators have been consulted on this risk and felt that it was low.
Incomplete 24 hour urine collections	Ambulant, uncatheterised patients and healthy participants 24-hour urine collections could be incomplete.	Ambulant, uncatheterised patients and healthy participants will receive careful education and written instructions of the importance of complete urine collections. In addition, documentation of incomplete collection, in addition to sampling collection times, will be recorded.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Phlebotomy for biomarkers (healthy participants)	Phlebotomy can be painful and result in bruising, bleeding and puncture site infection.	Phlebotomy from thermally injured participants will not be conducted as a part of this protocol. Healthy participants will undergo one blood draw during screening and a further draw on day one. Phlebotomy will be performed by an appropriately trained member of the site study team with aseptic non-touch technique to avoid infection.

3.3.2. Benefit Assessment

Study participants will not benefit directly from involvement in this study. However, the results of this study may contribute significantly to our understanding of changes in intestinal permeability and their relationship to morbidity and mortality in the context of thermal injury. This knowledge is paramount to designing future medicinal interventional studies, aiming to modulate intestinal permeability and, potentially, to improve outcomes for patients following thermal injury.

3.3.3. Overall Benefit:Risk Conclusion

The primary outcome measure of this study is the determination of intestinal permeability in healthy and thermal injury participants. Interventions in this study are the administration of STM by mouth or feeding tube (if one is site for routine clinical care) and the collection of urine and stool samples.

The risk of adverse events is minimised for the population being investigated in the proposed study as no drug intervention will be investigated and study assessments being conducted are non-invasive (with the exception of STM administration and phlebotomy in healthy participants).

4. OBJECTIVES AND ENDPOINTS

Objective	Endpoint
Co-Primary	
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time
Exploratory	
1. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry
2. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time

Objective	Endpoint
3. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of hemofiltration-free days • Number of episodes of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores
4. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios
5. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples
6. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission
7. To assess wound healing	• Time to wound recovery (e.g. 95%)
8. To characterise parameters that may influence drug PK/PD†	• Determine fluid input/output balance over time • Changes in serum albumin and plasma creatinine
9. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation

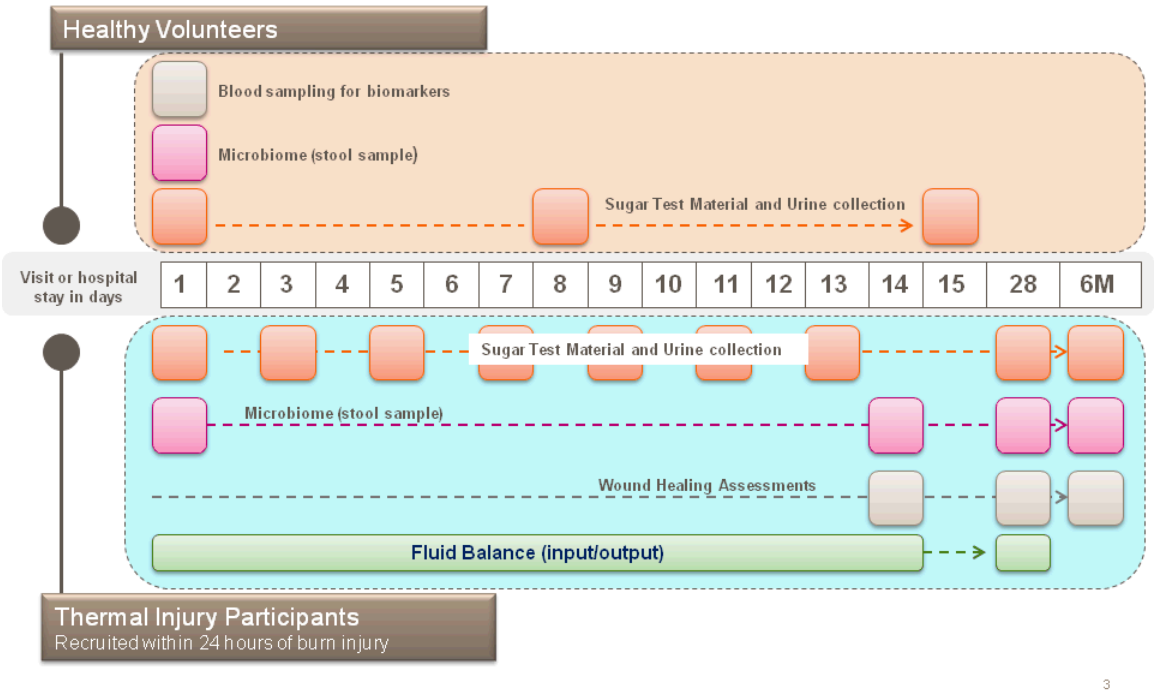
†Clinical data, routine laboratory results or blood/urine biomarker results obtained from the SIFTI-2 study will be used in the analysis of this exploratory endpoint see Section 5.4.1.

5. STUDY DESIGN

5.1. Overall Design

This is a longitudinal, prospective study of healthy participants and participants who have sustained a thermal injury. The following schematic summarises study measures and their frequency for healthy and thermal injury participants.

Figure 2 Study Schematic



5.2. Number of Participants

Table 1 describes the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Table 1 Recruitment Stratification

	Number of participants
Group 1 **Healthy participants**	15
Group 2 **Thermal Injury participants** **Percent Total Burn Surface Area (TBSA)** **≥15%**	25

The healthy participants (Group 1) will be recruited with an age range similar to that typical in thermal injury participants based on historic hospital admission data from the UK and data from the SIFTI1 study [Hampson, 2016].

If participants prematurely discontinue the study, additional replacement participants may be recruited at the discretion of the Sponsor in consultation with the investigator.

5.3. Participant and Study Completion

The full duration of the study for healthy participants is approximately two week and for thermal injury participants is approximately 6 months.

Thermal injury participants who withdraw prior to week 4 or healthy participants who withdraw prior to week 2 will be considered for analysis, although it is acknowledged that any missing data at later stages of the study may be related to outcome (either positive or negative). Given this is an exploratory study, the impact of missing data will be explored by assessing the sensitivity of results to different missing data approaches (for example, analyse all available data, analyse only complete data across time points and explore imputation of worst or best case scenarios).

Study withdrawals may also include participants who are consented to the study under Section 30 of the Mental Capacity Act 2005. In the event that participants do not re-confirm consent when they regain capacity, they will be withdrawn from the study.

The end of the study is defined as the date of the last visit of the last participant in the study.

5.4. Scientific Rationale for Study Design

5.4.1. Co-recruitment to the SIFTI-2 Study

Thermally injured participants who are eligible for this study must also be eligible for, and enrolled in a partner study named SIFTI-2.

SIFTI-2 is an observational study currently recruiting healthy participants and thermally injured participants and follows a successfully delivered predecessor study SIFTI1

[Hampson, 2016]. The objectives and endpoints of SIFTI-2 are included in the SIFTI-2 study protocol (reference number IRAS ID: 200366). The design of this study has been aligned with the SIFTI-2 study to support the strategy of co-consenting thermally injured participants to both studies. This will reduce the overall impact of research in this population in the following ways:

- There is sufficient residual blood from collection in SIFTI-2 to allow testing of blood biomarkers of interest for the HESTIA study. This strategy therefore limits impact on participants as no additional blood sampling is required for participation in HESTIA (with the exception of HIV, Hepatitis B and C testing at baseline). SIFTI-2 participants will be explicitly consented for their samples and data to be shared in this way.
- Sampling time points and study visits in SIFTI-2 and HESTIA have been aligned to reduce the impact of co-recruitment on thermally injured participants.

Clinical data from both studies can be generated from the same participant therefore allowing biomarker, microflora and intestinal permeability data to be compared. In contrast to thermally injured participants, healthy participants enrolling in this study will not be required to co-consent for participation in SIFTI-2.

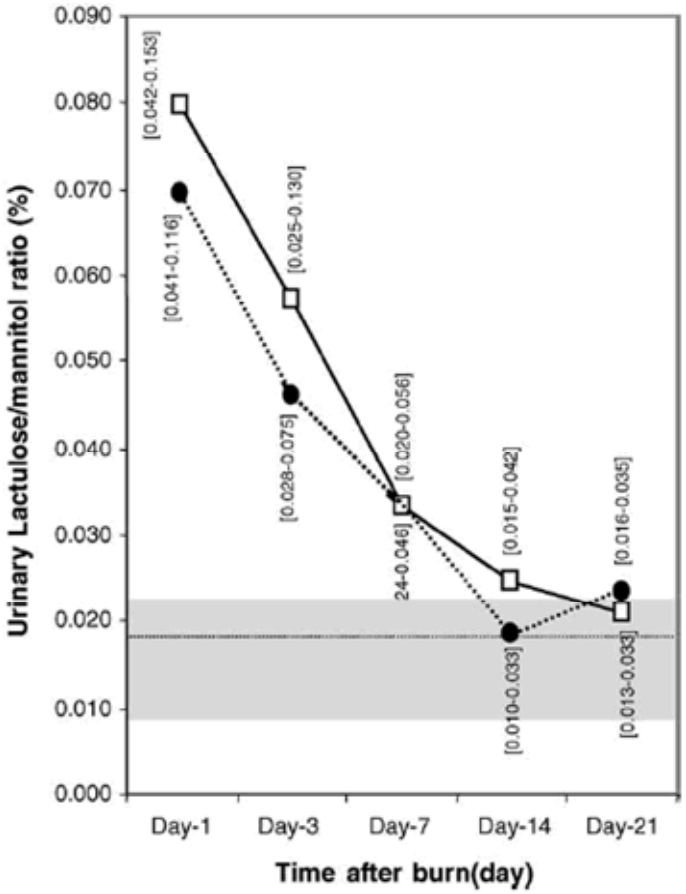
Data will be shared from the SIFTI-2 study with GSK through a secure electronic database.

A summary of the origin (HESTIA or SIFTI-2) of data and samples collected for the HESTIA study is available in Appendix 5 Section 12.5.

5.4.2. Recruitment and Sampling Schedule

Severely burned patients (with an injury affecting greater than 20% total body surface area), display a significant and rapid increase in intestinal permeability that has been shown to decline over time (Figure 3) [Olquin, 2005]. What is less well understood is whether a greater severity of thermal injury correlates with greater intestinal permeability. Moreover, the time to complete restoration of normal permeability and other factors which may influence permeability (other than the initial injury) are also not well understood.

Figure 3 Burn Injury Results in a Rapid Increase in Intestinal Permeability



This study aims to recruit participants as soon as possible following their admission in order to capture the initial changes in permeability. Serial measurement of intestinal permeability and sampling of the biomarkers of bacterial translocation, intestinal damage and inflammation are required during the acute phase (days 1-14) of admission in order to correlate them with clinical events (e.g. surgery), severity scores and clinical outcomes.

The convalescent time points (28 days and 6 months) are required to determine if intestinal permeability has returned to normal and to correlate observed changes on days 1-14 with longer-term clinical outcomes (e.g. wound healing).

Gut microbiome assessments will be made less frequently than intestinal permeability assessments as changes in the microbiome are predicted to evolve more slowly. Ideally a stool sample will be collected from thermal injury participants at study entry (limited, of course, by when participants first pass stool following admission). A convalescent sample is requested to assess if the gut microbiome is able to restore to a more normal composition (and will be compared with that of healthy participants to make that assessment).

Blood samples (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline) will not be taken from thermally injured participants during this study. Instead, biomarker data from blood samples taken during the SIFTI-2 study will be used. A single blood draw will be required from healthy participants on day 1 of participation.

5.4.3. Inclusion of Healthy Participants

Patient facing material (i.e. poster) will be used to facilitate recruitment of the healthy participants. Healthy participants will be recruited to this study to provide a baseline for endpoint measures on intestinal permeability and the gut microbiome.

Three measurements of intestinal permeability are required in order to define an average given the variability in healthy participants reported previously. The timing of the replicates follows the intense time course of the study to control for day-to-day variation over a 15 day period.

5.4.4. Preliminary Data Review

A preliminary review of the initial healthy participant's data will be performed to explore the practicality of intestinal permeability measurement and laboratory quantification of STM in urine and will also explore L/M ratio data at entry and over the time course of the study to assess whether the variability in L/M ratio is similar to that upon which the study was based. If the variability is much greater than expected, the number of subjects recruited may be increased.

The Reporting and Analysis Plan (RAP) will describe the planned preliminary review in greater detail.

5.4.5. The Use of the Sugar Test Materials (Lactulose, Mannitol and Sucralose)

As described in Section 3.2.2, lactulose, mannitol and sucralose will be administered to both thermally injured participants and healthy participants to measure permeability of the small and large intestine. The amount of each of the sugars to be used is based on previous successful clinical studies employing this method and balances having enough sugar present for detection in urine with their potential laxative effect (Del Valle-Pinero, 2013; Doig, 1998; Menzies, 1972).

6. STUDY POPULATION

The study population will comprise healthy and thermal injury participants presenting at enrolling study sites. Please note the following:

- Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.
- Where possible, written informed consent will be obtained from each subject prior to participation in this study. Recruitment of subjects who lack mental capacity is discussed in Section 6.1.
- Healthy participants will be consented to the HESTIA study only.

- Thermally injured participants are required to be co-consented to the SIFTI-2 and HESTIA studies outlined in Section 6.1. A diagrammatic overview of the SIFTI-2 and HESTIA thermal injury participant recruitment is given in Figure 4. (see Appendix 3).

6.1. Recruiting Participants with Differing Mental Capacity

Following evaluation of capacity (as outlined in the Mental Capacity Act (MCA) 2005), thermally injured participants enrolled in this study will fall into the following groups:

6.1.1. Adult Participants Determined to Have Mental Capacity at Study Entry and Throughout the HESTIA Study

Those participants who present with capacity and meet study entry criteria will be provided with a Participant Information Leaflet (PIL) outlining the study. If the participant agrees to consent to the HESTIA study following a discussion with the research team, they will be asked to sign a consent form.

Given the short (24 hour) window for recruitment, those patients who are acutely unwell will initially be presented with an abbreviated PIL. Once stable, this will be followed by the standard information leaflet and re-confirmation of consent.

6.1.2. Adult Participants Lacking Mental Capacity for the Duration of the HESTIA Study

It is anticipated that some subjects who meet eligibility criteria for this study will not be able to give informed consent due to their medical condition or its management (e.g. sedation, opioid analgesia, intubation). In such cases, participants may be enrolled in the study in accordance with Section 30- Section 34 of the MCA 2005. The decision to enrol the participant will be discussed with a legally acceptable representative (LAR) (also termed a ‘consultee’). This decision may or may not be witnessed by an independent witness according to the decision of the principal investigator.

6.1.3. Adult Participants Lacking Mental Capacity (either at Study Entry or During the Study) Who Later Regain Capacity and Are Required to Provide Informed Consent

As soon as is practically possible following a participant regaining capacity, participants will be asked to provide informed consent to remain in the study. If they decline, then they will be withdrawn from the study as soon as it is safe to do so (likely immediately given the design of this study). Samples and data collected prior to study withdrawal may be retained. The participant will be asked about this at the point of study withdrawal.

6.1.4. Adult Participants with Mental Capacity to Provide Consent at Study Entry Who are Later Deemed No Longer to have Mental Capacity

The decision for the participant to remain in the study will be discussed with a LAR and recorded. If the participant subsequently regains capacity again, they will be asked to re-consent to study participation.

When considering enrolment of participants who lack the mental capacity to consent, the following should be noted:

- Section 3.2.1 of the SIFTI-2 protocol describes the consent process for that study in detail and should be read in conjunction with this protocol. Please note that the SIFTI 2 protocol refers to a LAR as the Patient's Personal Consultee (PC) or Nominated Consultee (NC).
- A Study Information Leaflet will be provided to the LAR outlining the HESTIA trial before being asked to sign a form supporting the participant's enrolment in the study.
- The investigator and/or the site's IEC/IRB have responsibility for acting in accordance with the MCA 2005 in the matter of assessing who has the capacity to consent and who qualifies as a LAR of a potential subject. The investigator will also decide if an independent witness is required.
- Further information regarding the assessment of mental capacity and the appointing of LARs/PCs/NCs is given in Appendix 3 (Section 12.3.2 and Section 12.3.3).
- If a patient loses mental capacity subsequent to their consent and enrolment to the HESTIA study, samples and data collected prior to loss of capacity will be retained even if approval of continued study participation by a LAR is declined.

6.2. Inclusion Criteria for Healthy Participants (Group 1)

1. Males or Females must be ≥18 years of age at the time of signing informed consent.
2. Participants who are healthy as determined by the investigator following medical evaluation including medical history, physical examination, and laboratory tests (these are listed in Appendix 2).
3. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at screening or Day 1 as needed) and not breastfeeding.
4. Capable of giving signed informed consent as described in Appendix 3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.3. Inclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are eligible to be included in the study only if all of the following criteria apply:

6.3.1. Age

1. Participant must be ≥18 years of age.

6.3.2. Type of Participant and Disease Characteristics

2. Participants who have sustained a burn (thermal injury) with a TBSA ≥ 15%.

6.3.3. Other Inclusions

3. Admission to the burn centre (study site) ≤*24* hours of injury.
4. Able to take enteral fluids either orally or via a nasogastric tube (depends on facial burn damage).

6.3.4. Gender

5. Male and female.

   a. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at study entry) and not breastfeeding.

6.4. Exclusion Criteria for Healthy Participants (Group 1)

1. Healthy participants are excluded from this study if they are receiving anti-coagulation therapy.
2. Pregnancy or breastfeeding.
3. A body mass index >34kg/m^2
4. An active history of alcohol dependency
5. History of sensitivity to any of the STM, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator and/or GSK Medical Monitor, contraindicates their participation.
6. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody and confirmatory Hepatitis C PCR result within 3 months of screening.
7. A positive pre-study urine drug/alcohol screen.
8. A positive test for HIV antibody.
9. Participants unable to swallow large capsules (the capsules will be shown to participants at screening).
10. Galactosaemia or severe lactose intolerance.
11. Use of an antibiotic 2 weeks prior to study start (i.e. administration of the STM).
12. Gastroenteritis in the 2 weeks prior to study start (i.e. administration of the STM).

6.5. Exclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Chemical or electrical burn.
2. Multiple traumatic injuries with an Injury Severity Score (ISS) ≥16 (note: excludes burn in score system).

Prior/Concomitant Therapy

3. Patient received substantial undocumented management prior to arrival at the study site (burn centre) e.g. from paramedics or in a local accident and emergency department.
4. Systemic corticoidsteroid use.
5. Intravenous (IV) mannitol use.

Prior/Concurrent Conditions

6. Human immunodeficiency virus (HIV) infection.
7. Gastrointestinal disease (e.g. inflammatory bowel disease) which may affect intestinal permeability.
8. Previous bowel resection (e.g. hemicolectomy, small bowel resection)
9. Galatosaemia or severe lactose intolerance.
10. Bowel obstruction.
11. Renal dysfunction requiring renal replacement therapy (i.e. end-stage renal failure prior to thermal injury).
12. Active autoimmune disease and receiving immunomodulatory therapy e.g. rheumatoid arthritis anti-TNF.
13. Active chemotherapy for cancers or immunoremittive therapies (prednisolone, adalimumab) within 60 days of thermal injury.
14. Premorbid conditions of malignancy currently under treatment.
15. Previous bilateral lower extremity amputation.

Diagnostic assessments

16. Decision not to treat the patient due to futility.

6.6. Lifestyle Restrictions

6.6.1. Meals and Dietary Restrictions

- Participants will be fasted (or feed stopped) for **3 hours** prior to STM administration and for **3-5 hours** afterwards. For thermally injured participants these fasts should be aligned with those required for routine clinical care (feed holds, before surgical interventions) where possible.
- Refrain from consumption of the following for **24 hours** before and after the administration of STM:

- Foods/drinks/medicines and other products which contain sucralose, lactulose or mannitol as artificial sweeteners.

N.B. If cannot be avoided, then clear documentation of its administration is required and the current test to stop. If urine samples have been collected PRIOR to administration of the drug, then these can still be sent for analysis

6.6.2. Alcohol/Exercise/Aspirin (Healthy Participants only)

Alcohol, aspirin and vigorous exercise [Sequeira, 2014] are all known to cause transient increases in intestinal permeability. Healthy participants will therefore be requested to avoid alcohol, aspirin and physical exercise for **48 hours** before taking the STM and for the 24-hour urine collection period.

6.7. Screen Failures

There will be no screening period for thermal injury participants. Screening will be up to 28 days before Day 1 for healthy participants.

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

7. NON-INVESTIGATIONAL MEDICINAL PRODUCT (NIMP)

A study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. According to this definition, no GSK study treatment will be employed in this study.

The STM (comprising lactulose, mannitol and sucralose) will be intermittently administered enterally as a study challenge agent to measure permeability at different points along the GI tract. Lactulose and Mannitol assess small intestine permeability and sucralose to assess colonic permeability.

It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

7.1. STM Administered

Study Treatment Name	Lactulose (4-*o*-β-D-galactopyranosyl-D-fructofuranose)	Mannitol (D-mannitol) GRAS listed	Sucralose (1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside)
Dosage formulation	oral solution	oral solution	Capsules (powder)
Unit dose strength(s) Adults	5g	2g	2g (3 capsules to deliver total 2g sucralose)
Route of Administration	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal (capsules to be opened and contents added to lactulose and mannitol for tube administration)
Preparation and Dosing instructions	For oral administration, the lactulose and mannitol will be prepared as a 100ml drink to be taken with 3 sucralose capsules. For feeding tube administration, lactulose/mannitol/sucralose will be prepared as a 50ml solution and given via a feeding tube followed by an immediate 50ml drinking water flush Preparation refer to Study Reference Manual together with SoA tables (Section 2)		
Packaging and Labelling	Lactulose and Mannitol will be supplied pre-mixed in an amber bottle (or equivalent) for single use. Each container will be labelled as required per country requirement.		Sucralose will be provided as capsules in a storage container. Each container will be labelled as required per country requirement.
Manufacturer	Tayside Pharmaceuticals, UK		
Storage	Lactulose/Mannitol formulation should be stored under refrigerated conditions. The sucralose capsules should be stored at room temperature in a dry environment away from direct sunlight.		
Shelf-life	Lactulose/Mannitol pre-mix formulation and sucralose capsules supplied by Tayside Pharmaceuticals will have at least 3 month shelf-life when stored at the correct storage conditions.		

The preparation of the STM for oral use and nasogastric/nasojejunal tube administration can be found in the Study Reference Manual.

7.2. Dose Modification

Dose modification will not be required. Unit dose is described in Section 7.1.

7.3. Method of STM Administration: Treatment Assignment

There is no element of randomisation in the study and all study participants will receive the STM according to the relevant SoA. The method of administration can be found in the Study Reference Manual.

7.4. Blinding

No GSK study treatment will be employed in this study. All participants will receive the same STM and all thermal injury participants will perform the same study procedures.

7.5. Preparation/Handling/Storage/Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all STM received and any discrepancies are reported and resolved before use of the STM.
2. Only participants enrolled in the study may receive STM and only authorized site staff may supply or administer STM unless adequate training is provided such as in the case of healthy participants. All STM must be stored in a secure, temperature controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for STM accountability, reconciliation, and record maintenance, as needed.
4. Further guidance and information for the final disposition of unused STM are provided in the Study Reference Manual.
5. Under normal conditions of handling and administration, STM is not expected to pose significant safety risks to site staff.

7.6. STM Compliance

- When participants undergo intestinal permeability testing at the site, they will receive STM directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of STM and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the STM.
- If healthy participants need to prepare and administer the STM off-site such as at home, STM training will be provided and a record maintained by the investigator or designee.

7.7. Concomitant Therapy

- Refrain from consumption of the following for **24 hours** before and after the administration of STM:
  - Lactulose or mannitol-containing laxatives. Study sites will be asked to use movicol (polyethylene glycol) in place of lactulose.
  - Medicines with mannitol as an excipient (chlorthiazide sodium, some albumin preparations, some laxatives, tablets as a bulking agent).
  - Products containing sucralose.
- For healthy participants only, refrain from consumption of aspirin for **48 hours** before taking the STM and for the 24-hour urine collection period see Section 6.6.2.
- For healthy participants only, antibiotic use 2 weeks prior to STM administration and during the study is not permitted.
- Sennoside laxatives should be avoided. These can cause gastrointestinal irritation and may contribute to raised intestinal permeability.
- Additional Glutamine supplementation in excess of that delivered with a standard feeding protocol should be avoided during the first 28 days of study participation. If supplementation is given inadvertently, then the patient will remain in the study, but the total dose and duration of additional glutamine supplementation must be recorded in the CRF.
- Thermal injury participants that receive Intravenous (IV) mannitol for renal failure or raised intracranial pressure (testing to be delayed until 12 hours after last administration).

7.8. Treatment after the End of the Study

There will be no ongoing STM administration following the end of this study.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of STM administration

Discontinuation of STM administration can be considered by the investigator in the event that an adverse event to the STM is observed. Withdrawal of further STM administration does not require withdrawal from the study.

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

8.2.1. Other Withdrawal Criteria

- A participant will be withdrawn from the study following positive HIV test at screening; Serologic evidence of Hepatitis B (HB) infection based on the results of testing for HBsAg, and anti-HBc and positive test for Hepatitis C antibody confirmed by HCV RNA. If HCV RNA is not available, then the positive test for Hepatitis C antibody alone would be exclusionary. Results must be discussed with the medical monitor to withdraw the subject from the study and commence therapy according to local practice.
- Healthy participants that are treated with antibiotics during the duration of the study.
- Participants that experience signs and symptoms of gastro-intestinal infections during the duration of the study.
- Withdrawals related to mental capacity as described in Section 6 and Appendix 3.

