TITLE PAGE

Division: Worldwide Development
Information Type: Protocol Amendment

Title:	Phase I Trial to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Single Doses of Topical GSK1278863 in Healthy Volunteers and Diabetic Patients, and Repeat Doses of GSK1278863 in Diabetic Patients for the Treatment of Diabetic Foot Ulcer

Compound Number: GSK1278863

Development Phase: I

Effective Date: 31-JUL-2016

Protocol Amendment Number: 8

Author (s):

PPD

Clinical Pharmacology Science and Study Operations
Clinical Pharmacology Science and Study Operations
Discovery Medicine Heart Failure DPU, MPC
Discovery Medicine Heart Failure DPU, MPC
Clinical Statistics, MPC
Clinical Pharmacokinetics, Modeling and Simulations
Tahoma Research
Tahoma Research
University of Texas Southwestern Medical Center

Revision Chronology

GlaxoSmithKline Document Number	Date	Version
2011N130130_00	2012-SEP-07	Original
2011N130130_01	2012-NOV-21	Amendment No.:01
This amendment reflects changes to the patient eligibility criteria, stopping criteria and sampling time points per regulatory feedback.		
2011N130130_02	2013-FEB-14	Amendment No.: 02
This amendment reflects changes to DFU patient eligibility criteria number 7 and screening procedures in Part A as well as minor edits for clarity.		
2011N130130_03	2013-JUN-06	Amendment No.: 03
This amendment clarifies an eligibility criterion and DFU clinical assessments as well as minor edits for clarity.		
2011N130130_04	2014-JAN-29	Amendment No.: 04
This amendment reflects changes in the container type used to supply the bulk ointment. Minor edits are also included for clarity.		
2011N130130_05	2014-JUN-02	Amendment No. 05
This amendment reflects modifications to the DFU inclusion and exclusion criteria, hemoglobin stopping criteria and a change in biopsy collection expectations. Minor edits are also included for clarity.		
2011N130130_06	2014-JUN-09	Amendment No. 05
Amendment 5 was republished before distribution, with the DNG number of 2011N130130_06, in order to correct an error.		
2011N130130_07	2014-NOV-19	Amendment No. 06
Due to emerging data, specific areas of Part B of the protocol were redesigned to better meet the needs of the study. To date Part A GSK1278863 systemic exposure is below analytical limits of quantification and therefore the stand alone single dose assessement in Part B is no longer required. Eclusion criteria number 10 was adjusted to better reflect the patient population. Other minor edits were also included for clarity.		
2011N130130_08	2016-JAN-12	Amendment No. 07
This amendment contains modifications to inclusion/exclusion criteria. Other changes include updates to Part B that occurred in stream based on emerging data, but did not require a protocol amendment. Other minor edits for clarity.		
2011N130130_09	2016-JUL-31	Amendment No. 08
This amendment provides the addition of lactating/nursing females to the exclusion criteria and includes the rationale for the modifications to the inclusion/exclusion criteria in amendment 7 per regulatory feedback.		

SPONSOR SIGNATORY

PPD

31 July 2016

Laura Demopoulos, MD
SM Director,
MPC Performance Unit

Date

PPD

SPONSOR/MEDICAL MONITOR INFORMATION PAGE

Medical Monitor and Sponsor Contact Information:

Role	Name	Day Time Phone Number	After-hours Phone/Cell/ Pager Number	Fax Number	GSK Address
Primary Medical Monitor	PPD PPD MD	PPD			709 Swedeland Rd. King of Prussia, PA 19406
Secondary Medical Monitor	PPD MD				709 Swedeland Rd. King of Prussia, PA 19406
Tertiary Medical Monitor	PPD MD				UM2523 709 Swedeland Rd. King of Prussia, PA 19406

Sponsor Registered Address:

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

In some countries, the clinical trial sponsor may be the local GlaxoSmithKline affiliate company (or designee). If applicable, the details of the alternative Sponsor and contact person in the territory will be provided to the relevant regulatory authority as part of the clinical trial application.

Regulatory Agency Identifying Number(s): IND 114,886

INVESTIGATOR PROTOCOL AGREEMENT PAGE

For protocol number PWH115760

I confirm agreement to conduct the study in compliance with the protocol, as amended by this protocol amendment.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name:	
Investigator Address:	
Investigator Phone Number:	

Investigator Signature	Date

TABLE OF CONTENTS

PAGE

ABBREVIATIONS

2-D	Two dimensional
°C	Degrees Celsius
μg	Microgram
ABI	Ankle Brachial Index
ACS	Acute coronary syndrome
AE	Adverse Event
ALT	Alanine aminotransferase (SGPT)
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
AST	Aspartate aminotransferase (SGOT)
ATP	Antiprothrombin antibody
AUC	Area under concentration-time curve
AUC(0-∞)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
AUC(0-τ)	Area under the concentration-time curve over the dosing interval
AUC(0-t)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a subject across all treatments
BUN	Blood urea nitrogen
Cavg	Average concentration
CDC	Centers for Disease Control
Chem	Chemistry
CL	Systemic clearance of parent drug
cm^2	Centimeters squared
Cmax	Maximum observed concentration
CO_2	Carbon dioxide
COX 2	Cyclooxygenase 2
COX 4	Cyclooxygenase 4
CPK	Creatine phosphokinase
CPSSO	Clinical Pharmacology Science and Study Operations
C_{ss}	Concentration at steady-state
CVA	Cerebrovascular accident
CYP2C8	Cytochrome P4502C8
DBF	Database freeze
Db/db Mice	Leptin activity deficient mice which are obese and diabetic
DFO	Desferoxime
DFU	Diabetic foot ulcer
DMOG	4-Amino-4-deoxy-N10-methylpteroylglutamyl-γ-glutamate
DMPK	Drug Metabolism and Pharmacokinetics
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EGLN 1, 2, 3	Egl nine homolog 1, 2, 3

ELISA	Enzyme-linked immunosorbent assay
EPCs	Endothelial progenitor cells
EPO	Erythropoietin
F	Bioavailability
FDA	Food and Drug Administration
FGF	Fibroblast growth factors
Flux	Rate of drug transfer per unit of surface
FSH	Follicle Stimulating Hormone
FTIH	First time in humans
g/dL	Grams per deciliter
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilence
GGT	Gamma glutamyltransferase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GLUT1	Glucose transporter 1
GLUT3	Glucose transporter 3
GSK	GlaxoSmithKline
$^{2}H_2O$	Deuterated or heavy water
h/hr	Hour(s)
Hb	Hemoglobin
HbA1c	Glycosylated hemoglobin
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
Hema	Hematology
Hep B	Hepatitis B
Hep C	Hepatitis C
HIF	Hypoxia-inducible factor
HIFα	Hypoxia-inducible factor alpha
HIS	Hyperspectral imaging
HIV	Human Immunodeficiency Virus
HK1,2	Hexokinase-1, 2
HMOX1	heme oxygenase (decycling) 1
HO-1	Heme oxygenase 1
HPLC	High-performance liquid chromatography
HRT	Hormone replacement therapy
hsCRP	High sensitivity C-reactive protein
HIS	Hyperspectral imaging
HVT	Healthy volunteers
IB	Investigator's Brochure
ID	Identification
IC50	Half maximal inhibitory concentration
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDSL	Integrated Data Standards Library
IL	Interleukin (6,1b,8 and 10)

IgM	Immunoglobin M
IND	Investigational New Drug
INR	International normalized ratio
IP	Investigational Product
IRB	Institutional Review Board
IU	International Unit
IUD	Intrauterine device
IUS	Intrauterine system
IVRS	Interactive voice response system
L	Liter
LDH	Lactate dehydrogenase
LSLV	Last subject last visit
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
MI	Myocardial infarction
MIU/ml	Milli-international units per milliliter
mL	Milliliter
mm^3	Millimeters cubed
mmHg	Millimeters of mecury
$MMP_{1, 2, 9}$	Matrix metalloproteinase-1, 2 9
mRNA	Messenger RNA
MRSA	Methicillin-resistant Staphylococcus aureus
MSDS	Material Safety Data Sheet
Msec	Milliseconds
NCBP	Non-childbearing potential
ng.h/mL	Nanogram hours per milliliter
ng/mL	Nanograms per milliliter
NO	Nitric oxide
NOAEL	No observed adverse effect level
NOS	Nitric oxide synthase
PD	Pharmacodynamic
PDGF	Platelet-derived growth factor
PF4	Platelet factor 4
pg/mL	Picograms per milliliter
PHD	Prolyl hydroxylase
PK	Pharmacokinetic
pmol/L	Picomoles per liter
PO	Per os (by mouth)
PV	Pulse volume
PVR	Pulse volume recording
QT	QT interval
QTc	QT interval corrected for heart rate
RAP	Reporting and Analysis Plan

RBC	Red blood cells
RD	Repeat dose
RNA	Ribonucleic acid
rtPCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event(s)
SAS	Statistical Analysis Software
SD	Single dose
SD	Standard deviation
SE	Standard error
SDF-1α	Stromal cell-derived factor-1 alpha
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SoC	Standard of Care
SOP	Standard Operating Procedure
SPM	Study Procedures Manual
SPP	Skin perfusion pressure
t½	Terminal phase half-life
T-cell	T lymphocytes
TcPO2	Transcutaneous PO2
TE	Tris EDTA buffer solution
TGF	Stromal cell-derived factor-1
$TGF\beta_{1,2}$	Stromal cell-derived factor-1 beta 1 and beta 2
TIA	Transient Ischemic Attack
TIMP	Tissue inhibitors of metalloproteinase
Tlag	Lag time before observation of drug concentrations in sampled matrix
Tmax	Time of occurrence of Cmax
TNFα	Tumor necrosis factor
TNF9α	Tumor necrosis factor
TSP-1	Thrombospondin-1
ULN	Upper limit of normal
US	United States
VEGF	Vascular endothelial growth factor
ULN	Upper limit of normal
w/w	Weight for weight
WBC	White blood cells
T	Dosing interval

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
ARANZ Medical Silhouette
Chiron RIBA
OCT
SensiLase System
WinNonlin

1. INTRODUCTION

Diabetes is a leading cause of non-traumatic amputation. The estimated incidence of diabetes in the US exceeds 1.5 million new cases annually, with an overall prevalence of 23.6 million people or 7.8% of the nation's population [CDC, 2008]. One of the most common complications of diabetes in the lower extremity is diabetic foot ulcers (DFUs). There are over 850,000 diagnoses made in the US each year and an estimated 15% of patients with diabetes will develop a lower extremity ulcer during the course of their disease. In addition, 7% to 20% of patients with foot ulcers will subsequently require an amputation. Despite standardized protocols for wound care, chronic foot ulcers are the most common reason for hospitalization in the diabetic population. [Boulton, 2005; Reiber, 1999]. Furthermore, attempts to develop new pharmacologic therapies, mostly cytokine-based, have been largely unsuccessful. In addition to the associated physical symptoms and economic burden to the health care system, a diabetic foot ulcer can have detrimental effects on patient quality of life, impacting almost every life aspect including psychological, physical, social, and economic functioning [Goodridge, 2006]. DFUs are a medical, economic, and social problem today that is expected to increase exponentially over time.

Even in the absence of diabetes, wound healing is a complex, multi-stage process involving interactions among epidermal and dermal cells, gene signaling, and the extracellular matrix, as well as influences on angiogenesis and signaling between a multitude of local as well as plasma derived proteins. Effective healing requires a rapid, but transient inflammatory response which produces mediators including cytokines and growth factors, leading to the proliferation of dermal fibroblasts and keratinocytes. These cells migrate, differentiate and generate an extracellular matrix to form an initial barrier. Differentiation of dermal precursor cells, coupled with an angiogenic response, induces re-epithelialization and wound healing [Yamaguchi, 2010].

In diabetics, these healing processes are disrupted. Functional impairments include reductions in the proliferation of fibroblasts, endothelial cells, and keratinocytes; decreased macrophage function, decreased or impaired growth factor production [Galkowska, 2006], reduced collagen synthesis and granulation tissue, reduced angiogenesis, delayed re-epithelialization and impaired endothelial progenitor cell (EPC) homing [Falanga, 2005; Galiano, 2004; Mace, 2007; Gallagher, 2007]. An intervention that targets one or more of these pathways could convey significant improvements to wound healing in diabetic patients and thus represent a valuable therapeutic approach for the treatment of diabetic ulcers.

1.1. Background

Closer interrogation of the wound healing pathways impaired in the diabetic patient reveals a significant overlap with those induced during hypoxic stress and commonly attributed to the hypoxia-inducible factor (HIF). [Li, 2007; Thangarajah, 2009; Ceradini, 2004; Gallagher, 2007]. The transcriptional regulator, HIF, is a pivotal molecular switch that governs the adaptation to hypoxic insults. HIF regulates an array of target genes which induce erythropoiesis (erythropoietin/EPO), vasoregulation (NOS2, hemeoxygenase I), angiogenesis (VEGF, SDF-1α), and a switch to glycolytic

metabolism, as well as cellular/tissue preconditioning, cell survival and effects on inflammatory mediators [Li, 2007; Thangarajah, 2009; Ceradini, 2004; Semenza, 2004; Lukashev, 2006; Gallagher, 2007].

While the precise mechanisms that underpin impaired wound healing in the diabetic are unknown, a recent investigation has revealed a link between elevated glucose levels and diminished HIF response due to decreased binding of HIF to the co-activator p300 [Thangarajah, 2009]. The relationship between decreased HIF activity and impaired would healing is further demonstrated by the diminished angiogenic response and delayed burn wound healing in HIF heterozygote mice as compared to wild type littermates [Zhang, 2010]. Evidence also suggests that HIF is induced in wounds rapidly, and both local hypoxia and inflammatory cytokines such as TNFα may contribute to such increases [Albina, 2001]. Interestingly, the expression of HIF is impaired in diabetic animal models with chronic ischemia and with age. For example, Liu et al. demonstrated that 6 month old db/db mice have much lower HIF levels than 2 month old mice, and this deficiency is closely associated with an impaired ability in these older mice to heal a cutaneous wound [Liu, 2008]. Furthermore, HIF appears critical to migration of human dermal fibroblasts through regulation of heat shock protein-90alpha which promotes cell motility [Li, 2007]. Notably, HIF may play a regulatory role in T-cell function, serving to limit the secretion of inflammatory cytokines, and in adenosine receptor regulation, which is important to inflammatory signaling and supports tissue protection [Lukashev, 2006; Sitkovsky, 2004; Eltzschig, 2004].

Active HIF may also play a role in improving energy metabolism during ischemic conditions. Increased activation of HIF is linked to a shift toward glucose utilization, by evidence of increased enzyme expression found in the glycolytic pathways (GLUT 1, GLUT 3, LDH HK1, 2) as well as mitochondrial adaptations (e.g., increased expression of COX_{4-2}) that may maximize metabolic efficiency [Fukuda, 2007; Chang, 2007]. Notably, in genetic mouse (PHD1 knockout) studies, muscle tissue is protected in the midst of glycolytic shifts, suggesting the preservation of ATP generation may provide protective benefit during ischemic or low blood flow conditions such as in diabetic foot ulcers. [Aragonés, 2008; Liu, 2008]

Several therapeutic modalities have been explored in animal models to evaluate whether enhancement of HIF signaling improves the wound healing process. Intramuscular gene delivery of stabilized HIF DNA constructs in diabetic mice has been shown to increase circulating angiogenic cell number and accelerate healing [Liu, 2008]. Similar studies using viral delivery of stabilized HIF also demonstrate improved wound healing in diabetic mice [Botusan, 2008]. In addition to HIF over-expression studies, several investigators have evaluated the potential for a therapeutic role of prolyl hydroxylase (PHD) inhibition in these models. HIF prolyl hydroxylases play an important role linking oxygen sensing to HIF activity and provide a vital link to transmit hypoxia signals to HIF-mediated transcriptional machinery. Under normoxic conditions, HIFα subunits degraded by proteosomes lead to a decrease in target gene transcription. The degradation of HIFα subunits is dependent on the hydroxylation of two conserved proline residues in the subunits and subsequent interaction with von Hippel-Lindau tumor suppressor protein. This hydroxylation step is carried out by oxygen-requiring PHD enzymes (egl nine homologous proteins, e.g., EGLN 1, 2 and 3). During inhibition of proline

hydroxylation, HIFα escapes degradation allowing translocation to the nucleus and subsequent transcription of target genes.

Experimental evidence strongly suggests that PHD inhibitors can mimic many key aspects of hypoxia by stabilizing HIF, and these agents are thought to have therapeutic potential for a variety of ischemic diseases, including wound healing. [Bruick, 2001; Myllyharju, 2009; Myllyharju, 2008; Chowdhury, 2008]. Chang et al. demonstrated that desferoxime (DFO) can overcome the negative feedback due to age-related increases in PHD expression and enhance wound healing [Chang, 2007]. In this study, mobilization of angiogenic EPCs and skin flap healing were enhanced with DFO treatment. Furthermore, local administration of another PHD inhibitor, DMOG, was shown to enhance HIF responses and accelerate healing in db/db mice [Botusan, 2008]. Recruitment of circulating angiogenic precursor cells to wounded areas seems to be an important component of enhanced healing induced by DMOG.

Taken together, these data suggest that the HIF pathway is intimately associated with the wound healing process, and that this response is impaired in the diabetic setting. Targeting the inhibition of HIF-prolyl hydroxylase to increase active HIF levels would permit sustained or augmented production of HIF target genes and therefore be expected to have a positive impact on wound healing.

1.2. Clinical Experience with GSK1278863

This study will be the first experience with GSK1278863 administered as a topical agent. All previous clinical experience with the compound has been through oral administration, and the information related to oral administration may be found in the investigator brochure [GSK1278863 Investigator's Brochure GlaxoSmithKline Document Number RM2008/00267/07].

No human data are available to confirm which of the wound healing processes (e.g., inflammation, angiogenesis, cellular metabolism, or collagen production) is most affected by treatment with GSK1278863. An important objective for this first experience in humans will be to understand the kinetics of topical delivery of GSK1278863 and to correlate the clinical outcome with the kinetics and biologic effects of this investigational wound care product. This correlation will aid in establishing the appropriate use of GSK1278863 in the management of diabetic foot ulcers. This clinical-biologic model may also represent a useful tool for future evaluation of wound care products.

1.3. Rationale

The purpose of this study is to evaluate the safety/tolerability and pharmacokinetics of GSK1278863, and to describe the effects of GSK1278863 treatment on the clinical characteristics of DFUs as well as the components of the wound healing process (i.e., inflammation, bacterial load, angiogenesis, collagen formation and gene/protein expression for healing related pathways). These assessments are aimed at determining if one or more of these components are linked to therapeutic activity.

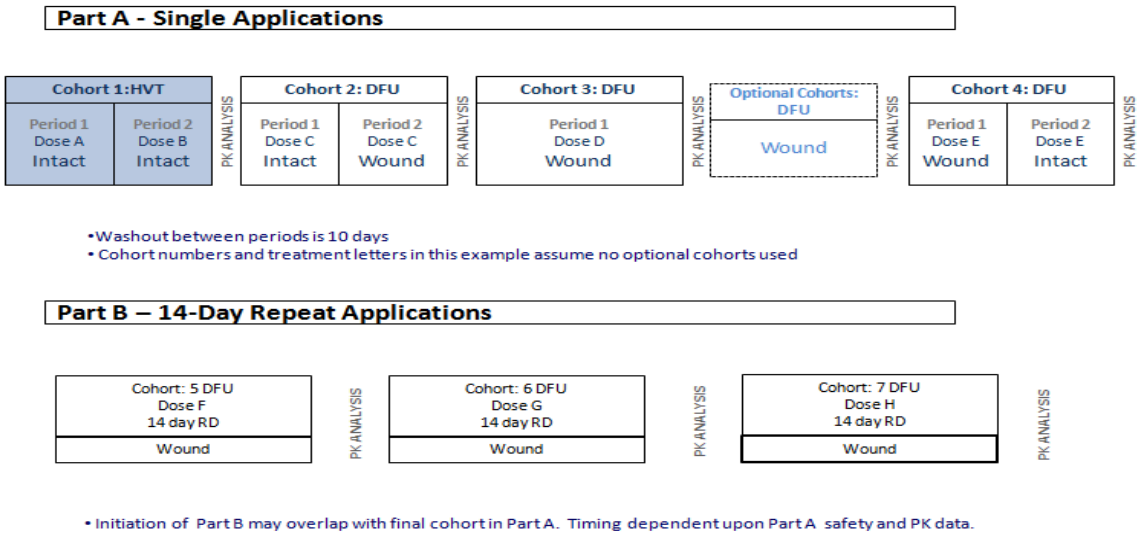
The delivery of the molecule to the site of action is expected to be optimized by local topical application, to elevate target tissue (i.e., skin) exposure while limiting systemic exposure, which could ultimately impart safety limitations due to the potent effect of the molecule on erythropoiesis.

While a number of pathways may be relevant to wound healing, the general hypothesis is that inhibition of HIF-prolyl hydroxylase by GSK1278863 will increase local concentrations of HIF and increase transcription of numerous target genes, notably those related to the angiogenesis, cellular metabolism, cell mobilization and reduction in pro-inflammatory cells (macrophage) or proinflammatory cytokines. Activation of these targets will improve cellular profiles for initial re-epithelialization, wound vascularization and perfusion and, ultimately, would closure.

1.4. Study Design Strategy

This first-time-in-human study of the topical formulation of GSK1278863 is designed as an initial exploration of the safety, tolerability, pharmacokinetic and pharmacodynamic effects. The study will be performed in a cohort of healthy volunteers and then in diabetics, including application both to healthy, intact skin, as well as to diabetic foot ulcers directly. This study proposes to move rapidly into diabetic patients with foot ulcers, as no equivalent model for this disorder exists to understand kinetics and tolerability of the compound. However, to provide some limited understanding of tolerability and kinetics, the study has been designed to gain initial information related to these parameters in healthy individuals with at least two doses of medication prior to entry into the diabetic cohorts. Furthermore, the first diabetic cohort will have the compound applied to intact skin to gauge tolerability in this patient population and to compare PK with that of healthy individuals. Subsequently, the same cohort of diabetic subjects will also receive equivalent dose(s) applied directly to the foot ulcer. A treatment period of two weeks was selected for the repeat dosing segment of the study to permit initial assessment of healing rate and biomarker analyses that are important to inform decisions related to progression of the compound for the indication (Figure 1).

Figure 1 Study Design/Schematic



1.4.1. Dose Rationale

1.4.1.1. Single Dose Strategy

As one of the main goals of this study is to determine the systemic exposure to GSK1278863 following topical administration, this study will follow a dose escalation paradigm. For the purposes of this study, 'dose' will be defined as the total mass of GSK1278863 applied, either to intact skin or DFU. The dose of GSK1278863 applied will be escalated based on safety/tolerability and the pharmacokinetic data from the preceding dose using petrolatum ointment formulations containing 0.05, 0.1, 0.5 and 1% GSK1278863.

Healthy volunteers and patients with DFUs will be recruited for this study, and drug will be applied to both intact skin (from healthy and DFU subjects) and to the DFU directly. There will be approximately six subjects on active drug and approximately two on placebo (6:2) for each single dose cohort. Dose escalation will proceed through the planned dose levels within each part of the study once preliminary safety/tolerability and PK data have been reviewed for at least 4 subjects/patients at the previous dose level. Blood samples will be drawn from pre-dose to approximately 72 hours post-dose in order to fully characterize the systemic pharmacokinetics. The initial sampling scheme can be found in the Time and Events table in Section 4.6.1, and may be changed if deemed necessary upon evaluating emergent pharmacokinetic data.

The decision to proceed to the next dose level of GSK1278863 will be made by the GSK Study Team and the Investigator based on safety (including plasma hemoglobin (Hb) levels), tolerability and preliminary pharmacokinetic and/or pharmacodynamic data obtained in at least 4 subjects for single dosing (SD) (Part A) and 12 subjects for repeat dosing (RD) (Part B) at the prior dose level. The actual doses to be administered may be adjusted based on safety, tolerability and preliminary pharmacokinetic and/or pharmacodynamic data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose. Pharmacokinetic data will be evaluated as appropriate throughout the study to continue dose escalation, or to adjust or discontinue dose escalation as necessary. The chosen doses will be appropriate to prevent the projected Cmax and AUC(0-t) from exceeding 1400ng/mL and 14,800ng.h/mL, respectively (NOAEL exposures from the three-month monkey safety assessment study). Notably, the systemic activity of the compound has robust effects on erythropoiesis. Therefore, doses will be selected to minimize systemic exposure of the compound to limit elevations in hemoglobin. Hence, the exposures will be held to a much lower than NOAEL threshold by selecting maximal dose levels which are predicted to limit systemic exposure at or below levels equivalent to a 15mg oral dose (Cmax 286ng/mL; AUC 650ng.h/mL). (Note: modeling efforts predict that average exposures of the currently targeted highest dose (3mg of the 1% formulation; Cmax 55.3ng/mL; AUC 334ng.h/mL) will be at or below the 10mg oral dose exposures (Cmax 135ng/mL; AUC 319ng.h/mL). The 15mg oral dose has been demonstrated to maintain average rates of hemoglobin elevation to <1g/dL over a 2 week period.

The first cohort of subjects will be healthy volunteers. The starting dose in this cohort will be 0.1% GSK1278863 petrolatum ointment at 25mg/cm^2 to a 12cm^2 section of intact skin (300mg total mass of the formulation) on the foot, which will deliver 300µg GSK1278863. The site of application will be covered with an occlusive dressing. The dosing vehicle will be removed from the skin by washing at 22.5 hours (Note: For practical reasons, a dosing period of approximately 22.5 hours will be followed for both the single and repeat dosing parts of this study. This allows for subject hygiene and dehydration of the skin prior to reapplication when necessary and will allow consistency between the SD and RD portion of this protocol). The second dose will be 10-fold greater, or a 3000µg GSK1278863 dose achieved by using 300mg of the 1% petrolatum ointment formulation. This will be applied to a different 12cm^2 section of intact skin on the lower limb in the same cohort of healthy volunteers following at least a 10 day washout period. Including a cohort of healthy volunteers allows for determination of the irritation potential of the formulation on intact skin before introducing the study drug to patients. Additionally, this will provide a baseline of GSK1278863 absorption kinetics in healthy skin. As petrolatum formulations have been on the market with numerous drugs, a low potential for irritation is expected in humans.

Diabetics with DFU no larger than 16cm^2 will be the second cohort of the study and diabetic patients will be used for the remainder of the dose escalation cohorts. Therefore, the largest anticipated amount of petrolatum ointment to be applied to a study wound is 300mg. In general, doses will be escalated by applying the same amount of ointment (25mg/cm^2) using a higher percentage ointment. However, if the need arises based on emergent data, doses may be escalated by increasing the amount of ointment applied per cm^2 skin/wound diameter (i.e., greater than 25mg/cm^2).

The first dose applied to the intact skin of DFU patients will be determined based on estimates from the systemic exposure observed in the healthy volunteer cohort and the known exposure-response with respect to a hemoglobin response (safety limitation). The first dose in patients will be applied on an area of intact skin (equal in size to their study wound) on the lower limb opposing the side with the study wound. A within subject comparison will be made after an appropriate washout period, by applying the same dose to the diabetic subject's wound. This will allow for pharmacokinetic comparisons between: 1) healthy volunteer and diabetics following administration to intact skin; and 2) intact skin and DFU applications in diabetics.

There will be at least two additional cohorts of diabetic patients. The dose in the second cohort of diabetics will be applied to their DFU only for one dosing session. The final single dose to the last diabetic cohort will be applied first to their DFU and following a sufficient washout (as determined from PK data), the same drug amount applied to the opposite leg on intact skin (matching the area of their DFU). The decision to include a within subject comparison between intact and wounded skin at a higher dose level will allow a test for linearity of pharmacokinetics from the lower dose that included intact and wounded skin. If more than three diabetic cohorts are required in the single dose phase of the study, only the last cohort will include a dosing session on intact skin.

1.4.1.2. Single Dose Selection Rationale

The starting doses and concentrations were chosen after performing theoretical simulations. The simulations assumed that, following application of GSK1278863 to a DFU, the absorption kinetics would be similar to a zero order infusion. The reason for this assumption is that dermal vasculature will be exposed in these wounds and therefore the drug will have direct access to dermal vasculature. The resultant systemic exposure predictions from these simulations are considered the worst case scenario with 90% of applied drug being available for systemic absorption (Table 1). Therefore, 10% of the drug is considered remaining at the site of application or within the applied formulation.

As skin is the site of action, it is assumed that drug will be in the proper vicinity of PHD enzymes and therefore, at concentrations adequate for PHD inhibition. Skin concentrations are predicted to exceed the in vitro derived IC50s (~5-31ng/mL). As the equivalent drug concentration in the ointment formulations range from 0.5 – 10 mg/mL and if similar penetration is attained as was attained from ex vivo human cadaver skin studies (0.21% total absorption from a 0.2% ointment formulation dose), approximately 0.3-6µg of GSK1278863 should penetrate the closest layers of skin. By assuming the wound volume is half a sphere and the compound needs to penetrate 1cm of skin beyond the wound, 10% of the lowest dose (150µg) applied to a 12cm^2 wound would result in a concentration of ~9.3ng/mL. The highest dose prediction resulted in skin concentrations of 186ng/mL. Therefore, local concentrations are expected to be as high as or higher than the IC50s of the three prolyl hydroxylase enzymes even at the lowest dose level. Evidence of positive pharmacology via this topical route has been observed in mice with increases in HIF target genes 8 hours after topical administration of petrolatum formulations containing 0.05 -2% GSK1278863.

To gain insight into the potential systemic exposure from topical administration, a comparison of exposures following administration of similar dose levels via the two routes of administration was performed on data sets available from animal studies. For intact skin, there is evidence of drug penetration from preclinical studies in the mouse and rabbit. A mouse study using topically administered GSK1278863 resulted in systemic exposures at 8 hours post dose for all drug concentrations studied (equivalent to ~0.02, 0.2, 1 and 4mg GSK1278863 applied). Additionally, there were equivalent exposures following application of drug to both wounded and non-wounded skin. Similarly, in the rabbit dermal irritation study, 0.5mL of the 2% formulation (equivalent to ~10mg of GSK1278863) resulted in similar systemic concentrations following administration to both intact and abraded skin. When comparing the Days 1 and 14 exposures (AUC) from this study (topical) to Day 5 exposures (4mg/kg/day which approximates 8-10mg/day) from an orally dosed study in pregnant rabbits, day 5 oral toxicokinetic exposure was approximately 10-fold and ≥100-fold the systemic exposure on Day 14 and Day 1, respectively, following topical administration . The change in ratio is accounted for by skin accumulation of the drug by day 14 compared to day one. There were somewhat lower Cmax ratios of approximately 60:1 and 5:1 on Days 1 and 14, respectively, when comparing oral to topical administration.

In an ex vivo human trunk skin flux study, 0.21% of the total dose of GSK1278863 administered from a 0.2% ointment formulation was absorbed and the flux was found to be variable across the 24 hour study. The mean flux ranged from 0.0002 to 0.0017μg/cm^2/h at 1 and 21 hours post dose, respectively. Given the preclinical skin penetration data from animal and human skin studies, the predicted systemic exposures following application to intact skin in this study should be quite minimal.

Using the fastest flux and the following systemic exposures were predicted.

$$Css = \frac{F \cdot Flux \cdot Area}{CL}$$

Equation 1.

Css = plasma concentration at steady-state

F = presumed bioavailability via the topical route

Flux = rate of drug transfer per unit area of surface

Area = treatment area

CL =systemic clearance

Bioavailability was presumed to be 1% (5-fold greater than ex vivo skin absorption) and CL was assumed to be observed human oral CL/F corrected by an oral F of 34%. This was the largest bioavailability observed in a preclinical species (monkey).

Additionally, simulations of human oral PK from an existing population PK model were performed to predict exposures from 0.15, 0.3, 1.5 and 3mg oral doses. These exposures

were divided by 100 and 60 for AUC and Cmax predictions, respectively, based on Day 1 rabbit PO:topical ratios. The predicted exposures from the above mentioned methods following topical application to intact skin are reported in Table 1 and depicted graphically in Figure 2. The intact and wounded skin predicted mean Cmax exposures at the presently assumed highest dose, even with the most conservative assumptions, are approximately 400- and 5-fold lower, respectively, than the Cmax exposure anticipated to raise Hb. The respective intact and wounded predicted mean AUC exposures are approximately 700- and 2-fold lower than the threshold AUC for Hb elevation.

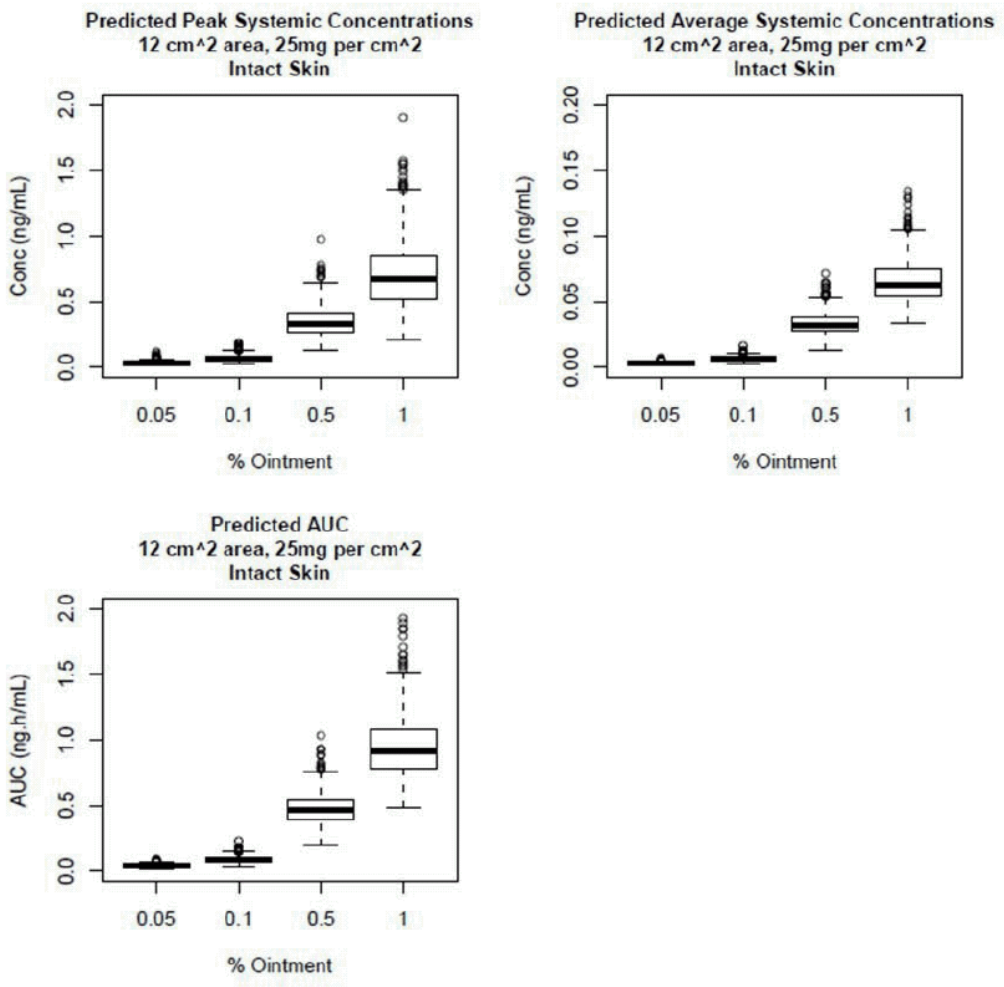
Table 1 Human Exposure Predictions Following Topical Application

Cohort Type	Ointment Strength (%)	Ointment Amount Applied (mg for 12cm^2 area)	Amount GSK1278863 Applied (mg)	Predicted SD Exposure (concentration (ng/mL))[1, 2]	Predicted SD Exposure (AUC (ng*hr/mL))[3,4]
Diabetic Patients (intact skin)	0.05	300	0.15	Css: 0.007 Cmax: 0.01-0.12 Cavg: 0.001-0.007	0.02-0.10
Healthy Volunteers or Diabetic Patients (intact skin)	0.1	300	0.3	Css: 0.013 Cmax: 0.03-0.19 Cavg: 0.003-0.02	0.04-0.23
Healthy Volunteers or Diabetic Patients (intact skin)	0.5	300	1.5	Css: 0.067 Cmax: 0.13-0.98 Cavg: 0.01-0.06	0.2-1.03
Healthy Volunteers or Diabetic Patients (intact skin)	1.0	300	3.0	Css: 0.13 Cmax: 0.22-1.9 Cavg: 0.03-0.13	0.49-1.93
Diabetic Patients (wound)	0.05	300	0.15	Cmax: 0.4-10.1 Cavg: 0.14-2.54	3.39-61.0
Diabetic Patients (wound)	0.1	300	0.3	Cmax: 1.08-17.3 Cavg: 0.28-6.73	6.62-162
Diabetic Patients (wound)	0.5	300	1.5	Cmax: 5.47-142 Cavg: 1.56-24.9	37.4-837
Diabetic Patients (wound)	1.0	300	3.0	Cmax: 7.49-210 Cavg: 3.12-67.5	74.9-1620

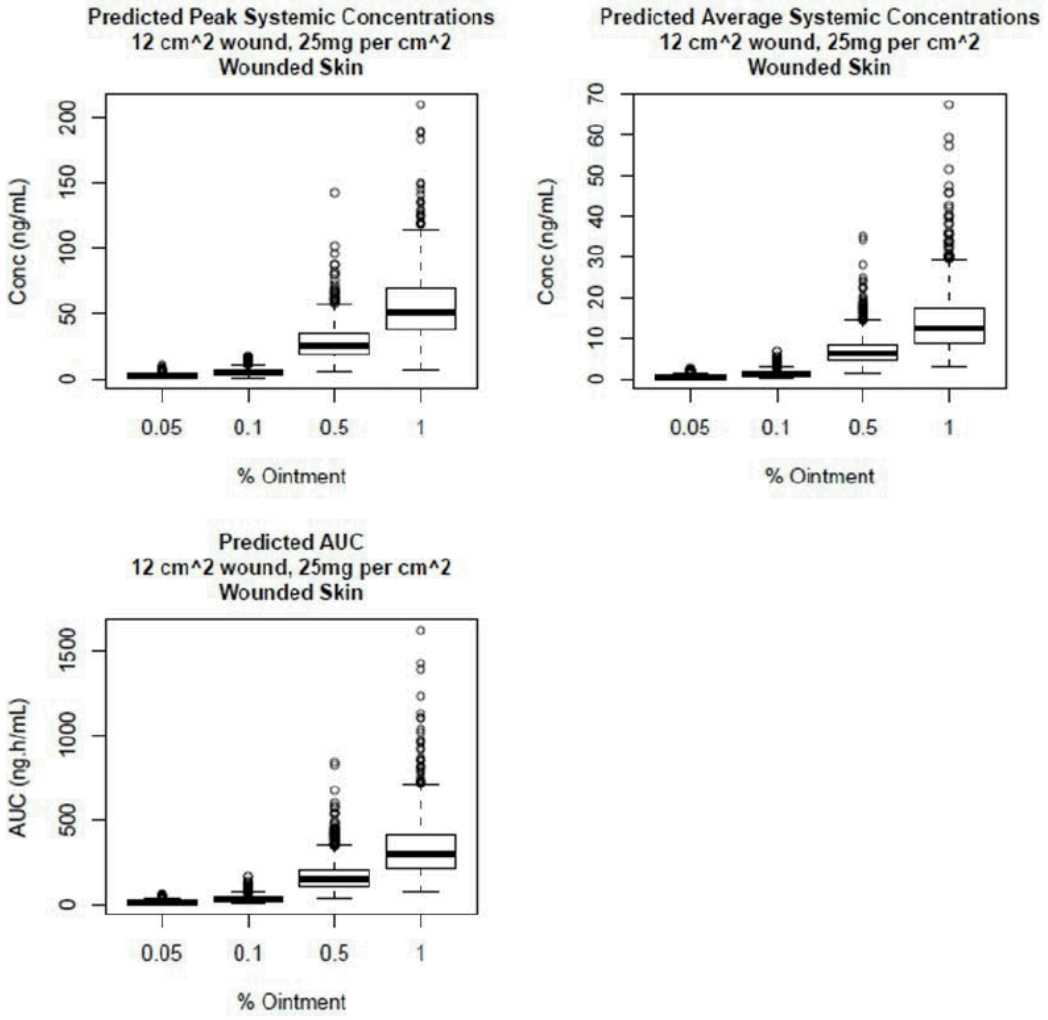
1. Intact skin Css: from human skin flux predictions; Intact skin Cmax and Cavg: from oral dose simulations and applied rabbit po:topical exposure ratios
2. Wounded Skin Cmax and Cavg: from zero order infusion simulations
3. Intact skin AUC: from oral dose simulations and applied rabbit po:topical exposure ratios
4. Wounded Skin AUC: from zero order infusion simulations

Figure 2 Graphical Representation of Systemic Exposure Predictions Following Topical Administration

Intact Skin



Wounded Skin



1.4.1.3. Repeat Dosing

Preliminary safety/tolerability and pharmacokinetic data from single dose applications to DFU subjects will be reviewed prior to the application of repeat doses to investigate the cumulative irritation potential and repeat dose pharmacokinetics of GSK1278863. Similarly, data from each repeat dose cohort will be reviewed prior to dose escalation. There will be approximately 12 subjects on active drug in addition to standard of care (SoC), approximately two on standard of care only, and approximately two on placebo (vehicle) for each repeat dose cohort. Doses will be identified based on pharmacokinetics, systemic exposure level, and its relevance to known effects on Hb, safety and tolerability. The same percent (strength) ointmentwill be applied daily for two weeks. The DFU subjects will have samples drawn for PK analysis at the beginning, midway through the treatment and at the end of the repeat dosing segment. The samples will be collected out to the appropriate time following the last dose on day 14. Additionally, one trough sample will be taken at pre-dose on day 11. The PK sampling

scheme can be found in the Time and Events tables in Section 4.6.2 and Section 4.6.3 and may be changed if deemed necessary upon determining real time pharmacokinetics data.

For practical reasons, a dosing period of approximately 24 hours could not be maintained and therefore a dosing period of ~22.5 hours will be followed for repeat dosing parts of this study. This allows for subject hygiene and dehydration of the skin prior to reapplication of the next dose. The amount of ointment applied to the DFUs may change based on wound size following initial dosing to maintain consistency of amount applied/cm^2 of wound size, which would also result in a change of the total mass of drug applied . If deemed appropriate, intermittent dosing, i.e., a dosing interval greater than 24 hours, may be implemented.

1.4.1.4. Repeat Dose Rationale

Predicting systemic exposures following repeat topical dosing to DFU involves several uncertainties. First, if there is a pharmacologic effect that improves wound healing, the DFU may decrease in size, and therefore less drug will be applied, and theoretically, there will be less penetration via the direct vascular route. Additionally, in rabbits, an approximate 15-fold increase in exposure from Day 1 to Day 14 due to accumulation from repeat daily application has been observed. There may be a depot effect contributing to this increase in systemic exposure over time. It is therefore expected that the systemic pharmacokinetics of GSK1278863 will not remain constant during the repeat dosing phase of this study due to: a) the changing wound characteristics; and b) this possible depot effect. The emergent pharmacokinetic data from the first repeat dose cohort of this study will allow for better predictions for the subsequent repeat dosing cohorts.

1.5. Summary of Risk Management

1.5.1. Risks Relating to GSK1278863

There has been no previous experience in man with topical administration of GSK1278863. The rationale for utilizing topical GSK1278863 therapy for DFU treatment is based on maximizing local concentrations of the active compound while limiting systemic exposure to reduce systemic liabilities. In the current study, a stepwise progression for the compound is utilized both for order of the subjects exposed and for dose level. Initial exposures will first take place in healthy subjects to gain initial tolerability and kinetic information prior to entry in the diabetic population. Further, initial application of the compound to diabetics will take place on intact skin prior to application to the wound. Dose levels will begin at low levels and be escalated only after adequate tolerability and kinetic understanding is obtained. Maximal dose levels that are selected in the escalation process will be those which are predicted to limit systemic exposure to levels at or below levels equivalent to a 15mg oral dose. The 15mg oral dose has been demonstrated to maintain average rates of hemoglobin elevation to <1g/dL over a 2 week period when compared to oral route of administration. In nonclinical studies using the petrolatum formulation to support topical administration to date, no new or unique safety concerns have been identified. Concentrations of GSK1278863 up to 2% w/w in petrolatum were not associated with dermal irritation or sensitization following topical application in rabbits.

In non-clinical studies, oral and intravenous administration of GSK1278863 was associated with gastrointestinal side effects (abnormal feces & emesis) and stomach erosions with bleeding. Thrombosis with ischemia secondary to polycythemia (excessive erythropoiesis) has also been observed.

All previous clinical studies in humans have utilized oral administration of GSK1278863. In these studies GSK1278863 has been generally well tolerated with no clinically significant safety-related findings attributable to the investigational product following doses up to 300mg single dose and up to 100mg once daily for 2 weeks in healthy subjects and 100mg once daily for 4 weeks in patients with anemia of chronic kidney disease, other than potential for robust erythropoietic responses.

Based on what is currently known of the possible roles for HIF-regulated pathways in mediating hypoxia associated pathophysiology, pulmonary artery hypertension and neo-vascularization (e.g., retinal, joint synovium) have been identified as areas of theoretical concern based on the mechanism of action of GSK1278863. No findings relevant to these theoretical concerns have been noted in animal or clinical studies conducted to date.

To minimize risks to participants, eligibility criteria, safety monitoring, and stopping criteria have been incorporated into the study design. Hematologic parameters will be closely monitored throughout the dosing period with guidance provided regarding discontinuation of dosing. Further, where appropriate, specific eligibility criteria or monitoring instructions relevant to theoretical concerns have been included in the study protocol. For example, subjects who are at high risk for thrombotic events or malignancy, or who have underlying retinal vascular disorders, significant pulmonary disease or inflammatory conditions such as rheumatoid arthritis are not eligible to participate. Stopping criteria and guidance for further evaluation are also provided (e.g., gastrointestinal, visual and pulmonary adverse events).

Please refer to the Investigator's Brochure for GSK1278863 for additional pre-clinical and clinical details and Guidance for Investigator [GSK1278863 Investigator's Brochure GlaxoSmithKline Document Number RM2008/00267/07].

2. OBJECTIVE(S)

2.1. Primary

- To evaluate the safety and tolerability of the topical application of GSK1278863 in healthy volunteers and patients with diabetic foot ulcer following single dose (SD) and repeat dose (RD) administration.
- To determine the pharmacokinetics of single and repeat doses of GSK1278863 and its metabolites following SD and RD administration.

2.2. Exploratory

2.2.1. To evaluate the effect of single doses of GSK1278863 on

- Gene expression for HIF targets and genes associated with wound healing in the DFU.
- Local levels of biomarkers (e.g., inflammatory panel for cytokines, proteases).
- Bioburden in the wound (e.g., rtPCR for bacterial load).
- Physical characteristics of the wound (e.g., rate of healing, wound volume).
- Clinical characteristics of the wound (e.g., exudate, granulation tissue, color).

2.2.2. To evaluate the effect of repeat doses of GSK1278863 on

- Gene expression for HIF targets and genes associated with wound healing in the DFU.
- Local levels of biomarkers (e.g., inflammatory panel for cytokines, proteases, inflammatory cell counts, collagen turnover).
- Bioburden in the wound (e.g., rtPCR for bacterial load).
- Biomarkers of angiogenesis via blood flow and oxygen availability [e.g., skin perfusion pressure (SPP) and pulse volume (PV), hyperspectral imaging].
- Tissue concentrations of GSK1278863 after repeat dosing in selected subjects, as site capabilities to perform the assessment permit.
- Circulating biomarkers for HIF activation (e.g., hemoglobin, hepcidin, EPO).
- Physical characteristics of the wound (e.g., rate of healing, wound volume).
- Clinical characteristics of the wound (e.g., exudate, granulation tissue, color)
- The dose-response and/or exposure-response relationships of repeat doses of GSK1278863 using PK/PD modeling for selected primary and exploratory endpoints (as data permit).

3. ENDPOINT(S)

3.1. Primary

- Clinical safety and tolerability data including spontaneous AE reporting, ECGs, vital signs, nurse/physician observation and clinical laboratory values after single and/or repeat doses of GSK1278863.
- Pharmacokinetic parameters of GSK1278863 and its metabolites: SD: Cmax, tmax, terminal t½, tlag, AUC(0-∞), AUC(0-t), (AUC(0-∞) and RD: Cmax, tmax, terminal t½, AUC(0-t), (AUC(0-∞) and accumulation ratio), after single and/or repeat doses of GSK1278863, as data permit.

3.2. Exploratory

3.2.1. After single doses of GSK1278863 (as data permit based on sampling feasibility)

- Change from baseline in gene expression (mRNA) in the DFU tissue.
- Change from baseline in biomarkers in the DFU (e.g., cytokine panel, protease panel).
- Change from baseline on bioburden in the DFU (e.g., rtPCR for bacterial load).
- Physical parameters of the wound (e.g., rate of healing, wound volume).
- Clinical characteristics of the wound (e.g., exudate, granulation tissue, color).

3.2.2. After repeat doses of GSK1278863 (as data permit based on sampling feasibility)

- Change from baseline in gene expression (mRNA) in the DFU tissue.
- Change from baseline in biomarkers of angiogenesis and blood flow and oxygen availability [e.g., skin perfusion pressure (SPP) and pulse volume (PV), hyperspectral imaging].
- Change from baseline in biomarkers in the DFU (e.g., cytokine panel, protease panel, macrophage quantification)
    a. If the methodology can be validated at the site(s) cell proliferation and collagen turnover will be assessed.
- Change from baseline on bioburden in the DFU (e.g., rtPCR for bacterial load).
- Tissue concentrations of GSK1278863 in selected subjects as determined from micro-dialysis during RD, if feasible to conduct the assessment.
- Change from baseline on circulating biomarkers of systemic HIF activation in the blood (e.g., hemoglobin, hepcidin, EPO).
- Physical parameters of the wound following repeat doses of GSK1278863, for example:
    a. Rate of wound healing (via planimetry).
    b. Change in wound volume (Silhouette software).
    c. Percentage of subjects with closure of the study DFU.
    d. Time to DFU closure.
    e. Time required to achieve a certain percent of wound closure.
    f. Incidence of DFU closure.
- Clinical characteristics of the wound (e.g., exudate, granulation tissue, color).
- Correlation between GSK1278863 dose and/or exposure and parameters of pharmacodynamic activity, safety, and tolerability, as data permit.

3.3. Descriptions of Selected Endpoints

Descriptions of selected endpoints related to wound healing are in the sections below.

3.3.1. Gene Expression Analysis

Expression of genes important in a range of dynamic cellular processes (including inflammation, angiogenesis and extracellular matrix deposition) is expected to change as wound healing progresses. Moreover, with active drug treatment, genes coding for direct downstream targets of HIF signaling (e.g., HMOX1, VEGF and NOS2) should be upregulated. Genetic markers of bioburden may also change with active drug treatment. Gene expression will therefore be assessed by RT-PCR using tissue samples taken from the wound area. Most of these genes will be probed for using a focused array covering approximately 600 genes. TaqMan PCR will be employed to validate gene expression for a number of signature genes for each of the signaling pathways/cellular processes of interest and for any other genes of interest not captured in the focused array.

3.3.2. Biomarker Analysis

The pattern of cytokines and growth factor levels as well as inflammatory cell levels are expected to show relationships with the healing process expressed by changes in ulcer characteristics.

3.3.2.1. Cytokines and Growth Factors

Subjects receiving the active drug are expected to show a pattern of pro-inflammatory protein production that is lower than that in subjects receiving placebo. Cytokine evaluation may include, but is not limited to: IL-6, IL-1b, IL-8, IL-10, GM-CSF, TNF-α, MMP-9, VEGF, HIF, TIMP, Fibrinogen, PDGF, and thrombospondin-1 (TSP-1), PF4 and FGF with multiplexed ELISA assay. Reductions in pro-inflammatory cytokines should correlate with reduction in wound dimensions.

3.3.2.2. Quantification of CD68+ Macrophages

Cellular inflammatory response at the tissue level will be studied in tissue samples from the wound site. Changes (decreases) in macrophage levels at week two are expected to coincide with improved clinical evidence for wound closure and with decreased inflammatory cytokines. It is hypothesized that a decrease in the hyper-inflamed state of the wound would be reflected in decreases in macrophage count by the end of treatment.

Fluorescence immunohistochemistry will be employed to quantify the number of CD68+ macrophage in frozen tissue sections. If methodology permits, macrophage subtype analysis may be performed by co-immunostaining sections for CD68 and CD206 (and potentially other markers) to identify and quantify the number of M2 macrophages.

3.3.2.3. Cell Proliferation and Collagen Turnover

An assessment to determine the level of cell proliferation and collagen turnover will be conducted on a selected group of patients if the methodology can be validated within the

timeframe of the study. Cell proliferation and collagen turnover are expected to increase at the wound site in patients receiving the active drug. These processes will be assessed by systemic labeling of subjects with "heavy water" (^2H_2O or deuterated water, i.e., the stable isotope deuterium incorporated into water) and subsequent measurement of deuterium in tissue samples from the wound site. If data confirm increased collagen turnover in patients receiving active drug, further analysis will be considered (e.g., collagen isoform identification to determine the form which contributes to the turnover).

3.3.3. Bacterial Load Determination

Bacterial load in the wound fluid will be determined by DNA-DNA checkerboard hybridization assay using specific DNA probes generated against potential pathogenic microbial species (e.g., S. aureas, P. aeruginosa, C. rectus, P. mirabilis, MRSA). Changes in the bacterial loads (decreases) in the wounds are expected to reflect changes in the characteristics of the wound during the healing process.

3.3.4. Tissue Concentration of GSK1278863

Assessment of tissue concentration of GSK1278863 will be assessed by skin microdialysis on a selected group of patients if the methodology can be validated within the timeframe of this study. Local concentration of drug in the skin tissue is predicted to more closely associate with the pharmacodynamic endpoints being assessed in the study. Furthermore, critical insight toward available drug for inhibition of skin prolyl hydroxylase enzyme may be gained that will assist in refinement of the dosing regimen.

3.3.5. Angiogenesis: Tissue Perfusion Assessment/Oxygen Availability

The ability of this therapeutic approach to impact blood flow within short term dosing will be explored using a laser Doppler SPP technique. SPP will be evaluated with the SensiLase System, which provides quantitative evaluation of microcirculatory perfusion in the skin. It is a non-invasive laser Doppler test combining SPP and pulse volume recording (PVR) to assess the capillary circulation and thereby assess wound healing potential. The system measures skin perfusion using a laser Doppler sensor located beneath a pressure cuff at or near the wound. It is hypothesised that angiogenic pathways will be activated by application of GSK1278863 and result in improved blood flow. Changes in flow are anticipated to coincide with changes in ulcer characteristics after two weeks of exposure. A second exploratory technique for indirect evidence of angiogenesis, hyperspectral imaging (HSI), may be employed if the technique can be established/validated at the investigative centers. Hyperspectral imaging uses a spectral separator to vary the wavelength of light admitted to a detector to assess both oxygen delivery (measured by oxyhemoglobin) and oxygen extraction (measured by deoxyhemoglobin) in tissues at or near the wounded tissue and would function as an indicator of a wound's capacity to heal. Higher levels of both molecules have shown correlation with a greater potential for healing (Khaodhia, 2007).

3.3.6. Physical Indicators of DFU Healing

Physical indicators of DFU healing will be assessed using planimetry (digital) as well as via acetate tracing methods. Images and tracings will be assessed by blinded personnel both at run-in to determine eligibility, and during the indicated timings throughout the study for endpoint analysis (including wound diameter, area and wound volume (Silhouette software). Changes in indicators of healing are expected to correlate with changes in assessments of angiogenesis, inflammation (cytokine levels), and macrophage number/type.

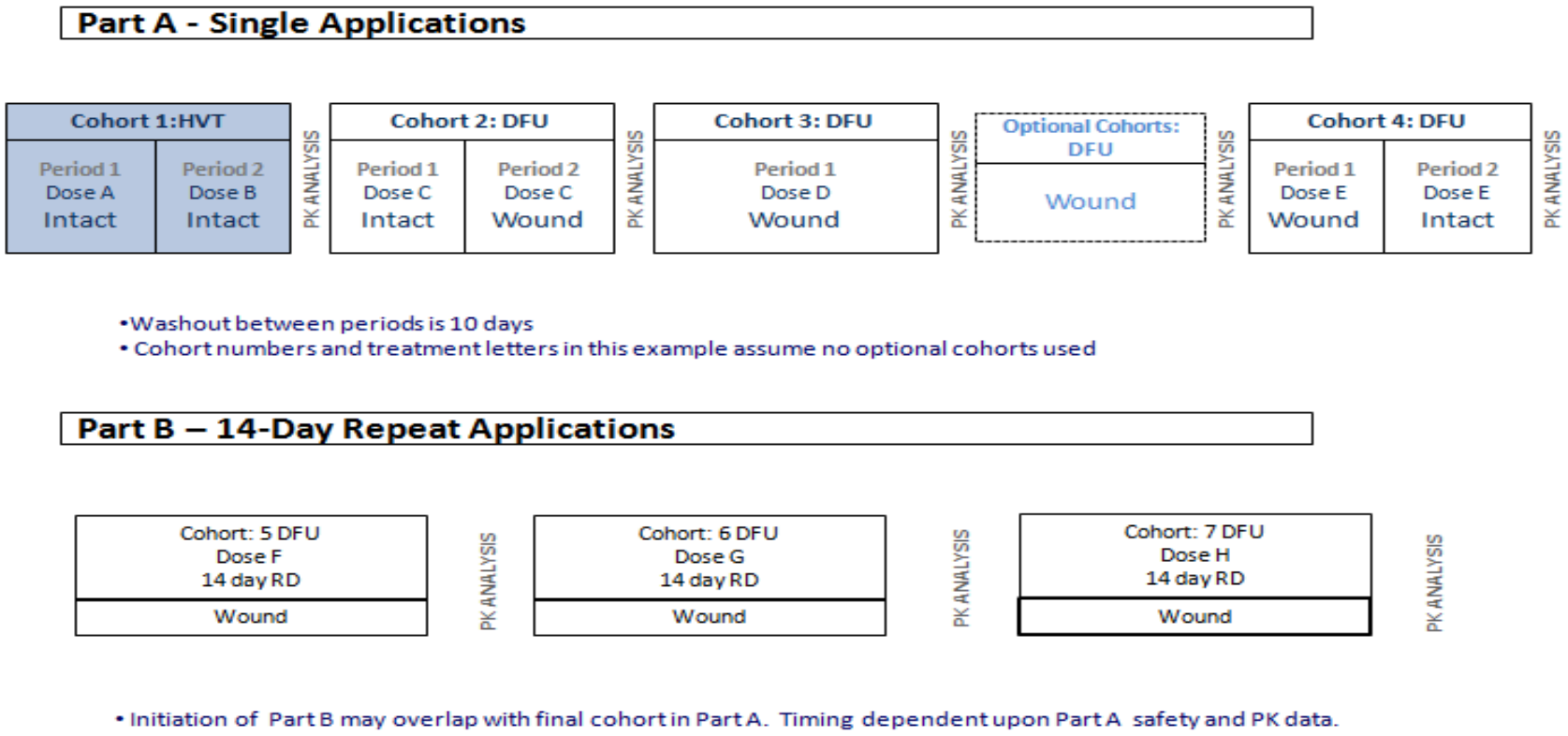
3.3.7. Clinical Characteristics of the Wound

The clinical characteristics of the wound will be assessed (Section 7.10) and recorded on clinical case report forms designed to capture information which describe the wound (e.g., redness, swelling etc).

4. INVESTIGATIONAL PLAN

4.1. Study Design/Schematic

Figure 3 Study Design/Schematic



Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the Time and Events Table are essential and required for study conduct.

4.2. Discussion of Design and Treatment Plan

This is a randomized, placebo-controlled, single-blind (subjects and investigators will be blinded, GSK internal personnel will not be blinded), parallel-group, two part (Part A, Part B) trial in healthy volunteers and subjects with diabetic foot ulcers. The study seeks to gain information related to safety/tolerability, pharmacokinetics and pharmacodynamics of the compound in this first administration to subjects via a topical formulation.

Part A is designed to evaluate single applications of GSK1278863. Part A will include one cohort of healthy volunteers (intact skin) and approximately 3 cohorts of diabetic subjects. Part B is designed to evaluate repeat applications of GSK1278863 in diabetics, both in the clinic and by subjects at home. Part B will include approximately 3 cohorts in which the concentration of drug applied will be determined by pharmacokinetic data from Part A and earlier cohorts in Part B.

For both parts of the protocol, additional cohorts may be added as necessary to obtain relevant safety, pharmacokinetic or pharmacodynamic data. Based on emerging data, the number of cohorts and subjects may be reduced to avoid unnecessary exposure.

Furthermore, for both Parts A and B, there will be at least one week between cohorts for review of safety, tolerability and PK data by the GSK study team (unblinded) and the Investigator (blinded). Pharmacodynamic data, where available, may be obtained for review.

Enrollment to each cohort may be staggered (i.e., cohorts do not have to be enrolled as one group).

Diabetic subjects, in either Part A or B, will require a two week run-in period in which standard of care (Section 7.4) is given before treatment of the DFU with GSK1278863or placebo in order to ensure the wound does not show a greater than 30% reduction in size.

4.2.1. Part A – Single Doses in Healthy Volunteers and DFU Patients (Cohorts 1 through 4)

Part A will enroll approximately 4 cohorts, moving from healthy volunteers (Cohort 1) to Type I or II diabetic patients with diabetic foot ulcer (3 or more cohorts), and will administer single applications of ascending doses of GSK1278863 or placebo. Each cohort in Part A will enroll enough subjects to complete approximately 6 on active drug and 2 on placebo (assumes ~10 subjects will be recruited per cohort to achieve 6:2 ratio). Diabetic subjects may participate in more than one cohort in Part A (requires a minimum of 14 days between cohorts to ensure a healing rate of less than 30%) and may also participate in Part B (requires a minimum of 14 days between cohorts to ensure a healing rate of less than 30%) provided the subject meets all selection criteria and this

participation will not result in blood withdrawal over the limit (Section 5.2.2). Participation in more than one cohort in Part A will not require the subject to be screened again.

4.2.1.1. Screening (Part A)

To determine subject eligibility for enrollment in the study, a screening visit will be performed within approximately 28 days of first dose administration. For the purposes of subject eligibility for enrollment in the study, fasting screening assessments are defined as any assessments performed prior to the first dose of study drug in each part. Ideally screening for diabetic patients would occur 14 days prior to Day 1 of the treatment of the DFU to accommodate the required run in period prior to wound treatment.

If a subject is not eligible for the study based on the Inclusion and Exclusion Criteria at the initial attempt, but becomes eligible at a later date during the enrollment period, the investigator should contact the GSK Medical Monitor to discuss the possibility of re-screening the subject.

4.2.1.2. Cohort 1 – Healthy Volunteers (Part A)

Cohort 1 will enroll healthy volunteers. Subjects will be randomized to receive single applications of GSK1278863 or placebo on intact skin in two escalating dosing periods for evaluation of safety and tolerability, PK of GSK1278863 and PD. The first application will be with a dose of 0.3mg (by utilization of 300mg of 0.1%). A review of preliminary safety and tolerability data will be conducted by the GSK study team (unblinded) and the Investigator (blinded). If GSK1278863 is well tolerated, subjects will receive a new dose as a single application to be determined based on data from the first application at least 10 days after the first application.

Subjects will come in to the clinic fasting early in the morning of Day 1 of each dosing period. They will receive their application of GSK1278863 or placebo after safety labs are drawn, and ECGs and vitals have been collected. Subjects may remain in the clinic post application for monitoring of safety and collection of blood for PK and PD sampling. Subjects may also be discharged from the clinic once all Day 1 assessments have been completed, and return for subsequent assessments. In this case, subjects will return to the clinic fasting early in the morning on Day 2, Day 3 and Day 4 for PK trough samples and safety assessments. This will be repeated at least 10 days later with a new dose of GSK1278863 in the second dosing period. Subjects will then return to the clinic 7 to 10 days and 1 month after dosing for follow-up visits. See Section 4.6.1 for detailed timing of assessments and sampling.

The duration of PK sampling may change dependent upon the kinetics observed.

The data obtained from this healthy cohort of subjects on intact skin will provide preliminary data on tolerability and initial PK following topical route of administration. These data will be used to select the dose for the first DFU cohort.

4.2.1.3. Run-In Period (Part A)

For two weeks prior to Day 1 of the DFU Treatment Period, subjects will receive standard-of-care. For all diabetic subjects in Part A, each subject's DFU will be measured at screening and Day 1; if, after 14 (±1 day) days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (Optional Baseline Run-In) will need to be scheduled prior to Day 1 to meet the Run-In requirement. Subjects that do not meet randomization eligibility will be discontinued from the study.

4.2.1.4. Cohort 2 (Part A)

Cohort 2 will enroll DFU subjects who fulfill screening criteria. Subjects will be randomized to receive a single application of GSK1278863 or placebo in two dosing periods. The two dosing periods are intended to provide a 'within patient' comparison of PK between application to intact skin and to DFU. The first application will be made on intact skin, followed by a 10-day washout period. Blood for serial PK sampling will be collected out to 72 hours post-dose. After review of safety, tolerability and PK data, these subjects will then receive an application of the same dose directly to the DFU.

Subjects will come in to the clinic fasting early in the morning of Day 1 of each dosing period and will follow the same schedule as Cohort 1, with the exception that the first application will be on intact skin, and the application in the second dosing period will be directly to the DFU. See Section 4.6.1 for detailed timing of assessments and sampling.

4.2.1.5. Cohorts 3 and 4 (Part A)

Cohorts 3 and 4 will enroll DFU subjects who fulfill screening criteria. Subjects will be randomized to receive either GSK1278863 or placebo. Each cohort will evaluate a new dose of GSK1278863, with applications made directly to the DFU. If subjects are re-enrolling from a previous cohort, they must complete the second Follow-up visit (Day 28-32 post last dose) and therefore the 14 day Run-in period may begin 14 days prior to this visit. If Cohort 4 is the final cohort, subjects will also receive application to intact skin.

Subjects in Cohort 3 will come in to the clinic fasting early in the morning of Day 1 and will follow the assessments as outlined in Section 4.6.1.

Cohort 4, or the final cohort if optional cohorts are enrolled, will have 2 dosing periods (10 day washout between periods), with GSK1278863 applied directly to the DFU in dosing period one and followed by application to intact skin in the second dosing period. Subjects will follow the same schedule as for Cohorts 1 and 2. See Section 4.6.1 for detailed timing of assessments and sampling.

4.2.1.6. Optional Cohorts (Part A)

Additional cohorts may be added as necessary to obtain relevant data for determination of starting doses for the repeat (Part B) portion of the study.

- If the data from Cohort 4 are considered sufficient to establish dosing for Part B, subjects from that cohort will be asked to return to the clinic for a SD administration of the compound to intact skin in order to make comparisons of systemic PK for linearity between low and high doses and a within-patient comparison of application of drug to intact skin versus DFU.
- If however, the data are NOT sufficient to establish dosing for the repeat dose section of the protocol, another cohort will be recruited for application of an additional dose to their DFU. This process will be repeated until sufficient data are collected to establish dosing for Part B. Once this is established, the last cohort will be asked to return for application of the compound to intact skin for the comparisons as described above.

Subjects of the cohort asked to return for application of the compound to intact skin will undergo PK sampling at time points established by the findings of the kinetics in the previous cohorts.

The timing of the final cohort, in which application to intact skin is assessed, may overlap with the first cohort of Part B, as long as all doses of GSK1278863 in previous cohorts are well tolerated and provide sufficient PK for Part B predictions. Systemic exposures may or may not permit predictions for dose selection and therefore dose determination will be selected based on available safety and tolerability data.

GSK1278863 PK data from the cohorts of Part A will be used for the predictions of the expected systemic exposures in Part B (data permitting). Preliminary safety, tolerability and PK data will be reviewed before initiating the randomized repeat-dosing period of Part B at approximately the same time as the final cohort in Part A.

4.2.1.7. Follow-up Visits

Follow-up visits will occur 7 to 10 days and 28 to 32 days following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete all follow-up procedures.

See Section 4.6 for details regarding all study procedures performed during the course of this trial.

4.2.2. Part B –Repeat Doses in Diabetic Foot Ulcer Subjects (Cohorts 5, 6 and 7)

Part B will include approximately 3 cohorts. Enough subjects will be enrolled in each cohort to complete approximately 16 diabetic subjects with diabetic foot ulcer. All subjects will receive standard of care (SoC; Section 7.4) up to randomization, and then will be randomized to one of three arms, a) 12 active drug + SoC, b) 2 SoC, and c) 2 placebo + SoC). The randomized repeat application in Part B may start after dosing in Cohort 4 of GSK1278863 has been found to be safe and well tolerated.

Doses for the cohorts in Part B will be determined from safety, tolerability and PK data from Part A. Each cohort will enroll a new group of subjects, randomized to either the

same dose, ascending dose or descending dose of GSK1278863. All applications in Part B will be made directly to the subject's DFU.

For each cohort, subjects will undergo repeat applications of GSK1278863 or placebo, at the dose chosen for that cohort for 14 days. The first day of the 14-day repeat application period will be conducted in the clinic. Subjects will return to the clinic as indicated in Section 4.6.2, and applications between visits will be performed by the subject at home.

Blood for PK sampling will be collected on Days 1, 7, 14 and 15. See Section 4.6.3 for specific time points for drawing samples.

There will be approximately one week between cohorts to allow for review of preliminary safety, tolerability and PK data before proceeding to the next cohort. Pharmacodynamic data, where available, may be obtained for review.

Subjects may not participate in more than one cohort in Part B. Subjects may participate in Part A and then Part B which will require the subject to be screened again.

4.2.2.1. Screening (Part B)

To determine subject eligibility for enrollment in the study, a screening visit will be performed within approximately 28 days of first dose administration. For the purposes of subject eligibility for enrollment in the study, screening assessments are defined as any assessments performed prior to the first dose of study drug in each part.

If a subject is not eligible for the study based on the Inclusion and Exclusion Criteria at the initial attempt, but becomes eligible at a later date during the enrollment period, the investigator should contact the GSK Medical Monitor to discuss the possibility of re-screening the subject.

4.2.2.2. Run-In Period (Part B)

For two weeks prior to Day 1 of the Treatment Period, subjects will receive standard-of-care as described in Section 7.4. For all subjects in Part B, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product and is considered a Run In Failure. If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (Optional Baseline Run In) will need to be scheduled prior to Day 1 to meet the Run In requirement. Subjects that do not meet randomization eligibility will be discontinued from the study.

4.2.2.3. Treatment Period – (Part B)

Cohorts 5, 6 and 7 (numbering assumes no optional cohorts are enrolled in Part A) will each enroll sufficient subjects so that approximately 48 DFU patients who fulfill screening criteria complete all study visits. Doses selected for these cohorts will be chosen based on preliminary safety data and PK results from Part A of this study.

14-Day Dosing (Part B)

Subjects will be randomized to receive applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of GSK1278863 and PD. Applications of study drug will be done in clinic on Days 1, 4, 7, and 14. Subjects are responsible for study drug application on Days 2-3, 5- 6 and 8-13.

It should be noted that on days where serial PK sampling occurs it is permissible to allow subjects to check out of the clinic after the 8 hour PK time point has been collected as long as the subject can return for sample collection within the 10 to 12 hour PK window. If subjects elect to stay overnight, they should be encouraged to apply the study ointment and complete the diary entries themselves before being discharged in the morning. Study staff can take this opportunity to provide additional instruction to subjects.

Subjects will be given detailed instructions for application of GSK1278863 at home, and enough study drug for applications at home between clinic visits. See Section 11.1 for further instructions on at-home dosing.

Doses for subsequent cohorts may be adjusted based on PK results, and/or safety and tolerability data.

4.2.2.4. Follow-up Visits

Follow-up visits will occur 7 to 10 days and 28 to 32 days following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete all follow-up procedures.

See Section 4.6 for details regarding all study procedures performed during the course of this trial.

4.3. Treatment Assignment

This will be a single-blind study. Treatments will be blinded to subjects and the investigator site staff only. GSK study staff is not blinded.

Subjects will be assigned to receive GSK1278863A or vehicle (placebo) in Part A and GSK1278863A or vehicle or SOC only (moist occlusion) in Part B in accordance with the randomization schedule generated by GSK Discovery Biometrics, prior to the start of the study, using validated internal software.

Subjects will be randomized into the study by means of an interactive voice response system (IVRS) to receive one of the treatment regimens listed in the below tables (Table 2 and Table 3).

Table 2 Part A Doses

Cohort	Regimen	Dose (mg)	Treatment Area by Period	
			1	2
1	P	Placebo[1]	Intact	Intact
	A	0.3mg		
	B	TBD[2]		
2	P	Placebo	Intact	Wound
	C	TBD		
3	P	Placebo	Wound	N/A
	D	TBD		
4(or final)	P	Placebo	Wound	Intact
	E	TBD		
O (Optional)[3]	P	Placebo	Wound	N/A
	F	TBD		

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

Table 3 Part B Doses

Cohort[1]	Regimen	Dose (mg)
5	P	Placebo[2]
	S	Standard of Care
	F[3]	TBD
6	P	Placebo
	S	Standard of Care
	G	TBD
7	P	Placebo
	S	Standard of Care
	H	TBD

1. Cohort numbering may be different based on the number of cohorts conducted in Part A
2. Placebo = vehicle
3. Starting dose may be adjusted based upon evaluation of PK data from Part A

In Part A

For Cohorts 2 through 4, drug volume to be administered will be based on wound area. See Section 11 for details.

An example of planned doses of GSK1278863 and regimen designation for Part A is in Table 4.