8.3. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the study site for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed.
- Safety concerns related to the STM should must be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue to be administered the STM.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the thermally injured participant's routine clinical management (e.g., weight measurement) and obtained before signing of ICF may be used for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA. Procedures (the administration of STM) are not part of routine care for either healthy or thermal injury participants.
- Healthy participants will be asked to donate a single blood sample on Day 1. No blood collection is specified in the SoA of this protocol for thermal injury participants (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline). The results of clinical laboratory blood tests will be recorded in the SIFTI-2 study and the data used in this study. Likewise, blood collection for exploratory biomarker detection will be included in the SIFTI-2 study and the data used in this study.

9.1. Efficacy Assessments

The administration of the STM is for the purpose of intestinal permeability measurement and is not therapeutic, therefore no efficacy will be assessed.

9.2. Adverse Events

9.2.1. Monitoring and reporting responsibilities

Healthy participants will be recruited to the HESTIA study alone and all AEs or SAEs occurring in this group should be managed according to this protocol.

Thermally injured participants recruited to this study will also be recruited to the SIFTI-2 study. The following guidance relates only to AEs or SAEs which the investigator reasonably believes to be the result of a procedure or requirement unique to this (the HESTIA) protocol. All other AEs or SAEs will be reported and managed in accordance with the SIFTI-2 protocol.

Unique procedures and requirements of HESTIA

1. The administration of STM
2. The collection of stool samples
3. Changes to standard of care for thermally injured participants:
    a. The fasts required during the measurement of intestinal permeability
    b. The use of alternative laxatives to lactulose and sennosides.

The definitions of an AE or SAE for this study can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious or that caused the participant to discontinue intestinal permeability measurement with the STM (see Section 8).

9.2.2. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2).
- All AEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2).
- Medical occurrences that begin before the start of unique HESTIA study procedures but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the STM administration or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.2.3. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. If the participants are conscious, open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence. For unconscious patients or participants not always able to provide valid verbal responses to open-ended questions, the investigator or designee will need to identify AEs and/or SAEs through relevant clinical signs and/or investigations.

9.2.4. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to follow proactively each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Appendix 4.

9.2.5. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of the study. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information e.g., summary or listing of SAE) from the sponsor will review and then file it and will notify the IRB/IEC, if appropriate according to local requirements.

9.2.6. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

The following broad disease related events (DREs) are common in thermally injured participants and can be serious/life threatening:

- Deterioration of condition.
- Death (may be expected in burns of a large surface area).
- Prolongation of hospital stay.
- Persistent or significant disability or incapacity.

Because these events are typically associated with the disease under study, they will not be reported according to the standard process for expedited reporting of SAEs to GSK (even though the event may meet the definition of a SAE). These events will be recorded on the DRE page in the participant's CRF within [the appropriate time frame *agreed upon by the SRT* for completion of DRE CRF pages]. These DREs will be monitored by clinical study team on a routine basis.

- *NOTE: However, if the investigator considers that there is a reasonable possibility that the event was related to administration of STM or another*

unique or required element of the study (as defined in Section 9.2.1) then the event must be recorded and reported as an SAE (instead of a DRE).

- A comprehensive list of further thermal injury related DREs can be found in Appendix 4 (Section 12.4).

9.3. Treatment of Overdose

For this study, an overdose is defined as any dose of STM greater than defined in Section 7.1. No specific treatment is recommended for an overdose and treatment is at the discretion of the investigator. The GSK medical monitor must be notified promptly.

9.4. Safety Assessments

Planned time points for all safety assessments are provided in the SoA and summarised here.

Safety Assessment	**When conducted**	
	Healthy Participants	**Thermally injured participants**
Laboratory tests	Screening. Only repeated if clinically indicated in the opinion of the investigator.	Only if clinically indicated.
Brief Physical Examination including Vital Signs Recording	Screening, Day 1, Day 8, Day 15	As a part of routine clinical care whilst admitted (not protocol specified). Following day 28 and 6 month intestinal permeability measurements (if patient not still admitted)
Detection of AEs	Day 1, Day 8, Day 15	Throughout the study
Assessment of health status	Screening, Day 1, Day 8, Day 15	Not required

9.4.1. Physical Examinations

- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

9.4.2. Vital Signs

- A **single** vital sign measurement will be obtained at each time point indicated in SoA Table, and will include systolic and diastolic blood pressure and heart rate. Any abnormalities and changes in measurements will be communicated to the medical monitor.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, mobile phones).
- Vital signs to be taken before blood collection for laboratory tests.
- Repeat or unscheduled measurements may be taken at the discretion of the investigator.

9.4.3. Clinical Safety Laboratory Assessment

- All study related laboratory assessments will be performed by a local laboratory. The laboratory reports must be reviewed by the investigator, this review documented and both report and review are to be filed with the source documents.
- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in participant management or are considered clinically significant by the investigator (e.g., SAE or AE), then the investigator must review the laboratory report, document this review, and record any clinically relevant in the AE section of the CRF.
- Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

9.5. Study Procedures

The following procedures will be carried out during the study.

9.5.1. Fluid Balance Measurement

All fluid input and output will be recorded every 24 hours for thermally injured participants.

9.5.2. Wound Healing

Assessment of wound healing will be the time to 95% wound healing. Physical parameters of the wound (e.g., rate of healing) will be recorded and collected as a part of both the HESTIA and the SIFTI-2 studies.

9.5.3. Other Clinical Responses

To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability the following will be recorded and collected as a part of the SIFTI-2 study. Details can be found in the SIFTI-2 study protocol.

- Number of ventilator-free days (ventilator start/restart/end date/time)
- Number of vasopressor-free days (medication chart review)
- Number of hemofiltration-free days (notes review)
- Number of episodes of confirmed infection and sepsis
- Number of surgical interventions
- Total length of hospital stay
- Calculate critical care and thermal injury severity scores

9.6. Pharmacokinetics

PK parameters are not evaluated in this study.

9.7. Pharmacodynamics

PD parameters are not evaluated in this study.

9.8. Intestinal Permeability Assessments

- Intestinal permeability will be determined by measuring the excretion of lactulose, mannitol and sucralose in urine following their enteral administration. It will be conducted in both healthy participants and thermally injured participants at the time points specified in the SoA.
- The complete method for administration of STM and measurement of intestinal permeability is detailed in the SRM.
- Urinary excretion of the orally ingested STM will be quantified using a technique such as capillary column gas chromatography.
- Urine samples will be collected in plastic bottles for analysis. Urine collection will begin immediately following STM administration. Urine samples will be collected over 24 hours post-STM administration. Accurate collection of the total volume voided during this 24 hour period is critical.

- **Sample Preparation**

Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

9.9. Genetics

Genetics are not evaluated in this study.

9.10. Sample Collection for Biomarker Analysis

The following biomarkers will be collected during the study. Details of sample processing, storage and shipping are included in the study reference manual.

Blood, stool and urine samples will be collected and stored. Timing of analyses and selected biomarkers will be dependent on the results of the intestinal permeability tests results.

9.10.1. Blood Biomarkers

Healthy participants

- Blood samples will be taken for healthy participants recruited in this study over the time period specified in the SOA. Blood will be taken adhering to standard operating procedure from venae puncture.
- The results of blood biomarker analysis will be evaluated in this study and compared to measures of intestinal permeability.
- Twenty (20) ml blood will be collected on Day 1 for biomarker analysis, and 20ml blood will be collected at screening for screening tests. The biomarkers to be measured may include, but are not limited to:
  - Markers of microbial translocation
  - Markers of intestinal damage
  - Inflammatory markers: e.g. C-Reactive Protein, Procalcitonin, cytokines (including TNF-a, IL-6, IL-8, IFN-g, IL-10, IL-1b, IL-12p70, IL-17, IL-4, IL13, IL1Ra, MIP1a, MIP1b, MIP2, GCSF, GMCSF, MCP-1, RANTES, HMGB1).

Thermally injured participants

- The blood required for this analysis in thermally injured participants will be collected as a part of the SIFTI-2 (IRAS 200366) study to which all thermally injured participants will be co-recruited. Details of the schedule for blood collection and the total volume of blood collected can be found in the SIFTI-2 study protocol.

9.10.2. Stool Sample Collection

- Stool samples will be collected from all participants in this study over the time period specified in Section 2, Schedule of activities (SOA). Stool samples will be collected adhering to standard operating procedure.
- For thermally injured participants, the initial sample will be taken as close to time of injury as possible ("first stool sample produced upon admission") and Day 14. Further samples will be taken on day 28 (± 3 days) and at month 6 (± 14 days).
- For healthy participants, a single sample will be collected at study entry (participants will be given a collection container at screening).

9.10.3. Urine Sample Collection

- Urine samples will be collected as a part of the measurement of intestinal permeability which is described in Section 9.4.
- Additional urine samples will be collected from patients as part of the SIFTI-2 study to which all thermally injured participants will be co-recruited. These will be used for, among other tests, the quantification of protein and microbial metabolites.
- It is standard practice that patients admitted with burns of TBSA ≥15% will have a urinary catheter inserted on admission to ensure the accurate maintenance of fluid balance. A clean urine sample will be taken from the appropriate port on the urinary catheter. In patients who are not catheterised, a mid-stream urine (MSU) should be collected in a clean universal container where possible.
- N.B. During the 24 hours following STM administration (during intestinal permeability measurement) urine samples must only be taken from the 5-hour of 24-hour urine collections **after** the aliquots for sugar quantification have been taken.

Sample Preparation

- Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

10. STATISTICAL CONSIDERATIONS

All statistical analyses will be performed using SAS (SAS Institute Inc., Cary, North Carolina, USA), version 9.2 or higher. Before database lock, a reporting and analysis plan (RAP) will be issued as a separate document, providing detailed methods for the analyses outlined below. Any deviations from the planned analyses will be described in a RAP addendum and justified in the final integrated clinical study report.

10.1. Hypotheses

As this is an enabling study designed to better understand the biomarkers of intestinal permeability and other biomarkers in participants with moderate to severe burns, the statistical analysis for this study will be exploratory in order to better understand the parameters to inform future investigational medicinal product studies.

The key factors of interest in this study are to understand (i) the nature of any differences at entry in intestinal permeability between healthy participants and thermal injury participants (ii) to understand the trajectory of changes in intestinal permeability over time.

The key endpoint to be explored is the lactulose:mannitol (L/M) ratio, but other permeability biomarkers will also be explored. The analysis approaches to address these questions are exploratory, but will initially be conducted as outlined in Section 10.5 and Section 10.6.

10.2. Sample Size Determination

Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (>15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Although the key aim is to estimate the variability and L/M ratio and assess the difference in L/M ratio between thermal injury and healthy participants, for illustration, a trial including 25 thermal injury and 15 healthy participants would have 89% power to detect a 3-fold difference in L/M ratio between thermal injury and healthy participants using a 2-sided significance level of $p<0.10$. This calculation uses a (log) b etween-subject SD of 1.15, as estimated from the literature [Olquin, 2005].

10.3. Data Analyses Consideration

In general, descriptive summaries will include number of subjects, mean, standard deviation, median, minimum, and maximum for continuous variables. If data are log-normally distributed data will be presented as number of subjects, geometric mean, coefficient of variation, minimum, and maximum; and percent for categorical variables. Summaries will present data by dose level and where appropriate, by assessment time.

10.4. Populations for Analyses

The **Safety Population** will consist of all subjects who receive at least 1 dose of STM and have at least on post-dose safety assessment.

The **Evaluable Population** will consist of all subjects who are entered into the study and have evaluable L/M ratio measurements.

10.5. Statistical Analyses

10.5.1. Safety Analyses

Administration of STM is for the measurement of intestinal permeability. The safety of this administration is not an endpoint of this study, but will be monitored and reported.

All safety data will be presented in data listings. Subject demographics, medical history, and prior and concomitant medications will be summarized using descriptive statistics. For continuous variables, these summaries will include number of subjects, mean, median, standard deviation, minimum, and maximum.

For categorical variables, the summaries will include frequencies and corresponding percentages. No inferential hypothesis testing will be performed on the safety variables.

Adverse events will be coded using the MedDRA classification system.

For healthy participants (who are recruited only to HESTIA), STM-emergent AEs will be defined as any AEs, regardless of relationship to STM administration, that occur after the first dose of STM until the final follow-up visit. The STM-emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. The total number of AEs will be summarized overall. The AEs will be further summarized by severity and relationship to STM. If relationship information is missing, the AE will be considered STM-related. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

For thermally-injured participants, STM-emergent AEs will be defined as any AE deemed related to STM administration that occurs after STM administration until the follow-up visit. The STM-related emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

As laboratory data and vital signs are only collected at screening for healthy participants, these data will be listed only. Clinical laboratory values that are outside of the reference ranges will be flagged and evaluated for clinical significance by the investigator. Physical examination findings will be listed. For thermally-injured participants, physical

examination findings and clinical laboratory values will be highly abnormal and as such any data collected will only be listed. Disease-related findings and changes will not be reported.

10.5.2. Other Analyses

Biomarker exploratory analyses will be described in the RAP.

10.5.3. Interim Analyses

A preliminary review of the initial healthy participant's data will be performed to explore the practicality of intestinal permeability measurement and laboratory quantification of STM in urine and will also explore L/M ratio data at entry and over the time course of the study to assess whether the variability in L/M ratio is similar to that upon which the study was based. If the variability is much greater than expected, the number of subjects recruited may be increased.

The RAP will describe the planned preliminary review in greater detail.

10.6. Analyses of lactulose/mannitol ratio

In all analyses the variable TBSA will be a categorical variable defined as "Yes" for thermally injured participants, and "No" for healthy participants.

Differences in permeability at entry

Intestinal permeability biomarkers will be summarised by TBSA group and overall. Data will summarised by geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio) = intercept + TBSA

Trajectory of the L/M ratio over time

Intestinal permeability biomarkers will be summarised over time, by TBSA group and overall. Data will summarise geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio at time X / L/M ratio at entry) = intercept + Time + TBSA + Time*TBSA.

This will be a repeated measurement analysis and will assess the rate of improvement in L/M ratio over time, and assess how this changes relative to healthy participants. If required, further modelling assessing more complex relationships between L/M ratio and time may be undertaken. Given this is an exploratory study the most appropriate variance-covariance matrix regarding the correlation of data over time will be explored as part of the statistical analysis.

Data from this model may also be used to estimate the time to 50% improvement (or other degrees of improvement) in L/M ratio in relation to L/M ratio values seen in

healthy participants. This will be used to assess the clinical relevance and sensitivity of such measures.

A model fitting log (AUC of L/M ratio) = intercept + TBSA will also be fitted. AUC will be calculated using all measurements taken over time. This will provide a summary of the weighted average L/M ratio value over time.

The use of %TBSA will also be assessed in the above analyses as a continuous covariate. The effects of age and Baux score will also be evaluated to understand differences in intestinal permeability in these groups [Osler, 2010].

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

ACR	Albumin/creatinine ratio
AE	Adverse Event
Anti-HBc	Anti-Hepatitis C
ART	Anti-retrovial treatment
CFR	Code of Federal Regulations
D	Day
G	Grams
eCRF	Electronic Case Report Form
ICU	Intensive Care Unit
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gatrointestinal
HB	Hepatitis B
HBs AG	Hepatitis B Antigen
HCV	Hepatitis C
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee

IRB	Institutional Review Board
ISS	Investigator Sponsored Study
L/M	Lactulose/mannitol ratio
LAR	Legally Authorised Representative
mL	Milliliter
MODS	Multi-organ dysfunction syndrome
NIMP	Non-investigational medicinal product
NC	Nominated Consultee
PC	Personal Consultee
PCR	Polymerase Chain Reaction
RAP	Reporting and Analysis Plan
SAE	Serious adverse event
SIFTI-2	A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injuries
SoA	Schedule of Activities
SRM	Study Reference Manual
STM	Sugar Test Material
TBSA	Total body surface area
TNF	Tumour Necrosis Factor
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
SAS

12.2. Appendix 2: Clinical Laboratory Tests

- All clinical laboratory tests will be performed in the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 2 Protocol-Required Safety Laboratory Assessments for Healthy Participants

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Hemoglobin Hematocrit		WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	Urea	Potassium		Total bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/	Total Protein
	Glucose nonfasting	Calcium	Alkaline phosphatase	
Routine Urinalysis	• pH, glucose, protein, blood, ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• Urine alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)] • Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)[1] • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :
1. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

12.3. Appendix 3: Study Governance Considerations

12.3.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- Any amendments to the SIFTI-2 protocol which impact on this protocol will be reviewed and may result in changes to this protocol being required. Any such changes will be subject to IEC/IRB approval before implementation.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.3.2. Recruiting participants under the Mental Capacity Act 2005

On admission into hospital the patient's capacity will be assessed. A patient may lack capacity due to the severity of their injury, arriving intubated and ventilated or due to a pre-existing co-morbidity.

Please note, the same process will also be followed for the SIFTI-2 study to which thermally injured participants will be co-recrruited.

If a patient does not have the capacity to make an informed decision, the research team will approach a patient's LAR, also known as a Personal Consultee. Examples of personal consultees include:

- A family member, carer or friend
- An attorney acting under a Lasting Power of Attorney
- A court appointed deputy, provided that they had a relationship with, or personal knowledge of, the person lacking capacity before their appointment as deputy.

There may be circumstances in which a personal consultee is not available, some examples of this are:

- Where no family member or friend is willing to act as a personal consultee
- Where the family member or friends live a long distance away and/or are not in frequent contact with the patient who lacks capacity
- Where the regular carers of the person who lacks capacity are doing so for payment or in a professional capacity (e.g. care home staff or nurses)
- Where someone is acting on a professional role (e.g. their GP or solicitor)

In this case, a nominated consultee will be required. A nominated consultee is considered to be a medical professional that has no connection to the research trial, but has an understanding of the implications of the research trial on the participant.

In these circumstances, examples of nominated consultees are:

- An emergency department doctor, preferably Consultant level.
- Intensive Care doctor, preferably Consultant level.
- Doctor from the burns team, not directly involved in the research study.

Once a personal or nominated consultee has been identified, they will be provided with a specific information leaflet about the trial. The personal and nominated consultee will be asked if they feel the study would be something the participant would have no objections to. If in their opinion the participant would have no objection to being recruited into a research trial the consultee will be asked to sign a declaration form.

12.3.3. Determining Whether a Participant has Capacity Under the Mental Capacity Act (2005)

Prior to deciding that a patient does not have the capacity to give informed consent the researcher must follow the Mental Capacity Act (2005) to ensure that the participant does not hold capacity. The principles of the MCA which we will adhere are as follows:

- A person must be assumed to have capacity unless it is established that he/she lacks capacity.
- A person is not to be treated as unable to make a decision unless all practical steps to help him/her to do so have been taken without success.
- A person is not to be treated as unable to make a decision merely because he/she makes an unwise decision.
- An act done or decision made, under this Act for or on behalf of a person who lacks capacity must be done, or made, in his best interests.

- Before the act is done, or the decision is made, regard must be had to whether the purpose for which it is needed can be as effectively achieved in a way that is less restrictive of the person's rights and freedom of action.

A decision to appoint a consultee on behalf of a patient will be made if the participant is unable to:

1. (a) understand the information relevant to the decision,
   (b) retain that information,
   (c) use or weigh that information as part of the process of making the decision, or
   (d) communicate his/her decision (whether by talking, using sign language or any other means).

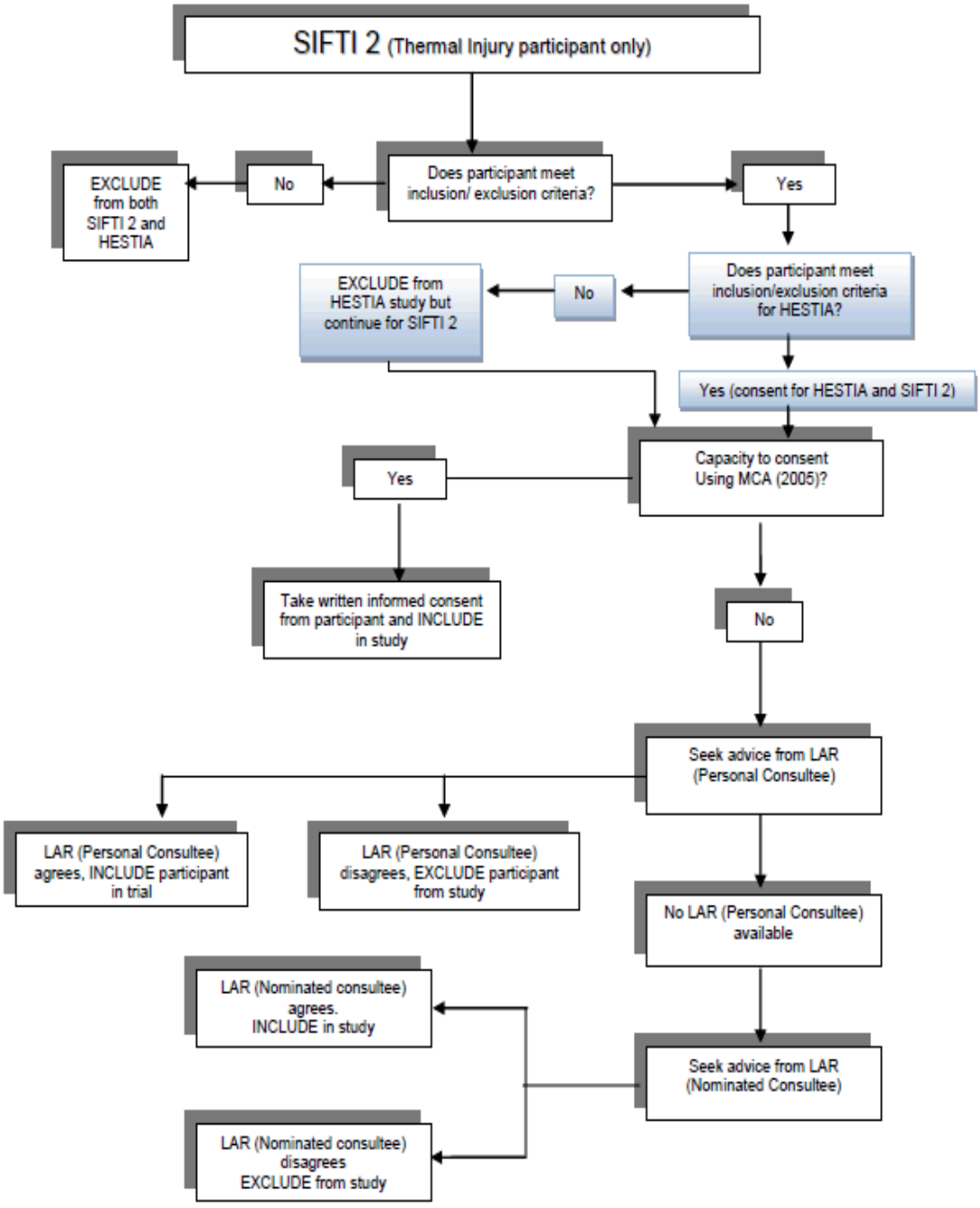
2. A person is not to be regarded as unable to understand the information relevant to a decision if he/she is able to understand an explanation of it given to him in a way that is appropriate to his circumstances (using simple language, visual aids or any other means).

3. The fact that a person is able to retain the information relevant to a decision for a short period only does not prevent him/her from being regarded as able to make the decision.

4. The information relevant to a decision includes information about the reasonably foreseeable consequences of

   (a) deciding one way or another, or
   (b) failing to make the decision.

Figure 4 HESTIA Thermal Injury Participant Recruitment



12.3.4. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.3.5. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Healthy participants who are rescreened are required to sign a new ICF.

12.3.6. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

12.3.7. Committees Structure

An Independent Data Monitoring Committee or similar review group will not be used in this study, but an internal preliminary data review will be conducted.

The Data Review team will consist of the GSK medical monitor, clinical and operational leads, statistician, early development lead and the safety officer. They will meet at intervals specified within the data review charter to review data relevant to the future conduct of the study, and will also assess any risk to study participants.

12.3.8. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

12.3.9. Dissemination of Clinical Study Data

- Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.
- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

12.3.10. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

12.3.11. Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the study specific Source Data Verification document.

12.3.12. Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with study participation, whether or not considered related to the study. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with study participation. • NOTE: As detailed in Section 9.2.1, only AEs deemed to be related to procedures or requirements unique to the HESTIA study will be recorded/reported for thermally injured participants. All other AEs will be recorded/reported through the SIFTI-2 study.

Events Meeting the AE Definition
• Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study STM administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. Table 3 provides a list of commonly occurring AEs in participants with severe thermal injury which may meet this definition. • The disease/disorder being studied or expected progression, signs, or symptoms of

the disease/disorder being studied, unless more severe than expected for the participant's condition.

- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Table 3 Complications of severe thermal injury which can be considered as associated with the underlying disease and do not require reporting as AEs unless judged to be more severe than expected for the participant's condition or related to HESTIA study procedures.

If any of the following are observed, then an AE will be recorded in the SIFTI2 study CRF.

If any of the following are observed and deemed to be related to STM administration or other unique requirements of the HESTIA study, then to be recorded in the HESTIA CRF and reported to GSK as per guidance below.