Table 4 Example Treatment Options

Cohort	Regimen/ Dosing Period	Dose (mg)	Treatment Area
1 (HVT)	P1	Placebo[1]	Intact Skin
	A	0.3 mg	Intact Skin
	P1	Placebo	Intact Skin
	B	TBD[2]	Intact Skin
2	P1	Placebo	Intact Skin
	C1	TBD	Intact Skin
	P2	Placebo	Wound
	C2	TBD	Wound
3	P2	Placebo	Wound
	D	TBD	Wound
4(or final)	P2	Placebo	Wound
	E1	TBD	Wound
	P1	Placebo	Intact Skin
	E2	TBD	Intact Skin
O (Optional)[3]	P2	Placebo	Wound
	F	TBD	Wound

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

In Part B

Drug volume to be administered will be based on wound area, which may vary during the treatment period. See Section 4.4 for details. Actual doses will be chosen based on preliminary PK data (data permitting, See Section 4.2.1.6) from Part A.

4.3.1. Subject Numbering

Subjects will be assigned a unique Subject Number at Screening for each cohort the subject opts to participate in. When a subject is known to qualify for randomization, the subject number will remain consistent for the duration of the participation in each cohort. GSK will provide the site with a unique set of Subject Numbers in sequential order beginning with the lowest number. Once a subject number is assigned to a subject, it cannot be re-assigned to any other subject during the study

If a subject is prematurely discontinued from the study and a replacement subject is to be recruited, a replacement treatment number will be used to assign the same treatment to the replacement subject.

4.4. Investigational Product and Other Study Treatment Dosage/Administration

	Study Treatment	
Product name:	**GSK1278863**	**Placebo**
Formulation description:	White to off-white smooth ointment	White to off-white smooth ointment
Dosage form:	Ointment	Ointment
Unit dose strength(s)/Dosage level(s):	0.05%w/w, 0.1%w/w, 0.5%w/w, 1.0%w/w	Placebo
Route/Administration/Duration:	Topical	Topical
Dosing instructions:	See Section 11 for Dosage and Administration Instructions	See Section 11 for Dosage and Administration Instructions
Device:	Part A: Amber Bottle or White Tube Part B: White Tube	Part A: Amber Bottle or White Tube Part B: White Tube
Manufacturer/ source of procurement:	Part A: GlaxoSmithKline Part B: DPT Laboratories, Ltd./GlaxoSmithKline	Part A: GlaxoSmithKline Part B: DPT Laboratories, Ltd./GlaxoSmithKline

4.5. Dose Adjustment/Stopping Criteria

This protocol allows some alteration from the outlined dosing schedule, but the predicted maximum exposure will not exceed systemic exposure to a 15mg oral dose. Modeling currently predicts that the highest dose levels will achieve exposures at or below a 10mg oral dose.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety and/or pharmacokinetic findings at a given dose level, or to add cohorts to evaluate up to sufficient additional dose levels. The study procedures for these additional subject(s) or cohort(s) will be the same as that described for other study subjects.

These dose adjustments may involve either an increase or a decrease in the planned dose, but all doses will be selected so as not to exceed mean a systemic exposure equivalent to a 15mg oral dose.

4.5.1. Dose Adjustment/Stopping Safety Criteria

An individual patient will be withdrawn from the study at the discretion of the Investigator and the study team. In the event one or more patients are withdrawn, additional patients may be enrolled to ensure an adequate number of patients complete each cohort.

4.5.1.1. Hemoglobin Stopping Criteria

A patient that experiences an absolute increase in hemoglobin of 1.5g/dL above their baseline level at any time will be withdrawn from the study.

Should hemoglobin values achieve the above set criteria, additional samples may be collected every 2-3 days and appropriate procedures (venesection or erythrocytapheresis) may be considered and implemented at the discretion of the investigator until the laboratory result returns to the patient's normal range.

4.5.1.2. Liver Chemistry Stopping Criteria

Liver chemistry threshold stopping criteria have been designed to assure subject safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

Study treatment will be stopped for a subject if the following liver chemistry stopping criteria is met:

- ALT ≥ 3xULN

Refer to Section 14, Liver Chemistry Follow-up Procedures, for details of the required assessments if a subject meets the above criteria.

4.5.1.3. QTc Withdrawal Criteria

Healthy Volunteers (Part A, Cohort 1)

A subject that meets criteria below will be withdrawn from the study. The QT correction formula used to determine discontinuation should be the same one used throughout the study.

- QTc > 500msec

Withdrawal decisions are to be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, obtain 2 more ECGs over a brief period, and then use the averaged QTc values of the 3 ECGs to determine whether the subject should be discontinued from the study.

DFU Patients (Part A Cohorts 2 through Final Cohort, Part B All Cohorts)

A subject who meets the criteria below will be withdrawn from the study. The QT correction formula used to determine discontinuation should be the same one used throughout the study.

- QTc > 530msec or uncorrected QT >600msec
- If subject has underlying bundle branch block then the QTc withdrawal criteria depends on the baseline value:

Baseline QTc value with underlying bundle branch block)	**QTc withdrawal criteria**
≤500msec	>530msec
≤530 msec	≥550msec

Withdrawal decisions are to be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, obtain 2 more ECGs over a brief period, and then use the averaged QTc values of the 3 ECGs to determine whether the subject should be discontinued from the study.

4.5.1.4. Other Dose Adjustment/Stopping Safety Criteria

- Any other occurrence that raises significant safety concern in the mind of the investigator.
- Any patient who has an increase from baseline in total wound area > 20% at any time will be withdrawn from treatment
- Any patient who develops at any time after randomization 3 of the following 6 signs at the study wound site will be withdrawn from treatment: local erythema, edema, pain, pus, local warmth, loss of function
- Diagnosis of cancer, with the exception of squamous cell and basal cell carcinoma (unless in proximity of the wound treated with GSK1278863).

The GSK Medical Monitor must be notified within 24 hours of any subject's withdrawal as a result of safety concerns.

4.6. Time and Events Table

4.6.1. Time and Events Table Part A

Study Population	Procedure	Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48h	72h		
All Subjects	Check in		X													
	Informed Consent	X														
	Demographics	X														
	Full Physical Exam	X														
	Brief Physical Exam		X									X	X	X	X	X
	Medical/medication/ drug/alcohol history	X	X													
	12-lead ECG[2]	X	X										X		X	
	Vital signs[3]	X	X										X		X	X
	Urine pregnancy(women)	X	X												X	
	HIV, Hep B and Hep C[4]	X														
	Hema/Chem Safety Labs[5]	X	X										X		X	
	Blood for HIFα Samples (e.g., hepcidin, EPO,VEGF)[6]		X							X	X[6]			X		
	Study treatment dosing			X												
	Pharmacokinetic Sampling[7]		X		X	X	X	X	X	X	X[7]	X	X	X		

Study Population	**Procedure**	**Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)**	**Study Day (each dosing period)**												**Follow Up**	
			Day 1									**Day 2**	**Day 3**	**Day 4**	**7-10 days post last dose**	**28-32 days post last dose**
			Pre-dose	**0h**	**0.25h**	**0.5h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**	**48h**	**72h**		
	Concomitant Medications		←==========→												X	
	Adverse Event Review[8]		←==========→												X	X
Subjects with DFUs[9] Only	Gene Expression (mRNA) Tissue Samples[10]		X											X	X	
	Biomarker fluid samples (e.g., for cytokines, growth factors, and bioburden)		X											X	X	
	Physical Characteristics (e.g., photos and wound measurement post-debridement, volume)	X	X											X	X	
	Clinical Characteristics[11]	X	X			X	X	X			X	X	X	X	X	
	Vascular Perfusion Eligibility Assessments[12]	X														

1. For subjects with DFUs to be eligible for the study or for entry into more than one cohort in Part A, rate of healing must be assessed in the 14 days prior to Day 1.
2. Single ECGs will be taken at Screening, pre-dose on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. HIV, Hep B and Hep C are only assessed in the Healthy Volunteer population.
5. Blood samples for safety analyses will be collected fasting at Screening, on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
6. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
7. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected immediately pre-dose and 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-dose. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile, but the total number of samples will not change.
8. Adverse events are to be collected from first dose through the final Follow-up Visit.
9. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin unless for screening/eligibility purposes.
10. Tissue sample collection at Pre-dose, Day 4 and the 7-10 Follow-up Visit is at the discretion of the PI or designee. Wounds that have healed such that biopsies would be disruptive to the continued healing process are exempt.
11. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. The 12 hr assessment can be done once within the 10-12 window for out-patients and at 12 hrs for in-patient subjects. Assessments are recorded in the eCRF
12. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.

4.6.2. Time and Events Table Part B

Procedures	**Screening**	**Optional Baseline Run In Visit[1]**	**Day 1**	**Day 4**	**Day 7**	**Day 14**	**Day 15**	**Follow-up**	
Visit Window (relative to Day 1)	-28 to -14 days		Exams/samples pre-dose					7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X	X	X	X	X	X
Informed Consent	X								
Demographics	X								
Complete physical	X								
Brief physical		X	X	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X	X							
12-lead ECG[2]	X		X				X		
Vital signs[3]	X		X		X		X	X	X
Urine pregnancy(females)	X		X					X	
Hema/Chem/ Safety Labs[4]	X		X		X		X	X	
Vascular Perfusion Eligibility Assessments[5]	X								
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF) [6]			X		X		X		
Standard of Care Treatment[7]	X	X	<-==========					X	X
Study Treatment/Dosing In the Clinic[8]			X	X	X	X			

Procedures	Screening	Optional Baseline Run In Visit[1]	Day 1	Day 4	Day 7	Day 14	Day 15	Follow-up	
PK serial blood sampling[9]			X		X	X	X		
PK Trough Sample[9]									
Tissue Biopsies (gene expression, macrophage and bioburden analysis)[10]			X		X		X		
Wound Fluid (cytokine and bioburden analysis)[11]			X		X		X	X	
SensiLase (Doppler blood flow)			X		X		X	X	
Physical Characteristics (assessed pre-dose)[12]	X	X	X	X	X		X	X	
Clinical Characteristics[13]	X		X	X	X	X	X	X	
Concomitant Medication Review			X	<-==========->				X	X
Adverse Event Assessment[14]			X	<-==========->				X	X

1. Optional Baseline Run In visit: This optional visit is for subjects that will exceed the 14 (±1 day) Run-in window between screening and Day 1.
2. Single ECGs will be taken at Screening and pre-dose on Day 1 and 24h post-last dose on Day15. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.

3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1, 7, 15 (24h post-last dose), and at Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1, 7, 15 (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI, toe pressure, Doppler ultrasound) is required for eligibility.
6. Blood samples for HIFα activation will be collected at pre-dose/equivalent time.
7. All subjects will be on SOC throughout the study (Section 7.4).
8. In between clinic visits, subjects will perform applications of study drug at home.
9. See separate Time and Events table for serial PK sampling details for Days 1, 7 and 14 and the associated 24 time point. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile.
10. Direct wound tissue biopsies will be collected at pre-dose/equivalent on Days 1, 7, and 15, however based on emerging data, time points may be added or deleted; wounds that have healed sufficiently may be exempt. Refer to Section 7.7.2 for additional details on the collection and processing of tissue biopsies.
11. Wound fluid will be collected at pre-dose/equivalent on Days 1, 7, 15 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7.1 for additional details on the collection and processing of wound fluid samples.
12. Physical characteristics of the wound (i.e., photos of the wound and wound measurement assessments) will be assessed at Screening (Optional Baseline Run In Visit), at pre-dose/equivalent time on Days 1, 4, 7, 15 and at the 7-10 day Follow-up Visit.
13. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
14. Adverse events are to be collected from first dose through the final Follow-up visit.

4.6.3. Serial PK Time and Events Table Part B

VIST DAY	Part B Serial PK Sampling[1,2]								
	Hours (h)								
	Pre-dose	1h	2h	4h	8h	10h	11h	12h	24h
Day 1	X		X						
Day 7	X		X						
Day 14 OUT-PATIENTS	X	X	X	X	X	X[3]			X
Day 14 IN-PATIENTS	X	X	X	X	X			X	X

1. Patients are permitted to either stay in clinic (in-patients) or be discharged and return for clinic visits (out-patients). Refer to Section 7.12 for details on the collection and processing of PK samples.
2. PK sampling times may be changed based on observed GSK1278863 PK profile.
3. Sample must be taken within the 10 to 12 hour time window.

5. STUDY POPULATION

5.1. Number of Subjects

Enough subjects will be enrolled in Part A (Single Doses) such that approximately 32 subjects (~8 healthy volunteers in Cohort 1, and ~24 DFU subjects in Cohorts 2, 3 and 4) complete dosing and critical assessments. If optional cohort(s) are utilized, each cohort will enroll approximately 8 subjects (6 on active drug, 2 on placebo).

In Part B, enough subjects will be enrolled such that approximately 48 subjects in Part B (~16 DFU subjects per cohort) complete dosing and critical assessments (assumes approximately 3 cohorts).

Additional subjects/cohorts may be enrolled to allow for evaluation of additional dose levels, based on emerging data. The IRB will be informed if any additional cohorts (including optional cohorts) are included in the study based on local site requirements.

If subjects prematurely discontinue the study, additional subjects may be enrolled as replacement subjects and assigned to the same treatment sequence at the discretion of the GSK Medical Monitor in consultation with the Investigator.

5.2. Eligibility Criteria – Healthy Volunteers (Part A Cohort 1)

5.2.1. Inclusion Criteria - Healthy Volunteers (Part A Cohort 1)

Specific information regarding warnings, precautions, contraindications, adverse events, and other pertinent information on the GSK investigational product or other study treatment that may impact subject eligibility is provided in the IB [GlaxoSmithKline Document Number RM2008/00267/07] Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

1. ALT, alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
2. Single QTc < 450msec.
3. Healthy as determined by a responsible and experienced physician, based on a medical evaluation including medical history, physical examination, laboratory tests and ECGs. A subject with a clinical abnormality or laboratory parameters outside the reference range for the population being studied may be included only if the Investigator and the GSK Medical Monitor agree that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. Subjects with Hb values higher than ULN the normal range should always be excluded from enrollment.

4. Male or female between 18 and 90 years of age inclusive, at the time of signing the informed consent.
5. A female subject is eligible to participate if she is of:

   1. Childbearing potential, and agrees to use one of the approved contraception methods as outlined in Section 8.1 from Screening until completion of the Follow-up Visit OR

   2. Non-childbearing potential (NCBP) defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40MlU/ml and estradiol < 40pg/mL (<147pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods in Section 8.1 if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT (consult with GSK Medical Monitor). Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

5.2.2. Exclusion Criteria – Healthy Volunteers (Part A Cohort 1)

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

1. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening
2. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
3. History of malignancy within 5 years of Screening or those with a strong family history of cancer (e.g., familial cancer disorders), with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
4. A history of drug or alcohol abuse, or a history of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >14 drinks for males or >7 drinks for females. One drink is equivalent to 12g of alcohol: 12 ounces (360mL) of beer, 5 ounces (150mL) of wine or 1.5 ounces (45mL) of 80 proof distilled spirits.
5. A positive test for HIV antibody.

6. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives (whichever is longer).
7. Unable to refrain from the use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety.
8. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.
9. Where participation in the study would result in donation of blood or blood products in excess of 500mL within a 56 day period
10. Pregnant females as determined by positive urine hCG test at screening or prior to dosing.
11. Unwillingness or inability to follow the procedures outlined in the protocol.
12. Subject is mentally or legally incapacitated.

5.3. Eligibility Criteria – DFU Subjects

5.3.1. Inclusion Criteria – DFU Subjects

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

A DFU subject will be eligible for inclusion in this study only if all of the criteria listed are met.

1. Diagnosed with Type I or Type II diabetes mellitus.
2. HbA1c $\leq$ 12%.
3. Single QTc $\leq$ 500msec, or $\leq$ 530msec in subjects with bundle branch block. No QTc exclusion for subjects with a predominantly paced rhythm.
4. Lower extremity diabetic foot ulcer of 30-364 days' duration.
5. DFU between 1cm^2 and 22cm^2 at screening.
6. Presence of at least one DFU that meets all of the following criteria:
    a. Ulcer has been diagnosed as a full-thickness, neuropathic DFU and is located at or distal to the malleolus (excluding ulcers between the toes but including those of the heel).

b. There is a minimum 2cm margin between the qualifying study ulcer and any other ulcers on the specified foot.

c. Ulcer size (area) ≥ 1cm^2 and ≤ 16cm^2 (post-debridement at time of randomization).

d. Wagner Grade 1.

e. Depth ≤ 5mm with no capsule, tendon or bone exposed and no tunneling, undermining, or sinus tracts.

Note: If the subject has more than one qualifying DFU, the ulcer designated as the study ulcer will be at the discretion of the Investigator. Non-study ulcers being treated during the course of the study will be treated with moist wound therapy Standard of Care (SoC) identified under this study).

7. Adequate vascular perfusion of the affected limb within 30 days of screening, as defined by at least one of the following:

   a) TcPO2 > 35mmHg.

   b) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2

   c) Toe pressure (plethysmography) > 50mmHg.

   d) Doppler ultrasound (biphasic or triphasic waveforms) consistent with adequate blood flow to the affected extremity, as determined by SoC.

8. ALT, alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).

9. Male or female between 18 and 90 years of age inclusive, at the time of signing the informed consent.

10. A female subject is eligible to participate if she is of:

    1. Childbearing potential, and agrees to use one of the approved contraception methods as outlined in Section 8.1 from Screening until completion of the Follow-up Visit OR,

    2. Non-childbearing potential (NCBP) defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40MlU/ml and estradiol < 40pg/mL (<147pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods in Section 8.1 if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT (consult with GSK Medical Monitor). Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

5.3.2. Exclusion Criteria – DFU Subjects

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

Diabetic subjects that are being considered for this study will not be eligible for inclusion in this study if any of the following criteria apply.

1. Subjects with:
   - Ulcers accompanied by infected cellulitis, osteomyelitis, or clinical signs or symptoms of infection,
   - Gangrene on any part of affected limb,
   - Active Charcot's foot on the study limb,
   - Planned vascular surgery, angioplasty or thrombolysis,
   - Ulcers involving exposure of tendon, bone, or joint capsule (It is acceptable to have ulcers extending through the dermis and into subcutaneous tissue with presence of granulation tissue),
   - Ulcers due to non-diabetic etiology.
2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) within 3 months prior to randomization.
3. Malignancy within 2 years of screening, with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
4. Other clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the Investigator or the GSK Medical Monitor, not stabilized or may otherwise impact the results of the study.
5. Patients undergoing hemodialysis.
6. Use of prohibited medications as described in Section 9.1 of the protocol.
7. A history of drug or alcohol abuse in the past year, or a history of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >14 drinks for males or >7 drinks for females. One drink is equivalent to 12g of alcohol: 12 ounces (360mL) of beer, 5 ounces (150mL) of wine or 1.5 ounces (45mL) of 80 proof distilled spirits.
8. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives (whichever is longer).
9. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

10. Where participation in the study would result in donation of blood or blood products in excess of 500mL within a 56 day period.
11. Pregnant females as determined by positive urine hCG test at screening or prior to dosing or lactating/nursing females.
12. Unwillingness or inability to follow the procedures outlined in the protocol.
13. Subject is mentally or legally incapacitated.

5.4. Screen and Baseline Failures

Data for screen and baseline failures will be collected in source documentation at the site but will not be transmitted to GSK.

6. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

6.1. Hypotheses and Treatment Comparisons

The primary objectives of this study are to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamics of single (Part A) and repeated (Part B) doses of GSK1278863A. No formal statistical hypotheses are to be tested. Instead, point estimates and confidence intervals will be constructed, where appropriate.

6.2. Sample Size Considerations

6.2.1. Sample Size Assumptions

There is no formal calculation of power or sample size in this study. Sample sizes are based on feasibility. A sample size of six subjects per active single dose (Part A), and twelve subjects per active repeated dose (Part B) completing the study are feasible sample sizes to provide sufficient safety and PK data for this FTIH study.

The target sample size is set at 8 subjects per cohort in Part A, (6 on active treatment and 2 on placebo), and 16 per cohort in Part B, (12 on active treatment , 2 on placebo and 2 receiving standard of care. The sample size for the placebo at each dose level is set at two to avoid subject bias in safety assessment.

6.2.2. Sample Size Re-estimation

No sample size re-estimation will be performed.

6.3. Data Analysis Considerations

Details of the statistical data analyses will be provided in the Reporting and Analysis Plan (RAP) separately. An outline of the statistical analyses is provided in the protocol.

6.3.1. Interim Analysis

There will be no formal interim analysis; however, all preliminary safety, tolerability, and available pharmacokinetic data will be reviewed internally at GSK prior to each dose escalation (GSK study staff are unblinded).

6.3.2. Final Analyses

Final analyses will be performed after Data Base Freeze (DBF) is declared by Clinical Pharmacology Science and Study Operations (CPSSO)

6.3.2.1. Safety Analyses

Safety data will be presented in tabular and/or graphical format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL) standards.

6.3.2.2. Pharmacokinetic Analyses

Listings will be generated and summary statistics (n, arithmetic mean, standard deviation, minimum, median, maximum, geometric mean, SD-log and 95% confidence intervals about the geometric mean) will be calculated for each derived plasma pharmacokinetic (PK) parameter by regimen and time. PK parameters are listed in Section 3.1.

For Part A, within group comparison (wound versus intact), comparisons of high dose versus low dose in DFU, and DFU versus HVT will be assessed used a mixed ANOVA model as data permit.

For Part B, dose proportionality will be assessed by using a power model as described below:

$$\log(\text{PK Parameter}) = \beta_0 + \beta_1 \cdot \log(\text{dose})$$

where β_0 is the intercept and β_1 is the slope. AUC and Cmax will be used for dose proportionality assessments.

If data permit, accumulation ratio will be assessed in Part B.

6.3.3. Pharmacodynamic Data Analyses

6.3.3.1. Wound Healing Endpoints (Part B)

Wound healing data will be summarized by regimen and time.

If data permit, rate of wound healing will be analyzed by analysis of covariance (ANCOVA) with dose group, visit, interaction of dose group and visit as fixed effects and the baseline assessments as covariates, appropriate variance-covariance structure may apply if data permit. Point estimates and 95% confidence intervals will be constructed for the comparisons of interest (i.e., Active – Placebo) for each dose of GSK1278863A. If appropriate, loge-transformation will be applied. In such cases, results will also be presented as ratios (Active : Placebo) with 95% confidence intervals.

Time to complete DFU closure will be assessed by time to event method (log rank test) as data permit.

Other wound healing related endpoints will analyzed as appropriate to the data, as data permit.

Mean/SE plot and box plot will be created as appropriate to the data.

6.3.3.2. mRNA, Protein and Biomarker Data Analysis

Biomarker data will be summarized by regimen and visit for Part A and Part B.

Biomarkers of HIF activation in Part A will be analyzed by 1) analysis of variance model (ANCOVA), 2) comparison of active dose vs. placebo, and 3) change from baseline within treatment group will be assessed, as data permit.

Biomarker data from Part B will be analyzed similarly. In the ANCOVA model, 1) visit, 2) interaction of dose and visit, and 3) comparison of active dose versus placebo at each visit time point will be assessed as data permit.

Mean/SE plots and box plots will be created as appropriate to the data.

6.3.3.3. Other Pharmacodynamic Endpoints

Pharmacodynamic (PD) endpoints will be summarized and analyzed as described in Section 6.3.3.1, as appropriate to the data.

6.3.3.4. Pharmacokinetic/Pharmacodynamic Analyses

If data permit, exploratory analyses will be performed to determine any potential relationships between systemic indices of exposure (Cmax and AUC) and various markers of efficacy and safety. Where potential relationships are observed, various established hierarchies of linear and non-linear models may be tested and fitted to evaluate any relationship seen.

A goal of this study is to identify a drug exposure (or dose) relationship with the primary endpoint, rate of wound healing. If data allow, the observed trajectories of wound healing from Day 1 until the end of repeat dosing (two weeks) will be modeled and the relationship with drug exposure (or dose) will be determined. This model will aid in designing the next long term study for this indication and will be implemented by performing simulations to chose doses, numbers of patients per dose, etc. In addition, any relationships/correlations between the wound healing rate and biomarkers will be identified and included in the model if deemed appropriate.

6.3.3.5. Pharmacodynamic/Biomarker Analyses

If data permit, the relationships between various biomarkers and wound healing endpoints, as well as other PD endpoints, will be explored similarly to Section 6.3.3.4.

7. STUDY ASSESSMENTS AND PROCEDURES

This section lists the parameters of each planned study assessment. The exact timing of each assessment is listed in the Time and Events Table (Section 4.6). Detailed procedures for obtaining each assessment are provided in the sections below. Whenever vital signs, 12-lead ECGs and blood draws are scheduled for the same nominal time, the assessments should occur in the following order: 12-lead ECG, vital signs, blood draws. The timing of the assessments should allow the blood draw to occur at the exact nominal time.

The timing and number of planned study assessments, including safety, pharmacokinetic and pharmacodynamic/biomarker/novel biomarker assessments may be altered during the course of the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. The change in timing or addition of time points for any planned study assessments must be approved and documented by GSK, but this will not constitute a protocol amendment. The IRB will be informed of any safety issues that require alteration of the safety monitoring scheme. No more than 500mL of blood will be collected over the duration of the study, including any extra assessments that may be required.

7.1. Demographic/Medical History Assessments

The following demographic parameters will be captured: date of birth, gender, race and ethnicity.

Medical/medication/alcohol history will be assessed as related to the eligibility criteria listed in Section 5.2. Cardiovascular medical history/risk factors will also be assessed at baseline.

7.2. Safety

Planned timepoints for all safety assessments are listed in the Time and Events Tables (Section 4.6). Additional time points for safety tests such as vital signs, physical exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

7.2.1. Physical Exams

The exam will be performed by a qualified licensed, medical professional (i.e., physician, physician assistant, or nurse practitioner) and include a thorough review of all body systems.

- A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities. Height and weight will also be measured and recorded.
- A brief physical examination will include assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

7.2.2. Vital Signs

Vital sign measurements will include systolic and diastolic blood pressure and pulse rate. The subject will be resting in a supine or semi-supine position for at least 5 minutes prior to the procedure.

7.2.3. Electrocardiogram (ECG)

Single 12-lead ECGs will be obtained at each timepoint during the study using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. The subject will be resting in a supine position for at least 5 minutes prior to the procedure. Refer to Section 4.5.1.3 for QTc withdrawal criteria and additional QTc readings that may be necessary.

7.2.4. Clinical Laboratory Assessments

Hematology, clinical chemistry, and additional parameters to be tested in all subjects are listed in Table 5.

Table 5 List of Clinical Laboratory Assessments

Hematology

Platelet Count	*RBC Indices*:	*Automated WBC Differential:*
RBC Count	MCV	Neutrophils
WBC Count (absolute)	MCH	Lymphocytes
Reticulocyte Count	MCHC	Monocytes
Hemoglobin		Eosinophils
Hematocrit		Basophils

Clinical Chemistry

BUN	Potassium	AST (SGOT)	Total and direct bilirubin
Creatinine	Chloride	ALT (SGPT)	Uric Acid
Glucose, fasting	Total CO_2	GGT	Albumin
Sodium	Calcium	Alkaline phosphatase	Total Protein
CPK	HbA1C	hsCRP	

Additional Lab Assessments for HIF activation

VEGF	Hepcidin	Iron	
EPO	Ferritin	Iron Binding Saturation	

Other screening tests

HIV
Hepatitis B (HBsAg)
Hepatitis C (Hep C antibody -- if second generation Hepatitis C antibody positive, a hepatitis C antibody Chiron RIBA immunoblot assay (or other third generation immunoassay) should be reflexively performed **on the same sample** to confirm the result, as sample availability permits.
FSH and estradiol (as needed in women of non-child bearing potential only)
Urine pregnancy test (females only, with the exception of subjects randomized to SoC)

7.3. DFU Assessments

The following assessments will be performed in Part A and Part B of the study according to site designation, capability and feasibility.

7.3.1. Part A

Blood samples for HIFα, (e.g., VEGF, EPO). All Cohorts
Biomarker Samples (e.g., for cytokines and bioburden) DFU subjects only
Gene Expression (mRNA) Tissue Samples DFU subjects only
Physical Indicators (e.g., photos and wound measurement) DFU subjects only
Clinical Indicators DFU subjects only

7.3.2. Part B

Blood samples for HIFα, (e.g., VEGF, EPO).
Biomarker Samples: (e.g., for cytokines and bioburden)
Gene Expression (mRNA) and Macrophages (Immunohistochemistry)Tissue Samples
Heavy Water (Site involvement will depend on site feasibility – selected subjects (one cohort) based on emerging data) (Repeat Dosing only.) Tissue samples via biopsy.
SensiLase (Dopplar blood flow)
Hyperspectral Imaging (oxygen dynamics) – if the technique can be established/validated. (Site involvement will depend on site feasibility – selected subjects (one cohort) based on emerging data) (Repeat Dosing only.)
Physical Indicators (e.g., photos and wound measurement). Silhouette for volume (record in eCRF) Digital photos (core) and acetate tracings (after screening scan and upload and send to core).
Clinical Indicators
Irritation/symptom Scale

7.4. Standard of Care Treatment

All subjects with DFU will receive Standard of Care treatment during this study. This will include active treatment arms, Run-in, Washout and for those randomized to the SoC treatment arm in the Treatment Period. Patients in this study will receive the same SoC-wound care that they would receive were they not in the study.

SoC consists of treatment plans that manage the wound environment through the phases of wound healing. Debridement and moist wound care are the fundamentals of wound care. A moist wound environment is promoted through the appropriate use of wound dressings and wound gels or ointments. Appropriate dressing application manages wound exudates and prevents degradation of epithelial cells and biochemical wound healing components.

SoC treatment arm is as follows:

- Cleanse wound with normal saline.
- Perform sharp debridement "as needed".
- Apply moist occlusion (0.9% sodium chloride gel plus a secondary dressing consisting of non-adherent foam and an outer gauze wrap) and offload using a removable cast or healing sandal, as appropriate.

7.4.1. Wound Bed Preparation and Debridement

Wound bed preparation is defined as the management of the ulcer to accelerate endogenous healing to facilitate the effectiveness of other therapeutic measures. The goal of wound bed preparation is to convert the molecular and cellular environment of a chronic wound to that of an acute wound. Necrotic tissue, excessive bacterial burden, senescent cells, and cellular debris can all inhibit wound healing. Surgical (sharp) debridement is the preferred method of debridement.

Wound debridement procedures:

Assess pain level and determine appropriate method of pain control.

- Cleanse wound with sterile saline.
- Utilize sterile tissue scissors, forceps, surgical blade and/or other appropriate surgical instruments to remove devitalized tissue, as clinically indicated.
- Following debridement, cleanse wound with sterile saline.
- Achieve hemostasis through use of pressure, topical coagulants or electrocautery as indicated.
- Apply appropriate wound dressing.

7.5. Gene Expression

Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with DFU or medically related conditions and/or the action of GSK1278863 will be identified by application of:

- Measurement of the levels of a subset of RNA species on blood and wound tissue samples.
- Proteome analysis of plasma and wound tissue samples.

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

7.5.1. RNA Expression Research of a Subset of RNA Species

RNA expression studies will be conducted using quantitative RT-PCR, and/or alternative equivalent technologies, which can facilitate the simultaneous measurement of the relative abundances of hundreds of RNA species resulting in a RNA expression profile for each blood and wound tissue sample. The RNAs assayed may be those involved with the pathogenesis or bioburden of DFUs, the absorption, distribution, metabolism, or excretion of GSK1278863 or in the subject's response to GSK1278863. In addition continuing research may identify other proteins or regulatory RNAs that may be involved in response to GSK1278863 or the pathogenesis of DFUs. The RNAs that code for these proteins and/or regulatory RNAs may also be studied. This will enable the evaluation of changes in RNA expression profiles that may correlate with biological response relating to DFUs, wounds, disorders related to the skin or the action of GSK127886.

7.5.2. Proteome Research

Wound tissue proteome studies may be performed by 2-D gel separation, and/or peptide mass mapping, or an alternative equivalent procedure. Proprietary algorithms and standard statistical techniques, such as ANOVA and ANCOVA, may be used to identify individual proteins exhibiting statistically acceptable changes in their levels between samples, and between groups of samples. These differentially expressed proteins may be identified by mass spectrometry or equivalent technology. This will enable the evaluation of changes in proteome profiles that may correlate with biological response relating to DFUs, wounds, other disorders of the skin and medically related conditions, or the action of GSK1278863.

The same samples may also be used to confirm findings by application of alternative technologies.

7.6. HIF Alpha

Blood samples for evidence of HIF activity (i.e., EPO, Hepcidin, VEGF) will be collected for all subjects, according to the table in Section 4.6. See the Study Procedures Manual (SPM) for processing, storage and shipping instructions.

7.7. Biomarkers (e.g., Cytokines, Bioburden and Gene Expression) Collection and Processing

With the subject's consent, wound biopsy tissue, wound fluid and blood sample(s) will be collected during this study to be used for the purposes of measuring novel biomarkers to identify factors that may influence wound healing and tissue repair and/or medically related conditions, as well as the biological and clinical responses to GSK.1278863. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events.

Samples will be collected at the timepoints indicated in Section 4.6. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with wound healing and tissue repair or medically related conditions and/or the action of GSK1278863 may be assessed in accordance with the objectives and endpoints of the study.

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

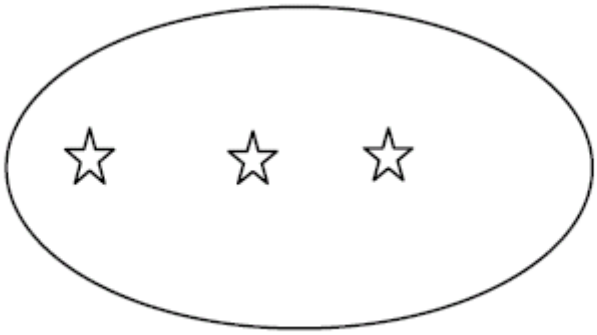
7.7.1. Wound Fluid Collection Directions

- All wound fluid samples are to be collected before wound debridement. The periwound area is to be prepped with an alcohol swab while being careful not to touch the wound base or the interior of the wound with the swab.
- The following wound fluid samples are to be collected at each visit using periopaper:
  - Three cytokines samples, placed in pre-labeled cryovials
  - Two bacterial samples, placed in pre-labelled cryovials containing Tris EDTA (TE) buffer solution (150µL/tube)
- Remove the periostrip from the sleeve by carefully holding only the orange section of the strip with a sterile forceps.
- Place the white end of the periostrip at appropriate collection site. Hold each periostrip in place for 30 seconds or until the entire white section of the periopstrip is visibly wet.
- Immediately after collection, using the forceps, transfer the periopstrip to the pre-labelled cryovial and then immediately drop the vial into liquid nitrogen to snap freeze samples.
- After snap freezing, the vials are to be transferred to a pre-labelled freezer box and then moved immediately into a -80°C freezer. Samples will be shipped to laboratory within 8 months of collection. Shipping instructions are in the SPM.

7.7.2. Punch Biopsy Directions

- Whenever feasible, tissue biopsies should be done at the time of debridement.
- Prior to obtaining biopsies, the wound is to be cleansed with saline solution.
- Obtain 3 tissue samples in the locations shown in Figure 4 by holding a punch biopsy instrument vertically over the wound then rotating down in a circular motion (using first two fingers). Once the instrument has penetrated the wound bed down to the hub, it can be removed. Using a needle held in the non-dominate hand, stretch the cylindrical tissue specimen away from the wound bed and use scissors held in the dominate hand to cut the specimen free. The use of forceps is discouraged because these instruments cause crush artifact that can render the sample unusable.

Figure 4 Sample locations within the DFU



- Place tissue samples in pre-labelled collection tubes for processing and storage.
- Obtain hemostasis as needed by method of choice of application of pressure, lumicain, or silver nitrate. Dress wound with appropriate dressing.
- Study wounds that have healed sufficiently will be exempt from further biopsies. This determination is at the discretion of the Principal Investigator or designee.

7.7.3. Macrophages

Macrophage analysis will be performed by immunostaining of sections of tissue prepared from biopsy samples. Tissue sections will be prepared for immunohistochemical analysis according to standard procedures, and immunostained with anti-CD68 antibody to detect macrophage. If methodology permits, CD68 immunostaining may be combined with markers of particular macrophage subtypes (for e.g., CD206 for M2 macrophage). This will enable the evaluation of changes in immune cell infiltration and function that may correlate with biological response relating to DFUs, wounds, diseases of the skin and medically related conditions or the action of GSK1278863.

7.7.4. Sample Collection for Macrophage Immunostaining

Upon collection, the biopsy will be placed in a pre-labelled cryovial and snap frozen in liquid nitrogen. The cryovials will be transferred to a pre-labelled freezer box and then moved immediately into a -80°C freezer. Samples will be shipped to laboratory within 8 months of collection. Shipping instructions are in the SPM.