System Assessment	Complication type	Action
Airway problems	Failed extubation	Record AE
	Tracheostomy complication	
Breathing Problems	Pneumothorax	Record AE
	Pulmonary Oedema	
	Respiratory Arrest	
	Pneumonia	
	VAP	
	Acute lung injury (ALI)	
	ARDS	
Circulatory Problems	Haemodynamic instability	Record AE
	Increasing vasoactive drug support	Record ionotrope dose in con-meds
	Arrhythmia	

System Assessment	Complication type	Action
	Endocarditis	
	Acute LVF/CCF	
	Cardiac Arrest	
	MI	
Neurological Problems	Reduced GCS (off sedation)	Record AE
	Intra-Cranial bleed	
	CVA	
	Acute confusion/Delirium	
	Meningitis-bacterial	
Oedema Complications	Abdominal Compartment Syndrome (ACS)	Record AE
	Acute Limb compartment syndrome	
Microbiological problems	Sepsis	Record AE Record in Microbiology form
	Chest Infection	
	Lower Respiratory Tract Infection	
	UTI	
	Bloodstream Infection (BSI)	
	Wound infection	
	Intra-vascular catheter (line) infection	
	Infective diarrhoea	
	Clostridium difficile infection/pseudomembranous colitis	
Renal/Urology problems	Acute rhabdomyolysis	Record AE

System Assessment	Complication type	Action
	Acute renal failure	Ensure biochemistry and CK results recorded n CRF
	Acute urinary retention	
	Renal replacement therapy	
Thromboembolic complications	Lower limb DVT	Record AE with location of thrombus
	Upper limb DVT	
	Pulmonary embolism	
	Other VTE	
	Fat embolism	
Wound complications	Major graft loss	Record AE with details
	Major skin substitute loss	
	Wound infection	
	Invasive wound infection	

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

Please note that, as described in Section 9.2.6 a, b, c and d below are considered as 'disease related events' as they occur commonly in patients following thermal injury unless, in the opinion of the investigator, they are directly related to STM administration or other unique requirements of the HESTIA study.

A SAE is defined as any untoward medical occurrence that:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually

involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before

submission to GSK.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between administration of the NIMP (STM) and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to STM administration will be considered and investigated.
- The investigator will also consult the Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data collection tool.
- If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section).
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor by telephone.
- Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to

complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in SRM.

12.5. Appendix 5

The following table of study objectives specifies, for each objective, the provenance of clinical data and samples which will be used to explore that endpoint

Objective	Endpoint	Origin of Data/Samples
Co-Primary		
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry	• HESTIA study
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time	• HESTIA Study
Exploratory		
3. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry	• HESTIA Study
4. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time	• HESTIA Study
5. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of	• All clinical data will be obtained from SIFTI2. • Permeability measurements will be obtained from HESTIA

Objective	Endpoint	Origin of Data/Samples
	hemofiltration-free days • Number of episodes of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores	
6. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios	• Blood biomarkers obtained from SIFTI-2 (and HESTIA for healthy participants) • Urine for microbial metabolite analysis, claudin 3 and KIM 1 obtained from SIFTI-2 (and HESTIA for Healthy participants) • Urine albumin:creatinine and protein:creatinine ratios obtained from SIFTI-2 (and HESTIA for healthy participants) • Permeability data (STM absorption) from HESTIA
7. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples	• Stool samples collected in HESTIA protocol • Permeability data from HESTIA
8. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission	• Medical History data from HESTIA • Permeability data from HESTIA

Objective	Endpoint	Origin of Data/Samples
9. To assess wound healing	• Time to wound recovery (e.g. 95%)	• Wound healing assessment data from clinical notes will be captured in HESTIA at 14 day, 28 day and 6 month visits.
10. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation	• Microbiome data from HESTIA study • Blood Biomarker data from SIFTI-2 (thermally injured participants) and HESTIA (healthy participants.

TITLE PAGE

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability and Systemic Inflammation (HESTIA)

Protocol Number: 206243/01

Short Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability and Systemic Inflammation (HESTIA)

Compound Number: NONE

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
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UK

Medical Monitor Name and Contact Information will be provided separately.

Regulatory Agency Identifying Number(s): NA

Approval Date: 15-AUG-2017

SPONSOR SIGNATORY:

PPD

15-8-2017

Craig Tipple, MBBS, MRCP, PhD
Director, Clinical Development,
R&D Infectious Disease, GSK

Date

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 1	*15-Aug-2017*
Original Protocol	*02-Mar-2017*

Amendment 1 15-Aug-2017

Overall Rationale for the Amendment: The interim analysis has been removed since lactulose and mannitol data in healthy controls was unlikely to change the required sample size in thermally injured participants.

Section # and Name	Description of Change	Brief Rationale
10.5.3 Interim Analysis	Removed preliminary review of lactulose/mannitol ratio data.	Analysis of lactulose and mannitol data in healthy controls would be unlikely to change the study size, and will no longer be performed.
5.2 Number of Participants	Included withdrawn participants may be replaced.	To ensure the target number of evaluable population is maintained.
5.4.4 Preliminary Data Review	Modified the preliminary data review.	To describe the change in preliminary data review.
Synopsis	Updated preliminary data review.	For consistency.
6.5 Exclusion Criteria	Revision to eligibility criteria to exclude hepatitis infection for thermally injured participants. Addition the personal consultee will not be notified if a patient lacking mental capacity was found to be HIV positive.	Consistency correction. Hepatitis screening will be performed. To provide Ethic Committee reassurance and address any ambiguity of this information.
8.2.1 Other Withdraw Criteria	Added the withdraw of participants receiving hemodialysis.	Renal replacement therapy (i.e. dialysis) may filter out lactulose and mannitol.
9.2.2 Time Period and Frequency for Collecting AE and SAE Information	Added: Any SAEs assessed as related to study participation will be recorded from the time a participant consents. All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours.	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April 2017.
9.4 Clinical Safety Laboratory Assessments	Removal of if laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory.	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April 2017.
Appendix 4: Adverse Events:	Reporting SAE to GSK if the electronic system is unavailable.	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April

Section # and Name	**Description of Change**	**Brief Rationale**
Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting		2017.

TABLE OF CONTENTS

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1. SYNOPSIS

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability, and Systemic Inflammation (HESTIA)

Short Title: Investigation of Thermal Injury and Intestinal Permeability

Rationale:

Patients with severe thermal injury [>20% total body surface area (TBSA)] are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

No GSK study treatment will be employed in this study.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients.

Previous literature demonstrates that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study, therefore, is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.

4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

In order to enter this study thermally injured participants will be required to co-enrol in this study and an allied study entitled: A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injury (SIFTI-2) (reference number IRAS ID: 200366). Clinical data, standard of care laboratory data and investigational biomarker data will be shared from the SIFTI-2 study to this study and the combined data from both studies will be used to address exploratory endpoints. Participants of the SIFTI-2 study will be appropriately consented for this data sharing.

Objectives and Endpoints:

Objective	Endpoint
Co-Primary	
• To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
• To characterise the effect of thermal injury on small intestinal permeability over time	• Changes in L/M ratio over time

Overall Design:

A prospective, longitudinal study will be conducted in adult (≥18 years old) men and women admitted to a hospital following thermal injury. Measurements of intestinal permeability, inflammation and microbial translocation will be taken over a six month period. A cohort of healthy participants will also be recruited in order to determine the reference against which post-burn permeability measurements and other biomarkers will be compared.

The lactulose-to-mannitol ratio is traditionally used to assess small intestinal permeability and sucralose to assess colonic permeability. Lactulose, mannitol and sucralose [henceforth referred to as sugar test material (STM)] will be intermittently administered enterally for the purpose of intestinal permeability measurement to examine permeability at different points along the GI tract and is described in Section 7. It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

An internal preliminary data review will be conducted. This review is described in Section 5.4.4.

Number of Participants:

Table 1 in Section 5.2 described the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Treatment Groups and Duration:

Group 1: Healthy Participants

The total duration of this study for healthy participants will be approximately two weeks, in addition to the screening window:

- Screening: up to 28 days before Day 1
- Day 1: study start and assessments will be performed
- Day 8 and Day 15

Group 2: Thermally injured participants

Thermal injury participants will be asked to participate for a total of 6 months (plus or minus 14 days).

- There will be no screening period. Thermal injury participants will be recruited within 24 hours of their admission to the burns unit at the study site.
- Intense monitoring phase: Assessments will be performed on alternate days for the first 14 days following study enrolment. If the participant is discharged prior to 14 days, the intense monitoring phase will end, but the participant will remain enrolled in the study.
- Convalescent monitoring phase: Assessments at 28 days and 6 months will be made on an outpatient basis if the participant has already been discharged from hospital.

Exception to monitoring periods:

- If a discharged participant attends the centre for routine clinical care on any of days 8-14, then study assessments will be made and samples will be taken

2. SCHEDULE OF ACTIVITIES (SOA)

2.1. Healthy Participants (Group 1)

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Review of inclusion/ exclusion criteria and informed consent	X				
Demography	X				
Medical history (includes substance and alcohol usage at screening) and Medication history	X				
Substance testing (urine)	X				Substances: [Recreational Drugs and Alcohol]
HIV, Hepatitis B and C screening	X				If test otherwise performed within 3 months prior to study entry, testing is not required
Laboratory assessments	X				
Pregnancy test (WOCBP only) (urine)	X	(X)			Only performed again on Day 1 if patient at risk of pregnancy at or since screening

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Blood sampling for biomarkers		X			20ml of blood will be sampled in a single draw on one day, preferably Day 1
Stool sample collection		X			Participants will be given a collection container at screening to bring with them on Day 1.
Brief physical examination including measurements of height and weight Vital Signs (systolic and diastolic blood pressure and heart rate)	X	X	X	X	BMI calculated from height and weight at screening only Examinations should be conducted the day after intestinal permeability measurement.
Medical review (assessment of health status)		X	X	X	Monitor for signs and symptoms of gastro-intestinal infections and other emergent issues
STM Training	X				Refresher training may be provided as needed
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection		X	X	X	Please refer to SRM for full information Intestinal permeability measurement to be performed at home the day before the study visit.

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
AE/SAE and Concomitant medication reviews	(X)	←==========		==========→	Day 1 will include concomitant medication review only

2.2. Thermal Injury Participants (Group 2)

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)													6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)	
Review of inclusion/ exclusion criteria and informed consent	X															Participants will be co-consented to the SIFTI-2 study
Medical history (includes substance and alcohol usage) and Medication history	X															
Substance testing (urine)	X															Substances: [Recreational Drugs, Alcohol]
Pregnancy test (WOCBP only) (urine)	X														X	To be repeated at 6 months prior to final STM administration

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)														6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
HIV, Hepatitis B and C screening	X																If test otherwise performed within 3 months prior to study entry, testing is not required
Initial assessment of Burns	X																Calculation of %TBSA Location of thermal injury and depth
Fluid balance (total input/output)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X*		Daily (over 24 hr) *only if participant still admitted
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection	X		X		X		X		X		X		X		X	X	Measurements every 48 hours from first measure. Preference: D1, 3, 5, 7, 9, 11, 13. Otherwise: D2, 4, 6, 8, 10, 12, 14. Please refer to SRM for full method.
Brief Physical Examination															X	X	Following final intestinal permeability measurement. Can be omitted if patient is still admitted to hospital.

Procedure	**D1 (≤ 24 hours of admission)**	**Treatment Period [ICU Days] (+/4 hours)**														**6 months (± 14 days)**	**Notes**
		D2	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**D28 (± 3 days)**		
Stool sample collection	←==========→ (A sample of the first stool produced following admission)													X	X	X	Time to first stool collection; preferably on Day 1. Then on Days 14 and 28 and 6 months
Wound Healing Assessments														X	X	X	This is to assess time to 95% wound healing
AE/SAE and Concomitant medication reviews	X	←==========→														X	AE/SAE monitoring will begin after the first administration of STM

- The timing and number of planned study assessments may be altered during the course of the study based on newly available data or factors outside of the study such as priority medical care to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol deviation or require a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

3. INTRODUCTION

3.1. Study Rationale

The purpose of this study is to describe the kinetics and magnitude of increases in intestinal permeability which are observed as a result of thermal injury. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

3.2. Background

3.2.1. Thermal Injury, Intestinal Permeability and Multi-Organ Dysfunction Syndrome

Patients with severe thermal injury >20% TBSA are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients (Figure 1).

Figure 1 Changes in Intestinal Permeability

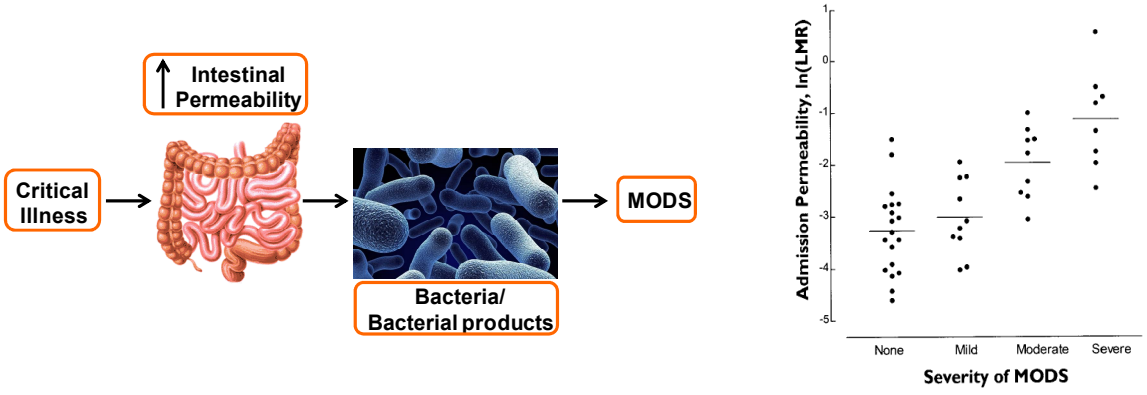


Figure 1 (Left panel) Hypothesis: The gut is the major driver of MODS in critical illness (Right panel) Correlation between intestinal permeability as measured by lactulose/mannitol ratio and the severity of MODS in critically ill patients. Intestinal permeability was determined by measuring the differential absorption of lactulose (increased in the damaged gut) and mannitol (freely absorbed in the normal and damaged

gut) following oral administration and expressed as the ratio of lactulose to mannitol (L/M) [Olquin; 2005].

There are some data showing that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.
4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

3.2.2. Intestinal Barrier Function and Its Measurement

The intestinal barrier combines a physical and immunological barrier. Epithelial cells are connected by tight junctions and prevent the passage of bacteria, toxins and antigens into the systemic circulation [Bjarnason, 1995]. Paneth cells, located in crypts of the small intestine, produce anti-microbial substances (e.g. lysozyme and defensins) and other immune cells patrol the lamina propria [Ayabe, 2000]. Barrier function can be disturbed by diseases such as inflammatory bowel disease; by drugs such as aspirin and alcohol; by ischaemia and has been observed following a number of acute injuries such as burns, trauma and radiation injury [Bjarnason, 1995; Derikx, 2006]. This disturbance results in the translocation of the intestinal flora (pathogenic or commensal) to the systemic circulation. Microbes are accompanied by proteins which normally form part of the tight intracellular junctions of the intestinal epithelium, such as claudins, and other enterocyte-derived proteins [Grootjans, 2010]. In this study, intestinal permeability will be measured directly using oligosaccharide absorption and indirectly by looking for micro-organisms and soluble markers of intestinal barrier dysfunction in the systemic circulation.

Since the 1970s, oligosaccharides have been used as test probes to measure the function of the intestinal barrier [Menzies, 1972]. Lactulose, a large polysaccharide, does not normally cross the intestinal barrier, but following damage can cross the epithelium and enter the systemic circulation. It is not metabolised, so is filtered in the kidney and excreted in the urine. The fractional excretion (amount administered / amount recovered in urine) of lactulose is therefore a measure of intestinal permeability. The amount of lactulose entering the urine is dependent on a number of factors including renal function,

gastric emptying, and degradation in the large bowel by commensal bacteria; thus, a monosaccharide such as mannitol, which passes freely across the healthy intestinal barrier, is often co-administered to 'normalise' lactulose measurements. Following administration of both lactulose and mannitol the fractional excretion of the sugars is expressed as a ratio where mannitol is the denominator. Lactulose and mannitol absorption occurs mainly in the proximal small intestine and is complete within approximately 5 hours of oral administration. This approach has been previously used successfully in patients with severe burn injury and intestinal permeability was found to correlate with episodes of sepsis [Doig, 1998].

In order to assess the permeability of the large bowel a third oligosaccharide, sucralose, will also form part of the sugar absorption test. This synthetic sweetener (marketed by Tate and Lyle as 'Splenda') is not subject to the same degradation by large bowel commensal flora as lactulose and is therefore a better measure of large bowel permeability than lactulose. Again, this has formed part of previously described studies aiming to measure intestinal permeability [Del Valle-Pinero, 2013].

Lactulose, mannitol and sucralose will be co-administered to both healthy participants and participants following thermal injury. In order to document accurately the time course of change in permeability, thermally injured participants will be asked to undergo the test on alternate days for 14 days (the intense monitoring phase) followed by two convalescent samples at day 28 and month 6 (thermal injury participants). In order to produce an accurate baseline measurement, healthy participants will be asked to undergo three measurements of intestinal permeability over approximately a two week period.

3.2.3. The Intestinal Microbiome and Thermal Injury

During the intense and convalescent monitoring phases of this study, samples of stool will be collected. These samples will undergo gene sequence analysis in order to determine the composition of the intestinal microbiota. These results will be compared with culture results from peripheral whole blood samples. The hypothesis is that raised intestinal permeability will correlate with an increased frequency of bacteraemia and that the particular bacteria detected in blood will correlate with the composition of the intestinal microbiota.

Additionally, it has been demonstrated that thermal injury alters the composition of the intestinal microbiome [Hammer, 2015]. The ultimate aim is to be able to block changes in intestinal permeability which might affect this change in composition and are therefore interested, in the current study, to assess the impact of thermal injury on the microbiome.

3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Non-investigational Medicinal Product Use		
Oral administration of sucralose in solution is unlikely to be palatable.	The dose of sucralose to be administered is 2g per test. A tolerable sweetness score (e.g. 11.3 (diet Pepsi)) would require the sucralose to be administered in 4 litres of water.	Sucralose is to be administered in capsules when given by mouth. If being administered by nasogastric or nasojejunal tube the capsules are to be emptied into the lactulose and mannitol solution
Depending on the volume of administration, the final STM solution may be hyper-osmolar. Administration directly into the jejunum via a nasojejunal tube may then result in osmotic movement of water into the intestine causing distention and discomfort.	This is based on the physiological principle that the stomach normally regulates the osmolality of its contents passing into the small intestine. Administration via nasojejunal tube (but not nasogastric tube) bypasses this process.	The lactulose, mannitol and sucralose will be delivered nasojejunally in 50ml of water and followed by a 50ml water flush. This makes the solution iso-osmolar (300 mosmol/Kg in situ). following nasojejunal administration
Lactulose and mannitol can produce an osmotic laxative effect following enteral administration.	Both lactulose and mannitol are used clinically as osmotic laxatives. The typical dose of lactulose (for the treatment of constipation) would be approximately 21g daily.	The amount of lactulose being used in this study is 5g, 75% below the standard laxative dose. The dose of mannitol is equally low compared to that contained in laxatives.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
STM administration for the measurement of intestinal permeability requires a hold in feeding which may be longer than a standard feeding hold.	Intestinal permeability assessment requires the administration of STM (see Section 7) and the collection of urine samples at defined time points (see the SRM). This procedure has previously been used in patients with severe thermal injury [Doig, 1998] but has not been studied extensively; participant fasting is required pre and post administration of these sugars; and repeated administration as per the SoA tables is also unexplored.	Where possible, tube feeding targets will be volume and not time-based to reduce the amount of feed missed on test days. Moreover, fasts for the tests will, where possible, be aligned with clinically indicated feeding holds (such as fasts required before theatre, or scheduled overnight feed holds).
Other		
The degree of injury sustained by some participants may be severe.	Participants with severe thermal injury experience significant morbidity and high levels of mortality. Therefore it is anticipated that these participants will be subject to multiple medical complications which may impact the study assessments and period (See SoA tables)	Ensure that routine care in the burns unit is not compromised by study participation. Prompt reporting of any adverse events which are related to study procedures and may affect study safety.
Omission of lactulose and sennosides which are frequently administered as a part of routine burn management.	Lactulose is a part of the STM and its administration for clinical reasons would complicate intestinal permeability measurement significantly. Equally, the mechanism of actions of sennosides is to cause irritation of the GI tract	Polyethylene glycol (e.g. Movicol) will be used as an alternative osmotic laxative. If senna is required for clinical purposes, then its use must be documented in the CRF.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	and may result in increased permeability.	
The STM will be prepared for administration on the assessment day and will not be sterile.	There is a risk that the sugar test material formulation may be contaminated with yeast or bacteria. The presence of contamination within the STM formulation delivered via the feeding tube in thermal injury participants could present an infection risk.	Microbiology release testing will be conducted by Tayside pharmaceuticals. The STM will be prepared on the assessment day to minimise this risk. Lactulose/Mannitol will be refrigerated from the point of manufacture and until use. The site investigators have been consulted on this risk and felt that it was low.
Incomplete 24 hour urine collections	Ambulant, uncatheterised patients and healthy participants 24-hour urine collections could be incomplete.	Ambulant, uncatheterised patients and healthy participants will receive careful education and written instructions of the importance of complete urine collections. In addition, documentation of incomplete collection, in addition to sampling collection times, will be recorded.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Phlebotomy for biomarkers (healthy participants)	Phlebotomy can be painful and result in bruising, bleeding and puncture site infection.	Phlebotomy from thermally injured participants will not be conducted as a part of this protocol. Healthy participants will undergo one blood draw during screening and a further draw on day one. Phlebotomy will be performed by an appropriately trained member of the site study team with aseptic non-touch technique to avoid infection.

3.3.2. Benefit Assessment

Study participants will not benefit directly from involvement in this study. However, the results of this study may contribute significantly to our understanding of changes in intestinal permeability and their relationship to morbidity and mortality in the context of thermal injury. This knowledge is paramount to designing future medicinal interventional studies, aiming to modulate intestinal permeability and, potentially, to improve outcomes for patients following thermal injury.

3.3.3. Overall Benefit:Risk Conclusion

The primary outcome measure of this study is the determination of intestinal permeability in healthy and thermal injury participants. Interventions in this study are the administration of STM by mouth or feeding tube (if one is site for routine clinical care) and the collection of urine and stool samples.

The risk of adverse events is minimised for the population being investigated in the proposed study as no drug intervention will be investigated and study assessments being conducted are non-invasive (with the exception of STM administration and phlebotomy in healthy participants).

4. OBJECTIVES AND ENDPOINTS

Objective	Endpoint
Co-Primary	
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time
Exploratory	
1. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry
2. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time

Objective	Endpoint
3. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of hemofiltration-free days • Number of episodes of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores
4. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios
5. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples
6. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission
7. To assess wound healing	• Time to wound recovery (e.g. 95%)
8. To characterise parameters that may influence drug PK/PD†	• Determine fluid input/output balance over time • Changes in serum albumin and plasma creatinine
9. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation

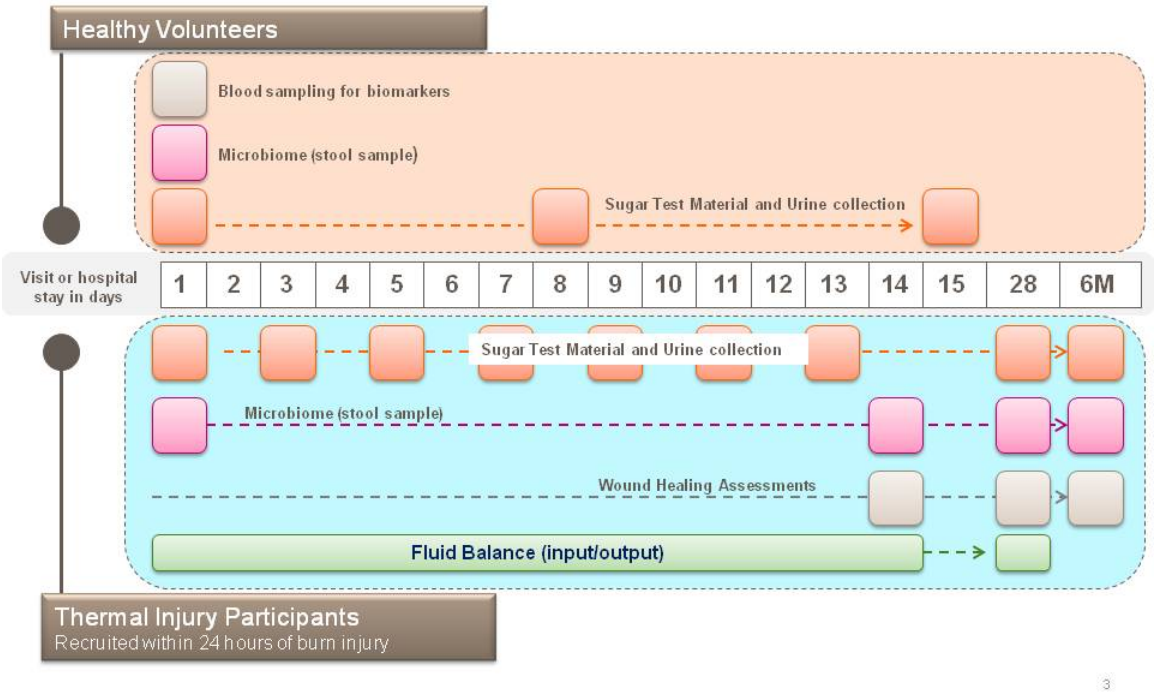
†Clinical data, routine laboratory results or blood/urine biomarker results obtained from the SIFTI-2 study will be used in the analysis of this exploratory endpoint see Section 5.4.1.

5. STUDY DESIGN

5.1. Overall Design

This is a longitudinal, prospective study of healthy participants and participants who have sustained a thermal injury. The following schematic summarises study measures and their frequency for healthy and thermal injury participants.

Figure 2 Study Schematic



5.2. Number of Participants

Table 1 describes the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Table 1 Recruitment Stratification

	Number of participants
Group 1 **Healthy participants**	15
Group 2 **Thermal Injury participants** **Percent Total Burn Surface Area (TBSA)** **≥15%**	25

The healthy participants (Group 1) will be recruited with an age range similar to that typical in thermal injury participants based on historic hospital admission data from the UK and data from the SIFTI1 study [Hampson, 2016].

If participants prematurely discontinue or are withdrawn from the study, additional replacement participants may be recruited at the discretion of the Sponsor in consultation with the investigator see Section 8.2.1.