7.8. Angiogenesis: Tissue Perfusion Assessment/Oxygen Availability

7.8.1. Vascular Perfusion

Assessments of vascular perfusion will be made according to the schedule in Section 4.6. Skin Perfusion Pressure (SPP) will be evaluated with the SensiLase System, which provides quantitative evaluation of microcirculatory perfusion in the skin. It measures skin perfusion (capillary perfusion pressure, or microcirculation) using a laser Doppler

sensor located beneath a pressure cuff (much like a blood pressure cuff). During the test the cuff is automatically inflated to the point the sensor determines that skin perfusion has stopped; the pressure is then released at a controlled rate while the cuff pressure and skin perfusion are measured. A laser detects the movement of blood upon release of the cuff; the pressure measured, in mmHG, is recorded at this instance giving the SPP reading. Subjects will have a plastic guide placed next to their wound and then a blood pressure cuff will be placed over the guide. The cuff will then be inflated and deflated while the reading is automatically captured. Software displays a graph of pressure and perfusion during cuff deflation and indicates the pressure at which skin perfusion is found to return.

7.8.1.1. Instructions for Assessing Vascular Perfusion with SensiLase

The subject is placed in a supine position. The laser wand is placed in the guide and the guide is placed adjacent to the wound, as close to the peri-wound area as possible while remaining on completely epithelialized tissue. A disposable cuff liner is placed on the cuff and the cuff is then placed snugly but not overly tight around the laser guide. One or two readings are to be taken: The first reading is to be taken at 12:00, with 12:00 being the most proximal edge of the wound. The next reading is also to be taken at the proximal side of the wound but within the angiosome tissue block, if position one is not within the angiosome tissue block. All relevant angiosomes necessary for appropriate interpretation of blood flow should also be captured. Angiosomes are designated locations found in the instruction manual for SensiLase.

7.8.2. Oxygen Dynamics/Angiogenesis

Oxygen dynamics and angiogenesis may be assessed by hyperspectral imaging as site feasibility allows and if the technique can be established/validated.

7.8.2.1. Hyperspectral Imaging Instructions

The hyperspectral imaging system illuminates the patient's tissue with light. Light that is reflected from the tissue is captured by a digital camera, and from a series of color images, spectral and statistical analyses are performed using computer software, to determine the chemistry of the tissue being imaged. A light and camera are placed several feet from the patient (None of this equipment ever touches the patient) and the image is captured in the system for analysis. The outcome of this study is for the resulting chemically encoded images to aid surgeons and clinicians assess and navigate the patient's condition and to make clinical decisions regarding treatment.

7.9. Physical Characteristics of the DFU

7.9.1. Location of the Study Ulcer

The location of the study ulcer will be recorded as foot (left or right), surface (plantar, dorsal, medial aspect of heel, medial aspect of great toe, posterior or lateral), and area [forefoot, midfoot, hindfoot (including the calcaneus), first through fifth metatarals or proximal to the foot on the ankle]. If more than one ulcer is located in the same proximity, be certain to clearly identify the specific study ulcer.

7.9.2. Instructions for Assessment of Physical Characteristics of the DFU - Wound Measurement and Photography

7.9.2.1. Photo Capture – Wound Images

Photographic documentation of the wound should be obtained after debridement. Randomization and weekly imaging of the wound should occur after cleansing and before treatment with study drug.

Photographic documentation should be sent electronically to the core lab as soon as possible, preferably on the same day the picture was taken.

Photographs of wounds will be stored locally at each site and uploaded to the wound core lab. Refer to the SPM for details.

7.9.2.2. Wound Tracings

Tracings of the wound should be obtained after debridement. To ensure the subject meets the randomization eligibility of the change in ulcer size between screening (or optional Baseline Run In visit, if different from day of screening) and randomization on Day 1, percent reduction of wound size must be calculated by the Investigator or designee using the method for measuring wound area provided in the SPM. The core lab may be consulted for assistance with area measurements or percent reduction determinations if necessary.

All wound tracings are to be scanned and sent to the core lab along with wound photographs (Section 7.9.2.1).

Original copies of the wound tracing are to be retained as source documents in the subjects' study files. Original tracings may be sent to the wound core lab when a quality scan cannot be obtained. Refer to the SPM for additional details.

7.9.2.3. Depth and Volume Measurements

The ARANZ Medical Silhouette system will be used for wound depth and volume measurement. All photographic parameters, including lighting, distance, and exposure, should comply with the ARANZ Silhouette Digital Camera Instruction Manual specifications. The volume measurement will be obtained directly from the camera and the measurement recorded on the case report form. Sites that do not have access to the ARANZ Medical Silhouette system will not be required to collect these exploratory data.

7.9.2.4. Ulcer Size Reduction

Percent change in study ulcer area will be determined by the site after the subject has completed the two-week Run-in period in order to determine if the subject qualifies for randomization. At the end of the Run-in period, the subject ulcer size reduction cannot exceed 30% in order for the subject to receive treatment. This assessment will be determined as an objective measurement, using the ulcer tracings collected at study visits.

7.10. Clinical Characteristics of the DFU

At each study visit, the investigator will assess the characteristics of the ulcer being treated. The investigator will assess, using the chart below (Table 6), the wound edge, base color, periwound condition, periwound color, degree of edema, amount of exudate (drainage), type of exudate and percent of granulation tissue present. The investigator will also assess whether the wound is healed or not healed.

Table 6 Ulcer Characteristics Chart

	N/A	
Wound Edge	☐	Viable Rolled Fibrotic Closed
Wound Base Color	☐	Red Yellow/Slough Black/Eschar
Periwound Condition	☐	Intact Macerated Denuded Reddened
Periwound Color	☐	Normal White/Grey Light Red/Pink Bright Red
Edema	☐	None Mild Moderate Severe
Drainage Amount	☐	None Minimal Moderate Heavy
Drainage Type	☐	Serous Sanguineous Serous-Sanguineous Purulent
Granulation Tissue	☐	0-25% 26-50% 51-75% 76-100%

7.10.1. Ulcer Exudate

Using the categories below as guidance, the Investigator will determine the amount and type of study ulcer exudate. The Investigator must take into account the amount of exudate absorbed into the study ulcer dressing. The following categories will be used to quantify the amount of ulcer exudate:

- No exudate
- Minimal amount: Light (scant) or small amount
- Moderate amount
- Heavy/large/copious amounts

The following categories will be used to describe the type of exudate:

- Not applicable: no exudate present
- Serous: clear or light yellow watery plasma
- Serosanguineous: pink to light-red watery plasma
- Sanguineous: red with fresh bleeding
- Purulent: thick and opaque exudate, of creamy yellow, green, white, or tan color

7.10.2. Ulcer Assessments

The presence/absence of the following signs of infection at the study ulcer site will be documented at each visit:

- Purulence
- Malodor
- Pain
- Increased warmth
- Erythema*
- Tenderness
- Induration
- Swelling*
- No symptoms present
- If Erythema or Swelling is selected, please complete the more detailed irritation assessments in Section 7.10.3.

7.10.3. Irritation Symptom Assessment

An irritation symptom assessment may be completed in both Part A and Part B of the study if ulcer assessments suggest that erythema and/or swelling are present.

Irritation Symptom Scale

Skin Irritation Scale:

Is infection present: Y / N

Erythema:

Score _____

Very slight erythema (barely perceptible) =1

Well-defined erythema = 2

Moderate to severe erythema = 3

Severe erythema[Severe erythema (beet redness) to slight eschar formation]= 4

Swelling/Edema:

Score_____

Very slight edema (barely perceptible) =1

Slight edema (edges are well defined by definite raising) = 2

Moderate (rasing approximately 1 millimeter) = 3

Severe edema (raising more than 1 millimeter and extending beyond the area of exposure = 4

7.11. Cell Proliferation and Collagen Turnover (Heavy Water Assessment)

Cell proliferation and collagen turnover will be assessed by incorporation of ^2H_2O (also called deuterated water or heavy water) into DNA and proteins in tissue biopsy samples.

Cell proliferation and collagen turnover is expected to increase at the wound site in patients receiving GSK1278863.

Complete instructions for heavy water assessment are not available at the time of this published protocol; if heavy water assessment is to be performed, based on feasibility (including site feasibility) and emerging data, an amendment will be issued prior to implementation.

7.12. Pharmacokinetics

7.12.1. PK Blood Sample Collection

Except for subjects randomized to Standard of Care, blood samples for pharmacokinetic analysis of GSK1278863 and metabolites will be collected into 2mL K3 EDTA tubes at the time points indicated in Section 4.6, Time and Events Tables. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring. Sample collection, processing, storage and shipping procedures are provided in the Study Procedures Manual (SPM).

7.12.2. PK Blood Sample Analysis

Plasma analysis will be performed under the management of Bioanalytical Science and Toxicokinetics, DMPK, GlaxoSmithKline. Concentrations of GSK1278863 and metabolites will be determined in plasma samples using the currently approved analytical methodology. Raw data will be stored in the archives at the site of bio-analysis. Once the plasma has been analyzed for GSK1278863 and metabolites any remaining plasma may be analyzed qualitatively for other circulating metabolites and the results reported under a separate DMPK protocol.

7.12.3. PK Sampling Windows

Best effort should be made to draw pharmacokinetic blood samples at exactly the scheduled time point. However, samples will be considered drawn per protocol if obtained within the following windows *following* each dose:

- Within ± 2 minute of target time, for samples from 0:30 to 1:30 post-dose
- Within ± 15 minutes of target time, for samples from 2:00 to 6:00 post-dose
- Within ± 30 minutes of target time, for samples after 6:00 post-dose
- Within ± 3 hours of target time for the 48 hour post-dose PK sample

7.12.4. Sample Analysis

Plasma analysis will be performed under the management of Worldwide Bioanalysis, DMPK, GlaxoSmithKline. Concentrations of GSK1278863 will be determined in plasma samples using the currently approved analytical methodology. Raw data will be stored in the GLP Archives, GlaxoSmithKline. Once the plasma has been analyzed for GSK1278863 any remaining plasma may be analyzed qualitatively for other circulating metabolites and the results reported under a separate DMPK protocol.

7.12.5. Microdialysis

Tissue samples may also be analyzed by micro-dialysis for concentrations of GSK1278863 (Part B repeat dosing). Microdialysis, if performed, will be conducted at one site only in selected subjects (one cohort), based on emerging data.

Instructions for microdialysis are not available at the time of this published protocol; if Microdialysis is to be performed, based on feasibility and emerging data, anamendment will be issued prior to implementation.

7.13. Pregnancy

7.13.1. Time Period for Collecting Pregnancy Information

Information on all pregnancies in female subjects will be collected after the start of dosing and through follow-up.

7.13.2. Action to be Taken if Pregnancy Occurs

The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE.

A spontaneous abortion is always considered to be an SAE and will be reported as such. Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in Section 12.9. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female subject who becomes pregnant while participating will discontinue study medication and be withdrawn from the study and complete all withdrawal procedures.

8. LIFESTYLE AND/OR DIETARY RESTRICTIONS

8.1. Contraception Requirements

8.1.1. Female Subjects

For female subjects who can become pregnant and for those on HRT and for whom menopausal status is not confirmed, at least one of these contraceptive methods, classified as highly effective, is required.

Abstinence

Abstinence from penile-vaginal intercourse must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Contraceptive Methods with a Failure Rate of < 1%

- Oral contraceptive, either combined or progestogen alone
- Injectable progestogen
- Implants of etonogestrel or levonorgestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches

- Intrauterine device (IUD) or intrauterine system (IUS) that meets the <1% failure rate as stated in the product label
- Male partner sterilization (vasectomy with documentation of azoospermia) prior to the female subject's entry into the study, and this male is the sole partner for that subject. For this definition, "documented" refers to the outcome of the investigator's/designee's medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records.
- Male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, cream or suppository).

These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring subjects understand how to properly use these methods of contraception.

8.2. Activity

Subjects will abstain from strenuous exercise for 48 hours prior to each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities during studies (e.g., watch television, read).

9. CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

9.1. Prohibited Medications

The healthy volunteer subjects must abstain from taking prescription or non-prescription drugs (including vitamins and dietary or herbal supplements), with the exception of those listed in Section 9.2, within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication until completion of the follow-up visit, unless in the opinion of the Investigator and sponsor the medication will not interfere with the study.

Diabetic subjects must abstain from taking potent CYP2C8 inhibitors and inducers such as gemfibrozil and rifampin/rifampicin. This is because the primary route of metabolism of GSK1278863 involves CYP2C8. Data from a study (PHI113634) to assess the interaction potential between GSK1278863 and a CYP2C8 inhibitor (gemfibrozil) showed a clinically significant increase in GSK1278863 exposure following co-administration. Therefore, inhibitors of CYP2C8 are prohibited from 7 days prior to the first dose of investigational product until 7 days after the last dose of investigational product and inducers of CYP2C8 are prohibited from 14 days prior to the first dose of investigational product until 7 days after the last dose of investigational product.

9.2. General Considerations

Occasional use of acetaminophen, defined as doses of ≤ 2 grams/day up to 48 hours prior to the first dose of study drug and until completion of follow-up procedures may be acceptable, at the discretion of the Principal Investigator or his/her designee.

Patients may not receive any erythropoietin stimulating agent for 3 months prior to screening, until after final study assessments during the Follow-Up Visit.

Patients may not receive systemic medication known to affect healing (e.g. corticosteroids (inhaled are permitted), methotrexate, TNF agents etc).

Women receiving HRT are permitted to continue therapy while on study. See Section 5.2.1 or Section 5.3.1.

All concomitant medications taken during the study will be recorded in the CRF. The minimum requirement is that drug name, dose administered and the dates of administration be recorded.

If the study ulcer is considered infected after the subject is enrolled the ulcer infection must be recorded as an Adverse Event. Subjects can remain in the study, however if infection occurs during washouts etc, the subjects must clear the infection prior to continuing.

10. COMPLETION OR EARLY WITHDRAWAL OF SUBJECTS

10.1. Subject Completion

A completed subject is one who has completed all phases of the study including the follow-up visit.

The end of the study is defined as the last subject's last visit.

10.2. Subject Withdrawal Criteria

Refer to Section 4.5 for dose adjustment/stopping criteria based on safety/PK/PD criteria.

A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral or administrative reasons.

10.3. Subject Withdrawal Procedures

10.3.1. Subject Withdrawal from Study

Subjects may withdraw from the study at any time and for any reason. They are not obliged to state the reason for withdrawal. However, the reasons for withdrawal, or failure to provide a reason, must be documented by the physician on the eCRF. Every effort should be made by the physician to follow up subjects who withdraw from the study, by adhering to follow-up procedures specified in Section 4.6.

10.3.2. Subject Withdrawal from Study Treatment

If a subject does not receive all doses of randomized study drug planned for that subject, the subject will be considered to have prematurely discontinued study drug. Every effort should be made by the physician to follow up subjects who withdraw from the study, by adhering to follow-up procedures specified in Section 4.6.

Decisions regarding replacement of subjects prematurely discontinued from study drug will be made by the Investigator and GSK Medical Monitor on a case-by-case basis.

10.4. Treatment After the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because other treatment options are available.

The investigator is responsible for ensuring that consideration has been given to the post-study care of the patient's medical condition, whether or not GSK is providing specific post-study treatment.

11. STUDY DRUG DOSAGE AND ADMINISTRATION

11.1. In-House Dosing

Subjects will receive investigational product directly from the Investigator or appropriate designee.

The dose of investigational product will be confirmed by a member of the study site staff other than the person administering the investigational product and prior to investigational product administration.

Instructions to sites:

1) Determine wound size in cm^2
2) Using proper ointment strength, determine amount (mg) of ointment to dispense for direct application to wound, for example:

$$Amount\ of\ ointment\ to\ dispense = 25mg \times wound\ size\ (cm^2)$$

3) Weigh ointment and transfer into dosing syringe
4) Weigh dosing syringe with ointment and record weight
5) Dispense ointment directly onto wound via syringe
6) After ointment is applied record the weight of empty dosing syringe to determine the precise amount of drug delivered

The amount of ointment (per wound size) applied may change as data is collected in order to adjust for exposure and/or safety.

11.1.1. At-Home Dosing

Pharmacists should dispense study drug for at-home dosing and label the container "Take as directed". A dosing card will be given to the subject, depending on the group they are randomized to, for instruction on at-home dosing. The dosing card will include detailed contact information for contacting study staff 24 hours per day. The patient's wound size will be determined and the amount of ointment for daily administration will be recorded on the dosing card. As above:

$$Amount\ of\ ointment\ to\ dispense = 25mg \times wound\ size\ (cm^2)$$

A conversion of ointment amount to ribbon length measured with the placard will be provided by GSK.

Compliance with study medication will be checked every time the subject returns for a study visit. The ointment tubes will be weighed prior to dispensing and during office visits to assess a daily averaged administered amount.

Patient instructions for dosing at home:

1. Site will provide a pre-marked placard to the patient indicating the amount of ointment to be applied.
2. Subjects will be directed to apply the ointment using the placard and to continue with their prescribed SoC.
3. Subjects will be instructed to avoid contact with the ointment other than at the wound site and to maintain aseptic techniques.
4. They will be instructed to return to the site with the tube(s) at each visit.
5. The sites will weigh the tube(s) to estimate the amount of ointment use by the patient.

11.2. Blinding

This will be a single-blind study, in that GSK study team members and the pharmacist at the site will be unblinded, but other site staff and subjects are blinded.

Because the GSK study team will be assessing data on a real-time basis while the study is ongoing, the team will be unblinded during the trial to allow accurate correlation of safety and tolerability information with PK and PD values. For this reason, this study is characterized as a single-blind study.

The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency**, when knowledge of the study treatment is important for the appropriate clinical management or welfare of the subject. Whenever possible, the Investigator must first discuss options with the GSK Medical Monitor or appropriate GSK study personnel **before** unblinding the subject's treatment assignment. If this is impractical, the investigator must notify GSK as soon as possible, but without revealing the treatment assignment of the unblinded subject, unless that information is important

for the safety of subjects currently in the study. The date and reason for the unblinding must be recorded in the appropriate data collection tool.

A subject will be withdrawn if the subject's treatment code is unblinded by the investigator or treating physician. The primary reason for discontinuation (the event or condition which led to the unblinding) will be recorded in the eCRF.

GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to clinical investigators in accordance with local regulations and/or GSK policy.

11.3. Packaging and Labeling

The contents of the label will be in accordance with all applicable regulatory requirements.

11.4. Preparation/Handling/Storage/Accountability

A description of the methods and materials required for preparation of GSK1278863 is provided in the SPM.

Study drug must be dispensed or administered according to procedures described herein. Only subjects enrolled in the study may receive study drug. Only authorized site staff may supply or administer study drug. All study drug must be stored in a secure area with access limited to the investigator and authorized site staff. Study drug is to be stored at **15-25°C and protected from light**. Maintenance of a temperature log (manual or automated) is required.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance. The investigator or the head of the medical institution (where applicable), or designated site staff (e.g., storage manager, where applicable) must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from and returned to GSK and the amount supplied and/or administered to and/or returned by subjects. The required accountability unit for this study will be tube, including tube weight. Discrepancies are to be reconciled or resolved. Procedures for final disposition of unused study treatment are listed in the SPM.

Investigational product is not expected to pose significant occupational safety risk to site staff under normal conditions of use and administration. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. Notify the monitor of any unintentional occupational exposure. A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

11.5. Assessment of Compliance

When the individual dose for a subject is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

When subjects are dosed at the study site, they will receive study treatment 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 study treatment 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 study treatment.

Instructions for checking drug compliance after at-home dosing are in the SPM.

11.5.1. Treatment of Study Treatment Overdose

GSK does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

12. ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The investigator or site staff is responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

AEs will be collected from the start of Study Treatment and until the follow-up contact.

SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed as related to study participation (e.g., study treatment, protocol-mandated procedures, invasive tests, or change in existing therapy) or related to a GSK product will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact. All SAEs will be recorded and reported to GSK within 24 hours, as indicated in Section 12.9.

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 subject has been discharged from the study, and he/she considers the event reasonably related to the study treatment or study participation, the investigator would promptly notify GSK.

12.1. Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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 the use of a medicinal product.

Events meeting the definition of an AE **include**:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgment of the investigator.
- 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 treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE unless this is an intentional overdose taken with possible suicidal/self-harming intent. This should be reported regardless of sequelae.).

Events that **do not** meet the definition of an AE include:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, **unless more severe than expected for the subject's condition, or otherwise meets the definition of an SAE**. For example, periodic treatment responsive hyperglycemia in patients with diabetes, ulcer: non-purulent exudates, transient inflammation near wound, some pain and local swelling with debridement.
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where 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.

12.2. Definition of Serious Adverse Events

If an event is not an AE per Section 12.1, 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, etc).

An SAE is any untoward medical occurrence that, at any dose:

a) Results in death

b) Is life-threatening

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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 hospitalization or prolongation of existing hospitalization

NOTE: In general, hospitalization signifies that the subject 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 out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills 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 disability/incapacity, or

NOTE: 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 or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect

f) Medical or scientific judgment should be exercised in deciding whether 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 subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are 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.

g) Is associated with liver injury **and** impaired liver function defined as:

- ALT ≥ 3xULN and total bilirubin* ≥ 2xULN (>35% direct), or
- ALT ≥ 3xULN and INR** > 1.5.

* Serum bilirubin fractionation should be performed if testing is available; if unavailable, measure urinary bilirubin via dipstick. If fractionation is unavailable and ALT ≥ 3xULN and total bilirubin ≥ 2xULN, then the event is still to be reported as an SAE.

** INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants. If INR measurement is obtained, the value is to be recorded on the SAE form.

For this study, any event that meets the definition of Serious Adverse Event, even if considered disease-related, must be reported as an SAE.

12.3. Cardiovascular Events

Investigators will be required to fill out event specific data collection tools for the following AEs and SAEs:

- Myocardial infarction/unstable angina
- Congestive heart failure
- Arrhythmias
- Valvulopathy
- Pulmonary hypertension
- Cerebrovascular events/stroke and transient ischemic attack
- Peripheral arterial thrombosis
- Deep venous thrombosis or pulmonary embolus
- Revascularization

This information should be recorded within one week of when the AE/SAE(s) are first reported.

12.4. Death Events

In addition, all deaths will require a specific death data collection tool to be completed. The death data collection tool includes questions regarding cardiovascular (including sudden cardiac death) and noncardiovascular death.

This information should be recorded within one week of when the death is first reported.

12.5. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrence. Appropriate questions include:

- "How are you feeling?"
- "Have you had any (other) medical problems since your last visit/contact?"
- "Have you taken any new medicines, other than those provided in this study, since your last visit/contact?"

12.6. Recording of AEs and SAEs

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) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the appropriate data collection tool.

It is not acceptable for the investigator to send photocopies of the subject's medical records to GSK in lieu of completion of the GSK, AE/SAE data collection tool. However, there may be instances when copies of medical records for certain cases are requested by GSK. In this instance, all subject identifiers, with the exception of the subject number, will be blinded on the copies of the medical records prior to submission of to GSK.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

12.7. Evaluating AEs and SAEs

12.7.1. Assessment of Intensity

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

Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

12.7.2. Assessment of Causality

The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence 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 natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated. The investigator will also consult the Investigator Brochure (IB) and/or Product Information, for marketed products, in the determination of his/her assessment [GlaxoSmithKline Document Number RM2008/00267/07]. 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.

12.8. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as may be indicated or as requested by GSK to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. This may include additional laboratory tests or investigations, histopathological examinations or consultation with other health care professionals. If a subject 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 data collection tool. The investigator will submit any updated SAE data to GSK within the designated reporting time frames.

12.9. Prompt Reporting of SAEs to GSK

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to GSK **within 24 hours**. Any follow-up information on a previously reported SAE will also be reported to GSK within 24 hours.

If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying GSK of the event and completing the appropriate data collection tool. The investigator will always provide an assessment of causality at the time of the initial report as described in Section 12.7.2, Assessment of Causality.

The primary mechanism for reporting SAEs to GSK will be the electronic data collection tool. If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the GSK Medical Monitor. Then the site will enter the serious adverse event data into the electronic system as soon as it becomes available.

The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE.

After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) 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, the site can report this information on a paper SAE form or to their GSK protocol contact by telephone.

GSK contacts for SAE receipt can be found at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

12.10. Regulatory Reporting Requirements for SAEs

Prompt notification of SAEs by the investigator to GSK is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSK has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. GSK will comply with country specific regulatory requirements relating to safety reporting to regulatory authorities, IRBs and investigators.

Investigator safety reports are prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and GSK policy and are forwarded to investigators as necessary. An investigator who receives an investigator safety report describing an SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from GSK will file it with the IB and will notify the IRB, if appropriate according to local requirements.

13. LIVER CHEMISTRY FOLLOW-UP PROCEDURES

Refer to the diagram in Appendix 1 for a visual presentation of the procedures listed below

The procedures listed below are to be followed if a subject meets the liver chemistry stopping criteria defined in Section 4.5.1.2:

- Immediately withdraw the subject from study treatment
- Notify the GSK medical monitor within 24 hours of learning of the abnormality to confirm the subject's study treatment cessation and follow-up.
- Complete the "Safety Follow-Up Procedures" listed below.
- Complete the liver event case report forms. If the event also meets the criteria of an SAE (See Section 12.2), the SAE data collection tool will be completed separately with the relevant details.
- Upon completion of the safety follow-up withdraw the subject from the study unless further safety follow up is required or GSK Medical Governance approval of drug restart is granted.
- Do not restart investigational product unless written approval is granted by GSK Medical Governance, whereupon the subject continues in the study after completion of the liver chemistry monitoring.

Safety Follow-Up Procedures for subjects with ALT ≥ 3xULN:

- Monitor subjects weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

Safety Follow-Up Procedures for subjects with ALT ≥3xULN and total bilirubin ≥2xULN (>35% direct bilirubin); or ALT ≥ 3xULN and INR[1] > 1.5:

- This event is considered an SAE (See Section 12.2). Serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).
- Make every reasonable attempt to have subjects return to the clinic within 24 hours for repeat liver chemistries, additional testing, and close monitoring (with specialist or hepatology consultation recommended).
- Monitor subjects twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values.

In addition, for all subjects with ALT ≥ 3xULN, every attempt must be made to also obtain the following:

- Viral hepatitis serology including:
  - Hepatitis A IgM antibody.
  - Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM).
  - Hepatitis C RNA.
  - Cytomegalovirus IgM antibody.
  - Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
  - Hepatitis E IgM antibody.
- Blood sample for pharmacokinetic (PK) analysis, obtained within 72 hours of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw in the eCRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, **do not obtain a PK sample**. Instructions for sample handling and shipping are included in the SPM.
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
- Fractionate bilirubin, if total bilirubin ≥ 2xULN.
- Assess eosinophilia
- Record the appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia) in the AE eCRF.

[1] INR testing not required per protocol and the threshold value does not apply to subjects receiving anticoagulants.

- Record use of concomitant medications, acetaminophen, herbal remedies, other over the counter medications, or putative hepatotoxins in the Concomitant Medications eCRF.
- Record alcohol use in the Liver Events eCRF.

The following are required for subjects with ALT ≥ 3xULN **and** bilirubin ≥ 2xULN (>35% direct) but are optional for other abnormal liver chemistries:

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies.
- Serum acetaminophen adduct HPLC assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [James, 2009]).
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
- The Liver Imaging and/or Liver Biopsy CRFs are also to be completed if these tests are performed.

14. STUDY CONDUCT CONSIDERATIONS

14.1. Posting of Information on Publicly Available Clinical Trial Registers

Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

14.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will also be conducted in accordance with ICH Good Clinical Practice (GCP), all applicable subject privacy requirements, and, the guiding principles of the 2008 Declaration of Helsinki. This includes, but is not limited to, the following:

- IRB review and favorable opinion/approval to conduct the study and of any subsequent relevant amended documents
- Written informed consent (and any amendments) to be obtained for each subject before participation in the study
- Investigator reporting requirements (e.g., reporting of AEs/SAEs/protocol deviations to IRB).

Written informed consent must be obtained from each subject prior to participation in the study.

14.3. Quality Control (Study Monitoring)

In accordance with applicable regulations including GCP, and GSK procedures, GSK monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and GSK requirements. When reviewing data collection procedures, the discussion will also include identification, agreement and documentation of data items for which the eCRF will serve as the source document.

GSK will monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents

14.4. Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance assessment and/or audit of the site records, and the regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study. In the event of an assessment, audit or inspection, the investigator (and institution) must agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventative actions to address any findings/issues identified.

14.5. Study and Site Closure

Upon completion or premature discontinuation of the study, the monitor will conduct closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations including GCP, and GSK procedures.

In addition, GSK reserves the right to temporarily suspend or prematurely discontinue this study at any time for reasons including, but not limited to, safety or ethical issues or severe non-compliance. If GSK determines such action is needed, GSK will discuss this with the investigator or the head of the medical institution (where applicable), including the reasons for taking such action. When feasible, GSK will provide advance notification to the investigator or the head of the medical institution, where applicable, of the impending action prior to it taking effect.

If the study is suspended or prematurely discontinued for safety reasons, GSK will promptly inform investigators or the head of the medical institution (where applicable) and the regulatory authorities of the suspension or premature discontinuation of the study and the reason(s) for the action. If required by applicable regulations, the investigator or

the head of the medical institution (where applicable) must inform the IRB promptly and provide the reason for the suspension or premature discontinuation.

14.6. Records Retention

Following closure of the study, the investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

GSK will inform the investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or GSK standards/procedures; otherwise, the retention period will default to 15 years.

The investigator must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility or transfer of ownership of the records in the event the investigator leaves the site.

14.7. Provision of Study Results to Investigators, Posting of Information on Publically Available Clinical Trials Registers and Publication

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. Investigators 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 investigators with the full summary of the study results. Investigators may share the summary results with the study subjects, as appropriate.

GSK will provide the investigator with the randomization codes for their site after completion of the full statistical analysis.

GSK aims to post a results summary to the GSK Clinical Study Register and other publicly available registers no later than 8 months after the last subject's last visit (LSLV) [this applies to each data analysis phase for studies with multiple phases, e.g., primary analysis, follow up analysis etc]. In addition, the aim is to submit a manuscript to a peer-

reviewed journal for publication within 18 months of LSLV. GSK also aims to publish the full study protocol on the GSK Clinical Study Register at the time the results of the study are published as a manuscript in the scientific literature.

When manuscript publication in a peer-reviewed journal is not feasible, further study information will be posted to the GSK Clinical Study Register to supplement the results summary.

14.8. Data Management

For this study subject data will be entered into GSK defined electronic case report forms (eCRFs), transmitted electronically to GSK or designee and combined with data provided from other sources in a validated data system.

Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medications terms will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and an internal validated medication dictionary, GSKDrug. eCRFs (including queries and audit trails) will be retained by GSK, and copies will be sent to the investigator to maintain as the investigator copy. **Subject initials will not be collected or transmitted to GSK according to GSK policy.**

15. REFERENCES

Albina JE, Mastrofrancesco B, Vessella JA, Louis CA, et al. HIF-1 expression in healing wounds: HIF-1a induction in primary inflammatory cells by TNF- a. *Am J Physiol Cell Physiol.* 2001;281:C1971-7.

Aragonés J, Schneider M, Van Geyte K, Fraisl P, et al. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat Genet.* 2008 Feb;40(2):170-80.

Botusan IR, Sunkari VG, Savu O, Catrina AI, et al. Stabilization of HIF-1α is critical to improve wound healing in diabetic mice. *PNAS.* 2008;105(49):19426-31.

Boulton AJM, Kirsner RS, Vileikyte L. Neuropathic diabetic foot ulcers. *N Engl J Med.* 2004;351:48-55.

Boulton AJM, Vileiktye L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet.* 2005;366:1719-24.

Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science.* 2001;294:1337-40.

Centers for Disease Control and Prevention (CDC). *National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2007.* US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA; 2008.

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med.* 2004;10(8):858-64.

Chang EI, Loh SA, Ceradini DJ, Chang SL. Age decreases endothelial progenitor cell recruitment through decreases in hypoxia-inducible factor 1α stabilization during ischemia. *Circulation.* 2007;116:2818-29.

Chowdhury R, Hardy A, Schofield CJ. The human oxygen sensing machinery and its manipulation. *Chem Soc Rev.* 2008;37(7):1308-19.

Eltzschig HK, Thompson LF, Karhausen J, Cotta RJ, et al. Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood.* 2004;104:3986-92.

Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet.* 2005;366(9498):1736-43.

Frykberg RG, et al. Diabetic foot disorders: a clinical practice guideline (rev. 2006). *J Foot ankle Surg.* 2006; 45(5):S-66.

Fukuda R, Zhang H, Kim J-W, Shimoda L, et al. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell.* 2007;129:111-22.

Galiano RD, Tepper OM, Pelo CR, Bhatt KA, et al. Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. *Am J Pathol.* 2004;164(6):1935-47.

Galkowska H, Wojewodzka U, Olszewski WL. Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. *Wound Repair Regen.* 2006;14(5):558-65.

Gallagher KA, Liu ZJ, Xiao M, Chen H, et al. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. *J Clin Invest.* 2007;117(5):1249-59.

GlaxoSmithKline Document Number 2011N115609_00 Study ID PHI113634. PHI113634 Clinical Pharmacology Study Report. Effective Date: May 2012

Goodridge D, Trepman E, Sloan J, et al. Quality of life of adults with unhealed and healed diabetic foot ulcers. *Foot Ankle Int.* 2006;27:274-80.

GSK1278863 Investigator's Brochure GlaxoSmithKline Document Number RM2008/00267/07 Effective Date: October 2015

James LP. Pharmacokinetics of acetaminophen - protein adducts **.** liver failure. *Drug Metab Disp.* 2009; 37:1779-84.

Khaodhia L, et al. The use of medical hyperspectral technology to evaluate microcirculatory changes in diabetic foot ulcers and to predict clinical outcomes. *Diabetes Care* , 2007; 30:903–910.

Li W, Guan S, Fan J, Cheng CF, et al. Extracellular heat shock protein-90α: linking hypoxia to skin cell motility and wound healing. *EMBO J.* 2007;26(5):1221-33.

Liu L, Marti G, Wei X, Zhang X, et al. Age-dependent impairment of HIF-1a expression in diabetic mice: correction with electroporation-facilitated gene therapy increases wound healing, angiogenesis, and circulating angiogenic cells. *J Cell Physiol.* 2008;217:319-27.

Lukashev D, Klebanov B, Kojima H, Grinberg A, et al. Cutting edge: hypoxia-inducible factor 1α and its activation-inducible short isoform I.1 negatively regulate functions of CD4+ and CD8+ T lymphocytes. *J Immunol.* 2006;177(8):4962-5.

Mace KA, Yu DH, Paydar KZ, Boudreau N, Young D. Sustained expression of HIF-1α in the diabetic environment promotes angiogenesis and cutaneous wound repair. *Wound Rep Reg.* 2007;15:636-45.

Myllyharju J. HIF prolyl 4-hydroxylases and their potential as drug targets. *Cur Pharm Design.* 2009;15:3878-85.

Myllyharju J. Prolyl 4-hydroxylases, key enzymes in the synthesis of collagens and regulation of the response to hypoxia, and their roles as treatment targets. *Ann Med.* 2008;40(6):402-17.

Reiber GE, Vileiktye L, Boyko EJ, delAguila M, Smith DG, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diab Care.* 1999;22(1):157-62.

Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. *Physiol.* 2004;19:176-82.

Sitkovsky MV, Lukashev D, Apasov S, Kojima H, et al. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annu Rev Immunol.* 2004;22:657-82.

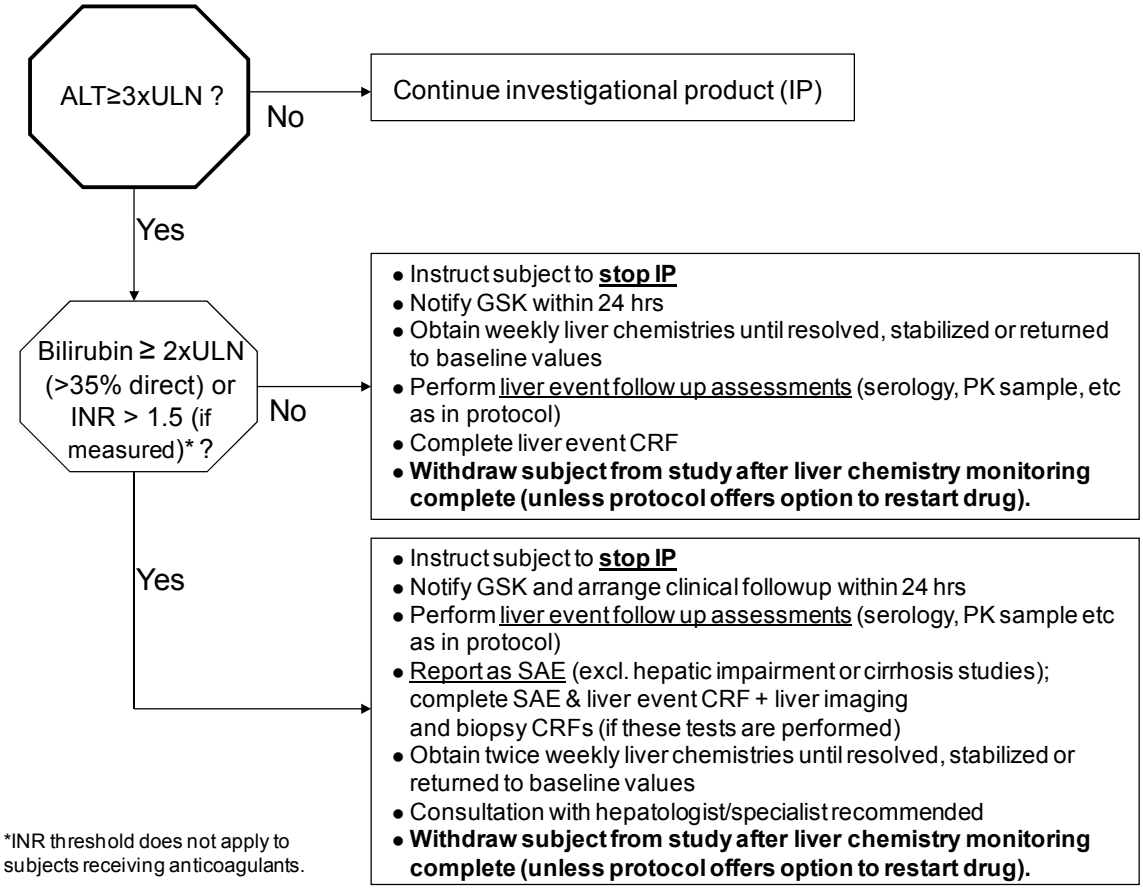
Thangarajah H, Yao D, Changa EI, Shia Y, et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. *PNQAS.* 2009;106(32):13505-10.