5.3. Participant and Study Completion

The full duration of the study for healthy participants is approximately two weeks and for thermal injury participants is approximately 6 months.

Thermal injury participants who withdraw prior to week 4 or healthy participants who withdraw prior to week 2 will be considered for analysis, although it is acknowledged that any missing data at later stages of the study may be related to outcome (either positive or negative). Given this is an exploratory study, the impact of missing data will be explored by assessing the sensitivity of results to different missing data approaches (for example, analyse all available data, analyse only complete data across time points and explore imputation of worst or best case scenarios).

Study withdrawals may also include participants who are consented to the study under Section 30 of the Mental Capacity Act 2005. In the event that participants do not re-confirm consent when they regain capacity, they will be withdrawn from the study.

The end of the study is defined as the date of the last visit of the last participant in the study.

5.4. Scientific Rationale for Study Design

5.4.1. Co-recruitment to the SIFTI-2 Study

Thermally injured participants who are eligible for this study must also be eligible for, and enrolled in a partner study named SIFTI-2.

SIFTI-2 is an observational study currently recruiting healthy participants and thermally injured participants and follows a successfully delivered predecessor study SIFTI1 [Hampson, 2016]. The objectives and endpoints of SIFTI-2 are included in the SIFTI-2 study protocol (reference number IRAS ID: 200366). The design of this study has been aligned with the SIFTI-2 study to support the strategy of co-consenting thermally injured participants to both studies. This will reduce the overall impact of research in this population in the following ways:

- There is sufficient residual blood from collection in SIFTI-2 to allow testing of blood biomarkers of interest for the HESTIA study. This strategy therefore limits impact on participants as no additional blood sampling is required for participation in HESTIA (with the exception of HIV, Hepatitis B and C testing at baseline). SIFTI-2 participants will be explicitly consented for their samples and data to be shared in this way.
- Sampling time points and study visits in SIFTI-2 and HESTIA have been aligned to reduce the impact of co-recruitment on thermally injured participants.

Clinical data from both studies can be generated from the same participant therefore allowing biomarker, microflora and intestinal permeability data to be compared. In contrast to thermally injured participants, healthy participants enrolling in this study will not be required to co-consent for participation in SIFTI-2.

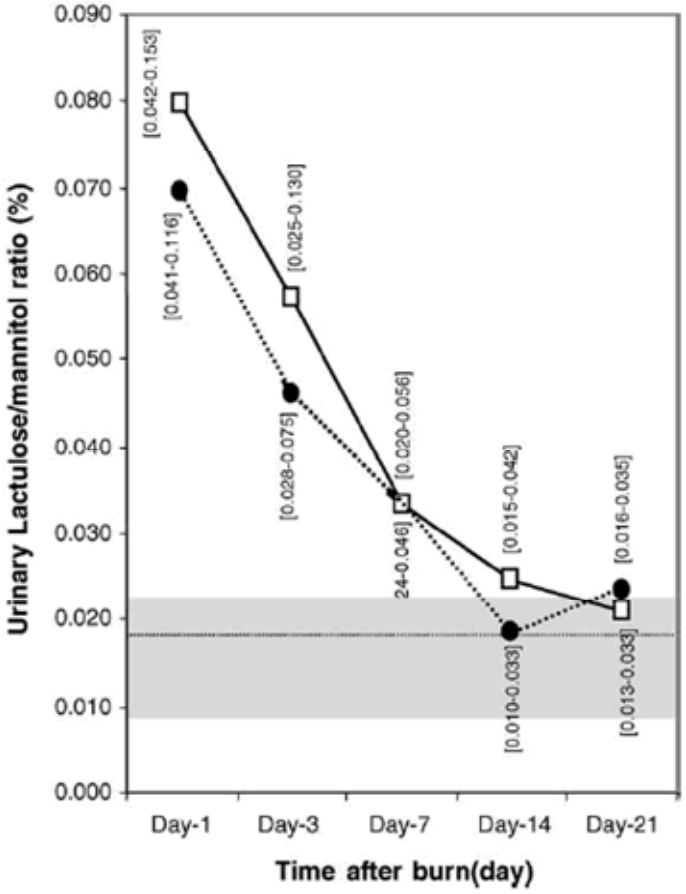
Data will be shared from the SIFTI-2 study with GSK through a secure electronic database.

A summary of the origin (HESTIA or SIFTI-2) of data and samples collected for the HESTIA study is available in Appendix 5 Section 12.5.

5.4.2. Recruitment and Sampling Schedule

Severely burned patients (with an injury affecting greater than 20% total body surface area), display a significant and rapid increase in intestinal permeability that has been shown to decline over time (Figure 3) [Olquin, 2005]. What is less well understood is whether a greater severity of thermal injury correlates with greater intestinal permeability. Moreover, the time to complete restoration of normal permeability and other factors which may influence permeability (other than the initial injury) are also not well understood.

Figure 3 Burn Injury Results in a Rapid Increase in Intestinal Permeability



This study aims to recruit participants as soon as possible following their admission in order to capture the initial changes in permeability. Serial measurement of intestinal permeability and sampling of the biomarkers of bacterial translocation, intestinal damage and inflammation are required during the acute phase (days 1-14) of admission in order to correlate them with clinical events (e.g. surgery), severity scores and clinical outcomes.

The convalescent time points (28 days and 6 months) are required to determine if intestinal permeability has returned to normal and to correlate observed changes on days 1-14 with longer-term clinical outcomes (e.g. wound healing).

Gut microbiome assessments will be made less frequently than intestinal permeability assessments as changes in the microbiome are predicted to evolve more slowly. Ideally a stool sample will be collected from thermal injury participants at study entry (limited, of course, by when participants first pass stool following admission). A convalescent sample is requested to assess if the gut microbiome is able to restore to a more normal composition (and will be compared with that of healthy participants to make that assessment).

Blood samples (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline) will not be taken from thermally injured participants during this study. Instead, biomarker data from blood samples taken during the SIFTI-2 study will be used. A single blood draw will be required from healthy participants on day 1 of participation.

5.4.3. Inclusion of Healthy Participants

Patient facing material (i.e. poster) will be used to facilitate recruitment of the healthy participants. Healthy participants will be recruited to this study to provide a baseline for endpoint measures on intestinal permeability and the gut microbiome.

Three measurements of intestinal permeability are required in order to define an average given the variability in healthy participants reported previously. The timing of the replicates follows the intense time course of the study to control for day-to-day variation over a 15 day period.

5.4.4. Preliminary Data Review

A safety review based on raw data will be conducted when approximately 10 participants have completed the study. This review would include data from any participant, either healthy or thermally injured, that has been collected at the time the review is conducted.

5.4.5. The Use of the Sugar Test Materials (Lactulose, Mannitol and Sucralose)

As described in Section 3.2.2, lactulose, mannitol and sucralose will be administered to both thermally injured participants and healthy participants to measure permeability of the small and large intestine. The amount of each of the sugars to be used is based on previous successful clinical studies employing this method and balances having enough sugar present for detection in urine with their potential laxative effect (Del Valle-Pinero, 2013; Doig, 1998; Menzies, 1972).

6. STUDY POPULATION

The study population will comprise healthy and thermal injury participants presenting at enrolling study sites. Please note the following:

- Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.
- Where possible, written informed consent will be obtained from each subject prior to participation in this study. Recruitment of subjects who lack mental capacity is discussed in Section 6.1.
- Healthy participants will be consented to the HESTIA study only.
- Thermally injured participants are required to be co-consented to the SIFTI-2 and HESTIA studies outlined in Section 6.1. A diagrammatic overview of the SIFTI-2 and HESTIA thermal injury participant recruitment is given in Figure 4. (see Appendix 3).

6.1. Recruiting Participants with Differing Mental Capacity

Following evaluation of capacity (as outlined in the Mental Capacity Act (MCA) 2005), thermally injured participants enrolled in this study will fall into the following groups:

6.1.1. Adult Participants Determined to Have Mental Capacity at Study Entry and Throughout the HESTIA Study

Those participants who present with capacity and meet study entry criteria will be provided with a Participant Information Leaflet (PIL) outlining the study. If the participant agrees to consent to the HESTIA study following a discussion with the research team, they will be asked to sign a consent form.

Given the short (24 hour) window for recruitment, those patients who are acutely unwell will initially be presented with an abbreviated PIL. Once stable, this will be followed by the standard information leaflet and re-confirmation of consent.

6.1.2. Adult Participants Lacking Mental Capacity for the Duration of the HESTIA Study

It is anticipated that some subjects who meet eligibility criteria for this study will not be able to give informed consent due to their medical condition or its management (e.g. sedation, opioid analgesia, intubation). In such cases, participants may be enrolled in the study in accordance with Section 30- Section 34 of the MCA 2005. The decision to enrol the participant will be discussed with a legally acceptable representative (LAR) (also termed a 'consultee'). This decision may or may not be witnessed by an independent witness according to the decision of the principal investigator.

6.1.3. Adult Participants Lacking Mental Capacity (either at Study Entry or During the Study) Who Later Regain Capacity and Are Required to Provide Informed Consent

As soon as is practically possible following a participant regaining capacity, participants will be asked to provide informed consent to remain in the study. If they decline, then they will be withdrawn from the study as soon as it is safe to do so (likely immediately given the design of this study). Samples and data collected prior to study withdrawal may be retained. The participant will be asked about this at the point of study withdrawal.

6.1.4. Adult Participants with Mental Capacity to Provide Consent at Study Entry Who are Later Deemed No Longer to have Mental Capacity

The decision for the participant to remain in the study will be discussed with a LAR and recorded. If the participant subsequently regains capacity again, they will be asked to re-consent to study participation.

When considering enrolment of participants who lack the mental capacity to consent, the following should be noted:

- Section 3.2.1 of the SIFTI-2 protocol describes the consent process for that study in detail and should be read in conjunction with this protocol. Please note that the SIFTI 2 protocol refers to a LAR as the Patient's Personal Consultee (PC) or Nominated Consultee (NC).
- A Study Information Leaflet will be provided to the LAR outlining the HESTIA trial before being asked to sign a form supporting the participant's enrolment in the study.
- The investigator and/or the site's IEC/IRB have responsibility for acting in accordance with the MCA 2005 in the matter of assessing who has the capacity to consent and who qualifies as a LAR of a potential subject. The investigator will also decide if an independent witness is required.
- Further information regarding the assessment of mental capacity and the appointing of LARs/PCs/NCs is given in Appendix 3 (Section 12.3.2 and Section 12.3.3).
- If a patient loses mental capacity subsequent to their consent and enrolment to the HESTIA study, samples and data collected prior to loss of capacity will be retained even if approval of continued study participation by a LAR is declined.

6.2. Inclusion Criteria for Healthy Participants (Group 1)

1. Males or Females must be ≥18 years of age at the time of signing informed consent.
2. Participants who are healthy as determined by the investigator following medical evaluation including medical history, physical examination, and laboratory tests (these are listed in Appendix 2).
3. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at screening or Day 1 as needed) and not breastfeeding.
4. Capable of giving signed informed consent as described in Appendix 3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.3. Inclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are eligible to be included in the study only if all of the following criteria apply:

6.3.1. Age

1. Participant must be ≥18 years of age.

6.3.2. Type of Participant and Disease Characteristics

2. Participants who have sustained a burn (thermal injury) with a TBSA ≥ 15%.

6.3.3. Other Inclusions

3. Admission to the burn centre (study site) ≤*24* hours of injury.
4. Able to take enteral fluids either orally or via a nasogastric tube (depends on facial burn damage).

6.3.4. Gender

5. Male and female.

   a. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at study entry) and not breastfeeding.

6.4. Exclusion Criteria for Healthy Participants (Group 1)

1. Healthy participants are excluded from this study if they are receiving anti-coagulation therapy.
2. Pregnancy or breastfeeding.
3. A body mass index >34kg/m^2
4. An active history of alcohol dependency
5. History of sensitivity to any of the STM, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator and/or GSK Medical Monitor, contraindicates their participation.
6. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody and confirmatory Hepatitis C PCR result within 3 months of screening.
7. A positive pre-study urine drug/alcohol screen.
8. A positive test for HIV antibody.
9. Participants unable to swallow large capsules (the capsules will be shown to participants at screening).
10. Galactosaemia or severe lactose intolerance.
11. Use of an antibiotic 2 weeks prior to study start (i.e. administration of the STM).
12. Gastroenteritis in the 2 weeks prior to study start (i.e. administration of the STM).

6.5. Exclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Chemical or electrical burn.
2. Multiple traumatic injuries with an Injury Severity Score (ISS) ≥16 (note: excludes burn in score system).

Prior/Concomitant Therapy

3. Patient received substantial undocumented management prior to arrival at the study site (burn centre) e.g. from paramedics or in a local accident and emergency department.
4. Systemic corticoidsteroid use.
5. Intravenous (IV) mannitol use.

Prior/Concurrent Conditions

6. Human immunodeficiency virus (HIV) infection.
7. Viral Hepatitis B or C infection.
8. Gastrointestinal disease (e.g. inflammatory bowel disease) which may affect intestinal permeability.
9. Previous bowel resection (e.g. hemicolectomy, small bowel resection)
10. Galatosaemia or severe lactose intolerance.
11. Bowel obstruction.
12. Renal dysfunction requiring renal replacement therapy (i.e. end-stage renal failure prior to thermal injury).
13. Active autoimmune disease and receiving immunomodulatory therapy e.g. rheumatoid arthritis anti-TNF.
14. Active chemotherapy for cancers or immunoremittive therapies (prednisolone, adalimumab) within 60 days of thermal injury.
15. Premorbid conditions of malignancy currently under treatment.
16. Previous bilateral lower extremity amputation.

NOTE: Due to the rapid recruitment period (within 24 hours of admission) for thermally injured participants, HIV and viral Hepatitis test results may not be available at the time of enrolment. In the event these tests return positive, the participant will be informed of the result(s) and withdrawn from the study (see Section 8.2.1). In the event that the participant has been recruited who lacked mental capacity to consent, the reason for their withdrawal (the positive HIV and/or viral Hepatitis test results) must not be shared with a personal consultee. According to UK law and the guidance of the British HIV association, if the participant's physician believes there is an overriding public interest to disclose the participant's results to a personal consultee who is a current or former sexual partner, then the result(s) may be disclosed without the participant's consent. This must be as a last resort. If the participant regains capacity, he/she will be informed of the positive test result(s).

Diagnostic assessments

17. Decision not to treat the patient due to futility.

6.6. Lifestyle Restrictions

6.6.1. Meals and Dietary Restrictions

- Participants will be fasted (or feed stopped) for **3 hours** prior to STM administration and for **3-5 hours** afterwards. For thermally injured participants these fasts should be aligned with those required for routine clinical care (feed holds, before surgical interventions) where possible.
- Refrain from consumption of the following for **24 hours** before and after the administration of STM:
    - Foods/drinks/medicines and other products which contain sucralose, lactulose or mannitol as artificial sweeteners.

N.B. If cannot be avoided, then clear documentation of its administration is required and the current test to stop. If urine samples have been collected PRIOR to administration of the drug, then these can still be sent for analysis

6.6.2. Alcohol/Exercise/Aspirin (Healthy Participants only)

Alcohol, aspirin and vigorous exercise [Sequeira, 2014] are all known to cause transient increases in intestinal permeability. Healthy participants will therefore be requested to avoid alcohol, aspirin and physical exercise for **48 hours** before taking the STM and for the 24-hour urine collection period.

6.7. Screen Failures

There will be no screening period for thermal injury participants. Screening will be up to 28 days before Day 1 for healthy participants.

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

7. NON-INVESTIGATIONAL MEDICINAL PRODUCT (NIMP)

A study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. According to this definition, no GSK study treatment will be employed in this study.

The STM (comprising lactulose, mannitol and sucralose) will be intermittently administered enterally as a study challenge agent to measure permeability at different points along the GI tract. Lactulose and Mannitol assess small intestine permeability and sucralose to assess colonic permeability.

It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

7.1. STM Administered

Study Treatment Name	Lactulose (4-*o*-β-D-galactopyranosyl-D-fructofuranose)	Mannitol (D-mannitol) GRAS listed	Sucralose (1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside)
Dosage formulation	oral solution	oral solution	Capsules (powder)
Unit dose strength(s) Adults	5g	2g	2g (3 capsules to deliver total 2g sucralose)
Route of Administration	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal (capsules to be opened and contents added to lactulose and mannitol for tube administration)
Preparation and Dosing instructions	For oral administration, the lactulose and mannitol will be prepared as a 100ml drink to be taken with 3 sucralose capsules. For feeding tube administration, lactulose/mannitol/sucralose will be prepared as a 50ml solution and given via a feeding tube followed by an immediate 50ml drinking water flush Preparation refer to Study Reference Manual together with SoA tables (Section 2)		
Packaging and Labelling	Lactulose and Mannitol will be supplied pre-mixed in an amber bottle (or equivalent) for single use. Each container will be labelled as required per country requirement.		Sucralose will be provided as capsules in a storage container. Each container will be labelled as required per country requirement.
Manufacturer	Tayside Pharmaceuticals, UK		
Storage	Lactulose/Mannitol formulation should be stored under refrigerated conditions. The sucralose capsules should be stored at room temperature in a dry environment away from direct sunlight.		
Shelf-life	Lactulose/Mannitol pre-mix formulation and sucralose capsules supplied by Tayside Pharmaceuticals will have at least 3 month shelf-life when stored at the correct storage conditions.		

The preparation of the STM for oral use and nasogastric/nasojejunal tube administration can be found in the Study Reference Manual.

7.2. Dose Modification

Dose modification will not be required. Unit dose is described in Section 7.1.

7.3. Method of STM Administration: Treatment Assignment

There is no element of randomisation in the study and all study participants will receive the STM according to the relevant SoA. The method of administration can be found in the Study Reference Manual.

7.4. Blinding

No GSK study treatment will be employed in this study. All participants will receive the same STM and all thermal injury participants will perform the same study procedures.

7.5. Preparation/Handling/Storage/Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all STM received and any discrepancies are reported and resolved before use of the STM.
2. Only participants enrolled in the study may receive STM and only authorized site staff may supply or administer STM unless adequate training is provided such as in the case of healthy participants. All STM must be stored in a secure, temperature controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for STM accountability, reconciliation, and record maintenance, as needed.
4. Further guidance and information for the final disposition of unused STM are provided in the Study Reference Manual.
5. Under normal conditions of handling and administration, STM is not expected to pose significant safety risks to site staff.

7.6. STM Compliance

- When participants undergo intestinal permeability testing at the site, they will receive STM directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of STM and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the STM.
- If healthy participants need to prepare and administer the STM off-site such as at home, STM training will be provided and a record maintained by the investigator or designee.

7.7. Concomitant Therapy

- Refrain from consumption of the following for **24 hours** before and after the administration of STM:
  - Lactulose or mannitol-containing laxatives. Study sites will be asked to use movicol (polyethylene glycol) in place of lactulose.
  - Medicines with mannitol as an excipient (chlorthiazide sodium, some albumin preparations, some laxatives, tablets as a bulking agent).
  - Products containing sucralose.
- For healthy participants only, refrain from consumption of aspirin for **48 hours** before taking the STM and for the 24-hour urine collection period see Section 6.6.2.
- For healthy participants only, antibiotic use 2 weeks prior to STM administration and during the study is not permitted.
- Sennoside laxatives should be avoided. These can cause gastrointestinal irritation and may contribute to raised intestinal permeability.
- Additional Glutamine supplementation in excess of that delivered with a standard feeding protocol should be avoided during the first 28 days of study participation. If supplementation is given inadvertently, then the patient will remain in the study, but the total dose and duration of additional glutamine supplementation must be recorded in the CRF.
- Thermal injury participants that receive Intravenous (IV) mannitol for renal failure or raised intracranial pressure (testing to be delayed until 12 hours after last administration).

7.8. Treatment after the End of the Study

There will be no ongoing STM administration following the end of this study.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of STM administration

Discontinuation of STM administration can be considered by the investigator in the event that an adverse event to the STM is observed. Withdrawal of further STM administration does not require withdrawal from the study.

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

8.2.1. Other Withdrawal Criteria

- A participant will be withdrawn from the study following positive (and confirmed) HIV test at screening; Serologic evidence of active Hepatitis B (HB) infection based on the results of testing for HBsAg, and anti-HBc or a positive test for Hepatitis C antibody confirmed by Hepatitis C RNA or antigen testing. If HCV RNA is not available, then the positive test for Hepatitis C antibody alone would be exclusionary. Results must be discussed with the medical monitor to withdraw the participant from the study and commence therapy according to local practice.
- Healthy participants that are treated with antibiotics during the duration of the study.
- Participants that experience signs and symptoms of gastro-intestinal infections during the duration of the study.
- Withdrawals related to mental capacity as described in Section 6 and Appendix 3.
- Participants that received haemodialysis during the first 48 hours of the study (i.e. during the first measurement of intestinal permeability) will be excluded from the evaluable population and a replacement will be recruited.
    - Participants that received haemodialysis during later time points will not be excluded, but consideration will be given to recruiting an additional participant if 3 or more intestinal permeability measurements occur concurrently with haemodialysis.

8.3. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the study site for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed.
- Safety concerns related to the STM should must be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue to be administered the STM.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the thermally injured participant's routine clinical management (e.g., weight measurement) and obtained before signing of ICF may be used for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA. Procedures (the administration of STM) are not part of routine care for either healthy or thermal injury participants.
- Healthy participants will be asked to donate a single blood sample on Day 1. No blood collection is specified in the SoA of this protocol for thermal injury participants (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline). The results of clinical laboratory blood tests will be recorded in the SIFTI-2 study and the data used in this study. Likewise, blood collection for exploratory biomarker detection will be included in the SIFTI-2 study and the data used in this study.

9.1. Efficacy Assessments

The administration of the STM is for the purpose of intestinal permeability measurement and is not therapeutic, therefore no efficacy will be assessed.

9.2. Adverse Events

9.2.1. Monitoring and reporting responsibilities

Healthy participants will be recruited to the HESTIA study alone and all AEs or SAEs occurring in this group should be managed according to this protocol.

Thermally injured participants recruited to this study will also be recruited to the SIFTI-2 study. The following guidance relates only to AEs or SAEs which the investigator reasonably believes to be the result of a procedure or requirement unique to this (the HESTIA) protocol. All other AEs or SAEs will be reported and managed in accordance with the SIFTI-2 protocol.

Unique procedures and requirements of HESTIA

1. The administration of STM
2. The collection of stool samples
3. Changes to standard of care for thermally injured participants:
    a. The fasts required during the measurement of intestinal permeability
    b. The use of alternative laxatives to lactulose and sennosides.

The definitions of an AE or SAE for this study can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious or that caused the participant to discontinue intestinal permeability measurement with the STM (see Section 8).

9.2.2. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2). However, any SAEs assessed as related to study participation (e.g., STM, protocol-mandated procedures, invasive tests, or change in existing therapy) will be recorded from the time a participant consents to participate in the study.
- All AEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2).
- Medical occurrences that begin before the start of unique HESTIA study procedures but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the STM administration or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.2.3. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. If the participants are conscious, open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence. For unconscious

patients or participants not always able to provide valid verbal responses to open-ended questions, the investigator or designee will need to identify AEs and/or SAEs through relevant clinical signs and/or investigations.

9.2.4. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to follow proactively each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Appendix 4.

9.2.5. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of the study. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information e.g., summary or listing of SAE) from the sponsor will review and then file it and will notify the IRB/IEC, if appropriate according to local requirements.

9.2.6. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

The following broad disease related events (DREs) are common in thermally injured participants and can be serious/life threatening:

- Deterioration of condition.
- Death (may be expected in burns of a large surface area).
- Prolongation of hospital stay.
- Persistent or significant disability or incapacity.

Because these events are typically associated with the disease under study, they will not be reported according to the standard process for expedited reporting of SAEs to GSK (even though the event may meet the definition of a SAE). These events will be recorded on the DRE page in the participant's CRF within [the appropriate time frame *agreed upon by the SRT* for completion of DRE CRF pages]. These DREs will be monitored by clinical study team on a routine basis.

- *NOTE: However, if the investigator considers that there is a reasonable possibility that the event was related to administration of STM or another unique or required element of the study (as defined in Section 9.2.1) then the event must be recorded and reported as an SAE (instead of a DRE).*
- A comprehensive list of further thermal injury related DREs can be found in Appendix 4 (Section 12.4).

9.3. Treatment of Overdose

For this study, an overdose is defined as any dose of STM greater than defined in Section 7.1. No specific treatment is recommended for an overdose and treatment is at the discretion of the investigator. The GSK medical monitor must be notified promptly.

9.4. Safety Assessments

Planned time points for all safety assessments are provided in the SoA and summarised here.

Safety Assessment	**When conducted**	
	Healthy Participants	**Thermally injured participants**
Laboratory tests	Screening. Only repeated if clinically indicated in the opinion of the investigator.	Only if clinically indicated.
Brief Physical Examination including Vital Signs Recording	Screening, Day 1, Day 8, Day 15	As a part of routine clinical care whilst admitted (not protocol specified). Following day 28 and 6 month intestinal permeability measurements (if patient not still admitted)
Detection of AEs	Day 1, Day 8, Day 15	Throughout the study
Assessment of health status	Screening, Day 1, Day 8, Day 15	Not required

9.4.1. Physical Examinations

- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

9.4.2. Vital Signs

- A **single** vital sign measurement will be obtained at each time point indicated in SoA Table, and will include systolic and diastolic blood pressure and heart rate. Any abnormalities and changes in measurements will be communicated to the medical monitor.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, mobile phones).
- Vital signs to be taken before blood collection for laboratory tests.
- Repeat or unscheduled measurements may be taken at the discretion of the investigator.

9.4.3. Clinical Safety Laboratory Assessment

- All study related laboratory assessments will be performed by a local laboratory. The laboratory reports must be reviewed by the investigator, this review documented and both report and review are to be filed with the source documents.
- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA.
- Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

9.5. Study Procedures

The following procedures will be carried out during the study.