Yamaguchi Y, Yoshikawa K. Cutaneous wound healing: an update. *J Dermatol.* 2010;28100:521-34.

Zhang X, Liu L, Wei X, Tan YS, et al. Impaired angiogenesis and mobilization of circulating angiogenic cells in HIF-1α heterozygous-null mice after burn wounding. *Wound Rep Reg.* 2010; 18:193-201.

16. APPENDICES

16.1. Appendix 1: Liver Safety Algorithms



16.2. Appendix 2: Definitions

For the purposes of this study **moist wound therapy** consists of the investigational drug vehicle, a non-adherent dressing (See SPM for dressing selection) and an outer gauze wrap.

100% re-epithelialization is defined as full re-epithelialization of the wound surface with no trace of exudate or drainage, as determined by the investigator.

Wound closure is defined as 100% re-epithelialization that is confirmed at follow-up.

The **date of complete wound closure** is defined as the date of the first assessment of 100% re-epithelialization.

16.3. Appendix 3: Protocol Amendment Changes

AMENDMENT 1

Where the Amendment Applies

This amendment applies to all sites participating in this study.

Summary of Amendment Changes with Rationale

- This amendment reflects changes to the patient eligibility criteria.
- Changes to the stopping criteria were made with the addition of 2 criteria and the modification of 2 existing criteria.
- The number of tissue biopsies obtained during the study was reduced from 8 to 5 (Days 1, 2, 8, 14 and 22).
- An additional follow-up visit was added.
- Additional (minor) wording changes were made to clarify existing instructions or to correct typographical errors.

List of Specific Changes

Section 4.2 Discussion of Design and Treatment Plan

PREVIOUS TEXT

This is a randomized, placebo-controlled, single-blind (subjects and investigators will be blinded, GSK internal personnel will not be blinded), parallel-group, two part (Part A, Part B) trial in healthy volunteers and subjects with diabetic foot ulcers~~, to be conducted at two sites~~.

REVISED TEXT

This is a randomized, placebo-controlled, single-blind (subjects and investigators will be blinded, GSK internal personnel will not be blinded), parallel-group, two part (Part A, Part B) trial in healthy volunteers and subjects with diabetic foot ulcers.

Section 4.2.1.7 Follow-up Visits

PREVIOUS TEXT

~~A~~ follow-up visit will occur 7 to 10 days following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete follow-up procedures ~~7-10 days after withdrawal~~.

REVISED TEXT

Follow-up visit**s** will occur 7 to 10 days **and 28 to 32 days** following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete all follow-up procedures.

Section 4.2.2.4 Follow-up Visits

PREVIOUS TEXT

~~A~~ follow-up visit will occur 7 to 10 days following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete follow-up procedures ~~7-10 days after withdrawal~~.

REVISED TEXT

Follow-up visit**s** will occur 7 to 10 days **and 28 to 32 days** following completion of dosing. Any subject withdrawing from the trial prematurely will also be asked to complete all follow-up procedures.

Section 4.5.1.1 Hemoglobin Stopping Criteria

PREVIOUS TEXT

A patient that experiences an absolute increase in hemoglobin of ~~2.5mg~~/dL above their baseline level at any time will be withdrawn from the study.

REVISED TEXT

A patient that experiences an absolute increase in hemoglobin of **1.0g**/dL above their baseline level at any time will be withdrawn from the study.

Section 4.5.1.4 Other Dose Adjustment/Stopping Safety Criteria

PREVIOUS TEXT

- Any other occurrence that raises significant safety concern in the mind of the investigator.
- ~~Upper GI bleeding or any GI bleeding resulting in clinically significant blood loss~~ (See Section 5.3 for additional guidance).
- Diagnosis of cancer, with the exception of squamous cell and basal cell carcinoma (unless in proximity of the wound treated with GSK1278863).
- Treatment emergent pulmonary hypertension, new onset or worsening retinopathy (e.g. proliferative retinopathy, or macular edema), or new onset or worsening non-traumatic joint inflammation (e.g., rheumatic or psoriatic arthritis).

REVISED TEXT

- Any other occurrence that raises significant safety concern in the mind of the investigator.
- **Any patient who has an increase from baseline in total wound area > 20% at any time will be withdrawn from treatment**
- **Any patient who develops at any time after randomization 3 of the following 6 signs at the study wound site will be withdrawn from treatment: local erythema, edema, pain, pus, local warmth, loss of function**
- **Subjects with documented GI bleeding, new onset positive fecal occult blood test, or abdominal pain (other than transient, minor abdominal pain).** See Section 5.3 for additional guidance.
- Diagnosis of cancer, with the exception of squamous cell and basal cell carcinoma (unless in proximity of the wound treated with GSK1278863).
- Treatment emergent pulmonary hypertension, new onset or worsening retinopathy (e.g. proliferative retinopathy, or macular edema), or new onset or worsening non-traumatic joint inflammation (e.g., rheumatic or psoriatic arthritis).

Section 4.6.1 Time and Events Table Part A

PREVIOUS TEXT

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)												Follow-up Visit
			Day 1									Day 2	Day 3	Day 4	7-10 days
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h	
All Subjects	Check in		X												
	Informed Consent	X													
	Demographics	X													
	Full Physical Exam	X													
	Brief Physical Exam		X									X	X	X	X
	Medical/medication/ drug/alcohol history	X	X												
	12-lead ECG[1]	X	X										X		X
	Vital signs[2]	X	X										X		X
	Urine pregnancy(women)	X													X
	HIV, Hep B and Hep C	X													
	Hema/Chem Safety Labs[3]	X	X										X		X
	Blood for HIFα Samples (e.g., hepcidin, EPO,VEGF)[4]	X	X							X	X[4]			X	

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)												Follow-up Visit
			Day 1									Day 2	Day 3	Day 4	7-10 days
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h	
	Study treatment dosing			X											
	Pharmacokinetic Sampling[5]		X		X	X	X	X	X	X	X[5]	X	X	X	
	Concomitant Medications		←==========→												X
	Adverse Event Review[6]		←==========→												X
Subjects with DFUs[7] Only	Gene Expression (mRNA) Tissue Samples[8]		X									X		X	X
	Biomarker fluid samples (e.g., for cytokines, growth factors, and bioburden)[9]		X											X	X
	Physical Characteristics (e.g., photos and wound measurement post-debridement, volume)	X	X											X	X
	Clinical Characteristics[10]	X	X			X	X	X			X	X	X	X	X

1. Single ECGs will be taken Screening, and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at Follow-up. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at Follow-up. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety analyses will be collected fasting at Screening and on Day 1 and at the 48h post-last-dose on Day 3, and at Follow-up. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
5. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected at immediately pre-dose (time 0), 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-'dose. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
6. Adverse events are to be collected from first dose through the Follow-up visit.
7. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin
8. Microarray and Taqman will be performed at all timepoints.
9. Wound fluid and tissue samples for biomarkers of cytokines and bioburden will be collected at pre-dose (time 0) on Days 1and 4 and at the Follow-up Visit.
10. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF

REVISED TEXT

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h		
All Subjects	Check in		X													
	Informed Consent	X														
	Demographics	X														
	Full Physical Exam	X														
	Brief Physical Exam		X									X	X	X	X	**X**
	Medical/medication/ drug/alcohol history	X	X													
	12-lead ECG[1]	X	X										X		X	
	Vital signs[2]	X	X										X		X	**X**
	Urine pregnancy(women)	X													X	
	HIV, Hep B and Hep C	X														
	Hema/Chem Safety Labs[3]	X	X										X		X	
	Blood for HIFα Samples (e.g., hepcidin, EPO,VEGF)[4]	X	X							X	X[4]			X		

Study Population	**Procedure**	**Screening (up to 28 days prior to Day 1)**	**Study Day (each dosing period)**												**Follow Up**	
			Day 1									**Day 2**	**Day 3**	**Day 4**	**7-10 days post last dose**	**28-32 days post last dose**
			Pre-dose	**0h**	**0.25h**	**0.5h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**	**48 h**	**72 h**		
	Study treatment dosing			X												
	Pharmacokinetic Sampling[5]		X		X	X	X	X	X	X	X[5]	X	X	X		
	Concomitant Medications		←==========											→	X	
	Adverse Event Review[6]		←==========											→	X	**X**
Subjects with DFUs[7] Only	Gene Expression (mRNA) Tissue Samples[8]		X									X		X	X	
	Biomarker fluid samples (e.g., for cytokines, growth factors, and bioburden)[9]		X											X	X	

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)														Follow Up	
			Day 1										Day 2	Day 3		Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h		24h	48 h		72 h		
	Physical Characteristics (e.g., photos and wound measurement post-debridement, volume)	X	X													X	X	
	Clinical Characteristics[10]	X	X			X	X	X			X	X		X	X	X	X	

1. Single ECGs will be taken Screening, and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at Follow-up. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at **the 7-10 day** Follow-up **Visit**. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety analyses will be collected fasting at Screening and on Day 1 and at the 48h post-last-dose on Day 3, and at **the 7-10 day** Follow-u p **Visit.** Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
5. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected at immediately pre-dose (time 0), 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-'dose. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
6. Adverse events are to be collected from first dose through the **final** Follow-up visit.
7. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin
8. Microarray and Taqman will be performed at all timepoints.
9. Wound fluid for biomarkers of cytokines and bioburden will be collected at pre-dose (time 0) on Days 1and 4 and at the **7- 10 day** Follow-up Visit.
10. Clinical characteristic assessments of the DFU begin with Section **7.1**1 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF

Section 4.6.2 Time and Events Table Part B

PREVIOUS TEXT

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up
		Day 1				Day 8								
Visit Window (relative to Day 1)	-28 to -1 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose
Clinic Visit		X	X	X		X		X	X	X	X	X	X	X
Informed Consent	X													
Demographics	X													
Complete physical	X													
Brief physical		X	X	X		X	X	X	X		X	X	X	X
Medical/medication/ drug/alcohol history	X													
12-lead ECG[1]	X	X										X		
Vital signs[2]	X	X				X		X				X		X
Urine pregnancy(females)	X	X												X
HIV, Hep B and Hep C screen	X													
Hema/Chem/ Safety Labs[3]	X	X				X		X				X		X
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)	X	X[4]				X[4]		X				X		
Standard of Care Treatment[5]	X		X	X	X									

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up
		Day 1				Day 8								
Study Treatment/Dosing In the Clinic[6]		X				X	X	X	X	X	X			
PK serial blood sampling[7]		X				X (truncated)		X	X		X	X	X	
PK Trough Sample[7]										X				
Gene Expression (mRNA) Samples Tissue Biopsies[8]		X	X			X	X	X			X	X		X
Biomarker Samples: Wound fluid for cytokines and bioburden[9]		X	X	X (Day 3 only[9])		X		X	X			X		X
Macrophages		X	X			X	X	X			X	X		X
SensiLase (Dopplar blood flow)		X				X		X				X		X
Physical Characteristics (assessed pre-dose)[11]	X[11]	X				X	X	X		X		X		X
Clinical Characteristics[12]	X	X	X	X		X	X	X	X	X	X	X	X	X
Concomitant Medication Review		X	←==========										→	X
Adverse Event Assessment[13]		X	←==========										→	X

1. Single ECGs will be taken at Screening and pre-dose on Day 1(SD) and 24h post-last-RD dose on Day 22. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1 (SD) and 8(RD), 15, 22 (24h post-last-RD dose) , and at Follow-up. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,8,14, 22 (24h post-last-dose), and at Follow-up. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
5. During washout times all subjects will be on SOC (Section 7.4).
6. In between clinic visits, subjects with perform applications of study drug at home.
7. See separate Time and Events table for serial PK sampling details for Days 1, 8, 14 and 21. Trough sample will be taken prior to dosing on Day 18. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
8. Direct wound tissue samples for analysis of gene expression will be collected at pre-dose on Days 1, 2, 8, 11, 14, 21, 22 and at the Follow-up Visit. TaqMan analysis will be performed on each day. Microarray analysis will be performed on the following Days 1, 2, 8, 14, 22 and Follow-up, however based on emerging data additional time points may be added. Refer to Section 7.4 for additional details on the collection and processing of gene expression samples.
9. Wound fluid and tissue samples for biomarkers of cytokines and bioburden will be collected at pre-dose on Days 1, 2. 3, 8, 14, 15, 22 and at the Follow-up Visit. Refer to the Section 7.5.3 for additional details on the collection and processing of biomarker tissue samples.
10. May be conducted at only one or both sites, dependent upon feasibility. Will be performed in a select cohort of patients. The precise procedure will be dependent upon the outcome of feasibility work.
11. Physical characteristics of the wound (e.g., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), and pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the Follow-up Visit. Any subject that is participating in additional cohorts during Part B would need to be assessed for wound healing rate for 14 days (as in the regular screen) to ensure healing rate is less than 30%.
12. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
13. Adverse events are to be collected from first dose through the Follow-up visit.

REVISED TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -1 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								**7-10 days post last dose**	**28-32 days post last dose**
Clinic Visit	**X**	X	X	X		X		X	X	X	X	X	X	X	X
Informed Consent	X														
Demographics	X														
Complete physical	X														
Brief physical		X	X	X		X	X	X	X	**X**	X	X	X	X	**X**
Medical/medication/ drug/alcohol history	X														
12-lead ECG[1]	X	X										X			
Vital signs[2]	X	X				X		X				X		X	**X**
Urine pregnancy(females)	X	X												X	
HIV, Hep B and Hep C screen	X														
Hema/Chem/ Safety Labs[3]	X	X				X		X				X		X	
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)	X	X[4]				X[4]		X				X			
Standard of Care Treatment[5]	X		X	X	X										
Study Treatment/Dosing In the Clinic[6]		X				X	X	X	X	X	X				

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up	
		Day 1				Day 8									
PK serial blood sampling[7]		X	**X**	**X**		X (truncated)		X	X		X	X	X		
PK Trough Sample[7]										X					
Gene Expression (mRNA) Samples Tissue Biopsies[8]		X	X			X		X				X			
Biomarker Samples: Wound fluid for cytokines and bioburden[9]		X	X	X (Day 3 only[9])		X		X	X			X		X	
Macrophages[10]		X	X			X	X	X			X	X		X	
SensiLase (Dopplar blood flow)		X				X		X				X		X	
Physical Characteristics (assessed pre-dose)[11]	X[11]	X				X	X	X		X		X		X	
Clinical Characteristics[12]	X	X	X	X		X	X	X	X	X	X	X	X	X	
Concomitant Medication Review		X	←==========											X	
Adverse Event Assessment[13]		X	←==========											X	**X**

1. Single ECGs will be taken at Screening and pre-dose on Day 1(SD) and 24h post-last-RD dose on Day 22. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1 (SD) and 8(RD), 15, 22 (24h post-last-RD dose) , and at Follow-up **Visits**. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,8,14, 22 (24h post-last-dose), and **at the 7-10 day** Follow-up **Visit**. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
5. During washout times all subjects will be on SOC (Section 7.4).
6. In between clinic visits, subjects with perform applications of study drug at home.
7. See separate Time and Events table for serial PK sampling details for Days 1, 8, 14 and 21 and **the associated 24, 48 and 72 hr time points**. Trough sample will be taken prior to dosing on Day 18. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
8. Direct wound tissue samples for analysis of gene expression will be collected at pre-dose on Days 1, 2, 8, 14 and 22, however based on emerging data additional time points may be added. Refer to Section 7.4 for additional details on the collection and processing of gene expression samples.
9. Wound fluid for biomarkers of cytokines and bioburden will be collected at pre-dose on Days 1, 2. 3, 8, 14, 15, 22 and at the **7-10 day** Follow-up Visit. Refer to the Section 7.5.3 for additional details on the collection and processing of biomarker tissue samples.
10. May be conducted at only one or both sites, dependent upon feasibility. Will be performed in a select cohort of patients. The precise procedure will be dependent upon the outcome of feasibility work.
11. Physical characteristics of the wound (e.g., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), and pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the **7-10 day** Follow-up Visit. Any subject that is participating in additional cohorts during Part B would need to be assessed for wound healing rate for 14 days (as in the regular screen) to ensure healing rate is less than 30%.
12. Clinical characteristic assessments of the DFU begin with Section **7.11** and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
13. Adverse events are to be collected from first dose through the **final** Follow-up visit.

Section 4.6.3 Serial PK Time and Events Table Part B

PREVIOUS TEXT

Procedure	Part B Serial PK Sampling[1,2]													
	Hours (h)													
	Pre-dose	0.25h	0.5h	1h	2h	4h	6h	8h	10h	11h	12h	24h	48h	72h
Single Dose Sampling OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	X
Single Dose Sampling IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	X
Repeated Dose Day 8 (Start of Repeated Dose)	X			X	X	X	X							
Repeated Dose Day ~~15~~ OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X		
Repeated Dose Day ~~15~~ IN-PATIENTS	X	X	X	X	X	X		X			X	X		
Repeated Dose Day 21 OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	
Repeated Dose Day 21 IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	

1. Patients are permitted to either stay in clinic (in-patients) or be discharged and return for clinic visits (out-patients). Refer to Section 7.13 for details on the collection and processing of PK samples.
2. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
3. Sample must be taken within the 10 to 12 hour time window.

REVISED TEXT

Procedure	**Part B Serial PK Sampling[1,2]**													
	Hours (h)													
	Pre-dose	**0.25h**	**0.5h**	**1h**	**2h**	**4h**	**6h**	**8h**	**10h**	**11h**	**12h**	**24h**	**48h**	**72h**
Single Dose Sampling OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	X
Single Dose Sampling IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	X
Repeated Dose Day 8 **Truncated** (Start of Repeated Dose)	X			X	X	X	X							
Repeated Dose Day **14** OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X		
Repeated Dose Day **14** IN-PATIENTS	X	X	X	X	X	X		X			X	X		
Repeated Dose Day 21 OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	
Repeated Dose Day 21 IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	

1. Patients are permitted to either stay in clinic (in-patients) or be discharged and return for clinic visits (out-patients). Refer to Section 7.13 for details on the collection and processing of PK samples.
2. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
3. Sample must be taken within the 10 to 12 hour time window.

Section 5.2.2 Exclusion Criteria – Healthy Volunteers (Part A Cohort 1)

PREVIOUS TEXT

8. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

Where participation in the study would result in donation of blood or blood products in excess of 500mL within a 56 day period.

9. Pregnant females as determined by positive urine hCG test at screening or prior to dosing.

10. Unwillingness or inability to follow the procedures outlined in the protocol.

11. Subject is mentally or legally incapacitated

REVISED TEXT

8. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

9. Where participation in the study would result in donation of blood or blood products in excess of 500mL within a 56 day period

10. Pregnant females as determined by positive urine hCG test at screening or prior to dosing.

11. Unwillingness or inability to follow the procedures outlined in the protocol.

12. Subject is mentally or legally incapacitated

Section 5.3.1 Inclusion Criteria –DFU Subjects

PREVIOUS TEXT

A DFU subject will be eligible for inclusion in this study only if all of the criteria listed for healthy volunteers are met (Section 5.2), ~~with the exception of criterion 3~~, as well as following criteria:

REVISED TEXT

A DFU subject will be eligible for inclusion in this study only if all of the criteria listed for healthy volunteers are met (Section 5.2) as well as following criteria:

Section 5.3.2 Exclusion Criteria –DFU Subjects

PREVIOUS TEXT

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

Diabetic subjects that are being considered for this study will not be eligible for inclusion in this study if any of the following criteria apply or if the exclusion criteria outlined in Section 5.2.2 apply, ~~with the exception of criterion 8~~.

1. Subjects with:

- Ulcers accompanied by infected cellulitis, osteomyelitis, or clinical signs or symptoms of infection,

- Gangrene on any part of affected limb,

- Active Charcot's foot on the study limb,

- Planned vascular surgery, angioplasty or thrombolysis,

- Ulcers involving exposure of tendon, bone, or joint capsule. (It is acceptable to have ulcers extending through the dermis and into subcutaneous tissue with presence of granulation tissue),

- Ulcers due to non-diabetic etiology.

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) and/or any major changes (per investigator's judgment) to related medications within ~~3~~ months prior to randomization.

3. ~~Active malignancy or last treatment for malignancy within 6 months of randomization (patients with basal or squamous cell carcinoma of the skin are not excluded).~~

4. Other clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the Investigator or the GSK Medical Monitor, not stabilized or may otherwise impact the results of the study.

5. Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within ~~three~~ months of randomization.

6. Patients undergoing hemodialysis.

7. History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within ~~6 months~~ prior to screening.

8. Active peptic, duodenal, or esophageal ulcer disease, or any gastrointestinal bleeding, within ~~12 weeks~~ prior to screening.

9. Patients with known pulmonary hypertension.

10. Use of prohibited medications as described in Section 9.1 of the protocol.

REVISED TEXT

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is required.

Diabetic subjects that are being considered for this study will not be eligible for inclusion in this study if any of the following criteria apply or if the exclusion criteria outlined in Section 5.2.2 apply.

1. Subjects with:

- Ulcers accompanied by infected cellulitis, osteomyelitis, or clinical signs or symptoms of infection,

- Gangrene on any part of affected limb,

- Active Charcot's foot on the study limb,

- Planned vascular surgery, angioplasty or thrombolysis,

- Ulcers involving exposure of tendon, bone, or joint capsule. (It is acceptable to have ulcers extending through the dermis and into subcutaneous tissue with presence of granulation tissue),

- Ulcers due to non-diabetic etiology.

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) and/or any major changes (per investigator's judgment) to related medications within **6** months prior to randomization.

3. **History of malignancy within 5 years of Screening or those with a strong family history of cancer (e.g., familial cancer disorders), with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.**

4. Other clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the Investigator or the GSK Medical Monitor, not stabilized or may otherwise impact the results of the study.

5. Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within **6** months of randomization.

6. Patients undergoing hemodialysis.

7. History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within **1 year** prior to screening.

8. Active peptic, duodenal, or esophageal ulcer disease, or any gastrointestinal bleeding, within **1 year** prior to screening.

9. Subjects with a platelet count <100,000/mm^3 at screening.

10. Subjects with an International Normalized Ratio (INR) >1.5 at screening.

11. Subjects with a hemoglobin level above the gender-specific upper limit of normal at screening.

12. Subjects with a history of non-traumatic joint inflammation (with the exception of inflammation due to osteoarthritis).

13. Patients with known pulmonary hypertension.

14. Use of prohibited medications as described in Section 9.1 of the protocol.

Section 7.5.3 Fluid and Tissue Collection for Biomarkers was deleted to enhance the flow of the document and improve clarity.

Section 7.5.3.1 Wound Fluid Collection Directions was moved to Section 7.7.1 to enhance the flow of the document and improve clarity.

Section 7.7 Biomarkers (e.g., Cytokines and Bioburden)

PREVIOUS TEXT

7.7. Biomarkers (e.g., Cytokines and Bioburden)

Wound tissue samples for biomarker assessments will be collected according to the table in Section 4.6.

REVISED TEXT

7.7. Biomarkers (e.g., Cytokines, Bioburden **and Gene Expression**) Collection and Processing)

Wound tissue **and fluid** samples for biomarker assessments will be collected according to the table in Section 4.6.

Section 7.9 Vascular Perfusion was renamed Angiogenesis: Tissue Perfusion Assessment/Oxygen Availability for consistency (A new heading of 7.9.1 was created for Vascular Perfusion)

7.9.2. Oxygen Dynamics/Angiogenisis

PREVIOUS TEXT

7.~~10~~ Oxygen Dynamics /Angiogenisis

Oxygen dynamics and angiogenesis may be assessed by hyperspectral imaging ~~according to the table in Section 4.6,~~ as site feasibility allows.

REVISED TEXT

7.**9.2**. Oxygen Dynamics/Angiogenisis

Oxygen dynamics and angiogenesis may be assessed by hyperspectral imaging as site feasibility allows.

Section 7.11 Clinical Characteristics of the DFU

PREVIOUS TEXT

Section 7.~~12~~ Clinical Characteristics of the DFU

At each study visit, the investigator will assess the characteristics of the ulcer being treated. The investigator will assess, using the chart below, the wound edge, base color, periwound condition, periwound color, degree of edema, amount of exudate, type of exudate and percent of granulation tissue present.

REVISED TEXT

Section 7.**11** Clinical Characteristics of the DFU

At each study visit, the investigator will assess the characteristics of the ulcer being treated. The investigator will assess, using the chart below **(Table 6)**, the wound edge, base color, periwound condition, periwound color, degree of edema, amount of exudate **(drainage)**, type of exudate and percent of granulation tissue present.

Section 7.11.2 Ulcer Exudate

PREVIOUS TEXT

7.~~12.1~~. Ulcer Exudate

The Investigator will determine the amount and type of study ulcer exudate. The Investigator must take into account the amount of exudate absorbed into the study ulcer dressing. The following categories will be used to quantify the amount of ulcer exudate:

• No exudate

• Minimal amount

~~• Light (scant) or small amount~~

• Moderate amount

• Heavy/large/copious amounts

REVISED TEXT

Section 7.**11.2** Ulcer Exudate

Using the categories below as guidance, the Investigator will determine the amount and type of study ulcer exudate. The Investigator must take into account the amount of exudate absorbed into the study ulcer dressing. The following categories will be used to quantify the amount of ulcer exudate:

- No exudate
- Minimal amount: **Light (scant) or small amount**
- Moderate amount
- Heavy/large/copious amounts

AMENDMENT 2

Where the Amendment Applies

This amendment applies to all sites.

Summary of Amendment Changes with Rationale

- This amendment reflects changes to Inclusion Criteria –DFU Subjects #7
- This amendment corrects the dosage found in Table 2 and Table 4 of Section 4.3. Treatment Assignment
- This amendment reflects changes to the Time and Events Tables for Part A and B.
- This amendment changes the screening procedures for subjects participating in more than one cohort in Part A
- Additional (minor) wording changes were made to clarify existing instructions or to correct typographical errors.

List of Specific Changes

Section 3.3.1.Gene Expression Analysis, Paragraph 1

PREVIOUS TEXT

Expression of genes important in a range of dynamic cellular processes (including inflammation, angiogenesis and extracellular matrix deposition) is expected to change as wound healing progresses. Moreover, with active drug treatment, genes coding for direct downstream targets of HIF signaling (e.g., HMOX1, VEGF and NOS2) should be upregulated. Gene expression will therefore be assessed by RT-PCR using tissue samples taken from the wound area. Most of these genes will be probed for using a focused array covering approximately 600 genes. TaqMan PCR will be employed to validate gene expression for a number of signature genes for each of the signaling pathways/cellular processes of interest and for any other genes of interest not captured in the focused array.

REVISED TEXT

Expression of genes important in a range of dynamic cellular processes (including inflammation, angiogenesis and extracellular matrix deposition) is expected to change as wound healing progresses. Moreover, with active drug treatment, genes coding for direct downstream targets of HIF signaling (e.g., HMOX1, VEGF and NOS2) should be upregulated. **Genetic markers of bioburden may also change with active drug treatment.** Gene expression will therefore be assessed by RT-PCR using tissue samples taken from the wound area. Most of these genes will be probed for using a focused array covering approximately 600 genes. TaqMan PCR will be employed to validate gene expression for a number of signature genes for each of the signaling pathways/cellular processes of interest and for any other genes of interest not captured in the focused array.

Section 4.2.1.Part A – Single Doses in Healthy Volunteers and DFU Patients (Cohorts 1 through 4), Paragraph 1, Sentence 4

PREVIOUS TEXT

Participation in more than one cohort in Part A, or participation in Part A then Part B, will require the subject to be screened again.

REVISED TEXT

Participation in more than one cohort in Part A, ~~or participation in Part A then Part B~~ will **not** require the subject to be screened again.

Section 4.2.1.1. Screening (Part A), Paragraph 1

PREVIOUS TEXT

For the purposes of subject eligibility for enrollment in the study, screening assessments are defined as any assessments performed prior to the first dose of study drug in each part.

REVISED TEXT

For the purposes of subject eligibility for enrollment in the study, **fasting** screening assessments are defined as any assessments performed prior to the first dose of study drug in each part.

Section 4.2.1.2. Cohort 1 – Healthy Volunteers (Part A), Paragraph 1, Sentence 3 and Paragraph 2, Sentence 7

PREVIOUS TEXT

The first application will be with a dose of 0.3mg (by utilization of 300mg of 0.01%).

Subjects will then return to the clinic 7 to 10 days after dosing for a follow-up visit.

REVISED TEXT

The first application will be with a dose of 0.3mg (by utilization of 300mg of ~~0.01%~~ **0.1%**).

Subjects will then return to the clinic 7 to 10 days **and 1 month** after dosing for ~~a~~ follow-up visit**s**.

Section 4.2.1.5. Cohorts 3 and 4 (Part A), Paragraph 1

PREVIOUS TEXT

If subjects are re-enrolling from a previous cohort ensure that there has been a minimum of a 10 day washout.

REVISED TEXT

.If subjects are re-enrolling from a previous cohort ensure that there has been a minimum of a 10 day washout **prior to initiating the 14 day run-in to assess healing rate**.

4.2.2. Part B – Single Dose/Repeat Doses in Diabetic Foot Ulcer Subjects (Cohorts 5, 6 and 7), Paragraph 6

PREVIOUS TEXT

Subjects may not participate in more than one cohort in Part B.

REVISED TEXT

Subjects may not participate in more than one cohort in Part B. **Subjects may participate in Part A and then Part B which will require the subject to be screened again.**

Section 4.3. Treatment Assignment, Table 2 Part A Doses

PREVIOUS TEXT

Cohort	Regimen	Dose (mg)	Treatment Area by Period	
			1	2
1	P	Placebo[1]	Intact	Intact
	A	0.150mg		
	B	TBD[2]		
2	P	Placebo	Intact	Wound
	C	TBD		
3	P	Placebo	Wound	N/A
	D	TBD		
4(or final)	P	Placebo	Wound	Intact
	E	TBD		
O (Optional)[3]	P	Placebo	Wound	N/A
	F	TBD		

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

REVISED TEXT

Cohort	Regimen	Dose (mg)	Treatment Area by Period	
			1	2
1	P	Placebo[1]	Intact	Intact
	A	~~0.150mg~~**0.3mg**		
	B	TBD[2]		
2	P	Placebo	Intact	Wound
	C	TBD		
3	P	Placebo	Wound	N/A
	D	TBD		
4(or final)	P	Placebo	Wound	Intact
	E	TBD		
O (Optional)[3]	P	Placebo	Wound	N/A
	F	TBD		

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

Section 4.3. Treatment Assignment, Table 4 Example Treatment Options

PREVIOUS TEXT

Cohort	Regimen/ Dosing Period	Dose (mg)	Treatment Area
1 (HVT)	P1	Placebo[1]	Intact Skin
	A	0.150mg	Intact Skin
	P1	Placebo	Intact Skin
	B	TBD[2]	Intact Skin
2	P1	Placebo	Intact Skin
	C1	TBD	Intact Skin
	P2	Placebo	Wound
	C2	TBD	Wound
3	P2	Placebo	Wound
	D	TBD	Wound
4(or final)	P2	Placebo	Wound
	E1	TBD	Wound
	P1	Placebo	Intact Skin
	E2	TBD	Intact Skin
O (Optional)[3]	P2	Placebo	Wound
	F	TBD	Wound

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

REVISED TEXT

Cohort	Regimen/ Dosing Period	Dose (mg)	Treatment Area
1 (HVT)	P1	Placebo[1]	Intact Skin
	A	~~0.150mg~~ **0.3mg**	Intact Skin
	P1	Placebo	Intact Skin
	B	TBD[2]	Intact Skin
2	P1	Placebo	Intact Skin
	C1	TBD	Intact Skin
	P2	Placebo	Wound
	C2	TBD	Wound
3	P2	Placebo	Wound
	D	TBD	Wound
4(or final)	P2	Placebo	Wound
	E1	TBD	Wound
	P1	Placebo	Intact Skin
	E2	TBD	Intact Skin
O (Optional)[3]	P2	Placebo	Wound
	F	TBD	Wound

1. Placebo = vehicle
2. Actual doses to be determined as preliminary PK data are reviewed and predicted exposures can be calculated.
3. More than one optional cohort may be used.

Section 4.6.1. Time and Events Table Part A

PREVIOUS TEXT

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48h	72h		
All Subjects	Check in		X													
	Informed Consent	X														
	Demographics	X														
	Full Physical Exam	X														
	Brief Physical Exam		X									X	X	X	X	X
	Medical/medication/ drug/alcohol history	X	X													
	12-lead ECG[1]	X	X										X		X	
	Vital signs[2]	X	X										X		X	X
	Urine pregnancy(women)	X													X	
	HIV, Hep B and Hep C	X														
	Hema/Chem Safety Labs[3]	X	X										X		X	
	Blood for HIFα Samples (e.g., hepcidin, EPO,VEGF)[4]	X	X							X	X[4]			X		
	Study treatment dosing			X												
	Pharmacokinetic Sampling[5]		X		X	X	X	X	X	X	X[5]	X	X	X		
	Concomitant Medications		←==========→												X	
	Adverse Event Review[6]		←==========→												X	X

Study Population	Procedure	Screening (up to 28 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48h	72h		
Subjects with DFUs[7] Only	Gene Expression (mRNA) Tissue Samples[8]		X									X		X	X	
	Biomarker fluid samples (e.g., for cytokines, growth factors, and bioburden)[9]		X											X	X	
	Physical Characteristics (e.g., photos and wound measurement post-debridement, volume)	X	X											X	X	
	Clinical Characteristics[10]	X	X			X	X	X			X	X	X	X	X	

1. Single ECGs will be taken Screening, and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the 7-10 day Follow-up Visit. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety analyses will be collected fasting at Screening and on Day 1 and at the 48h post-last-dose on Day 3, and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
5. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected at immediately pre-dose (time 0), 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-'dose. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
6. Adverse events are to be collected from first dose through the final Follow-up visit.
7. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin
8. Microarray and Taqman will be performed at all timepoints.
9. Wound fluid for biomarkers of cytokines and bioburden will be collected at pre-dose (time 0) on Days 1and 4 and at the 7-10 day Follow-up Visit.
10. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF

REVISED TEXT

Study Population	Procedure	Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h		
All Subjects	Check in		X													
	Informed Consent	X														
	Demographics	X														
	Full Physical Exam	X														
	Brief Physical Exam		X									X	X	X	X	X
	Medical/medication/ drug/alcohol history	X	X													
	12-lead ECG[2]	X	X										X		X	
	Vital signs[3]	X	X										X		X	X
	Urine pregnancy(women)	X													X	
	HIV, Hep B and Hep C	X														
	Hema/Chem Safety Labs[4]	X	X										X		X	
	Blood for HIFα Samples (e.g., hepcidin, EPO,VEGF)[5]		X							X	X[5]			X		
	Study treatment dosing			X												
	Pharmacokinetic Sampling[6]		X		X	X	X	X	X	X	X[5]	X	X	X		
	Concomitant Medications		←==========→												X	
	Adverse Event Review[7]		←==========→												X	X

Study Population	Procedure	Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h		
Subjects with DFUs[8] Only	Gene Expression (mRNA) Tissue Samples		X									~~X~~		X	X	
	Biomarker fluid samples (e.g., for cytokines, growth factors, and bioburden)[9]		X											X	X	
	Physical Characteristics (e.g., photos and wound measurement post-debridement, volume)	X	X											X	X	
	Clinical Characteristics[10]	X	X			X	X	X			X	X	X	X	X	
	Vascular Perfusion Eligibility Assessments[11]	**X**														

1. For subjects with DFUs to be eligible for the study or for entry into more than one cohort in Part A, rate of healing must be assessed in the 14 days prior to Day 1.
2. Single ECGs will be taken Screening, and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the 7-10 day Follow-up Visit. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety analyses will be collected fasting at Screening and on Day 1 and at the 48h post-last-dose on Day 3, and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.

6. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected at immediately pre-dose (time 0), 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-'dose. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
7. Adverse events are to be collected from first dose through the final Follow-up visit.
8. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin.
9. Microarray and Taqman will be performed at all timepoints.
10. Wound fluid for biomarkers of cytokines and bioburden will be collected at pre-dose (time 0) on Days 1and 4 and at the 7-10 day Follow-up Visit.
11. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF
12. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.

Section 4.6.2. Time and Events Table Part B

PREVIOUS TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -1 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X		X		X	X	X	X	X	X	X	X
Informed Consent	X														
Demographics	X														
Complete physical	X														
Brief physical		X	X	X		X	X	X	X	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X														
12-lead ECG[1]	X	X										X			
Vital signs[2]	X	X				X		X				X		X	X

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up	
		Day 1				Day 8									
Urine pregnancy(females)	X	X		`										X	
HIV, Hep B and Hep C screen	X														
Hema/Chem/ Safety Labs[3]	X	X				X		X				X		X	
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)	X	X[4]				X[4]		X				X			
Standard of Care Treatment[5]	X		X	X	X										
Study Treatment/Dosing In the Clinic[6]		X				X	X	X	X	X	X				
PK serial blood sampling[7]		X	X	X		X (truncated)		X	X		X	X	X		
PK Trough Sample[7]										X					
Gene Expression (mRNA) Samples Tissue Biopsies[8]		X	X			X		X				X			
Biomarker Samples: Wound fluid for cytokines and bioburden[9]		X	X	X (Day 3 only[9])		X		X	X			X		X	
Macrophages[10]		X	X			X	X	X			X	X		X	
SensiLase (Dopplar blood flow)		X				X		X				X		X	

Procedures	Screening	Single Dose		Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose								Follow-up	
		Day 1	Day 2			Day 8	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23		
Physical Characteristics (assessed pre-dose)[11]	X[11]	X				X	X	X		X		X		X	
Clinical Characteristics[12]	X	X	X	X		X	X	X	X	X	X	X	X	X	
Concomitant Medication Review		X	←==========→											X	
Adverse Event Assessment[13]		X	←==========→											X	X

1. Single ECGs will be taken at Screening and pre-dose on Day 1(SD) and 24h post-last-RD dose on Day 22. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1 (SD) and 8(RD), 15, 22 (24h post-last-RD dose) , and at Follow-up. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,8,14, 22 (24h post-last-dose), and at Follow-up. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
5. During washout times all subjects will be on SOC (Section 7.4).
6. In between clinic visits, subjects with perform applications of study drug at home.
7. See separate Time and Events table for serial PK sampling details for Days 1, 8, 14 and 21. Trough sample will be taken prior to dosing on Day 18. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
8. Direct wound tissue samples for analysis of gene expression will be collected at pre-dose on Days 1, 2, 8, 11, 14, 21, 22 and at the Follow-up Visit. TaqMan analysis will be performed on each day. Microarray analysis will be performed on the following Days 1, 2, 8, 14, 22 and Follow-up, however based on emerging data additional time points may be added. Refer to Section 7.4 for additional details on the collection and processing of gene expression samples.
9. Wound fluid and tissue samples for biomarkers of cytokines and bioburden will be collected at pre-dose on Days 1, 2. 3, 8, 14, 15, 22 and at the Follow-up Visit. Refer to the Section 7.5.3 for additional details on the collection and processing of biomarker tissue samples.
10. May be conducted at only one or both sites, dependent upon feasibility. Will be performed in a select cohort of patients. The precise procedure will be dependent upon the outcome of feasibility work.

11. Physical characteristics of the wound (e.g., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), and pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the Follow-up Visit. Any subject that is participating in additional cohorts during Part B would need to be assessed for wound healing rate for 14 days (as in the regular screen) to ensure healing rate is less than 30%.
12. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
13. Adverse events are to be collected from first dose through the Follow-up visit.

REVISED TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -14 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Informed Consent	X														
Demographics	X														
Complete physical	X														
Brief physical		X	X	X		X	X	X	X	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X														
12-lead ECG[1]	X	X										X			
Vital signs[2]	X	X				X		X				X		X	X
Urine pregnancy(females)	X	X												X	
HIV, Hep B and Hep C screen	X														
Hema/Chem/ Safety Labs[3]	X	X				X		X				X		X	
Vascular Perfusion	**X**														

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up	
		Day 1				Day 8									
Eligibility Assessments[4]															
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)	~~X~~	X[5]				X[5]		X				X			
Standard of Care Treatment[6]	X		X	X	X										
Study Treatment/Dosing In the Clinic[7]		X				X	X	X	X	X	X				
PK serial blood sampling[8]		X	X	X		X (truncated)		X	X		X	X	X		
PK Trough Sample[8]										X					
Gene Expression (mRNA) Samples Tissue Biopsies[9]		X	~~X~~			X		X				X			
Biomarker Samples: Wound fluid for cytokines and bioburden[10]		X	X	X (Day 3 only[10])		X		X	X			X		X	
Macrophages[11]		X	~~X~~			X	~~X~~	X			~~X~~	X		~~X~~	
SensiLase (Doppler blood flow)		X				X		X				X		X	
Hyperspectral Imaging[12]		**X**				**X**		**X**				**X**		**X**	

Procedures	Screening	Single Dose		Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose								Follow-up	
		Day 1	Day 2			Day 8	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23		
Physical Characteristics (assessed pre-dose)[13]	X[11]	X				X	X	X		X		X		X	
Clinical Characteristics[14]	X	X	X	X		X	X	X	X	X	X	X	X	X	
Concomitant Medication Review		X	←==========										→	X	
Adverse Event Assessment[15]		X	←==========										→	X	X

1. Single ECGs will be taken at Screening and pre-dose on Day 1(SD) and 24h post-last RD dose on Day 22. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1 (SD) and 8(RD), **14**, 22 (24h post-last-RD dose) , and at Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,8,14, 22 (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. **See Item** 7 **in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.**
5. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
6. During washout times all subjects will be on SOC (Section 7.4).
7. In between clinic visits, subjects with perform applications of study drug at home.
8. See separate Time and Events table for serial PK sampling details for Days 1, 8, 14 and 21 and the associated 24, 48 and 72 hr time points. Trough sample will be taken prior to dosing on Day 18. Refer to Section 7.13 for details on the collection and processing of PK samples. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
9. Direct wound tissue samples for analysis of gene expression will be collected at pre-dose/**equivalent** on Days 1, 8, 14 and 22, however based on emerging data additional time points may be added. Refer to Section 7.**5** for additional details on the collection and processing of gene expression samples.
10. Wound fluid for biomarkers of cytokines and bioburden will be collected at pre-dose/**equivalent** on Days 1, 2. 3, 8, 14, 15, 22 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7 for additional details on the collection and processing of biomarker tissue samples.

~~11. May be conducted at only one or both sites, dependent upon feasibility. Will be performed in a select cohort of patients. The precise procedure will be dependent upon the outcome of feasibility work.~~

11. **Will be performed based on hyperspectral imaging capabilities at each site.**

12. Physical characteristics of the wound (e.g., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), and pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the 7-10 day Follow-up Visit. ~~Any subject that is participating in additional cohorts during Part B would need to be assessed for wound healing rate for 14 days (as in the regular screen) to ensure healing rate is less than 30%.~~
13. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
14. Adverse events are to be collected from first dose through the final Follow-up visit.

5.3.1. Inclusion Criteria – DFU Subjects, Criteria #7

PREVIOUS TEXT

7. Adequate vascular perfusion of the affected limb, as defined by at least one of the following:
   a) TcPO2 > 35mmHg within 30 days of screening.
   b) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2, confirmed by TcPO2 >35mmHg.
   c) Toe pressure (plethysmography) > 50mmHg.
   d) Doppler ultrasound (biphasic or triphasic waveforms) consistent with adequate blood flow to the affected extremity, as determined by SoC.

REVISED TEXT

7. Adequate vascular perfusion of the affected limb within **30 days of screening**, as defined by at least one of the following:
   e) TcPO2 > 35mmHg ~~within 30 days of screening~~.
   f) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2, confirmed by TcPO2 >35mmHg.
   g) Toe pressure (plethysmography) > 50mmHg.
   h) Doppler ultrasound (biphasic or triphasic waveforms) consistent with adequate blood flow to the affected extremity, as determined by SoC.

7.8.1. Sample Collection for Macrophage Immunostaining, Paragraph 1

PREVIOUS TEXT

Upon collection, the biopsy will be placed in an embedding mold containing freezing medium (such as OCT embedding compound) and frozen immediately before storing at -80^{o}C prior to tissue sectioning and immunostaining according to standard procedures.

REVISED TEXT

Upon collection, the biopsy will be placed in ~~an embedding mold containing freezing medium (such as OCT embedding compound) and frozen immediately before storing at -80^{o}C prior to tissue sectioning and immunostaining according to standard procedures~~ a **pre-labeled cryovial and snap frozen in liquid nitrogen. The cryovials will be transferred to a pre-labelled freezer box and then moved immediately into a -80°C freezer. Samples will be shipped to laboratory within 8 months of collection. Shipping instructions are in the Study Procedures Manual.**

AMENDMENT 3

Where the Amendment Applies

This amendment applies to all sites.

Summary of Amendment Changes with Rationale

This amendment clarifies an eligibility criterion and DFU clinical assessments as well as minor edits for clarity.

List of Specific Changes

1.4.1.1 Single Dose Strategy, Paragraph 3, Sentence 2

PREVIOUS TEXT

The starting dose in this cohort will be 0.1% GSK1278863 petrolatum ointment at 25mg/cm^2 to a 12cm^2 section of intact skin (300mg total mass of the formulation) on the lower limb, which will deliver 300µg GSK1278863.

REVISED TEXT

The starting dose in this cohort will be 0.1% GSK1278863 petrolatum ointment at 25mg/cm^2 to a 12cm^2 section of intact skin (300mg total mass of the formulation) on the **foot** ~~lower limb~~, which will deliver 300µg GSK1278863.

4.2.1. Part A – Single Doses in Healthy Volunteers and DFU Patients (Cohorts 1 through 4), sentence 3

PREVIOUS TEXT

Diabetic subjects may participate in more than one cohort in Part A (requires a minimum of a 14 day washout between cohorts to ensure a healing rate of less than 30%) and may also participate in Part B (requires a minimum of a 14 day washout between cohorts to ensure a healing rate of less than 30%) provided the subject meets all selection criteria and this participation will not result in blood withdrawal over the limit (Section 5.2.2.).

REVISED TEXT

Diabetic subjects may participate in more than one cohort in Part A (requires a minimum of ~~a~~ 14 day**s** ~~washout~~ between cohorts to ensure a healing rate of less than 30%) and may also participate in Part B (requires a minimum of ~~a~~ 14 day**s** ~~washout~~ between cohorts to ensure a healing rate of less than 30%) provided the subject meets all selection criteria and this participation will not result in blood withdrawal over the limit (Section 5.2.2).

Section 4.2.1.3. Run-In period (Part A)

PREVIOUS TEXT

For all diabetic subjects in Part A, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. Subjects that do not meet randomization eligibility will be discontinued from the study.

REVISED TEXT
For all diabetic subjects in Part A, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. **If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (Baseline Run-In) will need to be scheduled prior to Day 1 to meet the Run-In requirement.** Subjects that do not meet randomization eligibility will be discontinued from the study.

Section 4.2.1.5. Cohorts 3 and 4 (Part A)

PREVIOUS TEXT

If subjects are re-enrolling from a previous cohort, ensure that there has been a minimum of a 10 day washout prior to initiating the 14 day run-in to assess healing rate.

REVISED TEXT

If subjects are re-enrolling from a previous cohort**,they must complete the second Follow-up visit (Day 28-32 post last dose) and therefore the 14 day Run-in period may begin 14 days prior to this visit.** ~~ensure that there has been a minimum of a 10 day washout prior to initiating the 14 day run-in to assess healing rate.~~

Section 4.2.2.2. Run-In Period (Part B), sentence 3

PREVIOU TEXT

For all subjects in Part B, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. Subjects that do not meet randomization eligibility will be discontinued from the study.

REVISED TEXT

For all subjects in Part B, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. **If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (Baseline Run In) will need to be scheduled prior to Day 1 to meet the Run In requirement.** Subjects that do not meet randomization eligibility will be discontinued from the study.

Section 4.3.1 Subject Numbering, Paragraph1, sentences 1 and 2

PREVIOUS TEXT

The site will assign screening numbers using their own unique schema. **Screening numbers are not collected by GSK**. Subjects will be assigned a unique Subject Number at admission to the clinic for each cohort the subject opts to participate in.

REVISED TEXT

~~The site will assign screening numbers using their own unique schema. **Screening numbers are not collected by GSK**.~~ Subjects will be assigned a unique Subject Number at ~~admission to the clinic~~**Screening** for each cohort the subject opts to participate in.

Section 5.3.1 Inclusion Criteria – DFU Subjects, Criteria 7. b)

PREVIOUS TEXT

b) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2, confirmed by TcPO2 >35mmHg.

REVISED TEXT

b) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2~~, confirmed by TcPO2 >35mmHg.~~

Section 7.10.2.1. Photo Capture – Wound Images

PREVIOUS TEXT

Photographic documentation of the wound should be obtained after debridement. Randomization and weekly imaging of the wound should occur after cleansing and before treatment with study drug.

When using the camera to photograph the target ulcer the base of the camera at its widest should lay perpendicular to the underlying bone as if they were cutting it. For toe wounds and very small wounds, this instruction should be used as far as possible. If this is not possible, the same position in which the first photograph was taken should be followed for all subsequent photos.

At each visit 2 photographs are to be taken of the subject card that records; subject number, date, visit number, study name and subject initials. Two photographs are to be taken approximately 8 inches from the wound. Two additional photographs are to be taken of the wound approximately 24 inches from the wound.

To ensure the subject meets the inclusion criteria of the change in ulcer size between screening and randomization, percent reduction of wound size must be calculated using a program provided to the sites and the guide in the case report form.

Photographic documentation should be sent electronically to the core lab as soon as possible, preferably on the same day the picture was taken.

Photographs of tracings and wounds will be stored on individual memory cards, as well as, downloaded to a computer at each site and uploaded to the wound core lab website. Refer to the SPM for details.

Photo 1- ID card

- Complete ID card with subjects study number and date then place in photographic field.
- View camera image to ensure complete ID card is in photograph.
- Take 2 photos.

Photo 2- Close-up view

- Locate ulcer to be photographed (if more than one ulcer on the target limb determine target ulcer.
- Identify target ulcer using sticker provided.
- Camera is to be oriented with the top of the camera directed towards the direction of the subject's head.
- Camera is to be held 8 inches from target ulcer.
- Depress focus button half way until the view of the ulcer is sharp.
- Take 2 photos.

Photo 3- Global view

- Camera is to be oriented with the top of the camera directed towards the direction of the subject's head.
- Camera is to be held at a distance that allows the entire limb from just below the knee and encompassing the entire foot to be viewed.
- The target ulcer must be visible in photo.
- Depress focus button half way until the view of the limb is sharp.
- Take 2 photos.

Saving Photos

Using the cable supplied connect the camera to a computer with a USB port, save photographs to computer desktop.

REVISED TEXT

Photographic documentation of the wound should be obtained after debridement. Randomization and weekly imaging of the wound should occur after cleansing and before treatment with study drug.

~~When using the camera to photograph the target ulcer the base of the camera at its widest should lay perpendicular to the underlying bone as if they were cutting it. For toe wounds and very small wounds, this instruction should be used as far as possible. If this is not possible, the same position in which the first photograph was taken should be followed for all subsequent photos.~~

~~At each visit 2 photographs are to be taken of the subject card that records; subject number, date, visit number, study name and subject initials. Two photographs are to be taken approximately 8 inches from the wound. Two additional photographs are to be taken of the wound approximately 24 inches from the wound.~~

~~To ensure the subject meets the inclusion criteria of the change in ulcer size between screening and randomization, percent reduction of wound size must be calculated using a program provided to the sites and the guide in the case report form.~~

Photographic documentation should be sent electronically to the core lab as soon as possible, preferably on the same day the picture was taken.

Photographs of tracings and wounds will be stored **locally** ~~on individual memory cards, as well as, downloaded to a computer~~ at each site and uploaded to the wound core lab ~~website~~. Refer to the SPM for details.

~~Photo 1- ID card~~

- ~~Complete ID card with subjects study number and date then place in photographic field.~~
- ~~View camera image to ensure complete ID card is in photograph.~~
- ~~Take 2 photos.~~

~~Photo 2- Close-up view~~

- ~~Locate ulcer to be photographed (if more than one ulcer on the target limb determine target ulcer.~~
- ~~Identify target ulcer using sticker provided.~~
- ~~Camera is to be oriented with the top of the camera directed towards the direction of the subject's head.~~
- ~~Camera is to be held 8 inches from target ulcer.~~
- ~~Depress focus button half way until the view of the ulcer is sharp.~~
- ~~Take 2 photos.~~

~~Photo 3- Global view~~

- ~~Camera is to be oriented with the top of the camera directed towards the direction of the subject's head.~~
- ~~Camera is to be held at a distance that allows the entire limb from just below the knee and encompassing the entire foot to be viewed.~~

- ~~The target ulcer must be visible in photo.~~
- ~~Depress focus button half way until the view of the limb is sharp.~~
- ~~Take 2 photos.~~

~~Saving Photos~~

~~Using the cable supplied connect the camera to a computer with a USB port, save photographs to computer desktop.~~

7.10.2.2. Wound Tracings

PREVIOUS TEXT

Each wound tracing is to be captured with a digital photograph using a camera provided to each site. The header information on the acetate tracing; study name, date, visit number, subject number, subject initials must be completed before the photograph is taken. Two pictures are to be taken of each tracing.

Printed copies of the photograph of the wound tracing are to be retained as source documents in the subjects' study files. Original tracings are to be sent to the wound core lab (see the Study Procedures Manual for shipping instructions).

- Complete header information on the Cover acetate (acetate with printed header information) using 0.5 mm Itoya pen including the subject's initials, unique study identifier, date and visit number.
- Place Blank acetate over the wound area.
- Then place Cover acetate over Blank acetate.
- Trace the ulcer using one continuous line around the wound edge (do not include any islands in the tracing).
- Ensure all gaps are closed and that the tracing is a solid line.
- Place the top film centered on the cardboard backer and discard the bottom film.
- Make a copy of the tracing and file the copy with the subject's source documentation.
- Using the camera take a picture of the Cover acetate:
    - Ensure the camera is set to C2.
    - Look through the viewfinder and ensure the entire acetate page will be captured in the photograph, and that the acetate page fills the majority of the picture.
    - Depress the focus button ½ way and ensure the acetate is in focus.
    - Take two pictures.
    - Using the cable supplied connect the camera to a computer with a USB port.

Save photograph to computer desktop.

REVISED TEXT

To ensure the subject meets the randomization eligibility of the change in ulcer size between screening (or optional Run In visit, if different from day of screening) and randomization on Day 1, percent reduction of wound size must be calculated by the Investigator or designee using the method for measuring wound area provided in the SPM. The core lab may be consulted for assistance with area measurements or percent reduction determinations if necessary.

All ~~Each~~ wound tracing **are** ~~is~~ to be **scanned and sent to the core lab along with wound photographs (Section 7.10.2.1).** ~~captured with a digital photograph using a camera provided to each site. The header information on the acetate tracing; **protocol number**, date, visit number, subject number, subject initials must be completed before **the acetate is scanned** photograph is taken. Two pictures are to be taken of each tracing.~~

Original ~~Printed~~ copies ~~of the photograph~~ of the wound tracing are to be retained as source documents in the subjects' study files. Original tracings may ~~are to~~ be sent to the wound core lab **when a quality scan cannot be obtained. Refer to the SPM for additional details** ~~(see the Study Procedures Manual for shipping instructions)~~.

- ~~Complete header information on the Cover acetate (acetate with printed header information) using 0.5 mm Itoya pen including the subject's initials, unique study identifier, date and visit number.~~
- ~~Place Blank acetate over the wound area.~~
- ~~Then place Cover acetate over Blank acetate.~~
- ~~Trace the ulcer using one continuous line around the wound edge (do not include any islands in the tracing).~~
- ~~Ensure all gaps are closed and that the tracing is a solid line.~~
- ~~Place the top film centered on the cardboard backer and discard the bottom film.~~
- ~~Make a copy of the tracing and file the copy with the subject's source documentation.~~
- ~~Using the camera take a picture of the Cover acetate:~~
  - ~~Ensure the camera is set to C2.~~
  - ~~Look through the viewfinder and ensure the entire acetate page will be captured in the photograph, and that the acetate page fills the majority of the picture.~~
  - ~~Depress the focus button ½ way and ensure the acetate is in focus.~~
  - ~~Take two pictures.~~
  - ~~Using the cable supplied connect the camera to a computer with a USB port.~~

~~Save photograph to computer desktop.~~

7.10.2.3. Volume Measurement and 7.10.2.4 Ulcer Depth

PREVIOUS TEXT

7.10.2.3 Volume Measurement

The ARANZ Medical Silhouette system will be used for wound volume measurement. All photographic parameters, including lighting, distance, and exposure, should comply with the ARANZ Silhouette Digital Camera Instruction Manual specifications. The volume measurement will be obtained directly from the camera and the measurement recorded on the case report form. .

7.10.2.4 Ulcer Depth

Ulcer Depth (Depth, D) will be measured at all study visits. Unit of measurement to be used is millimeters.

REVISED TEXT

7.10.2.3 Depth and Volume Measurements

The ARANZ Medical Silhouette system will be used for wound **depth and** volume measurement. All photographic parameters, including lighting, distance, and exposure, should comply with the ARANZ Silhouette Digital Camera Instruction Manual specifications. The volume measurement will be obtained directly from the camera and the measurement recorded on the case report form. **Sites that do not have access to the ARANZ Medical Silhouette system will not be required to collect these exploratory data.**

~~7.10.2.4 Ulcer Depth~~

~~The ARANZ Medical Silhouette system will be used for wound depth measurement. All photographic parameters, including lighting, distance, and exposure, should comply with the ARANZ Silhouette Digital Camera Instruction Manual specifications. The depth measurement will be obtained directly from the camera and the measurement recorded on the case report form. Sites that do not have access to the ARANZ Medical Silhouette system will not be required to collect these exploratory data. Ulcer Depth (Depth, D) will be measured at all study visits. Unit of measurement to be used is millimeters.~~

7.10.2.4. Ulcer Size Reduction

PREVIOUS TEXT

Percent change in study ulcer area will be determined by a central planimetry contractor after the subject has completed the two-week Run-in period in order to determine if the subject qualifies for randomization.

REVISED TEXT

Percent change in study ulcer area will be determined by ~~a central planimetry contractor~~ **the site** after the subject has completed the two-week Run-in period in order to determine if the subject qualifies for randomization.

7.11. Clinical Characteristics of the DFU, Paragraph 1

PREVIOUS TEXT

At each study visit, the investigator will assess the characteristics of the ulcer being treated. The investigator will assess, using the chart below (Table 6), the wound edge, base color, periwound condition, periwound color, degree of edema, amount of exudate (drainage), type of exudate and percent of granulation tissue present.

REVISED TEXT

At each study visit, the investigator will assess the characteristics of the ulcer being treated. The investigator will assess, using the chart below (Table 6), the wound edge, base color, periwound condition, periwound color, degree of edema, amount of exudate (drainage), type of exudate and percent of granulation tissue present. **The investigator will also assess whether the wound is healed or not healed.**

12.3 Cardiovascular Events, Added section

12.3 Cardiovascular Events

Investigators will be required to fill out event specific data collection tools for the following AEs and SAEs:

- **Myocardial infarction/unstable angina**
- **Congestive heart failure**
- **Arrhythmias**
- **Valvulopathy**
- **Pulmonary hypertension**
- **Cerebrovascular events/stroke and transient ischemic attack**
- **Peripheral arterial thrombosis**
- **Deep venous thrombosis or pulmonary embolus**
- **Revascularization**

This information should be recorded within one week of when the AE/SAE(s) are first reported.

AMENDMENT 4

Where the Amendment Applies

This amendment applies to all sites.

Summary of Amendment Changes with Rationale

This amendment reflects changes in the container type used to supply the bulk ointment. Minor edits are also included for clarity.

List of Specific Changes

Section 4.4 Investigational Product and Other Study Treatment Dosage/Administration, Line 8 of the table

PREVIOUS TEXT

Device:	Part A: Amber Bottle Part B: White Tube	Part A: Amber Bottle Part B: White Tube

REVISED TEXT

Device:	Part A: Amber Bottle **or White Tube** Part B: White Tube	Part A: Amber Bottle **or White Tube** Part B: White Tube

Section 4.6.1 Time and Events Table – Part A, Footnote # 8 and 9

PREVIOUS TEXT

8. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin.
9. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF

REVISED TEXT

8. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin **unless for screening/eligibility purposes**.
9. Clinical characteristic assessments of the DFU begin with Section 7.11 and contain instructions for completing the assessments including the irritation/symptom scale as needed. **The 12 hr assessment can be done once within the 10-12h window for out-patients and at 12 hrs for in-patient subjects**. Assessments are recorded in the eCRF

Section 5.3.1 Inclusion Criteria – DFU Subjects, Inclusion Criteria #5

PREVIOUS TEXT

5. DFU between 1cm² and 20cm² at screening.

REVISED TEXT

5. DFU between 1cm² and ~~20~~**16**cm² at screening.

AMENDMENT 5

Where the Amendment Applies

This amendment applies to all sites.

Summary of Amendment Changes with Rationale

This amendment reflects modifications to the DFU inclusion and exclusion criteria, hemoglobin stopping criteria and a change in biopsy collection expectations. Minor edits are also included for clarity.

List of Specific Changes

Section 4.5.1.1. Hemoglobin Stopping Criteria, Paragraph 1

PREVIOUS TEXT

A patient that experiences an absolute increase in hemoglobin of ~~1.0~~g/dL above their baseline level at any time will be withdrawn from the study.

REVISED TEXT

A patient that experiences an absolute increase in hemoglobin of **1.5**g/dL above their baseline level at any time will be withdrawn from the study.

Section 4.6.1 Time and Events Table – Part A, Urine pregnancy (women)

PREVIOUS TEXT

Study Population	Procedure	Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)	Study Day (each dosing period)												Follow Up	
			Day 1									Day 2	Day 3	Day 4	7-10 days post last dose	28-32 days post last dose
			Pre-dose	0h	0.25h	0.5h	1h	2h	4h	8h	12h	24h	48 h	72 h		
	Urine pregnancy(women)	X													X	

REVISED TEXT

Study Population	**Procedure**	**Screening[1] (HVT: -28 to -1 days; DFU: -28 to -14 days prior to Day 1)**	**Study Day (each dosing period)**												**Follow Up**	
			Day 1									**Day 2**	**Day 3**	**Day 4**	**7-10 days post last dose**	**28-32 days post last dose**
			Pre-dose	**0h**	**0.25h**	**0.5h**	**1h**	**2h**	**4h**	**8h**	**12h**	**24h**	**48 h**	**72 h**		
	Urine pregnancy(women)	X	**X**												X	

Section 4.6.1 Time and Events Table – Part A, Addition of footnote # 9 with associated re-numbering

PREVIOUS TEXT

1. For subjects with DFUs to be eligible for the study or for entry into more than one cohort in Part A, rate of healing must be assessed in the 14 days prior to Day 1.
2. Single ECGs will be taken at Screening, pre-dose on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety analyses will be collected fasting at Screening, on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
6. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected immediately pre-dose and 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-dose. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile, but the total number of samples will not change.
7. Adverse events are to be collected from first dose through the final Follow-up Visit.
8. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin unless for screening/eligibility purposes.
9. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. The 12 hr assessment can be done once within the 10-12 window for out-patients and at 12 hrs for in-patient subjects. Assessments are recorded in the eCRF
10. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.

REVISED TEXT

1. For subjects with DFUs to be eligible for the study or for entry into more than one cohort in Part A, rate of healing must be assessed in the 14 days prior to Day 1.
2. Single ECGs will be taken at Screening, pre-dose on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Day 1 and at the 48h post-last-dose on Day 3, and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety analyses will be collected fasting at Screening, on Day 1, at 48h post-last-dose on Day 3 and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. Blood samples HIFα activation on Day 1 will be collected at pre-dose, 8 and 10-12 hour window. Refer to Section 7.6 for additional details on the collection and processing of these samples.
6. Serial blood samples for the determination of the PK for GSK127886 and metabolites will be collected immediately pre-dose and 0.25, 0.5, 1, 2, 4, 8, 10-12 (once within the 10-12 window for out-patients only)/12 (in-patient subjects only), 24, 48 and 72hrs post-dose. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile, but the total number of samples will not change.
7. Adverse events are to be collected from first dose through the final Follow-up Visit.
8. These assessments are to be performed only when IP is applied to the ulcer not to the patient's intact skin unless for screening/eligibility purposes.
9. **Tissue sample collection at Pre-dose, Day 4 and the 7-10 Follow-up Visit is at the discretion of the PI or designee. Wounds that have healed such that biopsies would be disruptive to the continued healing process are exempt.**
10. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. The 12 hr assessment can be done once within the 10-12 window for out-patients and at 12 hrs for in-patient subjects. Assessments are recorded in the eCRF
11. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.

Section 4.6.1 Time and Events Table – Part B, Gene Expression Tissue Biopsies, Macrophages and Wound Fluid

PREVIOUS TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -14 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
Gene Expression (mRNA) Samples Tissue Biopsies[9]		X				X		X				X			
Biomarker Samples: Wound fluid for cytokines and bioburden[10]		X	X	X (Day 3 only[10])		X		X	X			X		X	
Macrophages		X				X		X				X			
SensiLase (Doppler blood flow)		X				X		X				X		X	

REVISED TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -14 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
~~Gene Expression (mRNA) Samples~~ Tissue Biopsies (gene expression, **macrophage and bioburden analysis**)[9]		X				X		X				X			
~~Biomarker Samples~~ Wound Fluid (cytokine and bioburden analysis)[10]		X	X	X (Day 3 only[10])		X		X	X			X		X	
~~Macrophages~~		~~X~~				~~X~~		~~X~~				~~X~~			
SensiLase (Doppler blood flow)		X				X		X				X		X	

Section 4.6.1 Time and Events Table – Part B, Footnote # 9

PREVIOUS TEXT

9. Direct wound tissue samples for analysis of gene expression will be collected at pre-dose/equivalent on Days 1, 8, 14 and 22, however based on emerging data ~~additional~~ time points may be added. Refer to Section 7.7.2 for additional details on the collection and processing of gene expression samples.

REVISED TEXT

9. Direct wound tissue samples for analysis ~~of gene expression~~ will be collected at pre-dose/equivalent on Days 1, 8, 14 and 22, however based on emerging data, time points may be added **or deleted; wounds that have healed sufficiently may be exempt**. Refer to Section 7.7.2 for additional details on the collection and processing ~~of gene expression samples~~ tissue biopsies.

Section 5.2.1 Inclusion Criteria – Healthy Volunteers (Part A, Cohort 1), Inclusion Criteria #5 and #6

PREVIOUS TEXT

5. A female subject is eligible to participate if she is of:
    1. Non-childbearing potential (NCBP) defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40MlU/ml and estradiol < 40pg/mL (<147pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods in Section 8.1 if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT (consult with GSK Medical Monitor). Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.
6. Male subjects with female partners of child-bearing potential must agree to use one of the contraception methods listed in Section 8.18.1. This criterion must be followed from the time of the first dose of study medication until Follow-up.

REVISED TEXT

5. A female subject is eligible to participate if she is of:
    1. **Childbearing potential, must agree to use one of the approved contraception methods as outlined in Section 8.1 from Screening until completion of the Follow-up Visit OR**

    2. Non-childbearing potential (NCBP) defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with

simultaneous follicle stimulating hormone (FSH) > 40MlU/ml and estradiol < 40pg/mL (<147pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods in Section 8.1 if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT (consult with GSK Medical Monitor). Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

6. ~~Male subjects with female partners of child-bearing potential must agree to use one of the contraception methods listed in Section 8.1~~8.1~~. This criterion must be followed from the time of the first dose of study medication until Follow-up~~.

Section 5.3.1 Inclusion Criteria – DFU Subjects

PREVIOUS TEXT

A DFU subject will be eligible for inclusion in this study only if all of the criteria listed for healthy volunteers are met (Section 5.2) as well as the following criteria:

1. Diagnosed with Type I or Type II diabetes mellitus.
2. HbA1c, ≤ 12%.
3. QTc < 480msec in subjects with bundle branch block.
4. Lower extremity diabetic foot ulcer of 30-364 days' duration.
5. DFU between 1cm² and 16cm² at screening.
6. Presence of at least one DFU that meets all of the following criteria:
    a. Ulcer has been diagnosed as a full-thickness, neuropathic DFU and is located at or distal to the malleolus (excluding ulcers between the toes but including those of the heel).
    b. There is a minimum 2cm margin between the qualifying study ulcer and any other ulcers on the specified foot.
    c. Ulcer size (area) ≥ 1cm2 and ≤ 12cm2 (post-debridement at time of randomization).
    d. Wagner Grade 1.
    e. Depth ≤ 5mm with no capsule, tendon or bone exposed and no tunneling, undermining, or sinus tracts.

Note: If the subject has more than one qualifying DFU, the ulcer designated as the study ulcer will be at the discretion of the Investigator. Non-study ulcers being treated during the course of the study will be treated with moist wound therapy Standard of Care (SoC) identified under this study).

7. Adequate vascular perfusion of the affected limb within 30 days of screening, as defined by at least one of the following:
    i) TcPO2 > 35mmHg.
    j) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2
    k) Toe pressure (plethysmography) > 50mmHg.
    l) Doppler ultrasound (biphasic or triphasic waveforms) consistent with adequate blood flow to the affected extremity, as determined by SoC.

REVISED TEXT

A DFU subject will be eligible for inclusion in this study only if all of the criteria listed ~~for healthy volunteers~~ are met. ~~(Section 5.2~~5.2~~) as well as the following criteria:~~

1. Diagnosed with Type I or Type II diabetes mellitus.
2. HbA1c, ≤ 12%.
3. **Single QTc < 450msec, or** ~~QTc~~ < 480msec in subjects with bundle branch block.
4. Lower extremity diabetic foot ulcer of 30-364 days' duration.
5. DFU between 1cm² and 16cm² at screening.
6. Presence of at least one DFU that meets all of the following criteria:
    f. Ulcer has been diagnosed as a full-thickness, neuropathic DFU and is located at or distal to the malleolus (excluding ulcers between the toes but including those of the heel).
    g. There is a minimum 2cm margin between the qualifying study ulcer and any other ulcers on the specified foot.
    h. Ulcer size (area) ≥ 1cm2 and ≤ 12cm2 (post-debridement at time of randomization).
    i. Wagner Grade 1.
    j. Depth ≤ 5mm with no capsule, tendon or bone exposed and no tunneling, undermining, or sinus tracts.

Note: If the subject has more than one qualifying DFU, the ulcer designated as the study ulcer will be at the discretion of the Investigator. Non-study ulcers being treated during the course of the study will be treated with moist wound therapy Standard of Care (SoC) identified under this study).

7. Adequate vascular perfusion of the affected limb within 30 days of screening, as defined by at least one of the following:

e) TcPO2 > 35mmHg.
    f) Ankle-Brachial Index (ABI) ≥ 0.6 and ≤ 1.2
    g) Toe pressure (plethysmography) > 50mmHg.

h) Doppler ultrasound (biphasic or triphasic waveforms) consistent with adequate blood flow to the affected extremity, as determined by SoC.

8. **ALT, alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).**
9. **Male or female between 18 and 90 years of age inclusive, at the time of signing the informed consent.**
10. **A female subject is eligible to participate if she is of:**

    1. Childbearing potential, and agrees to use one of the approved contraception methods as outlined in Section 8.1 from Screening until completion of the Follow-up Visit OR

    2. Non-childbearing potential (NCBP) defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea [in questionable cases a blood sample with simultaneous follicle stimulating hormone (FSH) > 40MIU/ml and estradiol < 40pg/mL (<147pmol/L) is confirmatory]. Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods in Section 8.1 if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks should elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT (consult with GSK Medical Monitor). Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.

Section 5.3.2 Exclusion Criteria – DFU Subjects

PREVIOUS TEXT

Diabetic subjects that are being considered for this study will not be eligible for inclusion in this study if any of the following criteria apply or if the exclusion criteria outlined in Section 5.2.2 apply.

1. Subjects with:
    - Ulcers accompanied by infected cellulitis, osteomyelitis, or clinical signs or symptoms of infection,
    - Gangrene on any part of affected limb,,
    - Active Charcot's foot on the study limb,
    - Planned vascular surgery, angioplasty or thrombolysis,
    - Ulcers involving exposure of tendon, bone, or joint capsule (It is acceptable to have ulcers extending through the dermis and into subcutaneous tissue with presence of granulation tissue),

- Ulcers due to non-diabetic etiology.