9.5.1. Fluid Balance Measurement

All fluid input and output will be recorded every 24 hours for thermally injured participants.

9.5.2. Wound Healing

Assessment of wound healing will be the time to 95% wound healing. Physical parameters of the wound (e.g., rate of healing) will be recorded and collected as a part of both the HESTIA and the SIFTI-2 studies.

9.5.3. Other Clinical Responses

To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability the following will be recorded and collected as a part of the SIFTI-2 study. Details can be found in the SIFTI-2 study protocol.

- Number of ventilator-free days (ventilator start/restart/end date/time)
- Number of vasopressor-free days (medication chart review)
- Number of hemofiltration-free days (notes review)
- Number of episodes of confirmed infection and sepsis
- Number of surgical interventions
- Total length of hospital stay
- Calculate critical care and thermal injury severity scores

9.6. Pharmacokinetics

PK parameters are not evaluated in this study.

9.7. Pharmacodynamics

PD parameters are not evaluated in this study.

9.8. Intestinal Permeability Assessments

- Intestinal permeability will be determined by measuring the excretion of lactulose, mannitol and sucralose in urine following their enteral administration. It will be conducted in both healthy participants and thermally injured participants at the time points specified in the SoA.
- The complete method for administration of STM and measurement of intestinal permeability is detailed in the SRM.
- Urinary excretion of the orally ingested STM will be quantified using a technique such as capillary column gas chromatography.
- Urine samples will be collected in plastic bottles for analysis. Urine collection will begin immediately following STM administration. Urine samples will be collected over 24 hours post-STM administration. Accurate collection of the total volume voided during this 24 hour period is critical.
- **Sample Preparation**

Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

9.9. Genetics

Genetics are not evaluated in this study.

9.10. Sample Collection for Biomarker Analysis

The following biomarkers will be collected during the study. Details of sample processing, storage and shipping are included in the study reference manual.

Blood, stool and urine samples will be collected and stored. Timing of analyses and selected biomarkers will be dependent on the results of the intestinal permeability tests results.

9.10.1. Blood Biomarkers

Healthy participants

- Blood samples will be taken for healthy participants recruited in this study over the time period specified in the SOA. Blood will be taken adhering to standard operating procedure from venae puncture.
- The results of blood biomarker analysis will be evaluated in this study and compared to measures of intestinal permeability.
- Twenty (20) ml blood will be collected on Day 1 for biomarker analysis, and 20ml blood will be collected at screening for screening tests. The biomarkers to be measured may include, but are not limited to:
  - Markers of microbial translocation
  - Markers of intestinal damage
  - Inflammatory markers: e.g. C-Reactive Protein, Procalcitonin, cytokines (including TNF-a, IL-6, IL-8, IFN-g, IL-10, IL-1b, IL-12p70, IL-17, IL-4, IL13, IL1Ra, MIP1a, MIP1b, MIP2, GCSF, GMCSF, MCP-1, RANTES, HMGB1).

Thermally injured participants

- The blood required for this analysis in thermally injured participants will be collected as a part of the SIFTI-2 (IRAS 200366) study to which all thermally injured participants will be co-recruited. Details of the schedule for blood collection and the total volume of blood collected can be found in the SIFTI-2 study protocol.

9.10.2. Stool Sample Collection

- Stool samples will be collected from all participants in this study over the time period specified in Section 2, Schedule of activities (SOA). Stool samples will be collected adhering to standard operating procedure.
- For thermally injured participants, the initial sample will be taken as close to time of injury as possible (“first stool sample produced upon admission”) and

Day 14. Further samples will be taken on day 28 (± 3 days) and at month 6 (± 14 days).

- For healthy participants, a single sample will be collected at study entry (participants will be given a collection container at screening).

9.10.3. Urine Sample Collection

- Urine samples will be collected as a part of the measurement of intestinal permeability which is described in Section 9.4.
- Additional urine samples will be collected from patients as part of the SIFTI-2 study to which all thermally injured participants will be co-recruited. These will be used for, among other tests, the quantification of protein and microbial metabolites.
- It is standard practice that patients admitted with burns of TBSA ≥15% will have a urinary catheter inserted on admission to ensure the accurate maintenance of fluid balance. A clean urine sample will be taken from the appropriate port on the urinary catheter. In patients who are not catheterised, a mid-stream urine (MSU) should be collected in a clean universal container where possible.
- N.B. During the 24 hours following STM administration (during intestinal permeability measurement) urine samples must only be taken from the 5-hour of 24-hour urine collections **after** the aliquots for sugar quantification have been taken.

Sample Preparation

- Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

10. STATISTICAL CONSIDERATIONS

All statistical analyses will be performed using SAS (SAS Institute Inc., Cary, North Carolina, USA), version 9.2 or higher. Before database lock, a reporting and analysis plan (RAP) will be issued as a separate document, providing detailed methods for the analyses outlined below. Any deviations from the planned analyses will be described in a RAP addendum and justified in the final integrated clinical study report.

10.1. Hypotheses

As this is an enabling study designed to better understand the biomarkers of intestinal permeability and other biomarkers in participants with moderate to severe burns, the statistical analysis for this study will be exploratory in order to better understand the parameters to inform future investigational medicinal product studies.

The key factors of interest in this study are to understand (i) the nature of any differences at entry in intestinal permeability between healthy participants and thermal injury participants (ii) to understand the trajectory of changes in intestinal permeability over time.

The key endpoint to be explored is the lactulose:mannitol (L/M) ratio, but other permeability biomarkers will also be explored. The analysis approaches to address these questions are exploratory, but will initially be conducted as outlined in Section 10.5 and Section 10.6.

10.2. Sample Size Determination

Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (>15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Although the key aim is to estimate the variability and L/M ratio and assess the difference in L/M ratio between thermal injury and healthy participants, for illustration, a trial including 25 thermal injury and 15 healthy participants would have 89% power to detect a 3-fold difference in L/M ratio between thermal injury and healthy participants using a 2-sided significance level of $p<0.10$. This calculation uses a (log) b etween-subject SD of 1.15, as estimated from the literature [Olquin, 2005].

10.3. Data Analyses Consideration

In general, descriptive summaries will include number of subjects, mean, standard deviation, median, minimum, and maximum for continuous variables. If data are log-normally distributed data will be presented as number of subjects, geometric mean, coefficient of variation, minimum, and maximum; and percent for categorical variables. Summaries will present data by dose level and where appropriate, by assessment time.

10.4. Populations for Analyses

The **Safety Population** will consist of all subjects who receive at least 1 dose of STM and have at least on post-dose safety assessment.

The **Evaluable Population** will consist of all subjects who are entered into the study and have evaluable L/M ratio measurements.

10.5. Statistical Analyses

10.5.1. Safety Analyses

Administration of STM is for the measurement of intestinal permeability. The safety of this administration is not an endpoint of this study, but will be monitored and reported.

All safety data will be presented in data listings. Subject demographics, medical history, and prior and concomitant medications will be summarized using descriptive statistics. For continuous variables, these summaries will include number of subjects, mean, median, standard deviation, minimum, and maximum.

For categorical variables, the summaries will include frequencies and corresponding percentages. No inferential hypothesis testing will be performed on the safety variables.

Adverse events will be coded using the MedDRA classification system.

For healthy participants (who are recruited only to HESTIA), STM-emergent AEs will be defined as any AEs, regardless of relationship to STM administration, that occur after the first dose of STM until the final follow-up visit. The STM-emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. The total number of AEs will be summarized overall. The AEs will be further summarized by severity and relationship to STM. If relationship information is missing, the AE will be considered STM-related. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

For thermally-injured participants, STM-emergent AEs will be defined as any AE deemed related to STM administration that occurs after STM administration until the follow-up visit. The STM-related emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

As laboratory data and vital signs are only collected at screening for healthy participants, these data will be listed only. Clinical laboratory values that are outside of the reference ranges will be flagged and evaluated for clinical significance by the investigator. Physical examination findings will be listed. For thermally-injured participants, physical

examination findings and clinical laboratory values will be highly abnormal and as such any data collected will only be listed. Disease-related findings and changes will not be reported.

10.5.2. Other Analyses

Biomarker exploratory analyses will be described in the RAP.

10.5.3. Interim Analyses

No formal interim analysis will be performed.

10.6. Analyses of lactulose/mannitol ratio

In all analyses the variable TBSA will be a categorical variable defined as "Yes" for thermally injured participants, and "No" for healthy participants.

Differences in permeability at entry

Intestinal permeability biomarkers will be summarised by TBSA group and overall. Data will summarised by geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio) = intercept + TBSA

Trajectory of the L/M ratio over time

Intestinal permeability biomarkers will be summarised over time, by TBSA group and overall. Data will summarise geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio at time X / L/M ratio at entry) = intercept + Time + TBSA + Time*TBSA.

This will be a repeated measurement analysis and will assess the rate of improvement in L/M ratio over time, and assess how this changes relative to healthy participants. If required, further modelling assessing more complex relationships between L/M ratio and time may be undertaken. Given this is an exploratory study the most appropriate variance-covariance matrix regarding the correlation of data over time will be explored as part of the statistical analysis.

Data from this model may also be used to estimate the time to 50% improvement (or other degrees of improvement) in L/M ratio in relation to L/M ratio values seen in healthy participants. This will be used to assess the clinical relevance and sensitivity of such measures.

A model fitting log (AUC of L/M ratio) = intercept + TBSA will also be fitted. AUC will be calculated using all measurements taken over time. This will provide a summary of the weighted average L/M ratio value over time.

The use of %TBSA will also be assessed in the above analyses as a continuous covariate. The effects of age and Baux score will also be evaluated to understand differences in intestinal permeability in these groups [Osler, 2010].

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

ACR	Albumin/creatinine ratio
AE	Adverse Event
Anti-HBc	Anti-Hepatitis C
ART	Anti-retrovial treatment
CFR	Code of Federal Regulations
D	Day
G	Grams
eCRF	Electronic Case Report Form
ICU	Intensive Care Unit
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gatrointestinal
HB	Hepatitis B
HBs AG	Hepatitis B Antigen
HCV	Hepatitis C
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISS	Investigator Sponsored Study
L/M	Lactulose/mannitol ratio
LAR	Legally Authorised Representative
mL	Milliliter
MODS	Multi-organ dysfunction syndrome
NIMP	Non-investigational medicinal product
NC	Nominated Consultee
PC	Personal Consultee
PCR	Polymerase Chain Reaction
RAP	Reporting and Analysis Plan
SAE	Serious adverse event
SIFTI-2	A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injuries
SoA	Schedule of Activities
SRM	Study Reference Manual

STM	Sugar Test Material
TBSA	Total body surface area
TNF	Tumour Necrosis Factor
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
SAS

12.2. Appendix 2: Clinical Laboratory Tests

- All clinical laboratory tests will be performed in the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 2 Protocol-Required Safety Laboratory Assessments for Healthy Participants

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Hemoglobin Hematocrit		WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	Urea	Potassium		Total bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/	Total Protein
	Glucose nonfasting	Calcium	Alkaline phosphatase	
Routine Urinalysis	• pH, glucose, protein, blood, ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• Urine alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)] • Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)[1] • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :
1. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

12.3. Appendix 3: Study Governance Considerations

12.3.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- Any amendments to the SIFTI-2 protocol which impact on this protocol will be reviewed and may result in changes to this protocol being required. Any such changes will be subject to IEC/IRB approval before implementation.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.3.2. Recruiting participants under the Mental Capacity Act 2005

On admission into hospital the patient's capacity will be assessed. A patient may lack capacity due to the severity of their injury, arriving intubated and ventilated or due to a pre-existing co-morbidity.

Please note, the same process will also be followed for the SIFTI-2 study to which thermally injured participants will be co-recrruited.

If a patient does not have the capacity to make an informed decision, the research team will approach a patient's LAR, also known as a Personal Consultee. Examples of personal consultees include:

- A family member, carer or friend
- An attorney acting under a Lasting Power of Attorney
- A court appointed deputy, provided that they had a relationship with, or personal knowledge of, the person lacking capacity before their appointment as deputy.

There may be circumstances in which a personal consultee is not available, some examples of this are:

- Where no family member or friend is willing to act as a personal consultee
- Where the family member or friends live a long distance away and/or are not in frequent contact with the patient who lacks capacity
- Where the regular carers of the person who lacks capacity are doing so for payment or in a professional capacity (e.g. care home staff or nurses)
- Where someone is acting on a professional role (e.g. their GP or solicitor)

In this case, a nominated consultee will be required. A nominated consultee is considered to be a medical professional that has no connection to the research trial, but has an understanding of the implications of the research trial on the participant.

In these circumstances, examples of nominated consultees are:

- An emergency department doctor, preferably Consultant level.
- Intensive Care doctor, preferably Consultant level.
- Doctor from the burns team, not directly involved in the research study.

Once a personal or nominated consultee has been identified, they will be provided with a specific information leaflet about the trial. The personal and nominated consultee will be asked if they feel the study would be something the participant would have no objections to. If in their opinion the participant would have no objection to being recruited into a research trial the consultee will be asked to sign a declaration form.

12.3.3. Determining Whether a Participant has Capacity Under the Mental Capacity Act (2005)

Prior to deciding that a patient does not have the capacity to give informed consent the researcher must follow the Mental Capacity Act (2005) to ensure that the participant does not hold capacity. The principles of the MCA which we will adhere are as follows:

- A person must be assumed to have capacity unless it is established that he/she lacks capacity.
- A person is not to be treated as unable to make a decision unless all practical steps to help him/her to do so have been taken without success.
- A person is not to be treated as unable to make a decision merely because he/she makes an unwise decision.
- An act done or decision made, under this Act for or on behalf of a person who lacks capacity must be done, or made, in his best interests.

- Before the act is done, or the decision is made, regard must be had to whether the purpose for which it is needed can be as effectively achieved in a way that is less restrictive of the person's rights and freedom of action.

A decision to appoint a consultee on behalf of a patient will be made if the participant is unable to:

1. (a) understand the information relevant to the decision,
   (b) retain that information,
   (c) use or weigh that information as part of the process of making the decision, or
   (d) communicate his/her decision (whether by talking, using sign language or any other means).

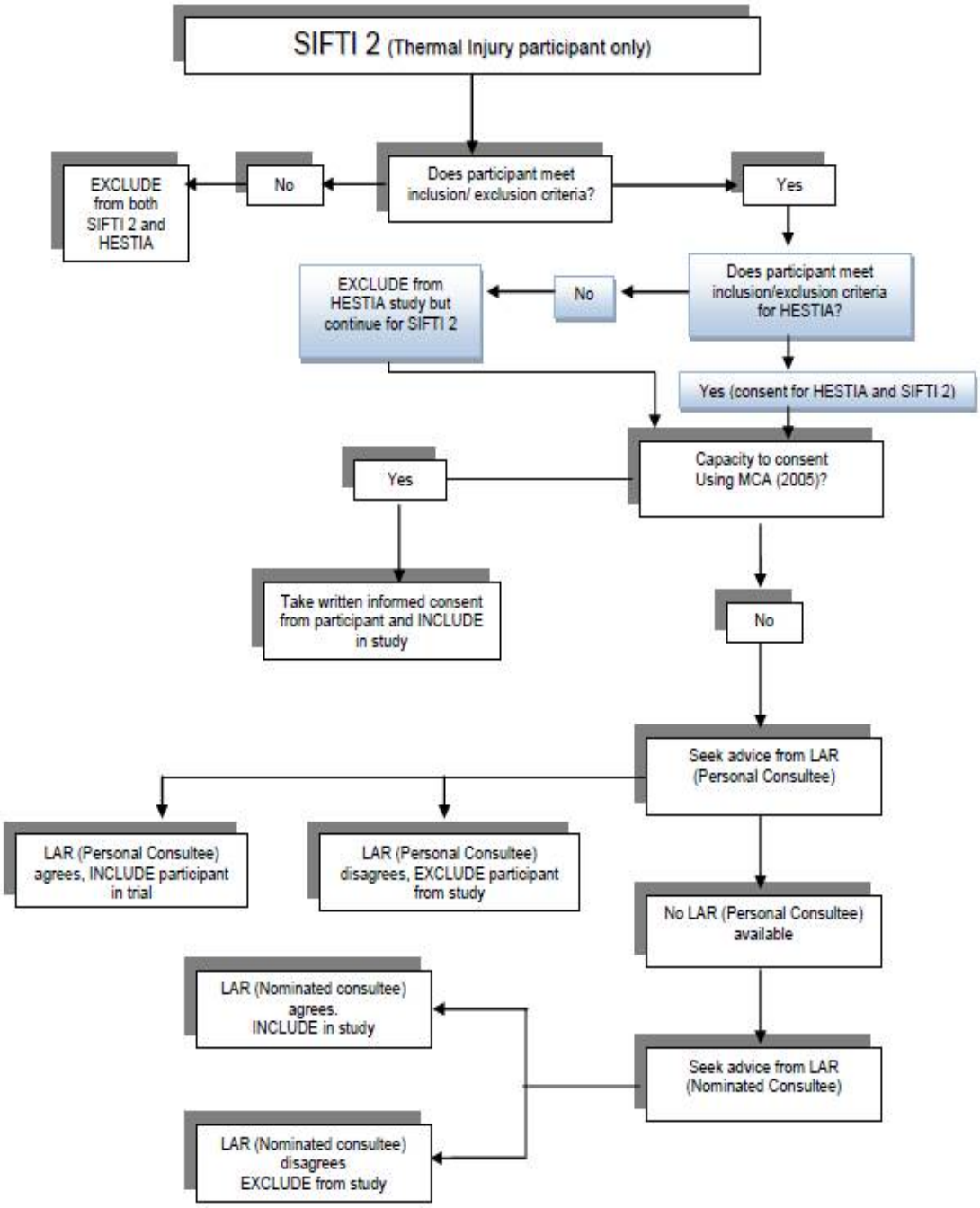
2. A person is not to be regarded as unable to understand the information relevant to a decision if he/she is able to understand an explanation of it given to him in a way that is appropriate to his circumstances (using simple language, visual aids or any other means).

3. The fact that a person is able to retain the information relevant to a decision for a short period only does not prevent him/her from being regarded as able to make the decision.

4. The information relevant to a decision includes information about the reasonably foreseeable consequences of

   (a) deciding one way or another, or
   (b) failing to make the decision.

Figure 4 HESTIA Thermal Injury Participant Recruitment



12.3.4. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.

Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.3.5. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Healthy participants who are rescreened are required to sign a new ICF.

12.3.6. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

12.3.7. Committees Structure

An Independent Data Monitoring Committee or similar review group will not be used in this study, but an internal preliminary data review will be conducted.

The Data Review team will consist of the GSK medical monitor, clinical and operational leads, statistician, early development lead and the safety officer. They will meet at

intervals specified within the data review charter to review data relevant to the future conduct of the study, and will also assess any risk to study participants.

12.3.8. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

12.3.9. Dissemination of Clinical Study Data

- Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.
- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

12.3.10. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

12.3.11. Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the study specific Source Data Verification document.

12.3.12. Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with study participation, whether or not considered related to the study. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with study participation. • NOTE: As detailed in Section 9.2.1, only AEs deemed to be related to procedures or requirements unique to the HESTIA study will be recorded/reported for thermally injured participants. All other AEs will be recorded/reported through the SIFTI-2 study.

Events Meeting the AE Definition
• Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study STM administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. Table 3 provides a list of commonly occurring AEs in participants with severe thermal injury which may meet this definition. • The disease/disorder being studied or expected progression, signs, or symptoms of

the disease/disorder being studied, unless more severe than expected for the participant's condition.

- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Table 3 Complications of severe thermal injury which can be considered as associated with the underlying disease and do not require reporting as AEs unless judged to be more severe than expected for the participant's condition or related to HESTIA study procedures.

If any of the following are observed, then an AE will be recorded in the SIFTI2 study CRF.

If any of the following are observed and deemed to be related to STM administration or other unique requirements of the HESTIA study, then to be recorded in the HESTIA CRF and reported to GSK as per guidance below.

System Assessment	Complication type	Action
Airway problems	Failed extubation	Record AE
	Tracheostomy complication	
Breathing Problems	Pneumothorax	Record AE
	Pulmonary Oedema	
	Respiratory Arrest	
	Pneumonia	
	VAP	
	Acute lung injury (ALI)	
	ARDS	
Circulatory Problems	Haemodynamic instability	Record AE
	Increasing vasoactive drug support	Record ionotrope dose in con-meds
	Arrhythmia	

System Assessment	Complication type	Action
	Endocarditis	
	Acute LVF/CCF	
	Cardiac Arrest	
	MI	
Neurological Problems	Reduced GCS (off sedation)	Record AE
	Intra-Cranial bleed	
	CVA	
	Acute confusion/Delirium	
	Meningitis-bacterial	
Oedema Complications	Abdominal Compartment Syndrome (ACS)	Record AE
	Acute Limb compartment syndrome	
Microbiological problems	Sepsis	Record AE Record in Microbiology form
	Chest Infection	
	Lower Respiratory Tract Infection	
	UTI	
	Bloodstream Infection (BSI)	
	Wound infection	
	Intra-vascular catheter (line) infection	
	Infective diarrhoea	
	Clostridium difficile infection/pseudomembranous colitis	
Renal/Urology problems	Acute rhabdomyolysis	Record AE

System Assessment	Complication type	Action
	Acute renal failure	Ensure biochemistry and CK results recorded n CRF
	Acute urinary retention	
	Renal replacement therapy	
Thromboembolic complications	Lower limb DVT	Record AE with location of thrombus
	Upper limb DVT	
	Pulmonary embolism	
	Other VTE	
	Fat embolism	
Wound complications	Major graft loss	Record AE with details
	Major skin substitute loss	
	Wound infection	
	Invasive wound infection	

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

Please note that, as described in Section 9.2.6 a, b, c and d below are considered as 'disease related events' as they occur commonly in patients following thermal injury unless, in the opinion of the investigator, they are directly related to STM administration or other unique requirements of the HESTIA study.

A SAE is defined as any untoward medical occurrence that:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually

involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before

submission to GSK.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between administration of the NIMP (STM) and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to STM administration will be considered and investigated.
- The investigator will also consult the Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.

- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor by telephone.
- Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to

complete and sign the SAE CRF pages within the designated reporting time frames.

- Contacts for SAE reporting can be found in SRM.

12.5. Appendix 5

The following table of study objectives specifies, for each objective, the provenance of clinical data and samples which will be used to explore that endpoint

Objective	Endpoint	Origin of Data/Samples
Co-Primary		
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry	• HESTIA study
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time	• HESTIA Study
Exploratory		
3. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry	• HESTIA Study
4. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time	• HESTIA Study
5. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of hemofiltration-free days • Number of episodes	• All clinical data will be obtained from SIFTI2. • Permeability measurements will be obtained from HESTIA

Objective	Endpoint	Origin of Data/Samples
	of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores	
6. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios	• Blood biomarkers obtained from SIFTI-2 (and HESTIA for healthy participants) • Urine for microbial metabolite analysis, claudin 3 and KIM 1 obtained from SIFTI-2 (and HESTIA for Healthy participants) • Urine albumin:creatinine and protein:creatinine ratios obtained from SIFTI-2 (and HESTIA for healthy participants) • Permeability data (STM absorption) from HESTIA
7. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples	• Stool samples collected in HESTIA protocol • Permeability data from HESTIA
8. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission	• Medical History data from HESTIA • Permeability data from HESTIA
9. To assess wound healing	• Time to wound recovery (e.g. 95%)	• Wound healing assessment data from clinical notes will be captured in HESTIA at 14

Objective	Endpoint	Origin of Data/Samples
		day, 28 day and 6 month visits.
10. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation	• Microbiome data from HESTIA study • Blood Biomarker data from SIFTI-2 (thermally injured participants) and HESTIA (healthy participants.

TITLE PAGE

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability and Systemic Inflammation (HESTIA)

Protocol Number: 206243/02

Short Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability and Systemic Inflammation (HESTIA)

Compound Number: NONE

Sponsor Name and Legal Registered Address:

GlaxoSmithKline Research & Development Limited
980 Great West Road
Brentford
Middlesex, TW8 9GS
UK

Medical Monitor Name and Contact Information will be provided separately.

Regulatory Agency Identifying Number(s): NA

Approval Date: 26-SEP-2017

SPONSOR SIGNATORY:

PPD

26-9-2017

Craig Tipple, MBBS, MRCP, PhD
Director, Clinical Development,
R&D Infectious Disease, GSK

Date

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 2	*26-Sep-2017*
Amendment 1	*15-Aug-2017*
Original Protocol	*02-Mar-2017*

Amendment 2: 26-Sep-2017

Overall Rationale for the Amendment: The Ethics Committee requested the legally acceptable representative be updated with personal or nominated consultee in the protocol.

Section # and Name	**Description of Change**	**Brief Rationale**
6.1.2	Legally acceptable representative was replaced with personal or nominated consultee.	At the request of the Ethics Committee.
6.1.4	Legally acceptable representative was replaced with personal or nominated consultee.	For consistency.
12	Removed LAR abbreviation	For consistency.
12.3.2, 12.3 and 12.5	Removed LAR	For consistency.
6.7	Screen failure will be assigned a new participant number.	Previous language was in conflict with FDA guidelines on subject numbering for re-screened subjects as defined in the Study Data Technical Conformance Guide v2 (issued in December, 2014).
Appendix 6	Protocol Amendment History was added.	Per template instructions.

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1. SYNOPSIS

Protocol Title: A Prospective, Longitudinal Study to Investigate the Effect of Thermal Injury on Intestinal Permeability, and Systemic Inflammation (HESTIA)

Short Title: Investigation of Thermal Injury and Intestinal Permeability

Rationale:

Patients with severe thermal injury [>20% total body surface area (TBSA)] are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

No GSK study treatment will be employed in this study.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients.

Previous literature demonstrates that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study, therefore, is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.

4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

In order to enter this study thermally injured participants will be required to co-enrol in this study and an allied study entitled: A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injury (SIFTI-2) (reference number IRAS ID: 200366). Clinical data, standard of care laboratory data and investigational biomarker data will be shared from the SIFTI-2 study to this study and the combined data from both studies will be used to address exploratory endpoints. Participants of the SIFTI-2 study will be appropriately consented for this data sharing.

Objectives and Endpoints:

Objective	Endpoint
Co-Primary	
• To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
• To characterise the effect of thermal injury on small intestinal permeability over time	• Changes in L/M ratio over time

Overall Design:

A prospective, longitudinal study will be conducted in adult (≥18 years old) men and women admitted to a hospital following thermal injury. Measurements of intestinal permeability, inflammation and microbial translocation will be taken over a six month period. A cohort of healthy participants will also be recruited in order to determine the reference against which post-burn permeability measurements and other biomarkers will be compared.

The lactulose-to-mannitol ratio is traditionally used to assess small intestinal permeability and sucralose to assess colonic permeability. Lactulose, mannitol and sucralose [henceforth referred to as sugar test material (STM)] will be intermittently administered enterally for the purpose of intestinal permeability measurement to examine permeability at different points along the GI tract and is described in Section 7. It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

An internal preliminary data review will be conducted. This review is described in Section 5.4.4.

Number of Participants:

Table 1 in Section 5.2 described the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Treatment Groups and Duration:

Group 1: Healthy Participants

The total duration of this study for healthy participants will be approximately two weeks, in addition to the screening window:

- Screening: up to 28 days before Day 1
- Day 1: study start and assessments will be performed
- Day 8 and Day 15

Group 2: Thermally injured participants

Thermal injury participants will be asked to participate for a total of 6 months (plus or minus 14 days).

- There will be no screening period. Thermal injury participants will be recruited within 24 hours of their admission to the burns unit at the study site.
- Intense monitoring phase: Assessments will be performed on alternate days for the first 14 days following study enrolment. If the participant is discharged prior to 14 days, the intense monitoring phase will end, but the participant will remain enrolled in the study.
- Convalescent monitoring phase: Assessments at 28 days and 6 months will be made on an outpatient basis if the participant has already been discharged from hospital.

Exception to monitoring periods:

- If a discharged participant attends the centre for routine clinical care on any of days 8-14, then study assessments will be made and samples will be taken

2. SCHEDULE OF ACTIVITIES (SOA)

2.1. Healthy Participants (Group 1)

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Review of inclusion/ exclusion criteria and informed consent	X				
Demography	X				
Medical history (includes substance and alcohol usage at screening) and Medication history	X				
Substance testing (urine)	X				Substances: [Recreational Drugs and Alcohol]
HIV, Hepatitis B and C screening	X				If test otherwise performed within 3 months prior to study entry, testing is not required
Laboratory assessments	X				
Pregnancy test (WOCBP only) (urine)	X	(X)			Only performed again on Day 1 if patient at risk of pregnancy at or since screening

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
Blood sampling for biomarkers		X			20ml of blood will be sampled in a single draw on one day, preferably Day 1
Stool sample collection		X			Participants will be given a collection container at screening to bring with them on Day 1.
Brief physical examination including measurements of height and weight Vital Signs (systolic and diastolic blood pressure and heart rate)	X	X	X	X	BMI calculated from height and weight at screening only Examinations should be conducted the day after intestinal permeability measurement.
Medical review (assessment of health status)		X	X	X	Monitor for signs and symptoms of gastro-intestinal infections and other emergent issues
STM Training	X				Refresher training may be provided as needed
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection		X	X	X	Please refer to SRM for full information Intestinal permeability measurement to be performed at home the day before the study visit.

Procedure	Screening	Treatment Period [Out patient days] (± 1 day)			Notes
		D1	D8	D15	
AE/SAE and Concomitant medication reviews	(X)	←==========		==========→	Day 1 will include concomitant medication review only

2.2. Thermal Injury Participants (Group 2)

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)														6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
Review of inclusion/ exclusion criteria and informed consent	X																Participants will be co-consented to the SIFTI-2 study
Medical history (includes substance and alcohol usage) and Medication history	X																
Substance testing (urine)	X																Substances: [Recreational Drugs, Alcohol]
Pregnancy test (WOCBP only) (urine)	X															X	To be repeated at 6 months prior to final STM administration

Procedure	D1 (≤ 24 hours of admission)	Treatment Period [ICU Days] (+/4 hours)														6 months (± 14 days)	Notes
		D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D28 (± 3 days)		
HIV, Hepatitis B and C screening	X																If test otherwise performed within 3 months prior to study entry, testing is not required
Initial assessment of Burns	X																Calculation of %TBSA Location of thermal injury and depth
Fluid balance (total input/output)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X*		Daily (over 24 hr) *only if participant still admitted
Intestinal permeability measurement: STM administration followed by a 24-hour urine collection	X		X		X		X		X		X		X		X	X	Measurements every 48 hours from first measure. Preference: D1, 3, 5, 7, 9, 11, 13. Otherwise: D2, 4, 6, 8, 10, 12, 14. Please refer to SRM for full method.
Brief Physical Examination															X	X	Following final intestinal permeability measurement. Can be omitted if patient is still admitted to hospital.

Procedure	**D1 (≤ 24 hours of admission)**	**Treatment Period [ICU Days] (+/4 hours)**														**6 months (± 14 days)**	**Notes**
		D2	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**D28 (± 3 days)**		
Stool sample collection	←==========→ (A sample of the first stool produced following admission)													X	X	X	Time to first stool collection; preferably on Day 1. Then on Days 14 and 28 and 6 months
Wound Healing Assessments														X	X	X	This is to assess time to 95% wound healing
AE/SAE and Concomitant medication reviews	X	←==========→														X	AE/SAE monitoring will begin after the first administration of STM

- The timing and number of planned study assessments may be altered during the course of the study based on newly available data or factors outside of the study such as priority medical care to ensure appropriate monitoring.
- Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol deviation or require a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

3. INTRODUCTION

3.1. Study Rationale

The purpose of this study is to describe the kinetics and magnitude of increases in intestinal permeability which are observed as a result of thermal injury. The results of this study will be used to inform the design of future drug studies of a novel medicinal product, which is predicted to reduce this increased permeability.

3.2. Background

3.2.1. Thermal Injury, Intestinal Permeability and Multi-Organ Dysfunction Syndrome

Patients with severe thermal injury >20% TBSA are at risk for organ dysfunction and may develop multiple organ dysfunction syndrome (MODS). This study aims to explore the relationship between thermal injury, changes in intestinal permeability and the onset of MODS.

One of the central hypotheses over the last two decades to explain the onset of MODS in the context of critical illness imputes that an increase in intestinal permeability results in the translocation of bacteria and bacterial products to the systemic circulation where they drive inflammation and injury to distal organs [Deitch, 2006]. This hypothesis is supported by data showing that there is a significant increase in intestinal permeability in critically ill patients, including patients with thermal injury, and that the degree of intestinal permeability correlates with the onset and severity of MODS [Doig, 1998]. As such, therapies directed at minimising these changes in intestinal permeability, thereby limiting the translocation of bacteria to the systemic circulation, are hypothesised to have an impact on clinical outcome in these patients (Figure 1).

Figure 1 Changes in Intestinal Permeability

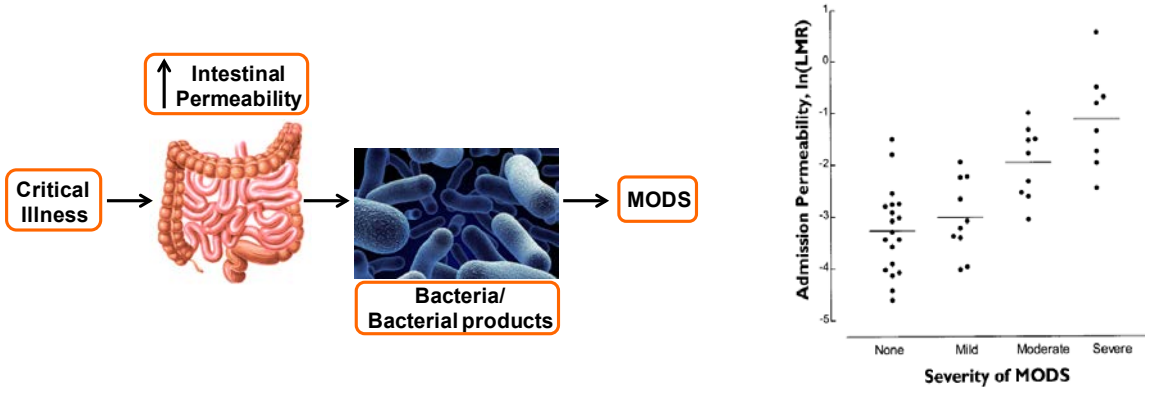

Figure 1 (Left panel) Hypothesis: The gut is the major driver of MODS in critical illness (Right panel) Correlation between intestinal permeability as measured by lactulose/mannitol ratio and the severity of MODS in critically ill patients. Intestinal permeability was determined by measuring the differential absorption of lactulose (increased in the damaged gut) and mannitol (freely absorbed in the normal and damaged

gut) following oral administration and expressed as the ratio of lactulose to mannitol (L/M) [Olquin, 2005].

There are some data showing that patients with severe thermal injury (>20% TBSA) display a significant and rapid increase in intestinal permeability that declines gradually over a two-week period following the injury [Olquin, 2005]. Additionally, these changes in intestinal permeability, correlate with the severity of sepsis observed in these patients [Ziegler, 1988].

The central hypothesis of this study is that thermal injury alters intestinal barrier function allowing the translocation of bacteria and bacterial products to the systemic circulation where they contribute to the onset of MODS.

The aims of the study are:

1. To establish the magnitude and time course of changes in intestinal permeability to inform timing and duration of future investigational medicinal product administration.
2. To establish the optimal method for assessment of intestinal permeability in patients with thermal injury.
3. To describe the patient population most likely to benefit from a new medicinal product which could prevent changes in intestinal permeability.
4. To improve our understanding of the links between intestinal damage, changes in the gut microbiome and microbial translocation to the systemic circulation following thermal injury.

3.2.2. Intestinal Barrier Function and Its Measurement

The intestinal barrier combines a physical and immunological barrier. Epithelial cells are connected by tight junctions and prevent the passage of bacteria, toxins and antigens into the systemic circulation [Bjarnason, 1995]. Paneth cells, located in crypts of the small intestine, produce anti-microbial substances (e.g. lysozyme and defensins) and other immune cells patrol the lamina propria [Ayabe, 2000]. Barrier function can be disturbed by diseases such as inflammatory bowel disease; by drugs such as aspirin and alcohol; by ischaemia and has been observed following a number of acute injuries such as burns, trauma and radiation injury [Bjarnason, 1995; Derikx, 2006]. This disturbance results in the translocation of the intestinal flora (pathogenic or commensal) to the systemic circulation. Microbes are accompanied by proteins which normally form part of the tight intracellular junctions of the intestinal epithelium, such as claudins, and other enterocyte-derived proteins [Grootjans, 2010]. In this study, intestinal permeability will be measured directly using oligosaccharide absorption and indirectly by looking for micro-organisms and soluble markers of intestinal barrier dysfunction in the systemic circulation.

Since the 1970s, oligosaccharides have been used as test probes to measure the function of the intestinal barrier [Menzies, 1972]. Lactulose, a large polysaccharide, does not normally cross the intestinal barrier, but following damage can cross the epithelium and enter the systemic circulation. It is not metabolised, so is filtered in the kidney and excreted in the urine. The fractional excretion (amount administered / amount recovered in urine) of lactulose is therefore a measure of intestinal permeability. The amount of lactulose entering the urine is dependent on a number of factors including renal function,

gastric emptying, and degradation in the large bowel by commensal bacteria; thus, a monosaccharide such as mannitol, which passes freely across the healthy intestinal barrier, is often co-administered to 'normalise' lactulose measurements. Following administration of both lactulose and mannitol the fractional excretion of the sugars is expressed as a ratio where mannitol is the denominator. Lactulose and mannitol absorption occurs mainly in the proximal small intestine and is complete within approximately 5 hours of oral administration. This approach has been previously used successfully in patients with severe burn injury and intestinal permeability was found to correlate with episodes of sepsis [Doig, 1998].

In order to assess the permeability of the large bowel a third oligosaccharide, sucralose, will also form part of the sugar absorption test. This synthetic sweetener (marketed by Tate and Lyle as 'Splenda') is not subject to the same degradation by large bowel commensal flora as lactulose and is therefore a better measure of large bowel permeability than lactulose. Again, this has formed part of previously described studies aiming to measure intestinal permeability [Del Valle-Pinero, 2013].

Lactulose, mannitol and sucralose will be co-administered to both healthy participants and participants following thermal injury. In order to document accurately the time course of change in permeability, thermally injured participants will be asked to undergo the test on alternate days for 14 days (the intense monitoring phase) followed by two convalescent samples at day 28 and month 6 (thermal injury participants). In order to produce an accurate baseline measurement, healthy participants will be asked to undergo three measurements of intestinal permeability over approximately a two week period.

3.2.3. The Intestinal Microbiome and Thermal Injury

During the intense and convalescent monitoring phases of this study, samples of stool will be collected. These samples will undergo gene sequence analysis in order to determine the composition of the intestinal microbiota. These results will be compared with culture results from peripheral whole blood samples. The hypothesis is that raised intestinal permeability will correlate with an increased frequency of bacteraemia and that the particular bacteria detected in blood will correlate with the composition of the intestinal microbiota.

Additionally, it has been demonstrated that thermal injury alters the composition of the intestinal microbiome [Hammer, 2015]. The ultimate aim is to be able to block changes in intestinal permeability which might affect this change in composition and are therefore interested, in the current study, to assess the impact of thermal injury on the microbiome

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3.3. Benefit/Risk Assessment

3.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Non-investigational Medicinal Product Use		
Oral administration of sucralose in solution is unlikely to be palatable.	The dose of sucralose to be administered is 2g per test. A tolerable sweetness score (e.g. 11.3 (diet Pepsi)) would require the sucralose to be administered in 4 litres of water.	Sucralose is to be administered in capsules when given by mouth. If being administered by nasogastric or nasojejunal tube the capsules are to be emptied into the lactulose and mannitol solution
Depending on the volume of administration, the final STM solution may be hyper-osmolar. Administration directly into the jejunum via a nasojejunal tube may then result in osmotic movement of water into the intestine causing distention and discomfort.	This is based on the physiological principle that the stomach normally regulates the osmolality of its contents passing into the small intestine. Administration via nasojejunal tube (but not nasogastric tube) bypasses this process.	The lactulose, mannitol and sucralose will be delivered nasojejunally in 50ml of water and followed by a 50ml water flush. This makes the solution iso-osmolar (300 mosmol/Kg in situ). following nasojejunal administration
Lactulose and mannitol can produce an osmotic laxative effect following enteral administration.	Both lactulose and mannitol are used clinically as osmotic laxatives. The typical dose of lactulose (for the treatment of constipation) would be approximately 21g daily.	The amount of lactulose being used in this study is 5g, 75% below the standard laxative dose. The dose of mannitol is equally low compared to that contained in laxatives.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
STM administration for the measurement of intestinal permeability requires a hold in feeding which may be longer than a standard feeding hold.	Intestinal permeability assessment requires the administration of STM (see Section 7) and the collection of urine samples at defined time points (see the SRM). This procedure has previously been used in patients with severe thermal injury [Doig, 1998] but has not been studied extensively; participant fasting is required pre and post administration of these sugars; and repeated administration as per the SoA tables is also unexplored.	Where possible, tube feeding targets will be volume and not time-based to reduce the amount of feed missed on test days. Moreover, fasts for the tests will, where possible, be aligned with clinically indicated feeding holds (such as fasts required before theatre, or scheduled overnight feed holds).
Other		
The degree of injury sustained by some participants may be severe.	Participants with severe thermal injury experience significant morbidity and high levels of mortality. Therefore it is anticipated that these participants will be subject to multiple medical complications which may impact the study assessments and period (See SoA tables)	Ensure that routine care in the burns unit is not compromised by study participation. Prompt reporting of any adverse events which are related to study procedures and may affect study safety.
Omission of lactulose and sennosides which are frequently administered as a part of routine burn management.	Lactulose is a part of the STM and its administration for clinical reasons would complicate intestinal permeability measurement significantly. Equally, the mechanism of actions of sennosides is to cause irritation of the GI tract	Polyethylene glycol (e.g. Movicol) will be used as an alternative osmotic laxative. If senna is required for clinical purposes, then its use must be documented in the CRF.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	and may result in increased permeability.	
The STM will be prepared for administration on the assessment day and will not be sterile.	There is a risk that the sugar test material formulation may be contaminated with yeast or bacteria. The presence of contamination within the STM formulation delivered via the feeding tube in thermal injury participants could present an infection risk.	Microbiology release testing will be conducted by Tayside pharmaceuticals. The STM will be prepared on the assessment day to minimise this risk. Lactulose/Mannitol will be refrigerated from the point of manufacture and until use. The site investigators have been consulted on this risk and felt that it was low.
Incomplete 24 hour urine collections	Ambulant, uncatheterised patients and healthy participants 24-hour urine collections could be incomplete.	Ambulant, uncatheterised patients and healthy participants will receive careful education and written instructions of the importance of complete urine collections. In addition, documentation of incomplete collection, in addition to sampling collection times, will be recorded.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Phlebotomy for biomarkers (healthy participants)	Phlebotomy can be painful and result in bruising, bleeding and puncture site infection.	Phlebotomy from thermally injured participants will not be conducted as a part of this protocol. Healthy participants will undergo one blood draw during screening and a further draw on day one. Phlebotomy will be performed by an appropriately trained member of the site study team with aseptic non-touch technique to avoid infection.

3.3.2. Benefit Assessment

Study participants will not benefit directly from involvement in this study. However, the results of this study may contribute significantly to our understanding of changes in intestinal permeability and their relationship to morbidity and mortality in the context of thermal injury. This knowledge is paramount to designing future medicinal interventional studies, aiming to modulate intestinal permeability and, potentially, to improve outcomes for patients following thermal injury.

3.3.3. Overall Benefit:Risk Conclusion

The primary outcome measure of this study is the determination of intestinal permeability in healthy and thermal injury participants. Interventions in this study are the administration of STM by mouth or feeding tube (if one is site for routine clinical care) and the collection of urine and stool samples.

The risk of adverse events is minimised for the population being investigated in the proposed study as no drug intervention will be investigated and study assessments being conducted are non-invasive (with the exception of STM administration and phlebotomy in healthy participants).

4. OBJECTIVES AND ENDPOINTS

Objective	Endpoint
Co-Primary	
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time
Exploratory	
1. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry
2. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time

Objective	Endpoint
3. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of hemofiltration-free days • Number of episodes of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores
4. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios
5. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples
6. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission
7. To assess wound healing	• Time to wound recovery (e.g. 95%)
8. To characterise parameters that may influence drug PK/PD†	• Determine fluid input/output balance over time • Changes in serum albumin and plasma creatinine
9. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation

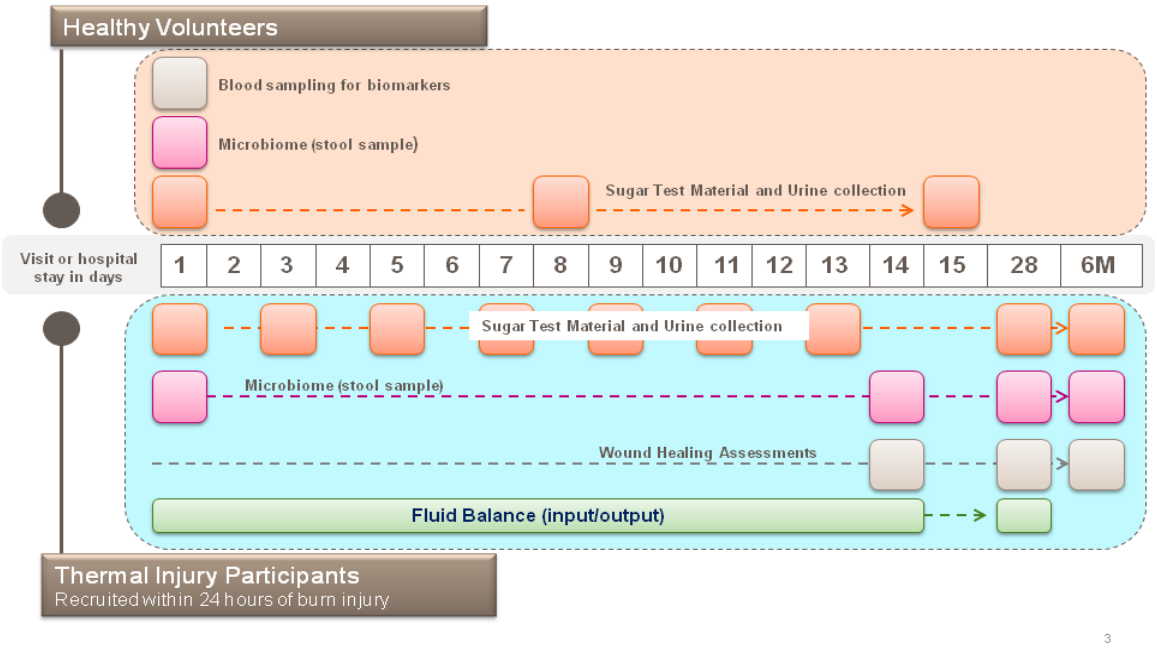
†Clinical data, routine laboratory results or blood/urine biomarker results obtained from the SIFTI-2 study will be used in the analysis of this exploratory endpoint see Section 5.4.1.

5. STUDY DESIGN

5.1. Overall Design

This is a longitudinal, prospective study of healthy participants and participants who have sustained a thermal injury. The following schematic summarises study measures and their frequency for healthy and thermal injury participants.

Figure 2 Study Schematic



5.2. Number of Participants

Table 1 describes the number of participants proposed for the study. Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (≥15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Table 1 Recruitment Stratification

	Number of participants
Group 1 **Healthy participants**	15
Group 2 **Thermal Injury participants** **Percent Total Burn Surface Area (TBSA)** **≥15%**	25

The healthy participants (Group 1) will be recruited with an age range similar to that typical in thermal injury participants based on historic hospital admission data from the UK and data from the SIFTI1 study [Hampson, 2016].

If participants prematurely discontinue or are withdrawn from the study, additional replacement participants may be recruited at the discretion of the Sponsor in consultation with the investigator see Section 8.2.1.

5.3. Participant and Study Completion

The full duration of the study for healthy participants is approximately two weeks and for thermal injury participants is approximately 6 months.

Thermal injury participants who withdraw prior to week 4 or healthy participants who withdraw prior to week 2 will be considered for analysis, although it is acknowledged that any missing data at later stages of the study may be related to outcome (either positive or negative). Given this is an exploratory study, the impact of missing data will be explored by assessing the sensitivity of results to different missing data approaches (for example, analyse all available data, analyse only complete data across time points and explore imputation of worst or best case scenarios).

Study withdrawals may also include participants who are consented to the study under Section 30 of the Mental Capacity Act 2005. In the event that participants do not re-confirm consent when they regain capacity, they will be withdrawn from the study.

The end of the study is defined as the date of the last visit of the last participant in the study.

5.4. Scientific Rationale for Study Design

5.4.1. Co-recruitment to the SIFTI-2 Study

Thermally injured participants who are eligible for this study must also be eligible for, and enrolled in a partner study named SIFTI-2.

SIFTI-2 is an observational study currently recruiting healthy participants and thermally injured participants and follows a successfully delivered predecessor study SIFTI1 [Hampson, 2016]. The objectives and endpoints of SIFTI-2 are included in the SIFTI-2 study protocol (reference number IRAS ID: 200366). The design of this study has been aligned with the SIFTI-2 study to support the strategy of co-consenting thermally injured participants to both studies. This will reduce the overall impact of research in this population in the following ways:

- There is sufficient residual blood from collection in SIFTI-2 to allow testing of blood biomarkers of interest for the HESTIA study. This strategy therefore limits impact on participants as no additional blood sampling is required for participation in HESTIA (with the exception of HIV, Hepatitis B and C testing at baseline). SIFTI-2 participants will be explicitly consented for their samples and data to be shared in this way.
- Sampling time points and study visits in SIFTI-2 and HESTIA have been aligned to reduce the impact of co-recruitment on thermally injured participants.

Clinical data from both studies can be generated from the same participant therefore allowing biomarker, microflora and intestinal permeability data to be compared. In contrast to thermally injured participants, healthy participants enrolling in this study will not be required to co-consent for participation in SIFTI-2.

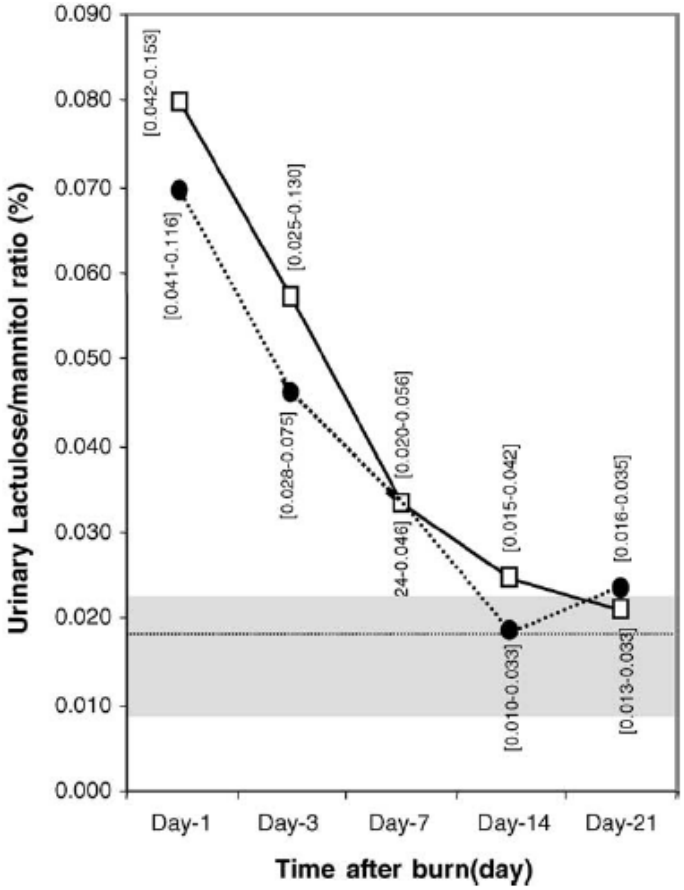
Data will be shared from the SIFTI-2 study with GSK through a secure electronic database.