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) and/or any major changes (per investigator's judgment) to related medications within 6 months prior to randomization.
3. History or malignancy within 5 years of screening or those with a strong family history of cancer (e.g. familial cancer disorders), with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
4. Other clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the Investigator or the GSK Medical Monitor, not stabilized or may otherwise impact the results of the study.
5. Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within 6 months of randomization.
6. Patients undergoing hemodialysis.
7. History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within 1 year prior to screening.
8. Active peptic, duodenal, or esophageal ulcer disease or any gastrointestinal bleeding, within 1 year prior to screening.
9. Subjects with a platelet count <100,000/mm^3 at screening.
10. Subjects with an International Normalized Ratio (INR) >1.5 at screening.
11. Subjects with a hemoglobin level above the gender-specific upper limit of normal at screening.
12. Subjects with a history of non-traumatic joint inflammation (with the exception of inflammation due to osteoarthritis).
13. Patients with known pulmonary hypertension.
14. Use of prohibited medications as described in Section 9.1 of the protocol.

REVISED TEXT

Diabetic subjects that are being considered for this study will not be eligible for inclusion in this study if any of the following criteria apply ~~or if the exclusion criteria outlined in Section 5.2.2 apply~~.

1. Subjects with:
    - Ulcers accompanied by infected cellulitis, osteomyelitis, or clinical signs or symptoms of infection,
    - Gangrene on any part of affected limb,,
    - Active Charcot's foot on the study limb,
    - Planned vascular surgery, angioplasty or thrombolysis,

- Ulcers involving exposure of tendon, bone, or joint capsule (It is acceptable to have ulcers extending through the dermis and into subcutaneous tissue with presence of granulation tissue),
- Ulcers due to non-diabetic etiology.

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) and/or any major changes (per investigator's judgment) to related medications within 6 months prior to randomization.
3. History or malignancy within 5 years of screening or those with a strong family history of cancer (e.g. familial cancer disorders), with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
4. Other clinically significant cardiovascular, pulmonary, renal, endocrine, hepatic, neurological, psychiatric, immunological, gastrointestinal, hematological, or metabolic disease that is, in the opinion of the Investigator or the GSK Medical Monitor, not stabilized or may otherwise impact the results of the study.
5. Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within 6 months of randomization.
6. Patients undergoing hemodialysis.
7. History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within 1 year prior to screening.
8. Active peptic, duodenal, or esophageal ulcer disease or any gastrointestinal bleeding, within 1 year prior to screening.
9. Subjects with a platelet count <100,000/mm^3 at screening.
10. Subjects with an International Normalized Ratio (INR) >1.5 at screening.
11. Subjects with a hemoglobin level above the gender-specific upper limit of normal at screening.
12. Subjects with a history of non-traumatic joint inflammation (with the exception of inflammation due to osteoarthritis).
13. Patients with known pulmonary hypertension.
14. Use of prohibited medications as described in Section 9.1 of the protocol.
15. **A history of drug or alcohol abuse in the past year, or a history of regular alcohol consumption within 6 months of the study defined as an average weekly intake of >14 drinks for males or >7 drinks for females. One drink is equivalent to 12g of alcohol: 12 ounces (360mL) of beer, 5 ounces (150mL) of wine or 1.5 ounces (45mL) of 80 proof distilled spirits.**
16. **The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives (whichever is longer).**

17. **History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.**
18. **Where participation in the study would result in donation of blood or blood products in excess of 500mL within a 56 day period.**
19. **Pregnant females as determined by positive urine hCG test at screening or prior to dosing.**
20. **Unwillingness or inability to follow the procedures outlined in the protocol.**
21. **Subject is mentally or legally incapacitated.**

Section 7.3 DFU Assessments, Paragraph 1

PREVIOUS TEXT

The following assessments will be performed in Part A and Part B of the study.

REVISED TEXT

The following assessments will be performed in Part A and Part B of the study **according to site designation, capability and feasibility.**

Section 7.7 Biomarkers (e.g., Cytokines, Bioburden and Gene Expression) Collection and Processing, Paragraph 1

PREVIOUS TEXT

Wound tissue and fluid samples for biomarker assessments will be collected according to the table in Section 4.6

REVISED TEXT

~~Wound tissue and fluid samples for biomarker assessments will be collected according to the table in Section 4.6~~

With the subject's consent, wound biopsy tissue, wound fluid and blood sample(s) will be collected during this study to be used for the purposes of measuring novel biomarkers to identify factors that may influence wound healing and tissue repair and/or medically related conditions, as well as the biological and clinical responses to GSK.1278863. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events.

Samples will be collected at the timepoints indicated in Section 4.6. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with wound healing and tissue repair or medically

related conditions and/or the action of GSK1278863 may be assessed in accordance with the objectives and endpoints of the study.

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

Section 7.7.2 Punch Biopsy Directions, Bullet point added

REVISED TEXT

- **Study wounds that have healed sufficiently will be exempt from further biopsies. This determination is at the discretion of the Principal Investigator or designee.**

Section 7.7.3 Exploratory Biomarkers, Paragraphs 1-4

PREVIOUS TEXT

Section 7.7.3 Exploratory Biomarkers

With the subject's consent, wound biopsy tissue, wound fluid and blood sample(s) will be collected during this study to be used for the purposes of measuring novel biomarkers to identify factors that may influence wound healing and tissue repair and/or medically related conditions, as well as the biological and clinical responses to GSK.1278863. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events.

Samples will be collected at the timepoints indicated in Section 4.6. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.

Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with wound healing and tissue repair or medically related conditions and/or the action of GSK1278863.

All samples will be retained for a maximum of 15 years after the last subject completes the trial.

REVISED TEXT

~~Section 7.7.3 Exploratory Biomarkers~~

~~With the subject's consent, wound biopsy tissue, wound fluid and blood sample(s) will be collected during this study to be used for the purposes of measuring novel biomarkers to identify factors that may influence wound healing and tissue repair and/or medically related conditions, as well as the biological and clinical responses to GSK.1278863. If relevant, this approach will be extended to include the identification of biomarkers associated with adverse events.~~

~~Samples will be collected at the timepoints indicated in Section 4.6. The timing of the collections may be adjusted on the basis of emerging PK or PD data from this study or other new information in order to ensure optimal evaluation of the PD endpoints.~~

~~Novel candidate biomarkers and subsequently discovered biomarkers of the biological response associated with wound healing and tissue repair or medically related conditions and/or the action of GSK1278863.~~

~~All samples will be retained for a maximum of 15 years after the last subject completes the trial.~~

Section 8.1.1 Female Subjects

PREVIOUS TEXT

For female subjects on HRT and menopausal status is not confirmed, these contraceptive methods, classified as highly effective, are required.

REVISED TEXT

For female subjects **who can become pregnant and for those** on HRT and **for whom** menopausal status is not confirmed, **at least one of** these contraceptive methods, classified as highly effective, is required.

Section 9.2 Permitted Medications

PREVIOUS TEXT

Section 9.2 Permitted Medications

Occasional use of acetaminophen, defined as doses of ≤ 2 grams/day up to 48 hours prior to the first dose of study drug and until completion of follow-up procedures may be acceptable, at the discretion of the Principal Investigator or his/her designee.

REVISED TEXT

~~Section 9.2 Permitted Medications~~

~~Occasional use of acetaminophen, defined as doses of ≤ 2 grams/day up to 48 hours prior to the first dose of study drug and until completion of follow-up procedures may be acceptable, at the discretion of the Principal Investigator or his/her designee.~~

Section 9.3 DFU – General Considerations, Heading title, Paragraph 4

PREVIOUS TEXT

Section 9.3 DFU –General Considerations

Women receiving HRT are permitted to continue therapy while on study. See Section 9.2.

REVISED TEXT

Section 9.2 ~~DFU –~~ General Considerations

Women receiving HRT are permitted to continue therapy while on study. See Section ~~9.2~~ **5.2.1 or Section 5.3.1**.

Section 11.1 At-Home Dosing, Sentence Added

PREVIOUS TEXT

Pharmacists should dispense study drug for at-home dosing and label the container "Take as directed". A dosing card will be given to the subject, depending on the group they are randomized to, for instruction on at-home dosing. The dosing card will include detailed contact information for contacting study staff 24 hours per day. The patients' wound size will be determined and the amount of ointment for daily administration will be recorded on the dosing card. As above:

$$Amount\ of\ ointment\ to\ dispense = 25mg \times wound\ size\ (cm^2)$$

Compliance with study medication will be checked every time the subject returns for a study visit. The ointment tubes will be weighed prior to dispensing and during office visits to assess a daily averaged administrated amount.

REVISED TEXT

Pharmacists should dispense study drug for at-home dosing and label the container "Take as directed". A dosing card will be given to the subject, depending on the group they are randomized to, for instruction on at-home dosing. The dosing card will include detailed contact information for contacting study staff 24 hours per day. The patients' wound size will be determined and the amount of ointment for daily administration will be recorded on the dosing card. As above:

$$Amount\ of\ ointment\ to\ dispense = 25mg \times wound\ size\ (cm^2)$$

A conversion of ointment amount to ribbon length measured with the placard will be provided by GSK.

Compliance with study medication will be checked every time the subject returns for a study visit. The ointment tubes will be weighed prior to dispensing and during office visits to assess a daily averaged administrated amount.

AMENDMENT 6

Where the Amendment Applies

This amendment applies to all sites participating in this study.

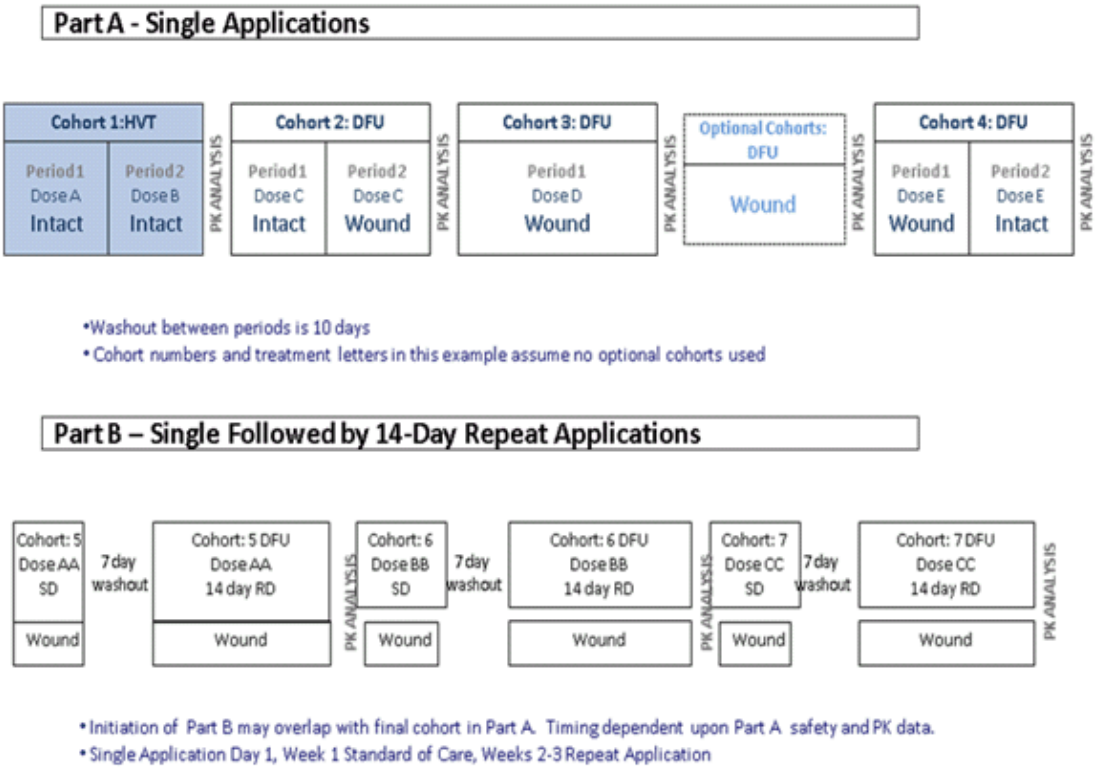
Summary of Amendment Changes with Rationale

Due to emerging data, specific areas of Part B of the protocol were redesigned to better meet the needs of the study. To date Part A GSK1278863 systemic exposure is below analytical limits of quantification and therefore the stand alone single dose assessement in Part B is no longer required. DFU exclusion criteria 10 was adjusted to better reflect the patient population. Other minor edits were also included for clarity.

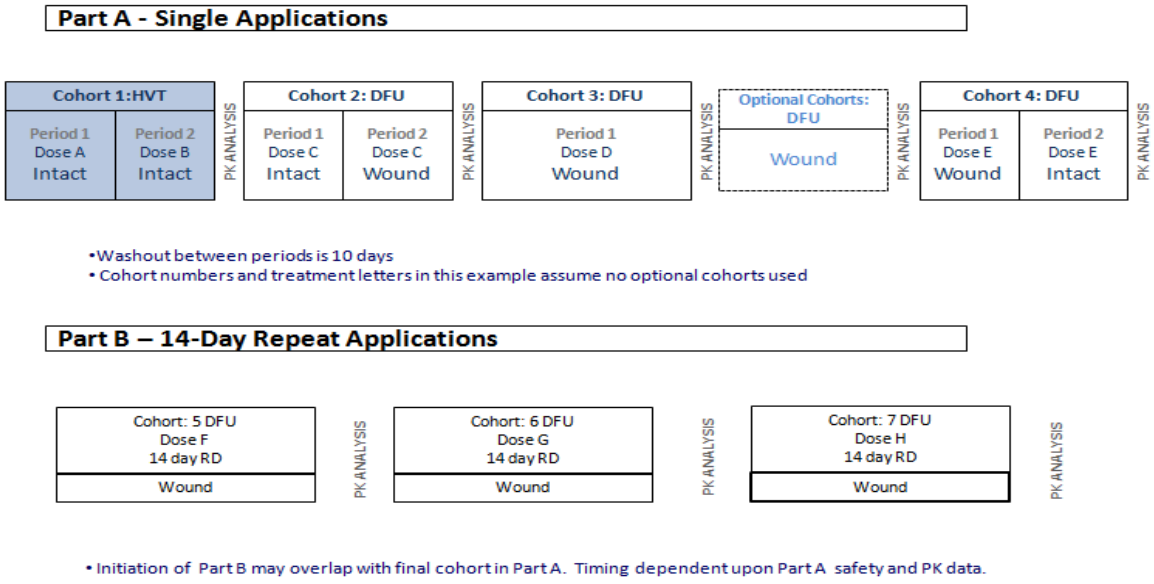
List of Specific Changes

SECTION 1.4 STUDY DESIGN STRATEGY AND SECTION 4.1 STUDY DESIGN/SCHEMATIC

Previous Text



Revised Text



SECTION 1.4.1.2 SINGLE DOSE SELECTION RATIONALE, EQUATION 1

PREVIOUS TEXT

Equation 1.

$$Css = \frac{F \cdot Flux}{CL}$$

Css = plasma concentration at steady-state

F = presumed bioavailability via the topical route

Flux = rate of drug transfer per unit area of surface

CL =systemic clearance

REVISED TEXT

$$Css = \frac{F \cdot Flux \cdot Area}{CL}$$

Equation 1.

Css = plasma concentration at steady-state

F = presumed bioavailability via the topical route

Flux = rate of drug transfer per unit area of surface

Area = treatment area

CL =systemic clearance

SECTION 1.4.1.3 REPEAT DOSING, PARAGRAPH 1 SENTENCES 4-6; PARAGRAPH 2

PREVIOUS TEXT

Three doses will be identified based on pharmacokinetics, systemic exposure level, and its relevance to known effects on Hb, safety and tolerability. There will be an initial single dose application and PK samples collected out to a time that has been deemed appropriate based on the single dose application to characterize the PK for accumulation calculations. After a one week washout period, the same dose will be applied daily for two weeks.

For practical reasons, a dosing period of approximately 24 hours could not be maintained and therefore a dosing period of ~22.5 hours will be followed for both the single and repeat dosing parts of this study. This allows for subject hygiene and dehydration of the skin prior to reapplication of the next dose. The amount of ointment applied to the DFUs may change based on wound size following one week of dosing to maintain consistency of amount applied/cm^2 of wound size, which would also result in a reduction of the total mass of drug applied . If deemed appropriate, intermittent dosing, i.e., a dosing interval greater than 24 hours, may be implemented.

REVISED TEXT

~~Three~~ Doses will be identified based on pharmacokinetics, systemic exposure level, and its relevance to known effects on Hb, safety and tolerability. ~~There will be an initial single dose application and PK samples collected out to a time that has been deemed appropriate based on the single dose application to characterize the PK for accumulation calculations~~. ~~After a one week washout period,~~ The same **percent (strength) ointment** ~~dose~~ will be applied daily for two weeks.

For practical reasons, a dosing period of approximately 24 hours could not be maintained and therefore a dosing period of ~22.5 hours will be followed for the ~~both the single and~~

repeat dosing ~~part~~s of this study. This allows for subject hygiene and dehydration of the skin prior to reapplication of the next dose. The amount of ointment applied to the DFUs may change based on wound size following ~~one week of~~ **initial** dosing to maintain consistency of amount applied/cm^2 of wound size, which would also result in a ~~reduction~~ **change** of the total mass of drug applied . If deemed appropriate, intermittent dosing, i.e., a dosing interval greater than 24 hours, may be implemented.

SECTION 4.2 DISCUSSION OF DESIGN AND TREATMENT PLAN, PARAGRAPH 2, SENTENCE 3; PARAGRAPH 3, SENTENCE 2 AND PARAGRAPH 5, SENTENCE 2

PREVIOUS TEXT

Part B is designed to evaluate first single, and then repeat applications of GSK1278863 in diabetics, both in the clinic and by subjects at home.

For both parts of the protocol, additional cohorts may be added as necessary to obtain relevant safety, pharmacokinetic or pharmacodynamic data.

Enrollment to each cohort may be staggered (i.e., cohorts do not have to be enrolled as one group). However, all enrollments should occur within ~4 weeks for each cohort.

REVISED TEXT

Part B is designed to evaluate ~~first single, and then~~ repeat applications of GSK1278863 in diabetics, both in the clinic and by subjects at home.

For both parts of the protocol, additional cohorts may be added as necessary to obtain relevant safety, pharmacokinetic or pharmacodynamic data. **Based on emerging data, the number of cohorts and subjects may be reduced to avoid unnecessary exposure.**

Enrollment to each cohort may be staggered (i.e., cohorts do not have to be enrolled as one group). ~~However, all enrollments should occur within ~4 weeks for each cohort.~~

4.2.1. PART A – SINGLE DOSES IN HEALTHY VOLUNTEERS AND DFU PATIENTS (COHORTS 1 THROUGH 4), PARAGRAPH 1, SENTENCE 2

PREVIOUS TEXT

Each cohort in Part A will enroll enough subjects to complete 6 on active drug and 2 on placebo (assumes ~10 subjects will be recruited per cohort to achieve 6:2 ratio).

REVISED TEXT

Each cohort in Part A will enroll enough subjects to complete **approximately** 6 on active drug and 2 on placebo (assumes ~10 subjects will be recruited per cohort to achieve 6:2 ratio).

4.2.1.6. OPTIONAL COHORTS (PART A), 1ST BULLET; PARAGRAPH 3; PARAGRAPH 4, SENTENCE 1

PREVIOUS TEXT

- If the data at the end of Cohort 4 are considered sufficient to establish dosing for Part B, subjects from that cohort will be asked to return to the clinic for a SD administration of the compound to intact skin in order to make comparisons of systemic PK for linearity between low and high doses and a within-patient comparison of application of drug to intact skin versus DFU.

The timing of the final cohort, in which application to intact skin is assessed, may overlap with the first cohort of Part B, as long as all doses of GSK1278863 in previous cohorts are well tolerated and provide sufficient PK for Part B predictions.

GSK1278863 PK data from the cohorts of Part A will be used for the predictions of the expected systemic exposures in Part B (data permitting).

REVISED TEXT

- If the data **from** ~~at the end of~~ Cohort 4 are considered sufficient to establish dosing for Part B, subjects from that cohort will be asked to return to the clinic for a SD administration of the compound to intact skin in order to make comparisons of systemic PK for linearity between low and high doses and a within-patient comparison of application of drug to intact skin versus DFU.

The timing of the final cohort, in which application to intact skin is assessed, may overlap with the first cohort of Part B, as long as all doses of GSK1278863 in previous cohorts are well tolerated and provide sufficient PK for Part B predictions. **Systemic exposures may or may not permit predictions for dose selection and therefore dose determination will be selected based on available safety and tolerability data.**

GSK1278863 PK data from the cohorts of Part A will be used for the predictions of the expected systemic exposures in Part B **(data permitting)**.

4.2.2. PART B – SINGLE DOSE/REPEAT DOSES IN DIABETIC FOOT ULCER SUBJECTS (COHORTS 5,6, AND 7), HEADING TITLE; PARAGRAPH 1, SENTENCE 2; PARAGRAPH 2; PARAGRAPH 3, SENTENCE 1 AND 2; PARAGRAPH 4

PREVIOUS TEXT

Section 4.2.2. Part B – Single Dose/Repeat Doses in Diabetic Foot Ulcer Subjects (Cohorts 5,6, and 7)

Enough subjects will be enrolled in each cohort to complete 16 diabetic subjects with diabetic foot ulcer.

Each cohort will enroll a new group of subjects, randomized descending doses of GSK1278863.

For each cohort, subjects will first be given a single application of GSK1278863 or placebo at the dose chosen for that cohort, followed by a 7-day washout period, and then repeat applications for 14 days, starting on Day 8. The initial application and the first day of the 14-day repeat application period will be conducted in the clinic.

Doses for the cohorts in Part B will be determined from safety, tolerability and PK data from Part A. Each cohort will enroll a new group of subjects, randomized to ascending doses of GSK1278863. All applications in Part B will be made directly to the subject's DFU. Doses may be adjusted based on wound size at each weekly visit. All applications in Part B will be made directly to the subject's DFU. Doses may be adjusted based on wound size at each weekly visit.

Blood for PK sampling will be collected on Days 1-4 (single dose) and Days 8, 14, 15 and Days 21-23(repeat dosing). See Section 4.6.3 for specific time points for drawing samples.

REVISED TEXT

Section 4.2.2. Part B – ~~Single Dose/~~Repeat Doses in Diabetic Foot Ulcer Subjects (Cohorts 5,6, and 7)

Enough subjects will be enrolled in each cohort to complete **approximately** 16 diabetic subjects with diabetic foot ulcer.

Doses for the cohorts in Part B will be determined from safety, tolerability and PK data from Part A. Each cohort will enroll a new group of subjects, randomized **to either the same dose, ascending dose or descending** dose**s** of GSK1278863. All applications in Part B will be made directly to the subject's DFU. ~~Doses may be adjusted based on wound size at each weekly visit.~~

For each cohort, subjects will **undergo repeat applications** ~~first be given a single application~~ of GSK1278863 or placebo, at the dose chosen for that cohort, ~~followed by a 7-day washout period, and then repeat applications~~ for 14 days~~, starting on Day 8~~. The ~~initial application and the~~ first day of the 14-day repeat application period will be conducted in the clinic.

Doses for the cohorts in Part B will be determined from safety, tolerability and PK data from Part A. Each cohort will enroll a new group of subjects, randomized to **the same dose,** ascending **dose or descending** dose~~s~~ of GSK1278863. All applications in Part B will be made directly to the subject's DFU. ~~Doses may be adjusted based on wound size at each weekly visit.~~

Blood for PK sampling will be collected on Days 1, **7**~~-4 (single dose)~~ ~~and Days 8~~, 14, 15 and **16**~~-Days 21-23(repeat dosing)~~. See Section 4.6.3 for specific time points for drawing samples.

4.2.2.2. RUN-IN PERIOD (PART B), PARAGRAPH 1, SENTENCE 2 AND 3

PREVIOUS TEXT

For all subjects in Part B, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product. If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (Baseline Run In) will need to be scheduled prior to Day 1 to meet the Run In requirement.

REVISED TEXT

For all subjects in Part B, each subject's DFU will be measured at screening and Day 1; if, after 14 days from screening, the subject's DFU has reduced in size by greater than 30 %, the subject will not be randomized to receive investigational product **and is considered a Run In Failure**. If Screening and Day 1 are not 14 days (±1 day) apart, an additional visit (**Optional** Baseline Run In) will need to be scheduled prior to Day 1 to meet the Run In requirement.

4.2.2.3 TREATMENT PERIOD – (PART B)

PREVIOUS TEXT

Cohorts 5, 6 and 7 (numbering assumes no optional cohorts are enrolled in Part A) will each enroll sufficient subjects so that 48 DFU patients who fulfill screening criteria complete all study visits.

Single Dose (Part B)

Subjects will be randomized to receive single applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of

GSK1278863 and PD. Subjects may be discharged from the clinic once all Day 1 assessments have been completed. Subjects will return to the clinic fasting early in the morning on Day 2 and Day 3 for PK trough samples and safety assessments.

See Section 4.6 for details regarding all study procedures performed during the course of this trial.

Washout Period

Subjects will receive standard-of-care (moist occlusion) treatment (Section 7.4) for the week between Day 1of single dosing and Day 8, which is the first day of repeat 14-day dosing.

14-Day Dosing (Part B)

Approximately one week after receiving the single dose (Day 8), subjects will return to the clinic to begin 14 days of dosing with the GSK1278863 dose they were randomized to for the previous single dose. Subjects may remain overnight (if elected), leaving in the morning of Day 9. Subjects will then perform the applications of study drug at home, returning on Day 11 for assessments and drug application. Patients will then apply study drug at home on days 12-13, and then return on day 14 for scheduled assessments and dosing. Subjects may again remain overnight if they elect for the purposes of serial PK sampling. After scheduled assessments on Day 15, subjects will again perform applications at home and return to the clinic on Day 18 and then again on days 21-23 (electing to stay overnight if desired) for scheduled assessments.

It should be noted that on days where serial PK sampling occurs it is permissible to allow subjects to check out of the clinic after the 8 hour PK time point has been collected as long as the subject can return for sample collection within the 10 to 12 hour PK window. Subjects that opt to remain in clinic will have PK samples drawn at 8 and 12 hours post dose.

Subjects will be given detailed instructions for application of GSK1278863 at home, and enough study for applications at home between clinic visits. See Section 11.1 for further instructions on at-home dosing.

Doses for subsequent cohorts may be adjusted based on PK results, and/or safety data.

REVISED TEXT

Cohorts 5, 6 and 7 (numbering assumes no optional cohorts are enrolled in Part A) will each enroll sufficient subjects so that **approximately** 48 DFU patients who fulfill screening criteria complete all study visits.

~~***Single Dose (Part B)***~~

~~Subjects will be randomized to receive single applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of GSK1278863 and PD. Subjects may be discharged from the clinic once all Day 1~~

~~assessments have been completed. Subjects will return to the clinic fasting early in the morning on Day 2 and Day 3 for PK trough samples and safety assessments.~~

~~See Section 4.6 for details regarding all study procedures performed during the course of this trial.~~

~~*Washout Period*~~

~~Subjects will receive standard-of-care (moist occlusion) treatment (Section 74) for the week between Day 1of single dosing and Day 8, which is the first day of repeat 14-day dosing.~~

14-Day Dosing (Part B)

~~Approximately one week after receiving the single dose (Day 8), subjects will return to the clinic to begin 14 days of dosing with the GSK1278863 dose they were randomized to for the previous single dose. Subjects may remain overnight (if elected), leaving in the morning of Day 9. Subjects will then perform the applications of study drug at home, returning on Day 11 for assessments and drug application. Patients will then apply study drug at home on days 12-13, and then return on day 14 for scheduled assessments and dosing. Subjects may again remain overnight if they elect for the purposes of serial PK sampling. After scheduled assessments on Day 15, subjects will again perform applications at home and return to the clinic on Day 18 and then again on days 21-23 (electing to stay overnight if desired) for scheduled assessments.~~ **Subjects will be randomized to receive applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of GSK1278863 and PD. Applications of study drug will be done in clinic on Days 1, 4, 7, 11, and 14. Subjects are responsible for study drug application on Days 2-3, 5- 6, 8-10, 12-13.**

It should be noted that on days where serial PK sampling occurs it is permissible to allow subjects to check out of the clinic after the 8 hour PK time point has been collected as long as the subject can return for sample collection within the 10 to 12 hour PK window. ~~Subjects that opt to remain in clinic will have PK samples drawn at 8 and 12 hours post dose.~~ **If subjects elect to stay overnight, they should be encouraged to apply the study ointment and complete the diary entries themselves before being discharged in the morning. Study staff can take this opportunity to provide additional instruction to subjects.**

Subjects will be given detailed instructions for application of GSK1278863 at home and enough study **drug** for applications at home between clinic visits. See Section 11.1 for further instructions on at-home dosing.

Doses for subsequent cohorts may be adjusted based on PK results, and/or safety **and tolerability** data.

4.3. TREATMENT ASSIGNMENT, PARAGRAPH 6

PREVIOUS TEXT

Drug volume to be administered will be based on wound area, which may vary during the treatment period. See Section 4.4 for details. Actual doses will be chosen based on preliminary PK data from Part A.

REVISED TEXT

Drug volume to be administered will be based on wound area, which may vary during the treatment period. See Section 4.4 for details. Actual doses will be chosen based on preliminary PK data **(data permitting, See Section 4.2.1.6)** from Part A.

4.6.1 TIME AND EVENT TABLE PART A, FOOTNOTE ADDED

REVISED TEXT

4. HIV, Hep B and Hep C are only assessed in the Healthy Volunteer population

4.6.3 TIME AND EVENTS TABLE PART B

PREVIOUS TEXT

Procedures	**Screening**	**Single Dose**	**Day 2**	**Days 3 and 4**	**Days 5 thru 7**	**14 Day Repeat Dose**	**Day 11**	**Day 14**	**Day 15**	**Day 18**	**Day 21**	**Day 22**	**Day 23**	**Follow-up**	
		Day 1				**Day 8**									
Visit Window (relative to Day 1)	-28 to -14 days	Exams/samples pre-dose	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Informed Consent	X														
Demographics	X														
Complete physical	X														
Brief physical		X	X	X		X	X	X	X	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X														
12-lead ECG[1]	X	X										X			
Vital signs[2]	X	X				X		X				X		X	X
Urine pregnancy(females)	X	X				X								X	
HIV, Hep B and Hep C screen	X														
Hema/Chem/ Safety Labs[3]	X	X				X		X				X		X	
Vascular Perfusion Eligibility Assessments[4]	X														
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)		X[5]				X[5]		X				X			
Standard of Care	X		X	X	X										

Procedures	Screening	Single Dose	Day 2	Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	Follow-up	
		Day 1				Day 8									
Treatment[6]															
Study Treatment/Dosing In the Clinic[7]		X				X	X	X	X	X	X				
PK serial blood sampling[8]		X	X	X		X (truncated)		X	X		X	X	X		
PK Trough Sample[8]										X					
Tissue Biopsies (gene expression, macrophage and bioburden analysis)[9]		X				X		X				X			
Wound Fluid (cytokine and bioburden analysis)[10]		X	X	X (Day 3 only[10])		X		X	X			X		X	
SensiLase (Doppler blood flow)		X				X		X				X		X	
Hyperspectral Imaging[11]		X				X		X				X		X	
Physical Characteristics (assessed pre-dose)[12]	X[11]	X				X	X	X		X		X		X	
Clinical Characteristics[13]	X	X	X	X		X	X	X	X	X	X	X	X	X	
Concomitant Medication Review		X	←==========											X	

Procedures	Screening	Single Dose		Days 3 and 4	Days 5 thru 7	14 Day Repeat Dose							Follow-up	
		Day 1	Day 2			Day 8	Day 11	Day 14	Day 15	Day 18	Day 21	Day 22	Day 23	
Adverse Event Assessment[14]		X	←==========→										X	X

1. Single ECGs will be taken at Screening and pre-dose on Day 1(SD) and 24h post-last RD dose on Day 22. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
2. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1 (SD) and 8(RD), 14, 22 (24h post-last-RD dose), and at Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
3. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,8,14, 22 (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
4. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.
5. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
6. During washout times all subjects will be on SOC (Section 7.4).
7. In between clinic visits during RD, subjects will perform applications of study drug at home.
8. See separate Time and Events table for serial PK sampling details for Days 1, 8, 14 and 21 and the associated 24, 48 and 72 hr time points. Trough sample will be taken prior to dosing on Day 18. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile, but the total number of samples will not change.
9. Direct wound tissue biopsies will be collected at pre-dose/equivalent on Days 1, 8, 14 and 22, however based on emerging data, time points may be added or deleted; wounds that have healed sufficiently may be exempt. Refer to Section 7.7.2 for additional details on the collection and processing of tissue biopsies.
10. Wound fluid will be collected at pre-dose/equivalent on Days 1, 2. 3, 8, 14, 15, 22 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7.1 for additional details on the collection and processing of wound fluid samples.
11. Will be performed based on hyperspectral imaging capabilities at each site.
12. Physical characteristics of the wound (i.e., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), at pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the 7-10 day Follow-up Visit.
13. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
14. Adverse events are to be collected from first dose through the final Follow-up visit.

REVISED TEXT

Procedures	Screening	~~Single Dose~~ **Optional Baseline Run In**[1] ~~Day 1~~	~~Day 2~~	~~Days 3 and~~ 4	~~Days 5 thru 7~~	~~14 Day Repeat Dose~~ Day ~~8~~ **1**	Day ~~11~~ **4**	Day ~~14~~ **7**	~~Day 15~~	Day ~~18~~ **11**	Day ~~21~~**14**	Day ~~22~~ **15**	Day ~~23~~ **16**	Follow-up	
Visit Window (relative to Day 1)	-28 to -14 days	~~Exams/samples pre-dose~~	Washout			Exams/samples pre-dose								7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	~~X~~	~~X~~		X	X	X	~~X~~	X	X	X	X	X	X
Informed Consent	X														
Demographics	X														
Complete physical	X														
Brief physical		X	~~X~~	~~X~~		X	X	X	~~X~~	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X	**X**													
12-lead ECG[12]	X	~~X~~										X			
Vital signs[23]	X	~~X~~				X		X				X		X	X
Urine pregnancy(females)	X	~~X~~				X								X	
~~HIV, Hep B and Hep C screen~~	~~X~~														
Hema/Chem/ Safety Labs[34]	X	~~X~~				X		X				X		X	
Vascular Perfusion Eligibility Assessments[4-5]	X														
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)		~~X~~				X[5-6]		X				X			

Procedures	Screening	~~Single Dose~~ **Optional Baseline Run In**[1] ~~Day 1~~	~~Day 2~~	~~Days 3 and~~ 4	~~Days 5 thru 7~~	~~14 Day Repeat Dose~~ Day ~~8~~ **1**	Day ~~11~~ **4**	Day ~~14~~ **7**	~~Day 15~~	Day ~~18~~ **11**	Day ~~21~~**14**	Day ~~22~~ **15**	Day ~~23~~ **16**	Follow-up	
Standard of Care Treatment[6][7]	X	**X**	~~X~~	~~X~~	~~X~~	←==========→								**X**	**X**
Study Treatment/Dosing In the Clinic[7][8]		~~X~~				X	X	X	~~X~~	X	X				
PK serial blood sampling[8][9]		~~X~~	~~X~~	~~X~~		X ~~(truncated)~~		X	~~X~~		X	X	X		
PK Trough Sample[8][9]										X					
Tissue Biopsies (gene expression, macrophage and bioburden analysis)[9][10]		~~X~~				X		X				X			
Wound Fluid (cytokine and bioburden analysis)[10][11]		~~X~~	~~X~~	~~X (Day 3 only[10])~~		X		X	~~X~~			X		X	
SensiLase (Doppler blood flow)		~~X~~				X		X				X		X	
Hyperspectral Imaging[11][12]		~~X~~				X		X				X		X	
Physical Characteristics (assessed pre-dose)[12][13]	X[11]	X				X	X	X		X		X		X	
Clinical Characteristics[13][14]	X	X	~~X~~	~~X~~		X	X	X	~~X~~	X	X	X	X	X	

Procedures	Screening	~~Single Dose~~ **Optional Baseline Run In**[1] ~~Day 1~~	~~Day 2~~	~~Days 3 and~~ 4	~~Days 5 thru 7~~	~~14 Day Repeat Dose~~ Day ~~8~~ **1**	Day ~~11~~ **4**	Day ~~14~~ **7**	~~Day 15~~	Day ~~18~~ **11**	Day ~~21~~**14**	Day ~~22~~ **15**	Day ~~23~~ **16**	Follow-up	
Concomitant Medication Review		~~X~~	←==========										==========→	X	
Adverse Event Assessment[14-15]		~~X~~	←==========										==========→	X	X

1. **Optional Baseline Run In visit: This optional visit is for subjects that will exceed the 14 (±1 day) Run-in window between screening and Day 1.**
2. Single ECGs will be taken at Screening and pre-dose on Day 1~~(SD)~~ and 24h post-last ~~RD~~ dose on Day ~~22~~ **15**. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1, **7** ~~(SD) and 8(RD)~~, ~~14~~ **15** (24h post-last-~~RD~~ dose), and at the Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1,~~8~~ **7**,~~14~~, ~~22~~ **15** (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI/TcPO2, toe pressure, Doppler ultrasound) is required for eligibility.
6. Blood samples for HIFα activation on Day 1 and Day 8 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
7. ~~During washout times~~ All subjects will be on SOC **throughout the study** (Section 7.4).
8. In between clinic visits during RD, subjects will perform applications of study drug at home.
9. See separate Time and Events table for serial PK sampling details for Days 1, ~~8~~**7**, 14 ~~and 21~~ and the associated 24~~,~~and 48 ~~and 72 hr~~ time points. Trough sample will be taken prior to dosing on Day ~~18~~ 11. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile, but the total number of samples will not change.
10. Direct wound tissue biopsies will be collected at pre-dose/equivalent on Days 1, 8, 14 and 22, however based on emerging data, time points may be added or deleted; wounds that have healed sufficiently may be exempt. Refer to Section 7.7.2 for additional details on the collection and processing of tissue biopsies.
11. Wound fluid will be collected at pre-dose/equivalent on Days 1, 2. 3, 8, 14, 15, 22 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7.1 for additional details on the collection and processing of wound fluid samples.
12. Will be performed based on hyperspectral imaging capabilities at each site.
13. Physical characteristics of the wound (i.e., photos of the wound and wound measurement assessments) will be assessed at Screening (acetate tracings only), at pre-dose/equivalent time on Days 1, 8, 11, 14, 18, 22 and at the 7-10 day Follow-up Visit.
14. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed.
15. Assessments are recorded in the eCRF.