A summary of the origin (HESTIA or SIFTI-2) of data and samples collected for the HESTIA study is available in Appendix 5 Section 12.5.

5.4.2. Recruitment and Sampling Schedule

Severely burned patients (with an injury affecting greater than 20% total body surface area), display a significant and rapid increase in intestinal permeability that has been shown to decline over time (Figure 3) [Olquin, 2005]. What is less well understood is whether a greater severity of thermal injury correlates with greater intestinal permeability. Moreover, the time to complete restoration of normal permeability and other factors which may influence permeability (other than the initial injury) are also not well understood.

Figure 3 Burn Injury Results in a Rapid Increase in Intestinal Permeability



This study aims to recruit participants as soon as possible following their admission in order to capture the initial changes in permeability. Serial measurement of intestinal permeability and sampling of the biomarkers of bacterial translocation, intestinal damage and inflammation are required during the acute phase (days 1-14) of admission in order to correlate them with clinical events (e.g. surgery), severity scores and clinical outcomes.

The convalescent time points (28 days and 6 months) are required to determine if intestinal permeability has returned to normal and to correlate observed changes on days 1-14 with longer-term clinical outcomes (e.g. wound healing).

Gut microbiome assessments will be made less frequently than intestinal permeability assessments as changes in the microbiome are predicted to evolve more slowly. Ideally a stool sample will be collected from thermal injury participants at study entry (limited, of course, by when participants first pass stool following admission). A convalescent sample is requested to assess if the gut microbiome is able to restore to a more normal composition (and will be compared with that of healthy participants to make that assessment).

Blood samples (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline) will not be taken from thermally injured participants during this study. Instead, biomarker data from blood samples taken during the SIFTI-2 study will be used. A single blood draw will be required from healthy participants on day 1 of participation.

5.4.3. Inclusion of Healthy Participants

Patient facing material (i.e. poster) will be used to facilitate recruitment of the healthy participants. Healthy participants will be recruited to this study to provide a baseline for endpoint measures on intestinal permeability and the gut microbiome.

Three measurements of intestinal permeability are required in order to define an average given the variability in healthy participants reported previously. The timing of the replicates follows the intense time course of the study to control for day-to-day variation over a 15 day period.

5.4.4. Preliminary Data Review

A safety review based on raw data will be conducted when approximately 10 participants have completed the study. This review would include data from any participant, either healthy or thermally injured, that has been collected at the time the review is conducted.

5.4.5. The Use of the Sugar Test Materials (Lactulose, Mannitol and Sucralose)

As described in Section 3.2.2, lactulose, mannitol and sucralose will be administered to both thermally injured participants and healthy participants to measure permeability of the small and large intestine. The amount of each of the sugars to be used is based on previous successful clinical studies employing this method and balances having enough sugar present for detection in urine with their potential laxative effect (Del Valle-Pinero, 2013; Doig, 1998; Menzies, 1972).

6. STUDY POPULATION

The study population will comprise healthy and thermal injury participants presenting at enrolling study sites. Please note the following:

- Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.
- Where possible, written informed consent will be obtained from each subject prior to participation in this study. Recruitment of subjects who lack mental capacity is discussed in Section 6.1.
- Healthy participants will be consented to the HESTIA study only.
- Thermally injured participants are required to be co-consented to the SIFTI-2 and HESTIA studies outlined in Section 6.1. A diagrammatic overview of the SIFTI-2 and HESTIA thermal injury participant recruitment is given in Figure 4. (see Appendix 3).

6.1. Recruiting Participants with Differing Mental Capacity

Following evaluation of capacity (as outlined in the Mental Capacity Act (MCA) 2005), thermally injured participants enrolled in this study will fall into the following groups:

6.1.1. Adult Participants Determined to Have Mental Capacity at Study Entry and Throughout the HESTIA Study

Those participants who present with capacity and meet study entry criteria will be provided with a Participant Information Leaflet (PIL) outlining the study. If the participant agrees to consent to the HESTIA study following a discussion with the research team, they will be asked to sign a consent form.

Given the short (24 hour) window for recruitment, those patients who are acutely unwell will initially be presented with an abbreviated PIL. Once stable, this will be followed by the standard information leaflet and re-confirmation of consent.

6.1.2. Adult Participants Lacking Mental Capacity for the Duration of the HESTIA Study

It is anticipated that some subjects who meet eligibility criteria for this study will not be able to give informed consent due to their medical condition or its management (e.g. sedation, opioid analgesia, intubation). In such cases, participants may be enrolled in the study in accordance with Section 30- Section 34 of the MCA 2005. The decision to enroll the participant will be discussed with a personal or nominated consultee (as defined in Section 12.3.2). This decision may or may not be witnessed by an independent witness according to the decision of the principal investigator.

6.1.3. Adult Participants Lacking Mental Capacity (either at Study Entry or During the Study) Who Later Regain Capacity and Are Required to Provide Informed Consent

As soon as is practically possible following a participant regaining capacity, participants will be asked to provide informed consent to remain in the study. If they decline, then they will be withdrawn from the study as soon as it is safe to do so (likely immediately given the design of this study). Samples and data collected prior to study withdrawal may be retained. The participant will be asked about this at the point of study withdrawal.

6.1.4. Adult Participants with Mental Capacity to Provide Consent at Study Entry Who are Later Deemed No Longer to have Mental Capacity

The decision for the participant to remain in the study will be discussed with a personal or nominated consultee and recorded. If the participant subsequently regains capacity again, they will be asked to re-consent to study participation.

When considering enrolment of participants who lack the mental capacity to consent, the following should be noted:

- Section 3.2.1 of the SIFTI-2 protocol describes the consent process for that study in detail and should be read in conjunction with this protocol.
- A Study Information Leaflet will be provided to the personal or nominated consultee outlining the HESTIA trial before being asked to sign a form supporting the participant's enrolment in the study.
- The investigator and/or the site's IEC/IRB have responsibility for acting in accordance with the MCA 2005 in the matter of assessing who has the capacity to consent and who qualifies as a personal or nominated consultee of a potential subject. The investigator will also decide if an independent witness is required.
- Further information regarding the assessment of mental capacity and the appointing of personal or nominated consultee is given in Appendix 3 (Section 12.3.2 and Section 12.3.3).
- If a patient loses mental capacity subsequent to their consent and enrolment to the HESTIA study, samples and data collected prior to loss of capacity will be retained even if approval of continued study participation by a personal or nominated consultee is declined.

6.2. Inclusion Criteria for Healthy Participants (Group 1)

1. Males or Females must be ≥18 years of age at the time of signing informed consent.
2. Participants who are healthy as determined by the investigator following medical evaluation including medical history, physical examination, and laboratory tests (these are listed in Appendix 2).
3. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at screening or Day 1 as needed) and not breastfeeding.
4. Capable of giving signed informed consent as described in Appendix 3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.3. Inclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are eligible to be included in the study only if all of the following criteria apply:

6.3.1. Age

1. Participant must be ≥18 years of age.

6.3.2. Type of Participant and Disease Characteristics

2. Participants who have sustained a burn (thermal injury) with a TBSA ≥ 15%.

6.3.3. Other Inclusions

3. Admission to the burn centre (study site) ≤*24* hours of injury.

4. Able to take enteral fluids either orally or via a nasogastric tube (depends on facial burn damage).

6.3.4. Gender

5. Male and female.

   a. Female participants:

   A female participant is eligible to participate if she is not pregnant (negative pregnancy testing at study entry) and not breastfeeding.

6.4. Exclusion Criteria for Healthy Participants (Group 1)

1. Healthy participants are excluded from this study if they are receiving anti-coagulation therapy.
2. Pregnancy or breastfeeding.
3. A body mass index >34kg/m^2
4. An active history of alcohol dependency
5. History of sensitivity to any of the STM, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator and/or GSK Medical Monitor, contraindicates their participation.
6. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody and confirmatory Hepatitis C PCR result within 3 months of screening.
7. A positive pre-study urine drug/alcohol screen.
8. A positive test for HIV antibody.
9. Participants unable to swallow large capsules (the capsules will be shown to participants at screening).
10. Galactosaemia or severe lactose intolerance.
11. Use of an antibiotic 2 weeks prior to study start (i.e. administration of the STM).
12. Gastroenteritis in the 2 weeks prior to study start (i.e. administration of the STM).

6.5. Exclusion Criteria for Thermal Injury Participants (Group 2)

Thermal injury participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Chemical or electrical burn.
2. Multiple traumatic injuries with an Injury Severity Score (ISS) ≥16 (note: excludes burn in score system).

Prior/Concomitant Therapy

3. Patient received substantial undocumented management prior to arrival at the study site (burn centre) e.g. from paramedics or in a local accident and emergency department.
4. Systemic corticoidsteroid use.
5. Intravenous (IV) mannitol use.

Prior/Concurrent Conditions

6. Human immunodeficiency virus (HIV) infection.
7. Viral Hepatitis B or C infection.
8. Gastrointestinal disease (e.g. inflammatory bowel disease) which may affect intestinal permeability.
9. Previous bowel resection (e.g. hemicolectomy, small bowel resection)
10. Galatosaemia or severe lactose intolerance.
11. Bowel obstruction.
12. Renal dysfunction requiring renal replacement therapy (i.e. end-stage renal failure prior to thermal injury).
13. Active autoimmune disease and receiving immunomodulatory therapy e.g. rheumatoid arthritis anti-TNF.
14. Active chemotherapy for cancers or immunoremittive therapies (prednisolone, adalimumab) within 60 days of thermal injury.
15. Premorbid conditions of malignancy currently under treatment.
16. Previous bilateral lower extremity amputation.

NOTE: Due to the rapid recruitment period (within 24 hours of admission) for thermally injured participants, HIV and viral Hepatitis test results may not be available at the time of enrolment. In the event these tests return positive, the participant will be informed of the result(s) and withdrawn from the study (see Section 8.2.1). In the event that the participant has been recruited who lacked mental capacity to consent, the reason for their withdrawal (the positive HIV and/or viral Hepatitis test results) must not be shared with a personal consultee. According to UK law and the guidance of the British HIV association, if the participant's physician believes there is an overriding public interest to disclose the participant's results to a personal consultee who is a current or former sexual partner, then the result(s) may be disclosed without the participant's consent. This must be as a last resort. If the participant regains capacity, he/she will be informed of the positive test result(s).

Diagnostic assessments

17. Decision not to treat the patient due to futility.

6.6. Lifestyle Restrictions

6.6.1. Meals and Dietary Restrictions

- Participants will be fasted (or feed stopped) for **3 hours** prior to STM administration and for **3-5 hours** afterwards. For thermally injured participants these fasts should be aligned with those required for routine clinical care (feed holds, before surgical interventions) where possible.
- Refrain from consumption of the following for **24 hours** before and after the administration of STM:
    - Foods/drinks/medicines and other products which contain sucralose, lactulose or mannitol as artificial sweeteners.

N.B. If cannot be avoided, then clear documentation of its administration is required and the current test to stop. If urine samples have been collected PRIOR to administration of the drug, then these can still be sent for analysis

6.6.2. Alcohol/Exercise/Aspirin (Healthy Participants only)

Alcohol, aspirin and vigorous exercise [Sequeira, 2014] are all known to cause transient increases in intestinal permeability. Healthy participants will therefore be requested to avoid alcohol, aspirin and physical exercise for **48 hours** before taking the STM and for the 24-hour urine collection period.

6.7. Screen Failures

There will be no screening period for thermal injury participants. Screening will be up to 28 days before Day 1 for healthy participants.

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants should be assigned a new participant number.

7. NON-INVESTIGATIONAL MEDICINAL PRODUCT (NIMP)

A study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. According to this definition, no GSK study treatment will be employed in this study.

The STM (comprising lactulose, mannitol and sucralose) will be intermittently administered enterally as a study challenge agent to measure permeability at different points along the GI tract. Lactulose and Mannitol assess small intestine permeability and sucralose to assess colonic permeability.

It is important to note that the administration of the STM is not therapeutic (lactulose and mannitol can be used as laxatives, however the amount in the STM is sub-therapeutic) and as such should be regarded as a non-investigational medicinal product (NIMP) [Guidance Documents Applying Investigational Medicinal Products (NIMPS), 2011].

7.1. STM Administered

Study Treatment Name	Lactulose (4-*o*-β-D-galactopyranosyl-D-fructofuranose)	Mannitol (D-mannitol) GRAS listed	Sucralose (1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside)
Dosage formulation	oral solution	oral solution	Capsules (powder)
Unit dose strength(s) Adults	5g	2g	2g (3 capsules to deliver total 2g sucralose)
Route of Administration	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal	Oral / nasogastric / nasojejunal (capsules to be opened and contents added to lactulose and mannitol for tube administration)
Preparation and Dosing instructions	For oral administration, the lactulose and mannitol will be prepared as a 100ml drink to be taken with 3 sucralose capsules. For feeding tube administration, lactulose/mannitol/sucralose will be prepared as a 50ml solution and given via a feeding tube followed by an immediate 50ml drinking water flush Preparation refer to Study Reference Manual together with SoA tables (Section 2)		
Packaging and Labelling	Lactulose and Mannitol will be supplied pre-mixed in an amber bottle (or equivalent) for single use. Each container will be labelled as required per country requirement.		Sucralose will be provided as capsules in a storage container. Each container will be labelled as required per country requirement.
Manufacturer	Tayside Pharmaceuticals, UK		
Storage	Lactulose/Mannitol formulation should be stored under refrigerated conditions. The sucralose capsules should be stored at room temperature in a dry environment away from direct sunlight.		
Shelf-life	Lactulose/Mannitol pre-mix formulation and sucralose capsules supplied by Tayside Pharmaceuticals will have at least 3 month shelf-life when stored at the correct storage conditions.		

The preparation of the STM for oral use and nasogastric/nasojejunal tube administration can be found in the Study Reference Manual.

7.2. Dose Modification

Dose modification will not be required. Unit dose is described in Section 7.1.

7.3. Method of STM Administration: Treatment Assignment

There is no element of randomisation in the study and all study participants will receive the STM according to the relevant SoA. The method of administration can be found in the Study Reference Manual.

7.4. Blinding

No GSK study treatment will be employed in this study. All participants will receive the same STM and all thermal injury participants will perform the same study procedures.

7.5. Preparation/Handling/Storage/Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all STM received and any discrepancies are reported and resolved before use of the STM.
2. Only participants enrolled in the study may receive STM and only authorized site staff may supply or administer STM unless adequate training is provided such as in the case of healthy participants. All STM must be stored in a secure, temperature controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for STM accountability, reconciliation, and record maintenance, as needed.
4. Further guidance and information for the final disposition of unused STM are provided in the Study Reference Manual.
5. Under normal conditions of handling and administration, STM is not expected to pose significant safety risks to site staff.

7.6. STM Compliance

- When participants undergo intestinal permeability testing at the site, they will receive STM directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of STM and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the STM.

- If healthy participants need to prepare and administer the STM off-site such as at home, STM training will be provided and a record maintained by the investigator or designee.

7.7. Concomitant Therapy

- Refrain from consumption of the following for **24 hours** before and after the administration of STM:
  - Lactulose or mannitol-containing laxatives. Study sites will be asked to use movicol (polyethylene glycol) in place of lactulose.
  - Medicines with mannitol as an excipient (chlorthiazide sodium, some albumin preparations, some laxatives, tablets as a bulking agent).
  - Products containing sucralose.
- For healthy participants only, refrain from consumption of aspirin for **48 hours** before taking the STM and for the 24-hour urine collection period see Section 6.6.2.
- For healthy participants only, antibiotic use 2 weeks prior to STM administration and during the study is not permitted.
- Sennoside laxatives should be avoided. These can cause gastrointestinal irritation and may contribute to raised intestinal permeability.
- Additional Glutamine supplementation in excess of that delivered with a standard feeding protocol should be avoided during the first 28 days of study participation. If supplementation is given inadvertently, then the patient will remain in the study, but the total dose and duration of additional glutamine supplementation must be recorded in the CRF.
- Thermal injury participants that receive Intravenous (IV) mannitol for renal failure or raised intracranial pressure (testing to be delayed until 12 hours after last administration).

7.8. Treatment after the End of the Study

There will be no ongoing STM administration following the end of this study.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of STM administration

Discontinuation of STM administration can be considered by the investigator in the event that an adverse event to the STM is observed. Withdrawal of further STM administration does not require withdrawal from the study.

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

8.2.1. Other Withdrawal Criteria

- A participant will be withdrawn from the study following positive (and confirmed) HIV test at screening; Serologic evidence of active Hepatitis B (HB) infection based on the results of testing for HBsAg, and anti-HBc or a positive test for Hepatitis C antibody confirmed by Hepatitis C RNA or antigen testing. If HCV RNA is not available, then the positive test for Hepatitis C antibody alone would be exclusionary. Results must be discussed with the medical monitor to withdraw the participant from the study and commence therapy according to local practice.
- Healthy participants that are treated with antibiotics during the duration of the study.
- Participants that experience signs and symptoms of gastro-intestinal infections during the duration of the study.
- Withdrawals related to mental capacity as described in Section 6 and Appendix 3.
- Participants that received haemodialysis during the first 48 hours of the study (i.e. during the first measurement of intestinal permeability) will be excluded from the evaluable population and a replacement will be recruited.
    - Participants that received haemodialysis during later time points will not be excluded, but consideration will be given to recruiting an additional participant if 3 or more intestinal permeability measurements occur concurrently with haemodialysis.

8.3. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the study site for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining

the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed.
- Safety concerns related to the STM should must be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue to be administered the STM.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the thermally injured participant's routine clinical management (e.g., weight measurement) and obtained before signing of ICF may be used for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA. Procedures (the administration of STM) are not part of routine care for either healthy or thermal injury participants.
- Healthy participants will be asked to donate a single blood sample on Day 1. No blood collection is specified in the SoA of this protocol for thermal injury participants (with the exception of HIV, Hepatitis B and Hepatitis C testing at baseline). The results of clinical laboratory blood tests will be recorded in the SIFTI-2 study and the data used in this study. Likewise, blood collection for exploratory biomarker detection will be included in the SIFTI-2 study and the data used in this study.

9.1. Efficacy Assessments

The administration of the STM is for the purpose of intestinal permeability measurement and is not therapeutic, therefore no efficacy will be assessed.

9.2. Adverse Events

9.2.1. Monitoring and reporting responsibilities

Healthy participants will be recruited to the HESTIA study alone and all AEs or SAEs occurring in this group should be managed according to this protocol.

Thermally injured participants recruited to this study will also be recruited to the SIFTI-2 study. The following guidance relates only to AEs or SAEs which the investigator reasonably believes to be the result of a procedure or requirement unique to this (the HESTIA) protocol. All other AEs or SAEs will be reported and managed in accordance with the SIFTI-2 protocol.

Unique procedures and requirements of HESTIA

1. The administration of STM
2. The collection of stool samples
3. Changes to standard of care for thermally injured participants:
    a. The fasts required during the measurement of intestinal permeability
    b. The use of alternative laxatives to lactulose and sennosides.

The definitions of an AE or SAE for this study can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious or that caused the participant to discontinue intestinal permeability measurement with the STM (see Section 8).

9.2.2. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2). However, any SAEs assessed as related to study participation (e.g., STM, protocol-mandated procedures, invasive tests, or change in existing therapy) will be recorded from the time a participant consents to participate in the study.
- All AEs will be collected from the start of unique HESTIA study procedures until the final visit at the time points specified in the SoA (Section 2).
- Medical occurrences that begin before the start of unique HESTIA study procedures but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the STM administration or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.2.3. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. If the participants are conscious, open-ended and non-leading verbal questioning of the

participant is the preferred method to inquire about AE occurrence. For unconscious patients or participants not always able to provide valid verbal responses to open-ended questions, the investigator or designee will need to identify AEs and/or SAEs through relevant clinical signs and/or investigations.

9.2.4. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to follow proactively each participant at subsequent visits/contacts. All SAEs, will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Appendix 4.

9.2.5. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of the study. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information e.g., summary or listing of SAE) from the sponsor will review and then file it and will notify the IRB/IEC, if appropriate according to local requirements.

9.2.6. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as SAEs

The following broad disease related events (DREs) are common in thermally injured participants and can be serious/life threatening:

- Deterioration of condition.
- Death (may be expected in burns of a large surface area).
- Prolongation of hospital stay.
- Persistent or significant disability or incapacity.

Because these events are typically associated with the disease under study, they will not be reported according to the standard process for expedited reporting of SAEs to GSK (even though the event may meet the definition of a SAE). These events will be recorded on the DRE page in the participant's CRF within [the appropriate time frame *agreed*

upon by the SRT for completion of DRE CRF pages]. These DREs will be monitored by clinical study team on a routine basis.

- *NOTE: However, if the investigator considers that there is a reasonable possibility that the event was related to administration of STM or another unique or required element of the study (as defined in Section 9.2.1) then the event must be recorded and reported as an SAE (instead of a DRE).*
- A comprehensive list of further thermal injury related DREs can be found in Appendix 4 (Section 12.4).

9.3. Treatment of Overdose

For this study, an overdose is defined as any dose of STM greater than defined in Section 7.1. No specific treatment is recommended for an overdose and treatment is at the discretion of the investigator. The GSK medical monitor must be notified promptly.

9.4. Safety Assessments

Planned time points for all safety assessments are provided in the SoA and summarised here.

Safety Assessment	**When conducted**	
	Healthy Participants	**Thermally injured participants**
Laboratory tests	Screening. Only repeated if clinically indicated in the opinion of the investigator.	Only if clinically indicated.
Brief Physical Examination including Vital Signs Recording	Screening, Day 1, Day 8, Day 15	As a part of routine clinical care whilst admitted (not protocol specified). Following day 28 and 6 month intestinal permeability measurements (if patient not still admitted)
Detection of AEs	Day 1, Day 8, Day 15	Throughout the study

Safety Assessment	When conducted	
	Healthy Participants	**Thermally injured participants**
Assessment of health status	Screening, Day 1, Day 8, Day 15	Not required

9.4.1. Physical Examinations

- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

9.4.2. Vital Signs

- A **single** vital sign measurement will be obtained at each time point indicated in SoA Table, and will include systolic and diastolic blood pressure and heart rate. Any abnormalities and changes in measurements will be communicated to the medical monitor.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, mobile phones).
- Vital signs to be taken before blood collection for laboratory tests.
- Repeat or unscheduled measurements may be taken at the discretion of the investigator.

9.4.3. Clinical Safety Laboratory Assessment

- All study related laboratory assessments will be performed by a local laboratory. The laboratory reports must be reviewed by the investigator, this review documented and both report and review are to be filed with the source documents.
- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA.
- Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

9.5. Study Procedures

The following procedures will be carried out during the study.

9.5.1. Fluid Balance Measurement

All fluid input and output will be recorded every 24 hours for thermally injured participants.

9.5.2. Wound Healing

Assessment of wound healing will be the time to 95% wound healing. Physical parameters of the wound (e.g., rate of healing) will be recorded and collected as a part of both the HESTIA and the SIFTI-2 studies.

9.5.3. Other Clinical Responses

To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability the following will be recorded and collected as a part of the SIFTI-2 study. Details can be found in the SIFTI-2 study protocol.

- Number of ventilator-free days (ventilator start/restart/end date/time)
- Number of vasopressor-free days (medication chart review)
- Number of hemofiltration-free days (notes review)
- Number of episodes of confirmed infection and sepsis
- Number of surgical interventions
- Total length of hospital stay
- Calculate critical care and thermal injury severity scores

9.6. Pharmacokinetics

PK parameters are not evaluated in this study.

9.7. Pharmacodynamics

PD parameters are not evaluated in this study.

9.8. Intestinal Permeability Assessments

- Intestinal permeability will be determined by measuring the excretion of lactulose, mannitol and sucralose in urine following their enteral administration. It will be conducted in both healthy participants and thermally injured participants at the time points specified in the SoA.
- The complete method for administration of STM and measurement of intestinal permeability is detailed in the SRM.
- Urinary excretion of the orally ingested STM will be quantified using a technique such as capillary column gas chromatography.

- Urine samples will be collected in plastic bottles for analysis. Urine collection will begin immediately following STM administration. Urine samples will be collected over 24 hours post-STM administration. Accurate collection of the total volume voided during this 24 hour period is critical.

- **Sample Preparation**

Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

9.9. Genetics

Genetics are not evaluated in this study.

9.10. Sample Collection for Biomarker Analysis

The following biomarkers will be collected during the study. Details of sample processing, storage and shipping are included in the study reference manual.

Blood, stool and urine samples will be collected and stored. Timing of analyses and selected biomarkers will be dependent on the results of the intestinal permeability tests results.

9.10.1. Blood Biomarkers

Healthy participants

- Blood samples will be taken for healthy participants recruited in this study over the time period specified in the SOA. Blood will be taken adhering to standard operating procedure from venae puncture.
- The results of blood biomarker analysis will be evaluated in this study and compared to measures of intestinal permeability.
- Twenty (20) ml blood will be collected on Day 1 for biomarker analysis, and 20ml blood will be collected at screening for screening tests. The biomarkers to be measured may include, but are not limited to:
  - Markers of microbial translocation
  - Markers of intestinal damage
  - Inflammatory markers: e.g. C-Reactive Protein, Procalcitonin, cytokines (including TNF-a, IL-6, IL-8, IFN-g, IL-10, IL-1b, IL-12p70, IL-17, IL-4, IL13, IL1Ra, MIP1a, MIP1b, MIP2, GCSF, GMCSF, MCP-1, RANTES, HMGB1).