4.6.3 SERIAL PK TIME AND EVENTS TABLE PART B

PREVIOUS TEXT

Procedure	**Part B Serial PK Sampling[1,2]**													
	Hours (h)													
	Pre-dose	**0.25h**	**0.5h**	**1h**	**2h**	**4h**	**6h**	**8h**	**10h**	**11h**	**12h**	**24h**	**48h**	**72h**
Single Dose Sampling OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	X
Single Dose Sampling IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	X
Repeated Dose Day 8 Truncated (Start of Repeated Dose)	X			X	X	X	X							
Repeated Dose Day 14 OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X		
Repeated Dose Day 14 IN-PATIENTS	X	X	X	X	X	X		X			X	X		
Repeated Dose Day 21 OUT-PATIENTS	X	X	X	X	X	X		X	X[3]			X	X	
Repeated Dose Day 21 IN-PATIENTS	X	X	X	X	X	X		X			X	X	X	

1. Patients are permitted to either stay in clinic (in-patients) or be discharged and return for clinic visits (out-patients). Refer to Section 7.12 for details on the collection and processing of PK samples.
2. PK sampling times may be changed based on observed GSK1278863 PK profile, but the total number of samples will not change.
3. Sample must be taken within the 10 to 12 hour time window.

REVISED TEXT

Procedure	**Part B Serial PK Sampling[1,2]**													
	Hours (h)													
	Pre-dose	**~~0.25h~~**	**0.5h**	**1h**	**2h**	**4h**	**6h**	**8h**	**10h**	**11h**	**12h**	**24h**	**48h**	**72h**
~~Single Dose Sampling OUT-PATIENTS~~ **Day 1**	X	~~X~~	X	X	X	X	**X**	X	X[3]			~~X~~	~~X~~	~~X~~
~~Single Dose Sampling IN-PATIENTS~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~		~~X~~			~~X~~	~~X~~	~~X~~	~~X~~
~~Repeated Dose Day 8 Truncated (Start of Repeated Dose)~~	~~X~~			~~X~~	~~X~~	~~X~~	~~X~~							
~~Repeated Dose~~ Day ~~14~~ **7** ~~OUT-PATIENTS~~	X	~~X~~	X	X	X	X		X	X[3]			~~X~~		
~~Repeated Dose Day 14 IN-PATIENTS~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~		~~X~~			~~X~~	~~X~~		

Procedure	Part B Serial PK Sampling[1,2]													
	Hours (h)													
	Pre-dose	~~0.25h~~	0.5h	1h	2h	4h	6h	8h	10h	11h	12h	24h	48h	72h
~~Repeated Dose~~ Day ~~21~~ **14** OUT-PATIENTS	X	~~X~~	X	X	X	X		X	X[3]			X	X	
~~Repeated Dose~~ Day ~~21~~ **14** IN-PATIENTS	X	~~X~~	X	X	X	X		X			X	X	X	

1. Patients are permitted to either stay in clinic (in-patients) or be discharged and return for clinic visits (out-patients). Refer to Section 7.12 for details on the collection and processing of PK samples.
2. PK sampling times may be changed based on observed GSK1278863 PK profile~~, but the total number of samples will not change~~.
3. Sample must be taken within the 10 to 12 hour time window.

5.1. NUMBER OF SUBJECTS, PARAGRAPH 2

PREVIOUS TEXT

In Part B, enough subjects will be enrolled such that approximately 48 subjects in Part B (~16 DFU subjects per cohort) complete dosing and critical assessments.

REVISED TEXT

In Part B, enough subjects will be enrolled such that approximately 48 subjects in Part B (~16 DFU subjects per cohort) complete dosing and critical assessments **(assumes approximately 3 cohorts)**.

5.3.2. EXCLUSION CRITERIA – DFU SUBJECTS, EXCLUSION CRITERIA # 10

PREVIOUS TEXT

10. Subjects with an International Normalized Ratio (INR) >1.5 at screening.

REVISED TEXT

10. Subjects with an International Normalized Ratio (INR) >~~1.5~~ **1.1** at screening. If on warfarin, then >2.5.

6.3.3.3. MRNA, PROTEIN AND BIOMARKER DATA ANALYSIS, PARAGRAPH 1

PREVIOUS TEXT

Biomarker data will be summarized by regimen for Part A, and regimen and visit for Part B.

REVISED TEXT

Biomarker data will be summarized by regimen ~~for Part A, and regimen~~ and visit for Part **A and Part** B.

7.7.2. PUNCH BIOPSY DIRECTIONS, BULLETED ITEM ADDED

PREVIOUS TEXT

- Prior to obtaining biopsies, the wound is to be cleansed with saline solution.

REVISED TEXT

- Whenever feasible, tissue biopsies should be done at the time of debridement.
- Prior to obtaining biopsies, the wound is to be cleansed with saline solution.

7.9.1.Location of the Study Ulcer

PREVIOUS TEXT

The location of the study ulcer will be recorded as foot (left or right), surface (plantar or dorsal), and area [forefoot, midfoot, hindfoot (including the calcaneus)]. If more than one ulcer is located in the same proximity, be certain to clearly identify the specific study ulcer.

REVISED TEXT

The location of the study ulcer will be recorded as foot (left or right), surface (plantar**,** ~~or~~ dorsal**, medial aspect of heel, medial aspect of great toe, posterior or lateral**), and area [forefoot, midfoot, hindfoot (including the calcaneus)**, first through fifth metatarals or proximal to the foot on the ankle**]. If more than one ulcer is located in the same proximity, be certain to clearly identify the specific study ulcer.

7.9.2.2 WOUND TRACINGS, PARAGRAPH 1

PREVIOUS TEXT

To ensure the subject meets the randomization eligibility of the change in ulcer size between screening (or optional Run In visit, if different from day of screening) and randomization on Day 1, percent reduction of wound size must be calculated by the Investigator or designee using the method for measuring wound area provided in the SPM. The core lab may be consulted for assistance with area measurements or percent reduction determinations if necessary.

REVISED TEXT

Tracings of the wound should be obtained after debridement. To ensure the subject meets the randomization eligibility of the change in ulcer size between screening (or optional **Baseline** Run In visit, if different from day of screening) and randomization on Day 1, percent reduction of wound size must be calculated by the Investigator or designee using the method for measuring wound area provided in the SPM. The core lab may be consulted for assistance with area measurements or percent reduction determinations if necessary.

7.13.3 Action to be Taken if Pregnancy Occurs in a Female Partner of a Male Study Subject, Section Title; Paragraph 1

PREVIOUS TEXT

7.13.3 Action to be Taken if Pregnancy Occurs in a Female Partner of a Male Study Subject, Section Title; Paragraph 1

The investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy. The partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

REVISED TEXT

~~The investigator will attempt to collect pregnancy information on any female partner of a male study subject who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the partner's pregnancy. The partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.~~

12. 9 PROMPT REPORTING OF SAES TO GSK

PREVIOUS TEXT

The primary mechanism for reporting SAEs to GSK will be the electronic data collection tool. If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the GSK Medical Monitor. Then the site will enter the serious adverse event 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 (e.g., InForm system) 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, the site can report this information on a paper SAE form or to their GSK protocol contact by telephone.

REVISED TEXT

The primary mechanism for reporting SAEs to GSK will be the electronic data collection tool. If the electronic system is unavailable for greater than 24 hours, the site will use the paper SAE data collection tool and fax it to the GSK Medical Monitor. Then the site will enter the serious adverse event data into the electronic system as soon as it becomes available.

The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box at the bottom of the eCRF page within 72 hours of submission of the SAE

After the study is completed at a given site, the electronic data collection tool (e.g., InForm system) 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, the site can report this information on a paper SAE form or to their GSK protocol contact by telephone.

AMENDMENT 7

Where the Amendment Applies

This amendment applies to all sites participating in this study.

Summary of Amendment Changes with Rationale

This amendment contains modifications to inclusion criteria 3, 5 and 6 and exclusion criteria 2, 3. Exclusion criteria 5 and 7 -13 were removed.

Rationale for Inclusion Criterion 3: The results of a thorough QT study utilizing oral doses of GSK1278863 support the conclusion that there appears to be no clinically significant effect on ΔΔQTcF after administration of doses up to 500 mg in healthy subjects. (GSK Document #2015N233454_00 PHI113635 14Dec 2015). These results, in addition to the limited systemic exposure demonstrated with topical application of GSK1278863, support the use of expanded QT enrolment criteria.

Rationale for Inclusion Criterion 5 and 6c: Systemic concentrations of GSK1278863 measured from patients administered 1% w/w (at 100mg/cm2) have been below the LLOQ of the analytical assay (0.10 ng/mL). A larger upper limit of screening/randomization ulcer size results in maximum systemic exposure predictions (following once daily application of ointment strengths up to 10% w/w GSK1278863 (at 100 mg/cm2) for 14 days) well below this study's intended systemic exposure limits (395 ng.h/mL and 198 ng/mL for AUC and Cmax, respectively). This prediction supports an increase in allowable ulcer size.

Rationale for Exclusion Criterion 2, 3, 5, 7-13: As of June 2016, GSK1278863 has been administered orally to over 1000 subjects, including those with chronic kidney disease (either not on dialysis or on dialysis), in single doses up to 500 mg, in repeat-doses up to 100 mg daily for 4 weeks and up to 25 mg daily for 24 weeks. Overall, the safety profile was consistent with the patient population and there were no adverse events that have been identified as related to treatment with GSK1278863. Planned phase 3 studies have thus adopted revised exclusion criteria consistent with those above in patients with anemia due to chronic kidney disease, a vulnerable population with respect to vascular events.

Systemic concentrations of GSK1278863 measured from DFU subjects administered 1% w/w (at 100mg/cm2) have been below the LLOQ of the analytical assay (0.10 ng/mL) and systemic exposures following once daily application of ointment strengths up to 10% w/w GSK1278863 (at 100 mg/cm2) in DFU subjects are predicted to be well below this study's intended systemic exposure limits (395 ng.h/mL and 198 ng/mL for AUC and Cmax, respectively).

The decision to modify the exclusion criteria was therefore based on the safety profile demonstrated in the oral program in patients with a similar or greater risk profile who are exposed to systemic concentrations of GSK1278863 which are well above those in the topical program.

Stopping and withdrawal criteria were also modified to align with the updated inclusion/exclusion criteria as appropriate.

Other changes include updates to Part B that occurred in stream based on emerging data, but did not require a protocol amendment. Other minor edits for clarity.

List of Specific Changes

4.2.2. Part B –Repeat Doses in Diabetic Foot Ulcer Subjects (Cohorts 5, 6 and 7), PARAGRAPH 4

Previous Text

Blood for PK sampling will be collected on Days 1, 7, 11, 14, 15 and 16. See Section 4.6.3 for specific time points for drawing samples.

Revised Text

Blood for PK sampling will be collected on Days 1, 7, ~~11~~, 14, **and** 15 ~~and 16~~. See Section 4.6.3 for specific time points for drawing samples.

4.2.2.3. Treatment Period – (Part B), PARAGRAPH 2

PREVIOUS TEXT

Subjects will be randomized to receive applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of GSK1278863 and PD. Applications of study drug will be done in clinic on Days 1, 4, 7, 11, and 14. Subjects are responsible for study drug application on Days 2-3, 5- 6, 8-10, 12-13.

REVISED TEXT

Subjects will be randomized to receive applications of GSK1278863 or placebo directly to the DFU on Day 1 for evaluation of safety and tolerability, PK of GSK1278863 and PD. Applications of study drug will be done in clinic on Days 1, 4, 7, ~~11~~, and 14. Subjects are responsible for study drug application on Days 2-3, 5- 6 **and** 8~~-10, 12~~13.

4.5.1.3. QTc Withdrawal Criteria, DFU Patients (Part A Cohorts 2 through Final Cohort, Part B All Cohorts)

PREVIOUS TEXT

- QTc > 500msec or uncorrected QT >600msec
- If subject has underlying bundle branch block then the QTc withdrawal criteria depends on the baseline value:

Baseline QTc value with underlying bundle branch block)	**QTc withdrawal criteria**
<450msec	>500msec
450-480msec	≥530msec

REVISED TEXT

- QTc > **530**~~500~~msec or uncorrected QT >600msec
- If subject has underlying bundle branch block then the QTc withdrawal criteria depends on the baseline value:

Baseline QTc value with underlying bundle branch block)	**QTc withdrawal criteria**
≤500 ~~450~~	>~~500msec~~**530msec**
≤530 ~~450-480~~msec	≥~~530msec~~**550msec**

4.5.1.4 Other Dose Adjustment/Stopping Safety Criteria; DELETED TEXT

PREVIOUS TEXT

- Subjects with documented GI bleeding, new onset positive fecal occult blood test, or abdominal pain (other than transient, minor abdominal pain). See Section 5.3 for additional guidance.
- Treatment emergent pulmonary hypertension, new onset or worsening retinopathy (e.g. proliferative retinopathy, or macular edema), or new onset or worsening non-traumatic joint inflammation (e.g., rheumatic or psoriatic arthritis).

REVISED TEXT

- ~~Subjects with documented GI bleeding, new onset positive fecal occult blood test, or abdominal pain (other than transient, minor abdominal pain). See Section 5.3 for additional guidance.~~
- ~~Treatment emergent pulmonary hypertension, new onset or worsening retinopathy (e.g. proliferative retinopathy, or macular edema), or new onset or worsening non-traumatic joint inflammation (e.g., rheumatic or psoriatic arthritis).~~

4.6.2. Time and Events Table Part B

PREVIOUS TEXT

Procedures	**Screening**	**Optional Baseline Run In Visit[1]**	**Day 1**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 15**	**Day 16**	**Follow-up**	
Visit Window (relative to Day 1)	-28 to -14 days		Exams/samples pre-dose							7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X	X	X	X	X	X	X	X
Informed Consent	X										
Demographics	X										
Complete physical	X										
Brief physical		X	X	X	X	X	X	X	X	X	X
Medical/medication/ drug/alcohol history	X	X									
12-lead ECG[2]	X		X					X			
Vital signs[3]	X		X		X			X		X	X
Urine pregnancy(females)	X		X							X	
Hema/Chem/ Safety Labs[4]	X		X		X			X		X	
Vascular Perfusion Eligibility Assessments[5]	X										
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)			X[6]		X			X			
Standard of Care Treatment[7]	X	X	←==========→							X	X

Procedures	**Screening**	**Optional Baseline Run In Visit**[1]	**Day 1**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 15**	**Day 16**	**Follow-up**	
Study Treatment/Dosing In the Clinic[8]			X	X	X	X	X				
PK serial blood sampling[9]			X		X		X	X	X		
PK Trough Sample[9]						X					
Tissue Biopsies (gene expression, macrophage and bioburden analysis)[10]			X		X			X			
Wound Fluid (cytokine and bioburden analysis)[11]			X		X			X		X	
SensiLase (Doppler blood flow)			X		X			X		X	
Hyperspectral Imaging[12]			X		X			X		X	
Physical Characteristics (assessed pre-dose)[13]	X[11]	X	X	X	X	X		X		X	
Clinical Characteristics[14]	X		X	X	X	X	X	X	X	X	
Concomitant Medication Review			X	←==========					==========→	X	X

Procedures	Screening	Optional Baseline Run In Visit[1]	Day 1	Day 4	Day 7	Day 11	Day 14	Day 15	Day 16	Follow-up	
Adverse Event Assessment[15]			X	←==========→						X	X

1. Optional Baseline Run In visit: This optional visit is for subjects that will exceed the 14 (±1 day) Run-in window between screening and Day 1.
2. Single ECGs will be taken at Screening and pre-dose on Day 1 and 24h post-last dose on Day15. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1, 7, 15 (24h post-last dose), and at Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1, 7, 15 (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI, toe pressure, Doppler ultrasound) is required for eligibility.
6. Blood samples for HIFα activation on Day 7 will be collected at pre-dose, 8 and 10-12 hour window. On all other indicated days one sample will be collected at pre-dose/equivalent time.
7. All subjects will be on SOC throughout the study (Section 7.4).
8. In between clinic visits , subjects will perform applications of study drug at home.
9. See separate Time and Events table for serial PK sampling details for Days 1, 7 and 14 and the associated 24and 48 hr time points. Trough sample will be taken prior to dosing on Day 11. Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile.
10. Direct wound tissue biopsies will be collected at pre-dose/equivalent on Days 1, 7, and 15, however based on emerging data, time points may be added or deleted; wounds that have healed sufficiently may be exempt. Refer to Section 7.7.2 for additional details on the collection and processing of tissue biopsies.
11. Wound fluid will be collected at pre-dose/equivalent on Days 1, 7, 15 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7.1 for additional details on the collection and processing of wound fluid samples.
12. Will be performed based on hyperspectral imaging capabilities at each site.
13. Physical characteristics of the wound (i.e., photos of the wound and wound measurement assessments) will be assessed at Screening (Optional Baseline Run In Visit), at pre-dose/equivalent time on Days 1, 4, 7, 11, 15 and at the 7-10 day Follow-up Visit.
14. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
15. Adverse events are to be collected from first dose through the final Follow-up visit.

REVISED TEXT

Procedures	**Screening**	**Optional Baseline Run In Visit[1]**	**Day 1**	**Day 4**	**Day 7**	**~~Day 11~~**	**Day 14**	**Day 15**	**~~Day 16~~**	**Follow-up**	
Visit Window (relative to Day 1)	-28 to -14 days		Exams/samples pre-dose							7-10 days post last dose	28-32 days post last dose
Clinic Visit	X	X	X	X	X	~~X~~	X	X	~~X~~	X	X
Informed Consent	X										
Demographics	X										
Complete physical	X										
Brief physical		X	X	X	X	~~X~~	X	X	~~X~~	X	X
Medical/medication/ drug/alcohol history	X	X									
12-lead ECG[2]	X		X					X			
Vital signs[3]	X		X		X			X		X	X
Urine pregnancy(females)	X		X							X	
Hema/Chem/ Safety Labs[4]	X		X		X			X		X	
Vascular Perfusion Eligibility Assessments[5]	X										
Blood samples for HIFα, (e.g., EPO, Hepcidin, VEGF)			X[6]		X			X			
Standard of Care Treatment[7]	X	X	←═══════════════════════════════════→							X	X
Study Treatment/Dosing In the Clinic[8]			X	X	X	~~X~~	X				

Procedures	**Screening**	**Optional Baseline Run In Visit**[1]	**Day 1**	**Day 4**	**Day 7**	~~**Day 11**~~	**Day 14**	**Day 15**	~~**Day 16**~~	**Follow-up**	
PK serial blood sampling[9]			X		X		X	X	~~X~~		
PK Trough Sample[9]						~~X~~					
Tissue Biopsies (gene expression, macrophage and bioburden analysis)[10]			X		X			X			
Wound Fluid (cytokine and bioburden analysis)[11]			X		X			X		X	
SensiLase (Doppler blood flow)			X		X			X		X	
~~Hyperspectral Imaging~~[12]			X		X			X		X	
Physical Characteristics (assessed pre-dose)[13]	X[11]	X	X	X	X	~~X~~		X		X	
Clinical Characteristics[14]	X		X	X	X	~~X~~	X	X	~~X~~	X	
Concomitant Medication Review			X	←==========→						X	X
Adverse Event Assessment[15]			X	←==========→						X	X

1. Optional Baseline Run In visit: This optional visit is for subjects that will exceed the 14 (±1 day) Run-in window between screening and Day 1.
2. Single ECGs will be taken at Screening and pre-dose on Day 1 and 24h post-last dose on Day15. ECGs should be taken while subject is supine for at least 5 minutes. Additional ECGs may be taken at the discretion of the investigator as needed based on symptoms or ECG findings.
3. Assessment of vital signs (including blood pressure and heart rate) will be performed at Screening and pre-dose on Days 1, 7, 15 (24h post-last dose), and at Follow-up Visits. Vital signs should be performed after resting in a supine or semi-supine position for at least 5 minutes.
4. Blood samples for safety will be collected fasting at Screening and pre-dose/equivalent time on Days 1, 7, 15 (24h post-last-dose), and at the 7-10 day Follow-up Visit. Refer to Section 7.2.4 for specific laboratory parameters to be tested.
5. See Item 7 in the Inclusion Criteria – DFU Subjects for acceptable perfusion parameters. Only 1 test of the 4 (TcPO2, ABI, toe pressure, Doppler ultrasound) is required for eligibility.
6. Blood samples for HIFα activation ~~on Day 7~~ will be collected at pre-dose~~, 8 and 10-12 hour window~~. ~~On all other indicated days one sample will be collected at pre-dose~~/equivalent time.
7. All subjects will be on SOC throughout the study (Section 7.4).
8. In between clinic visits, subjects will perform applications of study drug at home.
9. See separate Time and Events table for serial PK sampling details for Days 1, 7 and 14 and the associated 24~~and 48~~ hr time point~~s~~. ~~Trough sample will be taken prior to dosing on Day 11.~~ Refer to Section 7.12 for details on the collection and processing of PK samples. PK sampling times may be changed based on the observed GSK1278863 PK profile.
10. Direct wound tissue biopsies will be collected at pre-dose/equivalent on Days 1, 7, and 15, however based on emerging data, time points may be added or deleted; wounds that have healed sufficiently may be exempt. Refer to Section 7.7.2 for additional details on the collection and processing of tissue biopsies.
11. Wound fluid will be collected at pre-dose/equivalent on Days 1, 7, 15 and at the 7-10 day Follow-up Visit. Refer to the Section 7.7.1 for additional details on the collection and processing of wound fluid samples.
12. ~~Will be performed based on hyperspectral imaging capabilities at each site.~~
13. Physical characteristics of the wound (i.e., photos of the wound and wound measurement assessments) will be assessed at Screening (Optional Baseline Run In Visit), at pre-dose/equivalent time on Days 1, 4, 7,~~ 11~~, 15 and at the 7-10 day Follow-up Visit.
14. Clinical characteristic assessments of the DFU begin with Section 7.10 and contain instructions for completing the assessments including the irritation/symptom scale as needed. Assessments are recorded in the eCRF.
15. Adverse events are to be collected from first dose through the final Follow-up visit.

4.6.3. Serial PK Time and Events Table Part B

PREVIOUS TEXT

Procedure	**Part B Serial PK Sampling[1,2]**												
	Hours (h)												
	Pre-dose	**0.5h**	**1h**	**2h**	**4h**	**6h**	**8h**	**10h**	**11h**	**12h**	**24h**	**48h**	**72h**
Day 1	X	X	X	X	X	X	X	X[3]					
Day 7	X		X	X	X		X	X[3]					
Day 14 OUT-PATIENTS	X		X	X	X		X	X[3]			X	X	
Day 14 IN-PATIENTS	X		X	X	X		X			X	X	X	

REVISED TEXT

~~**Procedure**~~ **VISIT DAY**	**Part B Serial PK Sampling[1,2]**												
	Hours (h)												
	Pre-dose	**~~0.5h~~**	**1h**	**2h**	**4h**	**~~6h~~**	**8h**	**10h**	**11h**	**12h**	**24h**	**~~48h~~**	**~~72h~~**
Day 1	X	~~X~~	~~X~~	X	~~X~~	~~X~~	~~X~~	~~X[3]~~					
Day 7	X		~~X~~	X	~~X~~		~~X~~	~~X[3]~~					

~~Procedure~~ VISIT DAY	Part B Serial PK Sampling[1,2]												
	Hours (h)												
	Pre-dose	~~0.5h~~	1h	2h	4h	~~6h~~	8h	10h	11h	12h	24h	~~48h~~	~~72h~~
Day 14 OUT-PATIENTS	X		X	X	X		X	X[3]			X	~~X~~	
Day 14 IN-PATIENTS	X		X	X	X		X			X	X	~~X~~	

5.3.1. Inclusion Criteria – DFU Subjects; CRITERIA 3, 5, 6c

PREVIOUS TEXT

3. Single QTc < 450msec, or < 480msec in subjects with bundle branch block.
5. DFU between 1cm² and 16cm² at screening.
6. c. Ulcer size (area) ≥ 1cm^2 and ≤ 12cm^2 (post-debridement at time of randomization).

REVISED TEXT

3. Single QTc ~~< 450~~ **≤500msec**, or ~~< 480~~ **≤530**msec in subjects with bundle branch block. **No QTc exclusion for subjects with a predominantly paced rhythm.**
5. DFU between 1cm² and ~~16~~ **22**cm² at screening.
6. c. Ulcer size (area) ≥ 1cm^2 and ≤ ~~12~~ **16**cm^2 (post-debridement at time of randomization).

5.3.2. Exclusion Criteria – DFU Subjects; CRITERIA 2, 3, 5, 7, 8, 9, 10, 11, 12, 13

PREVIOUS TEXT

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) and/or any major changes (per investigator's judgment) to related medications within 6 months prior to randomization.
3. History or malignancy within 5 years of screening or those with a strong family history of cancer (e.g. familial cancer disorders), with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
5. Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within 6 months of randomization.
7. History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within 1 year prior to screening.
8. Active peptic, duodenal, or esophageal ulcer disease or any gastrointestinal bleeding, within 1 year prior to screening.
9. Subjects with a platelet count <100,000/mm^3 at screening.
10. Subjects with an International Normalized Ratio (INR) >1.1 at screening. If on warfarin, then >2.5.
11. Subjects with a hemoglobin level above the gender-specific upper limit of normal at screening.

12. Subjects with a history of non-traumatic joint inflammation (with the exception of inflammation due to osteoarthritis).
13. Patients with known pulmonary hypertension.

REVISED TEXT

2. Any unstable vascular syndromes (such as TIA, CVA, unstable angina, acute MI or ACS event) ~~and/or any major changes (per investigator's judgment) to related medications~~ within ~~6~~ **3** months prior to randomization.
3. ~~History or m~~Malignancy within ~~5~~ **2** years of screening ~~or those with a strong family history of cancer (e.g. familial cancer disorders),~~ with the exception of squamous cell or basal cell carcinoma of the skin that has been definitively treated.
5. ~~Patients with active treatment for retinal neovascularization (e.g., diabetic proliferative retinopathy or age related macular degeneration) within 6 months of randomization.~~
7. ~~History of venous thrombosis defined as deep vein thrombosis, pulmonary embolism or other venous thrombotic condition within 1 year prior to screening.~~
8. ~~Active peptic, duodenal, or esophageal ulcer disease or any gastrointestinal bleeding, within 1 year prior to screening.~~
9. ~~Subjects with a platelet count <100,000/mm^3 at screening.~~
10. ~~Subjects with an International Normalized Ratio (INR) >1.1 at screening. If on warfarin, then >2.5.~~
11. ~~Subjects with a hemoglobin level above the gender-specific upper limit of normal at screening.~~
12. ~~Subjects with a history of non-traumatic joint inflammation (with the exception of inflammation due to osteoarthritis).~~
13. ~~Patients with known pulmonary hypertension.~~

7.3.2. Part B

PREVIOUS TEXT

Blood samples for HIFα, (e.g., VEGF, EPO).
Biomarker Samples: (e.g., for cytokines and bioburden)
Gene Expression (mRNA) and Macrophages (Immunohistochemistry)Tissue Samples
Heavy Water (Site involvement will depend on site feasibility – selected subjects (one cohort) based on emerging data) (Repeat Dosing only.) Tissue samples via biopsy.
SensiLase (Dopplar blood flow)
Hyperspectral Imaging (oxygen dynamics)

Physical Indicators (e.g., photos and wound measurement). Silhouette for volume (record in eCRF) Digital photos (core) and acetate tracings (after screening scan and upload and send to core).
Clinical Indicators
Irritation/symptom Scale

REVISED TEXT

Blood samples for HIFα, (e.g., VEGF, EPO).
Biomarker Samples: (e.g., for cytokines and bioburden)
Gene Expression (mRNA) and Macrophages (Immunohistochemistry)Tissue Samples
Heavy Water (Site involvement will depend on site feasibility – selected subjects (one cohort) based on emerging data) (Repeat Dosing only.) Tissue samples via biopsy.
SensiLase (Dopplar blood flow)
Hyperspectral Imaging (oxygen dynamics) **– if the technique can be established/validated. (Site involvement will depend on site feasibility; selected subjects (one cohort) based on emerging data) (Repeat Dosing only.)**
Physical Indicators (e.g., photos and wound measurement). Silhouette for volume (record in eCRF) Digital photos (core) and acetate tracings (after screening scan and upload and send to core).
Clinical Indicators
Irritation/symptom Scale

7.8.1.1. Instructions for Assessing Vascular Perfusion with SensiLase

PREVIOUS TEXT

The subject is placed in a supine position. The laser wand is placed in the guide and the guide is placed adjacent to the wound, as close to the peri-wound area as possible while remaining on completely epithelialized tissue. A disposable cuff liner is placed on the cuff and the cuff is then placed snugly but not overly tight around the laser guide. One or two readings are to be taken: The first reading is to be taken at 12:00, with 12:00 being the most proximal edge of the wound. The next reading is also to be taken at the proximal side of the wound but within the angiosome tissue block, if position one is not within the angiosome tissue block. Angiosomes are designated locations found in the instruction manual for SensiLase.

REVISED TEXT

The subject is placed in a supine position. The laser wand is placed in the guide and the guide is placed adjacent to the wound, as close to the peri-wound area as possible while remaining on completely epithelialized tissue. A disposable cuff liner is placed on the cuff and the cuff is then placed snugly but not overly tight around the laser guide. One or two readings are to be taken: The first reading is to be taken at 12:00, with 12:00 being the most proximal edge of the wound. The next reading is also to be taken at the proximal side of the wound but within the angiosome tissue block, if position one is not

within the angiosome tissue block. **All relevant angiosomes necessary for appropriate interpretation of blood flow should also be captured.** Angiosomes are designated locations found in the instruction manual for SensiLase.

7.8.2. Oxygen Dynamics/Angiogenesis

PREVIOUS TEXT

Oxygen dynamics and angiogenesis may be assessed by hyperspectral imaging as site feasibility allows.

REVISED TEXT

Oxygen dynamics and angiogenesis may be assessed by hyperspectral imaging as site feasibility allows **and if the technique can be established/validated.**

7.12.3. PK Sampling Windows; BULLET POINT #4

PREVIOUS TEXT

- Within ± 3 hours of target time for the 48 and 72 hour post-dose PK sample

REVISED TEXT

- Within ± 3 hours of target time for the 48 ~~and 72~~ hour post-dose PK sample

9.1. Prohibited Medications; PARAGRAPH 2

PREVIOUS TEXT

Diabetic subjects must abstain from taking potent CYP2C8 inhibitors and inducers such as fluvoxamine, gemfibrozil, oral ketoconazole, trimethoprim, supplements containing quercetin and rifampin/rifampicin. This is because the primary route of metabolism of GSK1278863 involves CYP2C8. Data from a study (PHI113634) to assess the interaction potential between GSK1278863 and a CYP2C8 inhibitor (gemfibrozil) showed a clinically significant increase in GSK1278863 exposure following co administration. Therefore, inhibitors of CYP2C8 are prohibited from 7 days prior to the first dose of investigational product until 7 days after the last dose of investigational product and inducers of CYP2C8 are prohibited from 14 days prior to the first dose of investigational product until 7 days after the last dose of investigational product.

REVISED TEXT

~~Diabetic subjects must abstain from taking potent CYP2C8 inhibitors and inducers such as fluvoxamine, gemfibrozil, oral ketoconazole, trimethoprim, supplements containing quercetin and rifampin/rifampicin.~~ **Diabetic subjects must abstain from taking potent CYP2C8 inhibitors and inducers such as gemfibrozil and rifampin/rifampicin .** This is because the primary route of metabolism of GSK1278863 involves CYP2C8. Data from a study (PHI113634) to assess the interaction potential between GSK1278863 and a CYP2C8 inhibitor (gemfibrozil) showed a clinically significant increase in GSK1278863 exposure following co-administration. Therefore, inhibitors of CYP2C8 are prohibited from 7 days prior to the first dose of investigational product until 7 days after the last dose of investigational product and inducers of CYP2C8 are prohibited from 14 days prior to the first dose of investigational product until 7 days after the last dose of investigational product.

7.13.1. Time Period for Collecting Pregnancy Information

ADDED TEXT

Information on all pregnancies in female subjects will be collected after the start of dosing and through follow-up.

AMENDMENT 8

Where the Amendment Applies

This amendment applies to all sites participating in this study.

Summary of Amendment Changes with Rationale

This amendment contains the addition of an exclusion criterion for lactating or nursing females. This change was necessary since data on prenatal and post natal development are currently not available. The rationale for changes made in Amendment 7 was also updated due to omission in the previous amendment.

List of Specific Changes

5.3.2. Exclusion Criteria – DFU Subjects; CRITERIA 11

PREVIOUS TEXT

11. Pregnant females as determined by positive urine hCG test at screening or prior to dosing.

REVISED TEXT

11. Pregnant females as determined by positive urine hCG test at screening or prior to dosing **or lactating/nursing females**.

16.3 Appendix 3: Protocol Amendment Changes, Amendment 7, Summary of Changes with Rationale

PREVIOUS TEXT

This amendment contains modifications to inclusion criteria 3, 5 and 6 and exclusion criteria 2, 3. Exclusion criteria 5 and 7 -13 were removed. Other changes include updates to Part B that occurred in stream based on emerging data, but did not require a protocol amendment. Other minor edits for clarity.

REVISED TEXT

This amendment contains modifications to inclusion criteria 3, 5 and 6 and exclusion criteria 2, 3. Exclusion criteria 5 and 7 -13 were removed.

Rationale for Inclusion Criterion 3: The results of a thorough QT study utilizing oral doses of GSK1278863 support the conclusion that there appears to be no clinically significant effect on ΔΔQTcF after administration of doses up to 500 mg in healthy subjects. (GSK Document #2015N233454_00 PHI113635 14Dec 2015). These results, in addition to the limited systemic exposure demonstrated with topical application of GSK1278863, support the use of expanded QT enrolment criteria.

Rationale for Inclusion Criterion 5 and 6c: Systemic concentrations of GSK1278863 measured from patients administered 1% w/w (at 100mg/cm2) have been below the LLOQ of the analytical assay (0.10 ng/mL). A larger upper limit of screening/randomization ulcer size results in maximum systemic exposure predictions (following once daily application of ointment strengths up to 10% w/w GSK1278863 (at 100 mg/cm2) for 14 days) well below this study's intended systemic exposure limits (395 ng.h/mL and 198 ng/mL for AUC and Cmax, respectively). This prediction supports an increase in allowable ulcer size.

Rationale for Exclusion Criterion 2, 3, 5, 7-13: As of June 2016, GSK1278863 has been administered orally to over 1000 subjects, including those with chronic kidney disease (either not on dialysis or on dialysis), in single doses up to 500 mg, in repeat-doses up to 100 mg daily for 4 weeks and up to 25 mg daily for 24 weeks. Overall, the safety profile was consistent with the patient population and there were no adverse events that have been identified as related to treatment with GSK1278863. Planned phase 3 studies have thus adopted revised exclusion criteria consistent with those above in patients with anemia due to chronic kidney disease, a vulnerable population with respect to vascular events.

Systemic concentrations of GSK1278863 measured from DFU subjects administered 1% w/w (at 100mg/cm2) have been below the LLOQ of the analytical assay (0.10 ng/mL) and systemic exposures following once daily application of ointment strengths up to 10% w/w GSK1278863 (at 100 mg/cm2) in DFU subjects are predicted to be well below this study's intended systemic exposure limits (395 ng.h/mL and 198 ng/mL for AUC and Cmax, respectively).

The decision to modify the exclusion criteria was therefore based on the safety profile demonstrated in the oral program in patients with a similar or greater risk profile who are exposed to systemic concentrations of GSK1278863 which are well above those in the topical program.

Stopping and withdrawal criteria were also modified to align with the updated inclusion/exclusion criteria as appropriate.

Other changes include updates to Part B that occurred in stream based on emerging data, but did not require a protocol amendment. Other minor edits for clarity.