Thermally injured participants

- The blood required for this analysis in thermally injured participants will be collected as a part of the SIFTI-2 (IRAS 200366) study to which all thermally injured participants will be co-recruited. Details of the schedule for blood

collection and the total volume of blood collected can be found in the SIFTI-2 study protocol.

9.10.2. Stool Sample Collection

- Stool samples will be collected from all participants in this study over the time period specified in Section 2, Schedule of activities (SOA). Stool samples will be collected adhering to standard operating procedure.
- For thermally injured participants, the initial sample will be taken as close to time of injury as possible ("first stool sample produced upon admission") and Day 14. Further samples will be taken on day 28 (± 3 days) and at month 6 (± 14 days).
- For healthy participants, a single sample will be collected at study entry (participants will be given a collection container at screening).

9.10.3. Urine Sample Collection

- Urine samples will be collected as a part of the measurement of intestinal permeability which is described in Section 9.4.
- Additional urine samples will be collected from patients as part of the SIFTI-2 study to which all thermally injured participants will be co-recruited. These will be used for, among other tests, the quantification of protein and microbial metabolites.
- It is standard practice that patients admitted with burns of TBSA ≥15% will have a urinary catheter inserted on admission to ensure the accurate maintenance of fluid balance. A clean urine sample will be taken from the appropriate port on the urinary catheter. In patients who are not catheterised, a mid-stream urine (MSU) should be collected in a clean universal container where possible.
- N.B. During the 24 hours following STM administration (during intestinal permeability measurement) urine samples must only be taken from the 5-hour of 24-hour urine collections **after** the aliquots for sugar quantification have been taken.

Sample Preparation

- Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

10. STATISTICAL CONSIDERATIONS

All statistical analyses will be performed using SAS (SAS Institute Inc., Cary, North Carolina, USA), version 9.2 or higher. Before database lock, a reporting and analysis plan (RAP) will be issued as a separate document, providing detailed methods for the analyses outlined below. Any deviations from the planned analyses will be described in a RAP addendum and justified in the final integrated clinical study report.

10.1. Hypotheses

As this is an enabling study designed to better understand the biomarkers of intestinal permeability and other biomarkers in participants with moderate to severe burns, the statistical analysis for this study will be exploratory in order to better understand the parameters to inform future investigational medicinal product studies.

The key factors of interest in this study are to understand (i) the nature of any differences at entry in intestinal permeability between healthy participants and thermal injury participants (ii) to understand the trajectory of changes in intestinal permeability over time.

The key endpoint to be explored is the lactulose:mannitol (L/M) ratio, but other permeability biomarkers will also be explored. The analysis approaches to address these questions are exploratory, but will initially be conducted as outlined in Section 10.5 and Section 10.6.

10.2. Sample Size Determination

Sample size is based on feasibility. No formal calculation of power or sample size has been performed, but a sample size of 15 healthy participants and 25 thermal injury participants (>15% TBSA) should be sufficient to provide useful estimates of variability in lactutose:manitol ratios, and any change in L/M ratio over time.

Although the key aim is to estimate the variability and L/M ratio and assess the difference in L/M ratio between thermal injury and healthy participants, for illustration, a trial including 25 thermal injury and 15 healthy participants would have 89% power to detect a 3-fold difference in L/M ratio between thermal injury and healthy participants using a 2-sided significance level of $p<0.10$. This calculation uses a (log) b etween-subject SD of 1.15, as estimated from the literature [Olquin, 2005].

10.3. Data Analyses Consideration

In general, descriptive summaries will include number of subjects, mean, standard deviation, median, minimum, and maximum for continuous variables. If data are log-normally distributed data will be presented as number of subjects, geometric mean, coefficient of variation, minimum, and maximum; and percent for categorical variables. Summaries will present data by dose level and where appropriate, by assessment time.

10.4. Populations for Analyses

The **Safety Population** will consist of all subjects who receive at least 1 dose of STM and have at least on post-dose safety assessment.

The **Evaluable Population** will consist of all subjects who are entered into the study and have evaluable L/M ratio measurements.

10.5. Statistical Analyses

10.5.1. Safety Analyses

Administration of STM is for the measurement of intestinal permeability. The safety of this administration is not an endpoint of this study, but will be monitored and reported.

All safety data will be presented in data listings. Subject demographics, medical history, and prior and concomitant medications will be summarized using descriptive statistics. For continuous variables, these summaries will include number of subjects, mean, median, standard deviation, minimum, and maximum.

For categorical variables, the summaries will include frequencies and corresponding percentages. No inferential hypothesis testing will be performed on the safety variables.

Adverse events will be coded using the MedDRA classification system.

For healthy participants (who are recruited only to HESTIA), STM-emergent AEs will be defined as any AEs, regardless of relationship to STM administration, that occur after the first dose of STM until the final follow-up visit. The STM-emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. The total number of AEs will be summarized overall. The AEs will be further summarized by severity and relationship to STM. If relationship information is missing, the AE will be considered STM-related. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

For thermally-injured participants, STM-emergent AEs will be defined as any AE deemed related to STM administration that occurs after STM administration until the follow-up visit. The STM-related emergent AEs will be summarized for the overall number of AEs and the percentage of subjects who experience them. Listings for the subsets of SAEs and STM-related SAEs will be provided. The SAEs and number of AEs leading to discontinuation of STM administration will be summarized. The incidence of AEs will also be summarized by system organ class and preferred term.

As laboratory data and vital signs are only collected at screening for healthy participants, these data will be listed only. Clinical laboratory values that are outside of the reference ranges will be flagged and evaluated for clinical significance by the investigator. Physical examination findings will be listed. For thermally-injured participants, physical

examination findings and clinical laboratory values will be highly abnormal and as such any data collected will only be listed. Disease-related findings and changes will not be reported.

10.5.2. Other Analyses

Biomarker exploratory analyses will be described in the RAP.

10.5.3. Interim Analyses

No formal interim analysis will be performed.

10.6. Analyses of lactulose/mannitol ratio

In all analyses the variable TBSA will be a categorical variable defined as "Yes" for thermally injured participants, and "No" for healthy participants.

Differences in permeability at entry

Intestinal permeability biomarkers will be summarised by TBSA group and overall. Data will summarised by geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio) = intercept + TBSA

Trajectory of the L/M ratio over time

Intestinal permeability biomarkers will be summarised over time, by TBSA group and overall. Data will summarise geometric mean, CV, minimum, maximum and N. A model will be fitted, defined as

Log (L/M ratio at time X / L/M ratio at entry) = intercept + Time + TBSA + Time*TBSA.

This will be a repeated measurement analysis and will assess the rate of improvement in L/M ratio over time, and assess how this changes relative to healthy participants. If required, further modelling assessing more complex relationships between L/M ratio and time may be undertaken. Given this is an exploratory study the most appropriate variance-covariance matrix regarding the correlation of data over time will be explored as part of the statistical analysis.

Data from this model may also be used to estimate the time to 50% improvement (or other degrees of improvement) in L/M ratio in relation to L/M ratio values seen in healthy participants. This will be used to assess the clinical relevance and sensitivity of such measures.

A model fitting log (AUC of L/M ratio) = intercept + TBSA will also be fitted. AUC will be calculated using all measurements taken over time. This will provide a summary of the weighted average L/M ratio value over time.

The use of %TBSA will also be assessed in the above analyses as a continuous covariate. The effects of age and Baux score will also be evaluated to understand differences in intestinal permeability in these groups [Osler, 2010].

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

ACR	Albumin/creatinine ratio
AE	Adverse Event
Anti-HBc	Anti-Hepatitis C
ART	Anti-retrovial treatment
CFR	Code of Federal Regulations
D	Day
G	Grams
eCRF	Electronic Case Report Form
ICU	Intensive Care Unit
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GI	Gatrointestinal
HB	Hepatitis B
HBs AG	Hepatitis B Antigen
HCV	Hepatitis C
GSK	GlaxoSmithKline
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISS	Investigator Sponsored Study
L/M	Lactulose/mannitol ratio
mL	Milliliter
MODS	Multi-organ dysfunction syndrome
NIMP	Non-investigational medicinal product
NC	Nominated Consultee
PC	Personal Consultee
PCR	Polymerase Chain Reaction
RAP	Reporting and Analysis Plan
SAE	Serious adverse event
SIFTI-2	A Multi-centre, Prospective Study to Examine the Relationship between Neutrophil Function and Sepsis in Adults and Children with Severe Thermal Injuries
SoA	Schedule of Activities
SRM	Study Reference Manual
STM	Sugar Test Material

TBSA	Total body surface area
TNF	Tumour Necrosis Factor
WOCBP	Women of Child Bearing Potential

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
SAS

12.2. Appendix 2: Clinical Laboratory Tests

- All clinical laboratory tests will be performed in the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 2 Protocol-Required Safety Laboratory Assessments for Healthy Participants

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Hemoglobin Hematocrit		<u>WBC count with Differential</u>: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	Urea	Potassium		Total bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/	Total Protein
	Glucose nonfasting	Calcium	Alkaline phosphatase	
Routine Urinalysis	• pH, glucose, protein, blood, ketones by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• Urine alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)] • Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential)[1] • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :

1. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

12.3. Appendix 3: Study Governance Considerations

12.3.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- Any amendments to the SIFTI-2 protocol which impact on this protocol will be reviewed and may result in changes to this protocol being required. Any such changes will be subject to IEC/IRB approval before implementation.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.3.2. Recruiting participants under the Mental Capacity Act 2005

On admission into hospital the patient's capacity will be assessed. A patient may lack capacity due to the severity of their injury, arriving intubated and ventilated or due to a pre-existing co-morbidity.

Please note, the same process will also be followed for the SIFTI-2 study to which thermally injured participants will be co-recruited.

If a patient does not have the capacity to make an informed decision, the research team will approach a patient's personal consultee. Examples of personal consultees include:

- A family member, carer or friend
- An attorney acting under a Lasting Power of Attorney
- A court appointed deputy, provided that they had a relationship with, or personal knowledge of, the person lacking capacity before their appointment as deputy.

There may be circumstances in which a personal consultee is not available, some examples of this are:

- Where no family member or friend is willing to act as a personal consultee
- Where the family member or friends live a long distance away and/or are not in frequent contact with the patient who lacks capacity
- Where the regular carers of the person who lacks capacity are doing so for payment or in a professional capacity (e.g. care home staff or nurses)
- Where someone is acting on a professional role (e.g. their GP or solicitor)

In this case, a nominated consultee will be required. A nominated consultee is considered to be a medical professional that has no connection to the research trial, but has an understanding of the implications of the research trial on the participant.

In these circumstances, examples of nominated consultees are:

- An emergency department doctor, preferably Consultant level.
- Intensive Care doctor, preferably Consultant level.
- Doctor from the burns team, not directly involved in the research study.

Once a personal or nominated consultee has been identified, they will be provided with a specific information leaflet about the trial. The personal and nominated consultee will be asked if they feel the study would be something the participant would have no objections to. If in their opinion the participant would have no objection to being recruited into a research trial the consultee will be asked to sign a declaration form.

12.3.3. Determining Whether a Participant has Capacity Under the Mental Capacity Act (2005)

Prior to deciding that a patient does not have the capacity to give informed consent the researcher must follow the Mental Capacity Act (2005) to ensure that the participant does not hold capacity. The principles of the MCA which we will adhere are as follows:

- A person must be assumed to have capacity unless it is established that he/she lacks capacity.
- A person is not to be treated as unable to make a decision unless all practical steps to help him/her to do so have been taken without success.
- A person is not to be treated as unable to make a decision merely because he/she makes an unwise decision.
- An act done or decision made, under this Act for or on behalf of a person who lacks capacity must be done, or made, in his best interests.

- Before the act is done, or the decision is made, regard must be had to whether the purpose for which it is needed can be as effectively achieved in a way that is less restrictive of the person's rights and freedom of action.

A decision to appoint a consultee on behalf of a patient will be made if the participant is unable to:

1. (a) understand the information relevant to the decision,
   (b) retain that information,
   (c) use or weigh that information as part of the process of making the decision, or
   (d) communicate his/her decision (whether by talking, using sign language or any other means).

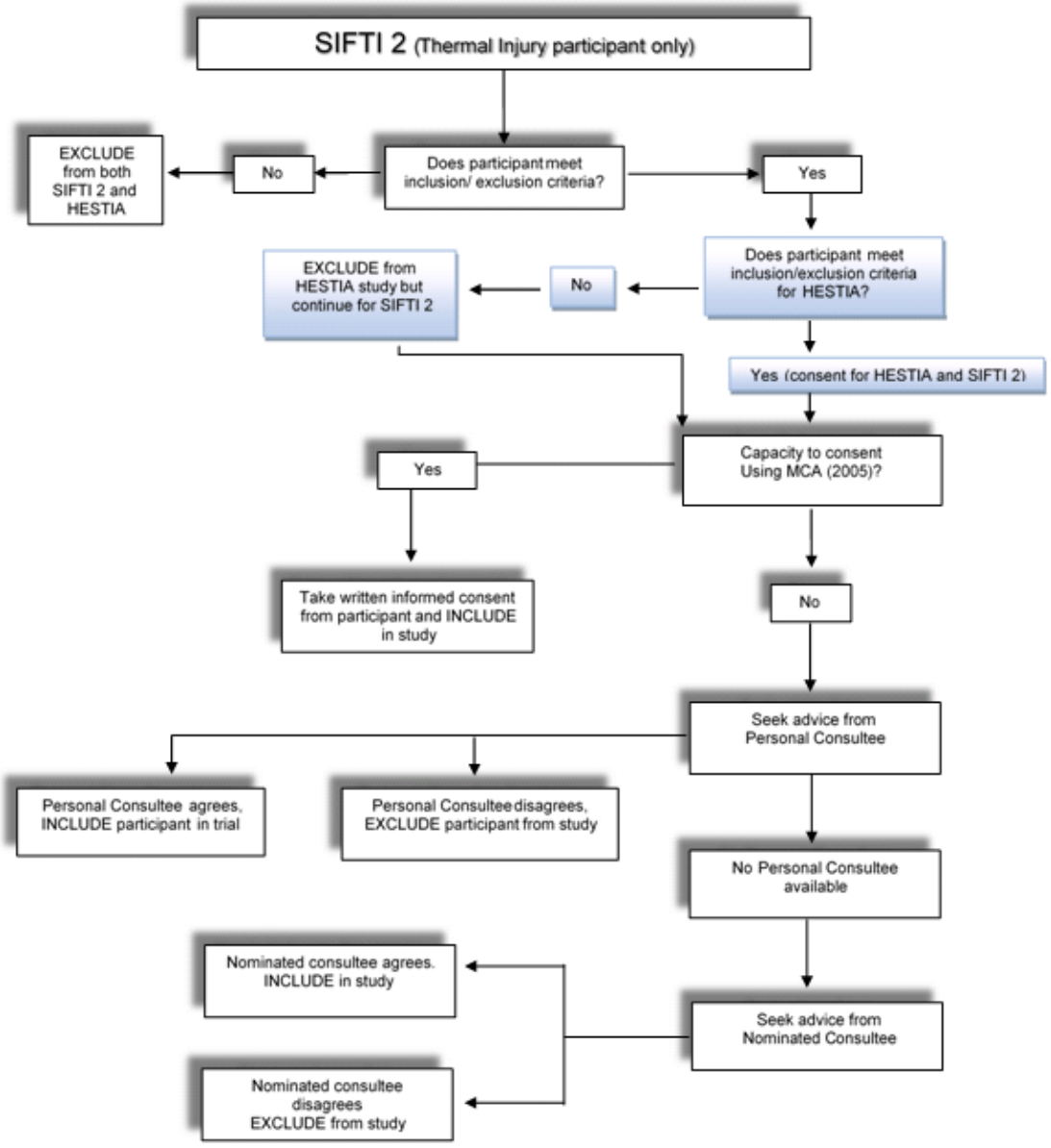
2. A person is not to be regarded as unable to understand the information relevant to a decision if he/she is able to understand an explanation of it given to him in a way that is appropriate to his circumstances (using simple language, visual aids or any other means).

3. The fact that a person is able to retain the information relevant to a decision for a short period only does not prevent him/her from being regarded as able to make the decision.

4. The information relevant to a decision includes information about the reasonably foreseeable consequences of

   (a) deciding one way or another, or
   (b) failing to make the decision.

Figure 4 HESTIA Thermal Injury Participant Recruitment



12.3.4. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.3.5. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her personal or nominated consultee and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants or their personal or nominated consultee will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's personal or nominated consultee.
- Healthy participants who are rescreened are required to sign a new ICF.

12.3.6. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

12.3.7. Committees Structure

An Independent Data Monitoring Committee or similar review group will not be used in this study, but an internal preliminary data review will be conducted.

The Data Review team will consist of the GSK medical monitor, clinical and operational leads, statistician, early development lead and the safety officer. They will meet at intervals specified within the data review charter to review data relevant to the future conduct of the study, and will also assess any risk to study participants.

12.3.8. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

12.3.9. Dissemination of Clinical Study Data

- Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.
- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.
- The procedures and timing for public disclosure of the results summary and for development of a manuscript for publication will be in accordance with GSK Policy.
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

12.3.10. Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the

currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

12.3.11. Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the study specific Source Data Verification document.

12.3.12. Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with study participation, whether or not considered related to the study. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with study participation. • NOTE: As detailed in Section 9.2.1, only AEs deemed to be related to procedures or requirements unique to the HESTIA study will be recorded/reported for thermally injured participants. All other AEs will be recorded/reported through the SIFTI-2 study.

Events Meeting the AE Definition
• Any abnormal laboratory test results (haematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study STM administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. Table 3 provides a list of commonly occurring AEs in participants with severe thermal injury which may meet this definition. • The disease/disorder being studied or expected progression, signs, or symptoms of

the disease/disorder being studied, unless more severe than expected for the participant's condition.

- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Table 3 Complications of severe thermal injury which can be considered as associated with the underlying disease and do not require reporting as AEs unless judged to be more severe than expected for the participant's condition or related to HESTIA study procedures.

System Assessment	Complication type	Action
Airway problems	Failed extubation	Record AE
	Tracheostomy complication	
Breathing Problems	Pneumothorax	Record AE
	Pulmonary Oedema	
	Respiratory Arrest	
	Pneumonia	
	VAP	
	Acute lung injury (ALI)	
	ARDS	
Circulatory Problems	Haemodynamic instability	Record AE
	Increasing vasoactive drug support	Record ionotrope dose in con-meds
	Arrhythmia	
	Endocarditis	
	Acute LVF/CCF	
	Cardiac Arrest	

System Assessment	Complication type	Action
	MI	
Neurological Problems	Reduced GCS (off sedation)	Record AE
	Intra-Cranial bleed	
	CVA	
	Acute confusion/Delirium	
	Meningitis-bacterial	
Oedema Complications	Abdominal Compartment Syndrome (ACS)	Record AE
	Acute Limb compartment syndrome	
Microbiological problems	Sepsis	Record AE Record in Microbiology form
	Chest Infection	
	Lower Respiratory Tract Infection	
	UTI	
	Bloodstream Infection (BSI)	
	Wound infection	
	Intra-vascular catheter (line) infection	
	Infective diarrhoea	
	Clostridium difficile infection/pseudomembranous colitis	
Renal/Urology problems	Acute rhabdomyolysis	Record AE Ensure biochemistry and CK results recorded n CRF
	Acute renal failure	
	Acute urinary retention	
	Renal replacement therapy	

System Assessment	Complication type	Action
Thromboembolic complications	Lower limb DVT	Record AE with location of thrombus
	Upper limb DVT	
	Pulmonary embolism	
	Other VTE	
	Fat embolism	
Wound complications	Major graft loss	Record AE with details
	Major skin substitute loss	
	Wound infection	
	Invasive wound infection	

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

Please note that, as described in Section 9.2.6 a, b, c and d below are considered as 'disease related events' as they occur commonly in patients following thermal injury unless, in the opinion of the investigator, they are directly related to STM administration or other unique requirements of the HESTIA study.

A SAE is defined as any untoward medical occurrence that:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE

should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

If any of the above are observed, then an AE will be recorded in the SIFTI2 study CRF.

If any of the above are observed and deemed to be related to STM administration or other unique requirements of the HESTIA study, then to be recorded in the HESTIA CRF and reported to GSK as per guidance below.

Recording AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the

participant number, will be redacted on the copies of the medical records before submission to GSK.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between administration of the NIMP (STM) and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to STM administration will be considered and investigated.
- The investigator will also consult the Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to GSK.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality

assessment.

- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor by telephone.
- Contacts for SAE reporting can be found in SRM.

SAE Reporting to GSK via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight

mail or courier service.

- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in SRM.

12.5. Appendix 5: Origin of Data/Samples

The following table of study objectives specifies, for each objective, the provenance of clinical data and samples which will be used to explore that endpoint

Objective	Endpoint	Origin of Data/Samples
Co-Primary		
1. To determine the impact of thermal injury on the magnitude of small intestinal permeability change as soon as possible following injury compared to healthy participants	• Lactulose/Mannitol (L/M) ratio at entry	• HESTIA study
2. To characterise the effect of thermal injury on small intestinal permeability over time and establish the clinical and demographic factors which can influence it	• Changes in L/M ratio over time	• HESTIA Study
Exploratory		
3. To determine the impact of thermal injury on colonic permeability as soon as possible following injury compared to healthy participants	• Fractional excretion of sucralose at entry	• HESTIA Study
4. To characterise the effect of thermal injury on colonic permeability over time	• Changes in the fractional excretion of sucralose over time	• HESTIA Study
5. To assess the relationship between severity of a participant's condition following thermal injury and changes in intestinal permeability†	• Number of ventilator-free days • Number of vasopressor-free days • Number of hemofiltration-free days • Number of episodes	• All clinical data will be obtained from SIFTI2. • Permeability measurements will be obtained from HESTIA

Objective	Endpoint	Origin of Data/Samples
	of confirmed infection and sepsis • Number of surgical interventions • Total length of hospital stay • Calculate critical care and thermal injury severity scores	
6. To assess plasma and urine biomarkers of intestinal permeability, bacterial translocation and renal tubular dysfunction following thermal injury†	• Change in markers of intestinal mucosal damage samples from blood • Change in urine protein:creatinine and urine albumin:creatinine ratios	• Blood biomarkers obtained from SIFTI-2 (and HESTIA for healthy participants) • Urine for microbial metabolite analysis, claudin 3 and KIM 1 obtained from SIFTI-2 (and HESTIA for Healthy participants) • Urine albumin:creatinine and protein:creatinine ratios obtained from SIFTI-2 (and HESTIA for healthy participants) • Permeability data (STM absorption) from HESTIA
7. To assess the impact of thermal injury and intestinal permeability on the intestinal microbiome compared to healthy participants	• Changes in microbiome of acute and convalescent stool samples	• Stool samples collected in HESTIA protocol • Permeability data from HESTIA
8. To assess the impact of pre-existing co-morbid conditions on intestinal permeability and clinical outcome following thermal injury†	• Medical history and drug history at the time of admission	• Medical History data from HESTIA • Permeability data from HESTIA
9. To assess wound healing	• Time to wound recovery (e.g. 95%)	• Wound healing assessment data from clinical notes will be captured in HESTIA at 14

Objective	Endpoint	Origin of Data/Samples
		day, 28 day and 6 month visits.
10. To characterise intestinal microbiota, and correlate its composition with both intestinal permeability and bacterial detection in blood†	• Changes in intestinal microbiome • Bacterial markers of translocation	• Microbiome data from HESTIA study • Blood Biomarker data from SIFTI-2 (thermally injured participants) and HESTIA (healthy participants.

12.6. Appendix 6: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

Amendment 01 (15-Aug-2017)

Overall Rationale for the Amendment: The interim analysis has been removes since lactuose and mannitol data in healthy controls was unlikely to change the required sample size in thermally injured participants.

Section # and Name	Description of Change	Brief Rationale
10.5.3 Interim Analysis	Removed preliminary review of lactulose/mannitol ratio data.	Analysis of lactulose and mannitol data in healthy controls would be unlikely to change the study size, and will no longer be performed.
5.2 Number of Participants	Included withdrawn participants may be replaced.	To ensure the target number of evaluable population is maintained.
5.4.4 Preliminary Data Review	Modified the preliminary data review.	To describe the change in preliminary data review.
Synopsis	Updated preliminary data review.	For consistency.
6.5 Exclusion Criteria	Revision to eligibility criteria to exclude hepatitis infection for thermally injured participants. Addition the personal consultee will not be notified if a patient lacking mental capacity was found to be HIV positive.	Consistency correction. Hepatitis screening will be performed. To provide Ethic Committee reassurance and address any ambiguity of this information.
8.2.1 Other Withdraw Criteria	Added the withdraw of participants receiving hemodialysis.	Renal replacement therapy (i.e. dialysis) may filter out lactulose and mannitol.
9.2.2 Time Period and Frequency for Collecting AE and SAE Information	Added: Any SAEs assessed as related to study participation will be recorded from the time a participant consents. All SAEs will be recorded and reported to the sponsor or designee immediately and under no	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April 2017.

Section # and Name	Description of Change	Brief Rationale
	circumstance should this exceed 24 hours.	
9.4 Clinical Safety Laboratory Assessments	Removal of if laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory.	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April 2017.
Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting	Reporting SAE to GSK if the electronic system is unavailable.	To align with the updates to Clinical Protocol Template (Vol 7) effective 7th April 2017.

DOCUMENT HISTORY	
Document	**Date**
Amendment 2	*26-Sep-2017*
Amendment 1	*15-Aug-2017*
Original Protocol	*02-Mar-2017*